Texas Forensic Science Commission

Justice Through Science

FINAL AUDIT REPORT FOR AUSTIN POLICE DEPARTMENT FORENSIC SERVICES DIVISION DNA SECTION

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I. SUMMARY OF APPLICABLE STATUTORY AUTHORITY

A. Legislative Background and Jurisdiction

The Texas Legislature created the Texas Forensic Science Commission ("Commission") during the 79th Legislative Session by passing House Bill 1068 (the "Act"). The Act amended the Texas Code of Criminal Procedure to add Article 38.01, which describes the composition and authority of the Commission.[1] During the 83rd and 84th Sessions, the Legislature further amended the Code of Criminal Procedure to clarify the Commission's jurisdictional authority.[2]

The Commission has nine members appointed by the Governor of Texas. Seven commissioners are scientists and two are attorneys (one prosecutor nominated by the Texas District and County Attorney's Association, and one defense attorney nominated by the Texas Criminal Defense Lawyer's Association). The Commission's Presiding Officer is Dr. Vincent J.M. Di Maio, as designated by the Governor.

1. Investigative Jurisdiction

Texas law requires the Commission to "investigate, in a timely manner, any allegation of professional negligence or professional misconduct that would substantially affect the integrity of the results of a forensic analysis conducted by an accredited laboratory, facility or entity."[3] The Act also requires the Commission to: (1) implement a reporting system through which accredited laboratories, facilities or entities may report professional negligence or professional

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[1] *See* Act of May 30, 2005, 79th Leg., R.S., ch. 1224, § 1, 2005.

[2] *See* Acts 2013, 83rd Leg., ch. 782 (S.B.1238), §§ 1 to 4, eff. June 14, 2013; Acts 2015, 84th Leg., ch. 1276 (S.B.1287), §§ 1 to 7, eff. September 1, 2015, (except TEX. CODE CRIM. PROC. art. 38.01 § 4-a(b) which takes effect January 1, 2019).

[3] TEX. CODE CRIM. PROC. art. 38.01 § 4(a)(3).

misconduct; *and* (2) require all laboratories, facilities or entities that conduct forensic analyses to report professional negligence or misconduct to the Commission.[4]

2. Accreditation Jurisdiction

The Commission is charged with accrediting crime laboratories that conduct forensic analyses of physical evidence for use in criminal proceedings.[5] The Commission's decision to recognize a particular laboratory as accredited is based upon the laboratory's accreditation status with certain approved national accrediting bodies.[6] Texas law exempts some forensic disciplines from the accreditation requirement—either by statute, by administrative rule, or by determination of the Commission.[7] Unless a forensic analysis is accredited or falls under an exemption, the evidence is not admissible in a criminal action in Texas courts.[8]

3. Important Limitations on the Commission's Authority

The Commission's authority contains important statutory limitations. For example, no finding by the Commission constitutes a comment upon the guilt or innocence of any individual.[9] The Commission's written reports are not admissible in civil or criminal actions.[10] The Commission also does not have the authority to issue fines or administrative penalties against any individual, laboratory or entity. The information the Commission receives during the course of any investigation is dependent upon the willingness of stakeholders to submit relevant documents and respond to questions posed. The information gathered in this report has ***not*** been

[4] *Id.* at §§ 4(a)(1) and 4(a)(2).

[5] TEX. CODE CRIM. PROC. art. 38.01 § 4-d(b).

[6] *See* 37 TEX. ADMIN. CODE Pt. 15 § 651.4

[7] *See* TEX. CODE CRIM. PROC. art. 38.35 § (a)(4); 37 TEX. ADMIN. CODE §§ 651.5 - 651.7; and TEX. CODE CRIM. PROC. art. 38.01 § 4-d(c).

[8] *See* TEX. CODE CRIM. PROC. art. 38.35 § (d)(1).

[9] *See* TEX. CODE CRIM. PROC. 38.01 at § 4(g).

[10] *Id*. at § 11.

subjected to the standards for admission of evidence in a courtroom. For example, no individual testified under oath, was limited by either the Texas or Federal Rules of Evidence (*e.g.*, against the admission of hearsay) or was subjected to cross-examination under a judge's supervision.

II. BACKGROUND

This report contains observations and recommendations of the Commission regarding the Austin Police Department Forensic Science Division's DNA Section ("APD DNA Lab") resulting from a May 2016 site audit conducted by the Commission and the American Society of Crime Laboratory Directors/Laboratory Accreditation Board ("ASCLD/LAB"). Lynn Garcia, the Commission's General Counsel and Dr. Bruce Budowle, Director of the University of North Texas Health Science Center's Center for Human Identification represented the Commission at the audit. (*See* Budowle CV at **Exhibit A**.) D. Jody Koehler, an ASCLD/LAB Assessor and the Manager of the DNA Section of the Department of Public Safety's Austin Laboratory represented ASCLD/LAB. (*See* Koehler CV at **Exhibit B**.) Because many of the issues identified during the audit stemmed from a broader statewide review of DNA mixture interpretation protocols and casework conducted by the Commission in December 2015, this report begins with basic educational information on DNA mixtures. It also provides historical background on developments statewide from May 2015 to the present before addressing the particular issues of concern in the APD DNA Lab.

A. DNA Mixture Interpretation Basics

The purpose of this section is to educate the criminal justice community (including non-scientists) on issues in DNA mixture interpretation. It favors simplicity over comprehensive technical and scientific detail. For a more thorough and scientifically rigorous explanation of

various considerations in DNA mixture interpretation, the Commission commends the reader's attention to the Reading List attached to this report as **Exhibit C**.

When a laboratory analyzes crime scene evidence to determine whether any DNA is present and whether it can be linked to a particular individual, it typically answers four important questions:

1. Is biological evidence present?
2. Could human DNA be extracted from the evidence?
3. If so, are the data of sufficient quality to enable reliable interpretation regarding who may have contributed, or who did not contribute the DNA to that piece of evidence?
4. If the known person cannot be excluded, what statistical significance can be placed on the possibility he or she actually contributed the DNA to that piece of evidence?

Depending on the case facts, the answer to the third and fourth questions may be critical to a jury's perception of innocence or guilt. For example, in a sexual assault where the assailant is unknown to the victim and little other physical evidence exists, the laboratory's conclusions regarding the relative strength of an association are extremely important. If the laboratory issues a report stating that 1 in 364 quadrillion people would be expected to have the same DNA profile as reported for a particular piece of evidence, such a massive statistic exceeding the inverse of the world's population provides a strong signal to the jury that it was most likely the suspect who contributed the DNA. But what about a statistic of 1 in 3,640 or 1 in 364 or 1 in 36? A prosecutor could attempt to use any of these statistics to persuade the jury that the suspect contributed the DNA to the evidence. However, as the statistical strength of the "match" decreases, the jury may be left with a weaker and weaker impression of whether the suspect's DNA is the only explanation for the evidence. Supportable statistics that *do not overstate the strength of the evidentiary data* are critical to the fair administration of justice. Historically, the

forensic DNA community has taken the view that understating the evidentiary data is acceptable and indeed preferable to overstating the significance of the results. Notwithstanding this cautionary position, laboratories should strive not to ignore meaningful evidentiary data when available and considered reliable and suitable for inclusion.

Laboratories calculate DNA statistics using commonly accepted statistical methods.[11] A laboratory selects which statistical method to use based on its analysts' ability to understand the method, its own internal protocols and resources, and the relative complexity of the evidence, including such issues as whether the analyst can determine how many people contributed DNA to the item in question and whether one contributor can be isolated as a single major or minor component of the mixture. Once the analyst decides how the data should be interpreted based on the statistical method applied, he or she uses data from population databases to calculate the particular statistic for inclusion in the report.

One key source of population data for these purposes is the FBI's allele frequency data on several population groups, which many crime laboratories use when analyzing DNA cases. In May 2015, the FBI notified the public that it had identified and corrected some minor errors in its population statistics. The revisions were attributable to human error in data entry and technology limitations at the time the database was created in the 1990's, but they were not expected to change any results from inclusion to an exclusion (or vice versa), or to have anything but a small and insignificant impact on the statistics provided in criminal cases. Indeed, empirical studies in and outside of Texas showed the statistical differences were minor. (*See* **Exhibit S**.) Nevertheless, in an abundance of caution, the Department of Public Safety and other publicly

[11] Commonly accepted statistical methods include the Random Match Probability, modified Random Match Probability, Likelihood Ratio and the Combined Probability of Inclusion/Exclusion (CPI/CPE). Each of these methods provides different information to the trier of fact. The community's recognition of widespread misunderstanding across the United States regarding the proper application of the CPI/CPE is what led to the statewide review of DNA mixture protocols and case samples for publicly funded laboratories in Texas.

funded laboratories in Texas sent notifications to the criminal justice community offering to re-calculate statistics upon request. (*See e.g.,* **Exhibit D**.)

In response to the notice, some prosecutors accepted the offer for recalculation of statistics in pending cases, not expecting any significant difference but making the request in an abundance of caution. Upon receiving the revised reports, some of these prosecutors observed significant statistical changes in certain items of evidence involving DNA mixtures. Having expected no meaningful changes at all, the prosecutors contacted the labs and sought the Commission's help in understanding what happened.

The laboratories explained that the significant statistical changes were not attributable to FBI database corrections, but rather to the fact that the evidence in these cases had been originally analyzed *before* the laboratories made certain important revisions to their mixture interpretation protocols to comply with recommendations made by the Scientific Working Group on DNA Analysis Methods (SWGDAM) in 2010 (*See* **Exhibit E**),[12] as well as to reflect the community's evolving understanding of how to properly apply statistical methods to increasingly complex biological samples. Though the process of DNA typing is based on sound science, a degree of subjective interpretation is required when analyzing DNA profiles containing multiple contributors and deciding which loci to include in statistical analysis, as well as what statistical weight to afford an association (if any). To address these challenges, forensic DNA analysts must have extensive expertise in the principles of profile interpretation and an appreciation for the complexity of the samples and the increasing possibility of missing data (allele dropout and other stochastic effects). This is especially true as the number of contributors increases and the

[12] *See* Scientific Working Group on DNA Analysis Methods (SWGDAM), *SWGDAM Interpretation Guidelines for Autosomal STR Typing by Forensic DNA Testing Laboratories* (2010), Available online as of July 3, 2016 at: https://www.fbi.gov/about-us/lab/biometric-analysis/codis/swgdam-interpretation-guidelines

quality of the profile decreases (due to low amounts of DNA, DNA degradation, PCR inhibition etc.). Forensic DNA analysts also require an understanding of key concepts in population genetics and statistics.

B. The Technical Issue in a Nutshell

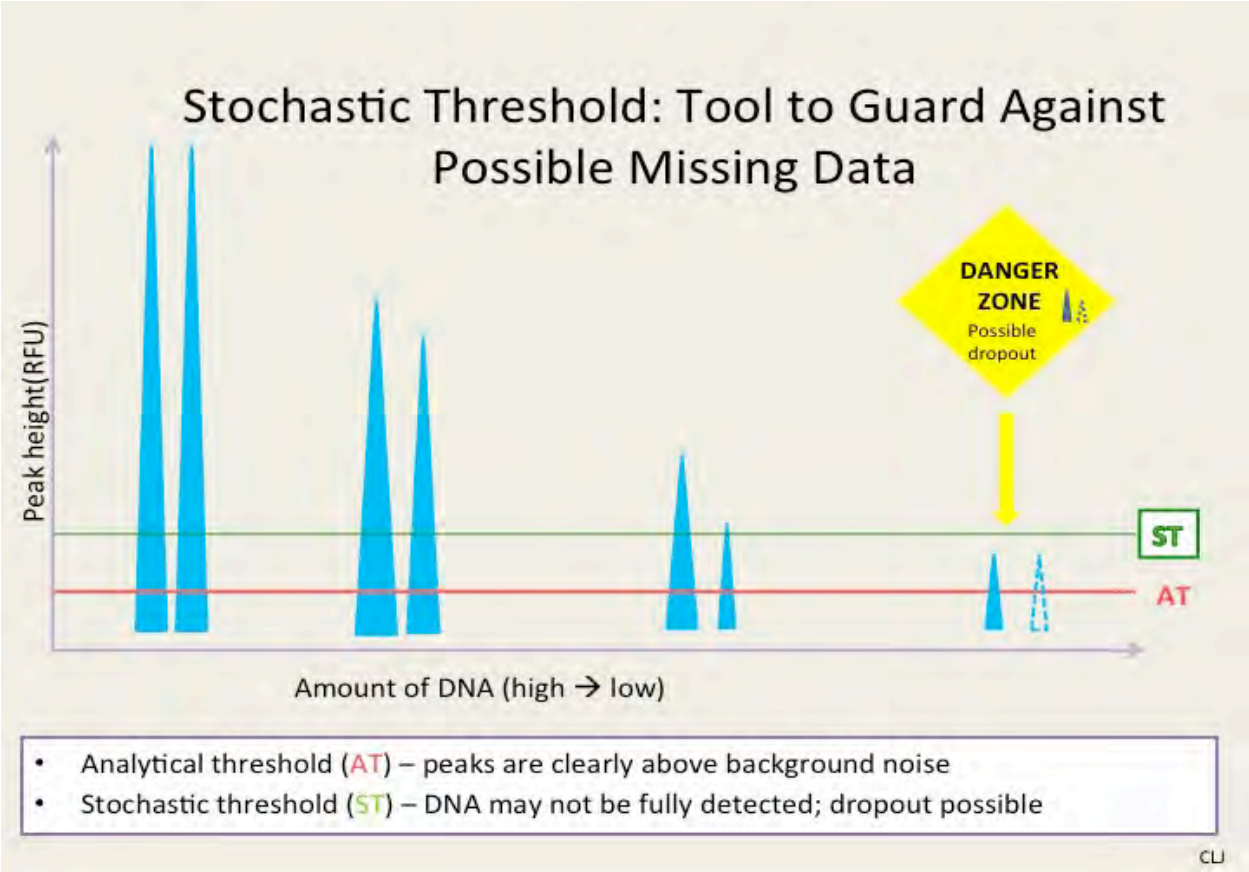
Among other things, the SWGDAM 2010 guidelines recommended that laboratories adopt two thresholds for evaluating DNA mixture data. The first threshold is an analytical threshold ("AT") which is used to distinguish between real DNA peaks and peaks that may be "noise" or artifacts occurring during the amplification and/or capillary electrophoresis processes. The second threshold is called a stochastic threshold ("ST"). The ST is higher than the AT and serves as a tool to help analysts have confidence that all data (alleles) are present in a particular sample, and to identify the potential for missing data in mixtures (and single source samples), often referred to as "allele dropout" or "allelic dropout." In a pristine evidentiary mixture sample where all DNA contributors are fully represented at all loci, an analyst would expect to find two alleles for each individual contributing DNA at each locus.[13] The problem is that crime scene evidence is rarely pristine. In addition, many factors including allele sharing between/among contributors, degradation, artifacts and other issues that occur during amplification of the DNA make it possible for some of the alleles to drop out of the data or be masked by shared alleles (overlapping peaks). The more alleles (peaks) observed at a locus, the greater the number of likely contributors to the mixture, and the more challenging it becomes to deconvolve the mixture, identify the individual contributors to that mixture, and flag the loci at which allele dropout and other stochastic effects may have occurred.

[13] The reason each individual has two alleles is because a person receives one allele from his/her mother and one from his/her father. Sometimes the two alleles received from the parents at a given locus are the same, which analysts refer to as a homozygote. The way analysts know the peak is homozygous is by the height of the peak—it is roughly double the height of what the individual alleles would have been if they were two distinct alleles (known as a heterozygote).

Because of these complexities, the SWGDAM 2010 guidelines recommended laboratories use a dual threshold as a tool for identifying possible missing data (*i.e.*, allele dropout). The dual threshold is pictured in the figure below.[14] The area between the AT (red line) and the ST (green line) is considered a zone of caution for interpretation because a peak found between these two lines may be missing its partner allele. When a partner allele may be missing, additional explanations for who contributed the DNA at the locus should be considered.

In the figure below, the yellow diamond points to a locus for which one allele appears as a blue peak (triangle) with a height falling in the "caution zone" between the AT and ST. Next to this peak is a second peak with dotted lines. This dotted peak represents the partner allele that dropped out (and would not be seen in the electropherogram). This allele does exist, but does not show up on the mock electropherogram because it was not successfully amplified during PCR due to degradation, inhibition, or stochastic effects.

[14] The Commission is grateful to Cassie L. Johnson at the Fort Worth Police Department Crime Laboratory DNA Section for granting permission to use this graphic.



To reiterate, the purpose for having a dual threshold is to provide analysts a tool for flagging situations in which data may be missing (such as the second partner allele in the far right of the graphic). Thus, anytime data appear between the AT and the ST, an analyst should proceed with caution and take great care in assessing all probable explanations considering the quality of the profile in its entirety.

It should be noted that a number of peer-reviewed journal articles discussed the concept of allele dropout well before the 2010 SWGDAM guidelines were published.[15] Some Texas

[15] *See e.g.*, Curran JM, Buckleton JS, "Inclusion Probabilities and Dropout," *Journal of Forensic Sciences* 55: 1171-1173 (2010); Budowle B, Onorato AJ, Callagham TF, Manna AD, Gross AM, et al. "Mixture Interpretation: Defining the Relevant Features for Guidelines for the Assessment of Mixed DNA Profiles in Forensic Casework," *Journal of Forensic Sciences* 54:810-821 (2009); Moretti T, Baumstark AL, Defenbaugh BS, Keys KM, Smerick JB, Budowle B. "Validation of Short Tandem Repeats (STRs) for Forensic Usage: Performance Testing of Fluorescent Multiplex STR Systems and Analysis of Authentic and Simulated Forensic Samples," *Journal of Forensic Sciences* 46:647-60 (2001); Moretti TR, Baumstark AL, Defenbaugh DA, Keys KM, Brown AL, Budowle B., "Validation of STR Tying by Capillary Electrophoresis," *Journal of Forensic Sciences*, 46:661-76 (2001); Gill

laboratories used dual thresholds even before the SWGDAM guidelines were published in 2010, but the majority adopted a dual threshold at some point between 2010 and 2015. The problem is that the primary statistical method used by many laboratories to calculate mixture statistics—the Combined Probability of Inclusion (CPI) — prohibits the use of any locus with a possibility of allele dropout. If the laboratory did not have sufficient tools in place to evaluate the possibility of dropout, analysts may have included a locus (or loci) that should have been excluded from the statistical calculations. Whenever a locus is eliminated from use, the resulting statistic becomes weaker. Once the laboratories incorporated tools to flag potential dropout and disregarded loci where dropout was possible (when previously they were included), the statistics changed in some cases, and in a few cases they changed several orders of magnitude (*e.g.*, from 1 in 1.4 billion to 1 in 38 or from 1 in 4,000 to uninterpretable). Those changes were reflected in revised reports and triggered notice and disclosure obligations by prosecutors as well as requests for Commission assistance in understanding key issues.

C. Commission Statewide Review: Training, Protocol and Case Sample Reviews

The results of the Texas recalculation requests raised awareness among lawyers and judges about issues that have been of concern within the forensic DNA community not just in Texas but nationwide (and indeed worldwide) for many years—DNA mixture interpretation is challenging and laboratories have not always interpreted complex mixtures properly. There can be substantial variation in the methods used for mixture interpretation from laboratory to laboratory and even within laboratories. Guidance and education on proper mixture

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P, Whitaker JP, Flaxman C, Brown N, Buckleton JS "An Investigation of the Rigor of Interpretation Rules for STR's Derived from less than 100 pg of DNA," *Forensic Science International* 112: 17-40 (2000); Budowle B, Lindsey JA, DeCou JA, Koons BW, Giusti AM, Comey CT, "Validation and Population Studies of the Loci LDLR, GYPA, HBGG, D7S8, and Gc (PM loci) and HLA-DQ-Alpha Using a Multiplex Amplification and Typing Procedure," *Journal of Forensic Sciences* 40:45-54 (1995); Walsh PS, Erlich HA, Higuchi R "Preferential PCR Amplification of Alleles: Mechanisms and Solutions," *PCR Methods Appl* 1: 241-250 (1992).

interpretation are contained in various journal publications, but the translation to the forensic community has not been as effective as one would hope. Efforts by the National Institute of Standards and Technology ("NIST") to train laboratories and raise red flags regarding DNA mixture interpretation problems they observed in two major studies (MIX05 and MIX13) have been extensive.[16] However, because NIST is not a regulatory or oversight body, its scientists are not in a position to determine whether the information they convey to analysts during conference presentations, workshops and training sessions is taken back to the laboratory and integrated in day-to-day casework.

In an attempt to address the potential impact of these issues within forensic DNA laboratories within the state of Texas, the Commission sought the assistance of DNA expert Dr. Bruce Budowle, Director of the University of North Texas Health Science Center's Center for Human Identification.[17] During November and December 2015, Dr. Budowle reviewed the DNA mixture protocols and mixture cases from publicly funded crime laboratories in Texas. In November and January 2015, the Commission also received further assistance from Drs. John Buckleton[18] and Simone Gittelson[19] who provided training regarding complex issues in mixture interpretation and statistical evaluation to DNA analysts working in crime laboratories in Texas.

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[16] Butler, John M., *Advanced Topics in DNA Typing: Interpretation,* Academic Press/Elsevier (2014) at 150-153.

[17] The Commission is grateful to Dr. Budowle for the countless hours he has provided to assist the Commission with both the statewide review and issues specific to the APD DNA Lab. Commission Staff also consulted at various points with internationally renowned experts including Drs. Frederick Bieber, John Buckleton, John Butler, Michael Coble, Simone Gittelson and Angela van Daal. The Commission owes a debt of gratitude to these experts for sharing their time and insight in an effort to improve the quality of forensic DNA analysis in Texas and nationwide.

[18] Dr. Buckleton is currently a guest researcher with NIST on temporary assignment from his permanent position as a Principal Scientist at the Institute of Environmental Science and Research in Auckland, New Zealand.

[19] Dr. Gittelson is a mathematical statistician in the Statistical Engineering Division of NIST with specialized expertise in forensic statistics, Bayesian inference, and decision theory.

After reviewing protocols and case examples, Dr. Budowle observed common issues in many (but not all) laboratory casework, including lack of incorporation of a dual threshold to evaluate stochastic effects in some cases, a misunderstanding of how to flag potential allele dropout in some cases, and protocols that were not robust enough to allow the laboratory to make the best possible use of available data through scientifically acceptable methods of re-introducing loci, subtracting assumed donors from intimate/indigenous samples, or isolating major/minor contributors.

These interpretive challenges are common and widespread within the forensic DNA community, and the vast majority of Texas laboratories immediately revised their protocols to incorporate the feedback they received during the review process. In addition, a statewide legal triage system was established with the support of the Texas Commission on Indigent Defense to ensure that any cases meriting reinterpretation are reviewed by lawyers and scientists equipped to identify potential issues and seek legal relief where appropriate.[20]

III. ISSUES SPECIFIC TO THE APD DNA LAB

A. Observations Related to DNA Mixture Interpretation

1. Establishment & Continuation of Quantification-Based Stochastic Threshold

Beginning in April 2010, the APD DNA Lab adopted a stochastic threshold (ST). However, unlike other laboratories that adopted a ST, the APD DNA Lab used the estimated quantity of input DNA into the amplification reaction as the primary method for determining potential stochastic effects such as allele dropout and did not account for allele stacking/sharing, stutter contribution, etc. This approach is referred to throughout this report as the APD DNA Lab's "quant-based ST," and was the primary catalyst for the site audit. Using a quant-based ST

[20] For information regarding the statewide mixture triage process, contact project director Bob Wicoff at Bob.Wicoff@pdo.hctx.net.

to determine potential stochastic effects in DNA mixtures is neither scientifically valid nor supported by the forensic DNA community. The review team is not aware of any peer-reviewed journal article citing the acceptance of a quant-based ST for mixture interpretation.

In adopting and continuing the use of a quant-based ST from 2010 to 2016, Technical Leaders (TLs) Jeff Sailus and Cassie Carradine and senior analysts in the APD DNA Lab appear to have interpreted language from the SWGDAM 2010 Mixture Guidelines referenced above, as well as from Dr. John M. Butler's textbook, "Advanced Topics in Forensic DNA Typing: Methodology" (*See* **Exhibit F**) as supporting the use of a quant-based ST for assessing stochastic effects in DNA mixture profiles. The significant confusion in the forensic DNA community regarding mixture interpretation from the inception of PCR-based methods in the mid-late 1990's to the present, coupled with audits by ASCLD/LAB and QAS during which the APD DNA Lab's quant-based ST was not flagged, contributed to the laboratory's misunderstanding. *Of greater concern, the analysts themselves were aware the quant-based ST was ineffective because they observed stochastic effects (e.g., allele dropout) in their casework even when the quantity of DNA in the sample exceeded the laboratory's own quant-based ST.* Indeed, Dr. Budowle and Ms. Koehler observed allele dropout above the quant-based ST in over 1/3 of the cases they reviewed.

While analysis of DNA quantitation is one step in determining whether to proceed with Polymerase Chain Reaction (PCR) amplification or determine how much DNA to place in an amplification reaction, the quantity of DNA is not an appropriate metric to assess potential stochastic effects that occur during amplification for DNA mixture evidence. An appropriate ST at the interpretation stage is based on the amount of the signal for each allele, measured in

relative fluorescence units (RFUs), which describes peak height and is captured on the resultant electropherogram.

As discussed earlier in this report, laboratories both in and outside of Texas may not have always employed a dual threshold to evaluate evidentiary profiles. However, the Commission identified no other laboratory that employed a quant-based ST after SWGDAM recommended in 2010 that a ST be incorporated as a tool for guarding against possible stochastic effects leading to allele dropout. Moreover, the APD DNA Lab continued to defend its decision to use the quant-based ST based on language cited from the SWGDAM 2010 guidelines and Dr. Butler's textbook. This was after being informed multiple times that the language was not meant to endorse the use of a quant-based ST for mixtures and the approach was scientifically indefensible. (*See e.g.,* **Exhibit G**.) The Lab also defended its SOP despite the fact that the analysts had raised concerns with both TLs that the quant-based ST was ineffective in casework. During interviews, the analysts explained that TL Sailus understood the concerns they raised regarding the quant-based ST and other issues and had begun efforts to optimize the laboratory's chemistry and update mixture interpretation methods to ensure the most accurate possible DNA statistics. (*See* **Exhibit H**.)

2. Validation Studies Lacked Data to Support Quant-Based Threshold

During the site audit, the team reviewed the APD DNA Lab's validation study dated April 26, 2010 which should have provided data-driven support for the quant-based ST used by the laboratory. (*See* **Exhibit I**.) However, the team's review found the validation study lacked sufficient data to support the selection of any ST. Only three samples were used with nine

different dilutions (1.75-0.0029296 ng).[21] The dilutions were incorrectly prepared, with the individual transferring sample volumes of 0.005 μL[22] of sample for amplification set-up. Attempting to transfer such small volumes will result in incorrect amounts of material being placed into reactions because the sample volume is too low to be accurately measured utilizing the tools available in the laboratory. These insufficiencies resulted in a quant-based ST validation study that was not supportable (and could not support any ST approach). The inadequate outcome was further demonstrated by subsequent analyst observations of stochastic effects even in cases where the quantity of DNA was higher than the very quant-based ST established as result of the improper validation study.

3. CPI Decisions Driven by the Known Profile (Suspect or Victim)

Analysts conducted an initial review of evidentiary profiles before reviewing suspect or victim reference profiles. Thus, the analysts believed there was no confirmation or other contextual bias in their interpretation of mixture evidence. However, the analysts decided whether a locus would be used for statistical calculations depending upon the alleles observed in the known profile (whether suspect or victim). The analysts did not determine *a priori* which loci had a high probability of allele dropout. Instead, they compared the evidence and known reference profiles, and then selected the loci to be used for statistical calculations based on whether the suspect or victim had "dropped out" at a particular locus. If an allele was missing, they invoked "allele dropout" as a reason for not observing the allele and then did not use the locus for statistical purposes. The appropriate approach is to *decide which locus (or loci) should be used first* based on whether there may be potential allele dropout *as indicated by analysis of*

[21] The team has concerns about whether the analysts performing the study understood the appropriate use of significant figures. The term "significant figure" refers to the number of important digits in an expression of scientific notation and indicates the confidence or precision with which a scientist states a quantity.

[22] One would think this is a typographical error and that it should be 0.5 μL because it is not possible to pipette such a small amount. However, this figure was taken directly from the validation data. (*See Exhibit I* at 10.)

the evidentiary sample in its entirety, not on which alleles are present or absent based on the victim or suspect reference profiles.

Dr. Budowle and Ms. Koehler observed results in 1/3 of the cases reviewed for which the only plausible explanation is that the analysts made decisions about the presence or absence of allele dropout based on the alleles in the known profile, contrary to the language in the SOP (*See* **Exhibit J**). This approach, commonly referred to as "suspect driven bias" was observed in the casework generally and was not limited to a particular analyst or analysts. It also should be noted that the interpretation bias described in this section is not unique to the APD DNA Lab. For example, a recent ASCLD/LAB review of the Sheriff's Crime Laboratory in Broward County, Florida highlighted similar issues in casework. (*See* **Exhibit K**.) These issues were also observed in April 2015 audits of the Department of Forensic Science in Washington, DC. (*See* **Exhibit L**.)

4. Unclear Use of Protocol Deviation

Setting aside the question of whether the use of a quant-based ST is scientifically supportable, Dr. Budowle and Ms. Koehler reviewed 26 cases in an attempt to confirm whether the analysts had correctly applied their quant-based ST as described in the SOP. In one case, the review team observed a deviation from protocol that did not appear to be supported by the documentation in the case file. The item in question had a quantity of DNA at 0.05025 ng (as recorded by the analyst), which was amplified, interpreted and reported. This amount of DNA is lower than the quant-based ST established in the APD DNA Lab's SOP. (*See* **Exhibit M**.) According to the SOP, for DNA quantities amplified below 0.0625 ng, the entire profile should be called uninterpretable if it is a mixture. Indeed, if a quant-based ST were employed according to the SOP, the sample should not have been consumed.

When first asked by prosecutors why she interpreted the data, the analyst asserted the quantity of DNA exceeded the quant-based ST set forth in the SOP because APD validated their quant-based ST using the amount of available DNA (or DNA in the entire extract), not the amount of DNA amplified in the PCR. This changed the multiplier used from 15 to 30, resulting in an amount of DNA that exceeded the quant-based ST in the SOP. (*See* **Exhibit R**.) However, during a conference call requested by prosecutors the following morning, the analyst provided a different explanation, agreeing the multiplier was the amplified DNA (15) and not the available DNA (30), and thus the amount of DNA at 0.05025 ng amplified was still below the quant-based ST of 0.0625 ng. She explained that the TL had signed a deviation allowing the profile to be interpreted as a major/minor mixture. While the written deviation did indeed permit the analyst to *consider* the profile as a major/minor mixture, it did not state the analyst could proceed and interpret the profile despite the SOP's clear guidance that a profile at 0.05025 ng amplified should be considered uninterpretable even for a major/minor mixture.

5. Significance of Observations Related to DNA Mixture Interpretation

In sum, the APD DNA Lab's choice of a quant-based ST, inadequate validation work and continuation of the quant-based approach despite evidence that it was ineffective raise concerns about the APD DNA Lab's understanding of foundational issues in DNA analysis, particularly with respect to the effects of amplification on evidentiary profiles, the importance of validation and data-driven protocols and the critical role of quality assurance in addressing concerns as they arise. It is difficult to determine how many criminal cases may have been affected by these issues as of the writing of this report. The Commission will seek guidance from the APD DNA Lab's Interim Technical Advisor on the number of cases potentially impacted. Recommendations on this issue are provided in **Section V** below.

B. Contamination Events

At the request of the Travis County District Attorney's office, the audit team reviewed a sexual assault case in which the issue of concern was possible carryover contamination from one sample (the victim's known DNA from a vaginal swab epithelial fraction) to another sample run at the same time (the penile swab of a person of interest who was later excluded). The APD analyst reported a three-person mixture from the epithelial fraction of the penile swab and concluded the victim could not be excluded as a contributor to the epithelial cell fraction. When retested by another laboratory, which included re-extraction of DNA from the penile swab, the results for the penile swab indicated a two-person mixture (although with relatively lower signal) and excluded the victim, thus supporting the possibility of carryover contamination between samples during the original testing by the APD analyst. Dr. Budowle and Ms. Koehler performed an extensive review of the case file and could not eliminate the possibility of carryover contamination from the epithelial cell fraction from the victim's vaginal swab to the epithelial cell fraction from the penile swab.

The reason the carryover contamination is described as "possible" is because it is impossible to conclude with 100% certainty that the results were attributable to carryover contamination. However, there are a number of red flags in the case that lend support to carryover contamination as the most likely explanation for the results, including the discordant results from the independent laboratory.

From a fact investigation perspective, the APD DNA Lab's results contradicted *both* the victim *and* the suspect's accounts. The victim described being assaulted by a single individual—an African American male. The suspect from whom the penile swab was taken was a Hispanic male. The reason APD officers requested the Hispanic male provide a penile swab is because he

was driving a vehicle in the vicinity of the assault that matched the victim's description of her assailant's vehicle. The analysis of the victim's sexual assault kit yielded a two-person mixture comprised of the victim and an unknown male contributor (the major contributor). A CODIS search of the unknown male contributor profile resulted in a "hit" for an African American male. In addition to the penile swab, APD officers took the bed sheet from the hotel room where the Hispanic male admitted to having sex with a female the same night the victim was assaulted, but maintained it was a different female he met at a bar and not the victim. The DNA analysis showed a major female contributor on the Hispanic male's penile swab that was the same as the DNA profile from the stain on the bed sheet, providing support for the Hispanic male's description of having sexual relations with another female. The only physical evidence connecting the Hispanic male to the victim was a low-level profile consistent with the victim's DNA from the penile swab identified by the APD DNA Lab.

From a scientific perspective, a number of factors lend support to the possibility of contamination. First, the DNA profile from the vaginal epithelial fraction of the sexual assault kit was essentially a single-source profile that was consistent with the reference profile of the victim. This sample was immediately adjacent to the epithelial fraction of the Hispanic male's penile swab throughout the entire analytical process. The epithelial fraction of the sexual assault kit contained 174 times (20.19/0.116 ng/μL) more DNA than the epithelial fraction from the penile swab. Thus, there was a risk of carryover contamination between the "high-level" sample and the "low-level" sample which could explain the observation of a minor profile consistent with the victim appearing on the Hispanic male suspect's penile swab. Though it is not always possible to run high levels of DNA separately from low levels of DNA, some laboratories

attempt to run "high plates" separately from "low plates" when possible to minimize the potential effects of carryover contamination.

A laboratory should exercise caution in relying on factual accounts from a victim or suspect conveyed by attorneys in the case. Laboratories are often cautioned to avoid "task-irrelevant" information that could have a biasing effect on data interpretation.[23] The laboratory must maintain independence from its clients to provide findings that are not biased by case circumstances or a desired investigative outcome. However, when an end-user of the DNA analysis raises concerns regarding possible contamination, as the Travis County District Attorney's Office did in this case as early as 2009, the laboratory must take the issue seriously and investigate possible explanations thoroughly. Section 4.8 of the ISO/IEC General Requirements for the Competence of Testing and Calibration Laboratories states:

> *The laboratory shall have a policy and procedure for the resolution of complaints received from customers or other parties. Records shall be maintained of all complaints and of the investigations and corrective actions taken by the laboratory (see also Sections 4.9, 4.11).*

Even if the concern expressed by the DA's office was not in the context of a formal complaint, it should have been thoroughly vetted under the ISO standards to determine whether contamination might have been possible due to the adjacent positions of the high-level and low-level samples throughout the analytical process, especially in light of the results from the independent laboratory which showed no indication of the victim's DNA on the Hispanic male's penile swab. The case file provides no documentation that questions were raised by the DA's office or vetted by the APD DNA Section. Complete documentation in the case file is critical

[23] For discussions on the subject of "task-relevance" in forensic science, *see e.g.*, Thompson, WC, "Determining the Proper Evidentiary Basis for an Expert Opinion: What Do Experts Need to Know and When Do They Know Too Much?" in *Blinding as a Solution to Bias*, First Ed., Elsevier Inc. 133-150 (2016); National Commission on Forensic Science Views Document: "Ensuring that Forensic Analysis Is Based Upon Task-Relevant Information," (December 8, 2015); Thompson, WC, "What Role Should Investigative Facts Play in the Evaluation of Scientific Evidence?" *Australian Journal of Forensic Sciences*, 2011; 43(2-3): 123-134.

not only to ensure compliance with accreditation standards but also because the parties may have notification and disclosure requirements under *Brady v. Maryland* or Section 39.14(h) of the Texas Code of Criminal Procedure.

During interviews, Dr. Budowle asked the analyst what should be done to address the possible contamination in the case. The analyst responded that if called to testify in the case, she would explain that carryover contamination was a possible explanation for the low-level victim DNA on the penile swab. This response conveys an inadequate understanding of the laboratory's responsibility to address the possible carryover contamination through the quality assurance system, including issuing an amended report if necessary, describing the nonconformity, conducting a root cause analysis and implementing a corrective action plan.

Finally, even setting aside the suspected contamination, Dr. Budowle and Ms. Koehler concluded the APD DNA Lab should not have provided a CPI statistic due to the low-level victim DNA on the penile swab and the high possibility of allele dropout at every locus. No loci were suitable for a statistical calculation using the CPI and thus the result should have been called uninterpretable.

In sum, this case raises many important issues, including whether:

1. The APD DNA Lab employs evidence handling practices sufficient to guard against carryover contamination;

2. There could have been carryover contamination in any other cases by this analyst or other analysts;

3. All analysts understand how to properly calculate a CPI statistic;

4. All analysts understand the importance of documenting case-related communications in the file especially from attorney end-users who express concerns;

5. All analysts understand the role of the quality assurance process in vetting possible contamination and concerns from end-users in the criminal justice community; and

6. All analysts understand their role in the disclosure and notification obligations imposed by *Brady v. Maryland* and/or Section 39.14(h) of the Texas Code of Criminal Procedure.

In addition, in a separate case review the audit team observed another 10 cases in which a reagent blank was contaminated but results were reported for the evidentiary samples processed in the same batch. The reagent blank contained 8 peaks above the analytical threshold (75 RFUs). Peaks ranged in height from 103-744 RFUs. APD DNA Lab Staff traced the contamination back to the analyst's extraction reagents. Results from the 10 cases were reported, under the theory that because the alleles observed in the contaminated reagent were not observed in the evidentiary samples, the contamination in the reagent blank must not have affected the evidentiary samples. The APD DNA Lab's SOP allowed the TL to sign off on reporting the evidentiary samples despite significant reagent blank contamination without providing any defined criteria for when such signoff was appropriate. Clearly defined criteria in the SOP would remove the subjectivity in decision-making for contamination incidents involving reagent blanks.

C. Use of AP Reagent Outside Manufacturer's Instructions

The forensic biology screening analysts routinely use a SERI Acid Phosphatase (AP) reagent beyond the "make fresh daily" instructions on the reagent bottle. APD DNA Lab analysts are instructed to make the AP reagent when needed and are allowed to use the reagent anywhere from a few days to 2-4 weeks or until they run out of prepared reagent. Though analysts perform a quality check of the reagent daily, there is no supporting documentation on the criteria (*e.g.,* time frame for development of color reaction or intensity of the color reaction) for assessing whether the AP reagent is performing optimally. In one study by the Ohio Bureau

of Criminal Identification, loss of activity of the AP reagent was observed when exceeding the "make fresh daily" instructions. (*See* **Exhibit N**.)

Subjectivity in analysis and possible loss of sensitivity in an AP reagent could lead analysts to miss potential semen stains when those stains are present, but significantly weaker than the positive control (or negative). As provided in the FBI's Quality Assurance Guidelines (QAS), if chemical reagents are to be used beyond expiration dates (or in this case outside the manufacturer's instructions), such use should be supported by validation data. When asked to supply the validation data to support the extended use of the reagent, the APD DNA Lab was unable to do so. The potential impact of the extended use of the AP Reagent should be examined through a comparative study, and the APD DNA Lab should take corrective action if weaker color reactions are observed with extended use vs. following the "make fresh daily" instructions. Without support that the AP reagent was performing appropriately, the APD DNA Lab will need to conduct a thorough root cause analysis and implement a corrective action plan regarding semen stains that may have been missed during casework screening.

D. Leadership and Training Issues

The APD Forensic Services Division underwent an organizational and management assessment by the Major Cities Chiefs Association in April 2016 (*See* **Exhibit O**.) The review was not intended to be a technical assessment of scientific protocols or the laboratory's quality assurance system. The assessment team looked at overall organizational structure and made recommendations. One recommendation was the addition of an Assistant Director level position to oversee the scientific analysis in the laboratory. A second recommendation was the addition of a senior forensic scientist to fill the Quality Assurance Coordinator position. The Commission agrees with these recommendations. The APD DNA Lab (including the future TL) will need a

strong scientific lead within the laboratory to provide scientific expertise, anticipate potential issues, and provide technical guidance in resolving questions should they arise.

While the APD DNA section has a written training program, it needs to be updated and strengthened for both forensic biology screening and all parts of the DNA analysis process from sample handling to statistical calculations. Through interviews, the team observed that analysts lacked understanding regarding important quality assurance procedures including internal reviews of customer concerns and critical evaluation of data. Training regarding basic issues such as proper validation and use of pipettes within approved manufacturer ranges is necessary. Some analysts struggled in responding to questions regarding foundational DNA topics such as the Hardy-Weinberg equilibrium, how one calculates a Random Match Probability, and how stutter occurs. Refresher training in basic molecular biology, forensic genetics and statistics will be a critical component of moving the laboratory forward.

Through interviews, the audit team observed that the APD DNA Lab's most recent TL was not a proficiency-tested DNA analyst in the technologies being utilized in the laboratory. While he did not sign reports or perform technical review of casework, he made technical decisions and signed off on deviations from the SOP. While he had many technical capabilities including but not limited to an excellent understanding of validation work, emerging technologies and their applications, he did not have the practical casework experience and understanding needed to effectively resolve legacy issues. The previous TL, while a proficiency-tested DNA analyst, did not have the depth of scientific or technical knowledge to make necessary adjustments to the training manual or the DNA SOP. She also conducted and signed off on validation studies that were inadequate, an observation her successor made and corrected

with a far more robust approach to validation and mixture interpretation, for example, introducing probabilistic genotyping software.

The importance of an effective TL in the DNA section of a modern crime laboratory cannot be overstated. To effectively lead the section, the TL must have adequate exposure to applied scientific research, statistical methods, practical casework experience, and an appreciation of the role of the laboratory in the criminal justice system. Current QAS Guidelines do not require Ph.D.s for the TL position, and the Commission recognizes that a Ph.D. does not necessarily guarantee quality nor is someone with a Master's degree incapable of being an exemplary TL. However, the audit team has observed that in addition to basic education requirements to be an effective TL, a candidate should be able to demonstrate experience with research/problem solving, an understanding of the requirements of validation studies and methodology implementation, statistics, applied scientific methods, and a working knowledge of quality assurance practices.

As laboratories across the country—including the APD DNA Lab—transition to probabilistic genotyping methods, DNA analysts must have sufficient depth of knowledge to understand how to properly deconvolute a mixture, assign the number of contributors and perform statistical calculations. Probabilistic genotyping software should not be viewed as a "black box" solution for challenging mixture interpretation cases. Analysts must be able to manually interpret a profile as well as understand the underlying validation and the analytical basis upon which the software performs calculations. They also must have a strong understanding of the statistical underpinnings of likelihood ratios (the output of probabilistic genotyping software) to be able to use the statistical method effectively in casework and to explain the statistics to triers of fact and other stakeholders.

IV. RELIANCE ON ACCREDITATION AND SWGDAM

As previously stated, ASCLD/LAB participated in the audit of the APD DNA Lab and released a letter on June 22, 2016 outlining eight nonconformities under ISO/IEC 17025. (*See* **Exhibit P**.) These nonconformities will need to be resolved in order for the APD DNA Lab to resume forensic biology screening and DNA analysis.

DNA laboratories are required to undergo external audits (by individuals external to the lab) every other year. On the off years, they are required to perform an internal audit (by individuals associated with the lab). ASCLD/LAB performed on-site assessments at the laboratory every five years, and the quant-based ST was either not identified or not questioned. Moreover, no deficiencies in validation studies were observed, though such problems were obvious. The depth of the DNA training program for analysts was also not questioned.

In 2010, the ASCLD/LAB assessor either did not review the ST validation study or did not appreciate that the quantity of DNA amplified was an inappropriate way to establish an ST for mixture interpretation. More than one analyst stated the quant-based ST was discussed with an auditor but it is unclear with which auditor this discussion occurred. After the quant-based ST was established in 2010, there does not appear to have been another external review of the ST study until the one conducted in May 2016 by the Commission and ASCLD/LAB. Though ASCLD/LAB conducted an assessment in 2015, validation studies were not reviewed by the assessors because they had already been reviewed either by ASCLD/LAB in 2010 or during external QAS audits.

The same lack of findings were present with external QAS audits (these QAS audits were performed by the National Forensic Science Technology Center ("NFSTC")) during the relevant time period. QAS audits by NFSTC were performed in June 2012 and November 2014 with no

findings related to the quant-based ST or other validation studies. The NFSTC auditors reviewed the laboratory's PowerPlex® Fusion 30 cycle validation data and no findings were made even though there appear to have been deficiencies in that validation as well.

Laboratory staff and criminal justice stakeholders rely on accreditation as an indication that the quality of the laboratory's work is sound. (*See e.g.*, **Exhibit G**.) Indeed, analysts often testify that the accreditation process assesses scientific validity and acceptance. As an example, consider the following testimony:

> *Q. Now, when we hear something like accredited, that sounds good, but what does that actually mean as far as the protocols that y'all have to follow in order to maintain that certification?*
>
> *A. Well, to be accredited, you're actually inspected by the accrediting agency, and they review your procedures to make sure that the procedures that you're following are scientifically valid, as well as accepted in the forensic community. They will come in and check out all of your operations, and then they routinely check—the accreditation cycle is actually a five-year cycle, but they do routinely check every year, or two years to make sure that you're following their guidelines and practices.*

Similarly, the assessment team from the Major Cities Chiefs Association made the following statement in the report issued after the organization structure audit: "The APD is an ISO 17025 accredited laboratory, compliant with all relevant forensic standards and the FBI QAS, as demonstrated by its current accreditation. While the assessment team did not conduct an ISO assessment, it was readily apparent that the laboratory and its staff upheld the high standards of accreditation." Statements like these provide strong indicators of the emphasis stakeholders place on the accreditation process as an indicator of quality and correctness of scientific procedures and policy.

The checks and balances that most stakeholders in the criminal justice system (including laboratory management) assume are provided by the QAS and ASCLD/LAB accreditation were not present in this case. No auditor raised concerns about the quant-based ST, the inadequacy of

the validation studies and the various other issues observed during three days of audits in May 2016. The observations in this report raise legitimate questions about the role and limitations of accreditation and the consistency of assessor teams. Specifically:

1. Are the scope and limitations of accreditation well understood by the criminal justice community, including laboratory analysts and management, attorneys and judges?

2. Do assessors consistently consider whether a laboratory's protocols and underlying validation are based on sound scientific principles or do they limit their review to determining whether the laboratory has a protocol in place that it follows?

3. If it is not the accrediting body's role to assess the foundational scientific validity of the analytical and interpretive methodologies used by DNA labs, which entities are responsible?

4. Should assessors re-review validation data from prior years considering that one validation study may be relied upon to build subsequent studies and protocols?

5. Are assessors properly educated and trained to undertake the task of reviewing and assessing the reliability of the methods actually implemented in the laboratory?

The forensic DNA community also relies heavily on SWGDAM for guidance on how to address complex issues that arise concerning mixture interpretation and many other issues. Though SWGDAM guidelines provide a tremendous amount of necessary and helpful information to the community, it would be a mistake to believe either the organization as a whole or its individual members view their role as intervening in the protocol decisions and practices of individual forensic DNA laboratories. Indeed, a senior analyst in the APD DNA Lab participates in SWGDAM and raised the APD DNA Lab's quant-based ST during a roundtable discussion at a SWGDAM meeting. She received no feedback from the membership indicating any problem with the approach.

It should also be noted the SWGDAM 2010 Guidelines include the following language: "The revised guidelines are not meant to be applied retroactively." Notwithstanding this language, if a laboratory did not employ scientifically valid methods before the release of any

particular SWGDAM guideline, it may in fact be necessary for the guideline (or concepts expressed within the guideline) to be applied retroactively to correct any inaccurate results, especially if the inaccuracies could lead to miscarriages of justice. SWGDAM should consider clarifying or deleting this language altogether to avoid misinterpretation. Laboratories must work with end-users in the criminal justice system (judges and lawyers) to assess whether retroactive application of a scientific principle is necessary or appropriate depending upon the facts and circumstances of a given situation.

The National Institute of Standards and Technology ("NIST") Organization of Scientific Area Committees ("OSAC") was established in 2014 for the purpose of creating forensic science standards and guidelines, including in the area of forensic DNA analysis. The hope is that these standards and guidelines will be scientifically rigorous, well understood and properly implemented by the forensic community across the United States. Though the OSAC is charged with developing the standards and guidelines, it bears no responsibility for whether or how they are implemented by individual laboratories. Currently, oversight for forensic DNA laboratories remains entirely within the umbrella of the existing accreditation bodies (ASCLD/LAB & ANAB), the FBI's QAS and state-level forensic commissions to the extent they exist. These entities will be responsible for ensuring the OSAC standards and guidelines are implemented correctly by the forensic community as part of the accreditation process. For this system to work properly, the standards and guidelines themselves must be based on sound scientific principles and the individuals chosen to audit against the standards and guidelines must have sufficient depth of scientific knowledge to fulfill their auditing responsibilities effectively.

V. RECOMMENDATIONS

The APD DNA Lab management has amended the scope of its accreditation to temporarily suspend forensic DNA analysis (*See* **Exhibit Q**), including forensic biology screening, which will allow the APD DNA Lab the necessary time to address findings as well as to re-train and re-qualify its analysts to acceptable standards, and/or hire additional highly qualified DNA analysts. This proactive approach should allow the APD DNA Lab to emerge as a stronger forensic DNA laboratory in the long-term. Following are the Commission's recommendations regarding next steps for the APD DNA Lab:

1. Retain a technical expert to advise the laboratory on an interim basis. The expert should have extensive experience in casework, training, validation and the laboratory quality assurance process. The expert should be on site to work with the analysts at least three days per week. The expert may at his or her discretion choose to bring other experts in to assist with training.

2. Conduct a national search for a permanent Technical Leader. The Technical Leader should have at least a Master's degree in biology, chemistry or forensic science with demonstrated expertise in applied scientific research, statistical methods and sufficient practical DNA casework and management experience.

3. Every analyst in the APD DNA Lab should go through a new training program and competency testing for all core competencies from forensic biology screening and forensic genetics through complex mixture interpretation. Training should include effective use of the quality assurance process and core legal issues as they apply to casework.

4. The laboratory needs to hire high-level management personnel (Assistant Director or higher) with scientific expertise so all Technical Leaders in the laboratory have a scientific resource to consult when questions of a technical nature arise.

5. The City of Austin must work with the Travis County District Attorney's Office to assess backlogs and prioritize cases to protect the public safety while the laboratory retools. This necessarily means the City will be required to spend a significant amount of funds to outsource casework. Realistically, the work required to address the issues described herein and bring the APD DNA Lab back online could take six months to a year. Though the Department of Public Safety may be able to absorb some of the caseload in the interim, it is not realistic to believe they can absorb it all absent a major infusion of additional resources.

6. The Interim Technical Expert will need to work closely with the Travis County District Attorney's Office and other affected stakeholders to ensure an open line of communication regarding progress and resolution of case backlogs through outside agencies.

7. The Interim Technical Expert should conduct a full root cause analysis and corrective action plan for possible carryover contamination described in Section III.B. above.

8. The Interim Technical Expert should conduct a comparison study on the use of the AP Reagent following the "make fresh daily" instructions vs. continued extended use.

9. The Interim Technical Expert should conduct a review of the laboratory's validation work to determine whether inadequacies in early validations impacted subsequent validation work, understanding that some studies build on each other. If deemed necessary, new validation studies should be performed to support methods in use.

10. The APD DNA lab should establish a RFU-based ST for the interpretation of all DNA casework. Adequate validation studies must be conducted in order to determine a suitable ST. Laboratory SOPs should be amended accordingly.

11. The Interim Technical Expert should assess whether the PowerPlex® Fusion 30-cycle validation study was performed properly. If there are issues with the study, what is the potential impact on casework (both mixtures and single source) and what is the recommended corrective action plan? Do any other STR amplification kits used by the laboratory before the PowerPlex® Fusion 30-cycle require review?

12. The APD DNA Lab should submit to an audit by the Commission and ASCLD/LAB before returning to casework.

13. The APD DNA Lab (and all laboratories in Texas) should consult regularly with their peers in other laboratories and with experts in and outside of Texas. The State has a wealth of resources in forensic DNA analysis, and laboratories (large and small) should take advantage of these resources in resolving questions and addressing complex issues as they arise.

14. The Commission should work with the laboratory's accrediting body to assess why the issues discussed herein were not identified during the accreditation process (including site audits). This assessment should include a discussion of the appropriate role and limitations of accreditation, a root cause analysis and corrective action plan for future DNA audits as appropriate.

In addition to the recommendations above, the Interim Technical Expert should report back to the Commission at each quarterly Commission meeting regarding the following questions:

1. Is there a possibility of crossover contamination in any cases other than the one discussed in **Section III.B** above based on review of 6 months of the analyst's casework before and after the incident? What, if any initiatives are necessary to address this issue?

2. Was the Interim Technical Expert able to assess at what stage(s) in the case preparation/analysis the contamination was likely to have occurred?

3. What are results of the comparison study of AP Spot reagent? Does the study of the AP reagent indicate that semen stains could have been missed during the screening process? If so, what is the recommended corrective action plan?

4. What is an estimate of the number of cases in which the reliability of interpretation was impacted by the laboratory's use of the quant-based ST and/or suspect or victim-driven CPI?

5. What are the results of knowledge-based competency testing to date?

6. What are the effects (if any) of inadequacies in early validation studies on subsequent validation studies? This assessment should include the ST study, the PowerPlex® Fusion 30-cycle validation, and any other applicable validation studies as determined by the Interim Technical Expert.

7. What recommendations are there regarding revisions to forensic biology and DNA training programs?

8. Is the legal triage system used statewide to review DNA mixture cases sufficient to review the APD DNA Lab's mixture cases for which a quant-based ST was employed (assuming no other problems with the case)?

9. Should mixture cases before 2010 be reviewed? After 2010 only? Upon request for recalculation by the defendant or in all cases?

10. What is the timeline of addressing potentially affected casework?

11. Any other recommendations? What additional resources are needed?

VI. RECOGNITION

The audit team is grateful to the staff and leadership of the APD for their willingness to participate in interviews, provide documents and respond openly to questions posed. We also commend Assistant Chief Troy Gay and Commander Nick Wright of the Austin Police Department for providing unwavering support and commitment to the process throughout. The

team is grateful for the tireless efforts of Assistant District Attorneys Brandon Grunewald and Robert Smith including their willingness to closely examine complex scientific issues. Last but not least, the Texas Department of Public Safety and the University of North Texas Health Science Center should be commended for assisting the APD DNA Lab by helping to relieve the burden for urgent cases where possible and by providing training materials and model SOPs.

EXHIBITS INDEX

Exhibit	Description
A	Curriculum Vitae of Bruce Budowle
B	Curriculum Vitae of Jody Koehler
C	Suggested Reading List (1991-2015)
D	Example of FBI PopStat Notification Letter
E	SWGDAM Autosomal STR Interpretation Guidelines (January 2010)
F	Excerpt from *Advanced Topics in Forensic DNA Typing: Methodology* by John M. Butler (2014)
G	Emails re Quant Based ST & Sample Informational Report (April 21, 2016)
H	APD Memo from TL Jeff Sailus to Lab Manager Bill Gibbens re DNA Section Update (November 24, 2014)
I	APD Stochastic Threshold Validation Study and Supporting Documents
J	Pages 40-53 of APD's Serology/DNA Section Technical Manual (March 10, 2015)
K	ASCLD/LAB Report re Broward Co. Florida Sheriff's Department (April 12,2016)
L	Final Report on Audit of Department of Forensic Sciences DNA Section in Washington, DC (April 22, 2015); Surveillance and Remote Surveillance Report of ANAB (April 24, 2015)
M	APD Serology/DNA Section Technical Manual p. 50, (March 10, 2015)
N	Ohio Study re: Detecting the Presence of Semen/Acid Phosphatase
O	Report of APD Forensic Services Audit performed by Major Cities Chiefs Association Forensic Science Committee (February 2016)
P	Letter from ASCLD/LAB Executive Director Pamela Bordner to Lab Manager Bill Gibbens (June 22, 2016)

Q Letter from APD Chief Art Acevedo to ASCLD/LAB Executive Director Pamela Bordner re: APD's Voluntary Discontinuation of Biological Services (June 13, 2016)

R Statements of Travis County Assistant District Attorneys Robert Smith and Brandon Grunewald (May 5, 2016)

S Notice of Amendment of the FBI's STR Population Data Published in 1999 and 2001; Harris County Institute of Forensic Sciences Validation Summary: ID Plus EXCEL PopStats Template v1.2 (June 1, 2015)

EXHIBIT A

NAME: Bruce Budowle

TITLE:

Executive Director, Institute of Applied Genetics
Professor, Department of Molecular and Medical Genetics

BUSINESS ADDRESS, PHONE NUMBER, and EMAIL ADDRESS:

Institute of Applied Genetics
Department of Molecular and Medical Genetics
University of North Texas Health Science Center
3500 Camp Bowie Boulevard
Ft. Worth, Texas 76107

Bruce.budowle@unthsc.edu

BIRTH DATE: October 13, 1953

BIRTH PLACE: San Pedro, California

MARITAL STATUS: Married

EDUCATION:

King College — B.A. - 1975 (Biology)
Bristol, Tennessee

Virginia Polytechnic Institute and State University — Ph.D. - 1979 (Genetics)
Blacksburg, Virginia

DISSERTATION: Phase Change in *Hedera helix L*.

RESEARCH AND/OR PROFESSIONAL EXPERIENCE:

1974	Undergraduate Research Scientist, King College, Bristol, Tennessee
1976 - 1979	Graduate Teaching Assistantship in Biology, Virginia Polytechnic Institute and State University, Blacksburg, Virginia
1979 - 1982	Postdoctoral Fellow in Immunogenetics, Awarded by the National Cancer Institute, University of Alabama in Birmingham, Alabama
1982	Consultant to Department of Criminal Justice University of Alabama in Birmingham
1983 - 1985	Consultant to Beckman Instruments, Inc. Palo Alto, California
1983 - 1994	Research Chemist, Forensic Science Research and Training Center, Laboratory Division, FBI Academy, Quantico, Virginia
1994 - 1997	Chief, Forensic Science Research Unit, Laboratory Division, FBI Academy, Quantico, Virginia

1985 - 2008	Adjunct Professor, School of Continuing Education, University of Virginia, FBI Academy Campus
1987 - 1988	Council Member, International Electrophoresis Society
1987 - 1988	Vice President, America's Branch of the Electrophoresis Society
1988 - 1990	Vice President, International Electrophoresis Society
1989 - 1991	Council Member, American Electrophoresis Society
1989 - 1998	Associate Editor, Applied and Theoretical Electrophoresis
1990 - present	Editorial Board, BioTechniques
1990 - 1991	Visiting Instructor, Rush Presbyterian - St. Luke's Medical Center.
1990 - present	Editorial/Advisory Board, International Journal of Legal Medicine
1991 - 2005	Chairman of the DNA Commission of the International Society of Forensic Haemogenetics
1994	Defense Science Board, Mitochondrial DNA, AFDIL
1994 - 1998	Editor, Crime Laboratory Digest
1995 - 2000	Chairman of The Scientific Working Group on DNA Analysis Methods
1995 - 2000	DNA Advisory Board, DNA Identification Act, Federal Bureau of Investigation
1995 - 2005	Editorial Board, Genetic Analysis: Biomolecular Engineering
1998 - 2001	The Research and Development Working Group, National Commission on the Future of DNA Evidence, National Institute of Justice
1998 - 2009	Senior Scientist - Biology, Laboratory Division, Federal Bureau of Investigation
1999 - present	Editorial Board, Forensic Science Communications
1999 - present	Editorial Board, Legal Medicine (Japanese Society of Legal Medicine)
1999 - 2003	Research Professor, Institute for Biosciences, Bioinformatics, and Biotechnology, George Mason University, Manassas, Virginia
1999 - 2003	Affiliate Professor, Department of Biology, George Mason University, Fairfax, Virginia

2000	Outside Reviewer for German Proficiency Testing System (GEDNAP)
2001-2002	Celera DNA Advisory Board, Mitochondrial DNA/WTC
2001-2003	Kinship and Data Analysis Panel for WTC Victim Identification
2002	Steering Committee, Colloquium on Microbial Forensics, American Society of Microbiology
2002 - 2004	Chair of Scientific Working Group Microbial Genetics and Forensics
2002	Co-organizer of Microbial Forensics Meeting, The Banbury Center, Cold Spring Harbor Laboratory
2002 - 2008	Adjunct Faculty, Department of Pathology, University of North Texas Health Science Center, Ft. Worth, Texas
2003 - 2008	National Biodefense Analysis and Countermeasures Center Advisory Group
2002 - 2007	National Interagency Genomics Science Coordinating Committee, National Science Foundation
2003	Disease Informatics Senior Coordinating Committee, National Science Foundation
2004	Co-organizer of Second Microbial Forensics Meeting, Identifying Gaps, sponsored by the Department of Homeland Security, The Banbury Center, Cold Spring Harbor Laboratory
2004 - 2007	Editorial Board, Forensic Science International
2004	Participant in Expert Meeting on Microbial Forensics, National Academy of Sciences, Washington, D.C., June 22-25, 2004
2004	Participant in Biosecurity Threats in the 21st Century: Re-examining how we define the “problem” and mitigate the effects, National Academy of Sciences, Minneapolis, MN, July 15, 2004
2004	Invited Lecturer, Post Graduate Course in Forensic Genetics, Finish Graduate School in Population Genetics and department of Forensic Medicine, University of Helsinki, Finland, September 20-21, 2004
2004	Member of Steering Committee on the Animal Forensics Working Group of the International Society of Animal Genetics
2004 - present	Member of Scientific Working Group for the NIAID-funded Bioinformatics Resource Center (BRC) at The Institute for Genomic Research (TIGR)

2005	Co-organizer of Third Microbial Forensics Meeting, sponsored by the Department of Homeland Security, Evidence Collection, Storage, and Extraction, The Banbury Center, Cold Spring Harbor Laboratory
2006	Participant in Advancing the International Biosecurity Dialogue: Clarifying Definitions, National Academy of Sciences, Washington, D.C., January 27, 2006
2006	Participant in Genomics and Global Pathogens, The American Academy of Microbiology, Washington, D.C., September 27-28, 2006
2006	Lecturer in Science Exposition and Ethics Course, Watson School of Biological Science, Cold Spring Harbor, New York, November 29, 2006
2006	International Fellow, Institute of Environmental Science and Research, New Zealand, December 1-13, 2006
2006 - 2008	Steering Committee Member, Scientific Working Group on Chemical, Biological, Nuclear and Radiological Analyses
2007	Member of National Planning Committee for Workshop on Plant Pathogen Forensics: Filling the Gaps, sponsored by Oklahoma State University, Oklahoma City, Oklahoma, January 11-13, 2007
2007 - present	Editorial Board, Forensic Science International Genetics
2007	Co-organizer of Fourth Microbial Forensics Meeting, Enduring Research Pathways, sponsored by the Department of Homeland Security, The Banbury Center, Cold Spring Harbor Laboratory
2008	Invited Outside Reviewer on DNA Technology for National Research Institute of Police Science, National Police Agency, Chiba, Japan, January 15-16, 2008
2008	Visiting Fellow, Faculty of Health Science and Medicine, Bond University, Gold Coast, Australia, June 23-July 5, 2008
2008 - present	Visiting Professor, Faculty of Health Science and Medicine, Bond University, Gold Coast, Australia
2008 - present	Member, Expert Working Group on Human Factors in Latent Print Analysis, NIST and NIJ
2009 - present	Professor, Department of Forensics and Investigative Genetics, University of North Texas Health Science Center, Ft. Worth, Texas
2009 - present	Executive Director, Institute of Investigative Genetics, University of North Texas Health Science Center, Ft. Worth, Texas

2009	Invited Speaker, Overview of Microbial Forensics and the Concepts of Validation, Committee on Review of the Scientific Approaches used during the FBI's Investigation of the 2001 *Bacillus anthracis* Mailings, First Meeting, National Academy of Sciences, July 30-31, 2009
2009	Invited Speaker, Low Copy Number Typing Issues, Mixture Interpretation Issues, Committee on Science, Technology and Law, National Academy of Sciences, October 19, 2009
2009 - present	Co-Editor-in-Chief, BMC Investigative Genetics
2010	Member of Steering Committee for Forensic Death Investigation Symposium, National Institute of Justice, Scottsdale, AZ, June 7-9, 2010
2010	Consultant to Cyprus Institute of Neurology and Genetics Laboratory of Forensic Genetics UN Missing Persons Identification Program, Cyprus, September 20-24, 2010
2010 - present	Adjunct Faculty, Department of Biological Sciences, University of North Texas, Denton, TX
2010	Co-organizer of Fifth Microbial Forensics Meeting, Microbial Forensics in the Era of Genomics, sponsored by the Department of Homeland Security, The Banbury Center, Cold Spring Harbor Laboratory, November 7-10, 2010
2011	Co-organizer of Lyme Disease Diagnostics in the Proteomics-Genomics Era, The Banbury Center, Cold Spring Harbor Laboratory, April 10-13, 2011
2011	Visiting Professor, Department of Forensic Medicine, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand, June 2011.
2011	Member of Organizing Committee, Microbial Evolution and Cutting Edge Tools for Outbreak Investigations, Center for Disease Control and Prevention, Atlanta, GA, September 14-16, 2011.
2011 - present	Editorial Board, American Journal of Forensic Medicine and Pathology
2012 - present	Member of Academic Committee, Key Laboratory of Forensic Genetics, Institute of Forensic Science of Ministry of Public Security, Beijing, China
2012	Member of planning committee for the Forum on Microbial Threats Workshop: The science and applications of microbial genomics: predicting, detecting, and tracking novelty in the microbial world, Institute of Medicine, Board on Global Health, National Academy of Sciences, June 12-13 2012.
2012- present	Member of the Technical Advisory Group to the Board of the Houston Forensic Science Center, LGC, inc.

2013-2016	Member of the International Expert Committee for the Biology Division of Health Sciences Authority, Singapore
2013- present	Appointment with Center of Excellence in Genomic Medicine Research(CEGMR), King Abdulaziz University, Jeddah, Saudi Arabia.
2013-2014	Member of Committee for the Science Needs for Microbial Forensics: Developing an Initial International Science Roadmap, Institute of Medicine, Board on Global Health, National Academy of Sciences.
2013-2015	Visiting Professor, Science Without Borders, Universidade Federal Do Rio De Janeiro, Centro De Ciências Da Saúde, Instituto De Biofísica Carlos Chagas Filho
2014-2015	Member of Committee on PCR Standards for the BioWatch Program, Board of Life Sciences, Division on Earth and Life Sciences, Board of Health Sciences Policy, Institute of Medicine, Board on Global Health, National Research Council, National Academy of Sciences.
2014 – present	Associate Editorial Board of Biosafety and Biosecurity of Frontiers in Bioengineering and Biotechnology
2016 – present	Director of the Center for Human Identification, University of North Texas Health Science Center, Ft. Worth, Texas
2016	GAO Meeting on Gaps in Capabilities for Attributing the Source of a Biological Attack, Washington, DC, April 20-21, 2016.
2016	Tackling Low Cost Nucleic Acid Test for the Developing World: Catalyzing Innovation in Sample Preparation, Scientific Advisory Board, Bill & Melinda Gates Foundation, Seatttle, WA, May 25, 2016.

MEMBERSHIPS IN PROFESSIONAL AND SCHOLARLY ORGANIZATIONS:

International Society for Forensic Genetics

HONORS AND OTHER SPECIAL COMMENTS:

1) Pi Alpha Sigma (1972)
2) Undergraduate Research Award (1974)
3) Graduate State Tuition Scholarship (1976 - 1979)
4) Phi Kappa Phi (1976)
5) Sigma Xi (1978)
6) American Academy of Forensic Sciences Recognition Award (1981)
7) Attorney General's Award for Exceptional Service (1991)
8) Jefferson Award, University of Virginia (1991)
9) Forensic Scientist of the Year, MAAFS (1996)
10) Honorary Member of the Finnish Society of Forensic Medicine (1998)
11) Director’s Award for Excellence in Investigative Support (2000)
12) Paul L. Kirk Award, Criminalistics Section, American Academy of Forensic Sciences (2001)
13) University of Alabama at Birmingham’s 2004 Ireland Distinguished Visiting

Scholar
14) Honorary Member of the Mediterranean Academy of Forensic Sciences (2004)
15) Health Care Hero Award, Dallas Business Journal (2010)
16) GSA Outstanding Faculty Awawrd 2016, GSBS, UNTHSC

RESEARCH INTERESTS:

Forensic Science
Genetic Marker Systems
Technique Development
Molecular Biology
Population Genetics
Human Genetics
Microbial Forensics
Pharmacogenomics

PUBLICATIONS:

1. Budowle, B., Go, R. C. P. and Acton, R. T.: Isoelectric focusing of hair proteins. In: Electrophoresis '81 (Allen, R. C. and Arnaud, P., eds.) Walter de Gruyter, Berlin, pp. 585-590, 1981.

2. Budowle, B., Go, R. C. P., Barger, B. O. and Acton, R. T.: Properdin factor B polymorphism in black Americans. J. Immunogenetics 8:519-521, 1981.

3. Budowle, B. and Acton, R. T.: A technique for the detection of variable electrophoretic patterns of hair proteins. Electrophoresis 2:333-334, 1981.

4. Budowle, B., Acton, R. T. and Barger, B. O.: A method for the dialysis of micro-samples. Anal. Biochem. 118:399-400, 1981.

5. Budowle, B., Reitnauer, P. J., Barger, B. O., Go, R. C. P., Roseman, J. M. and Acton, R. T.: Properdin factor B in type 1 (insulin-dependent) diabetic patients. Diabetologia 22(6):483-485, 1982.

6. Acton, R. T., Balch, C. M., Budowle, B., Go, R. C. P., Roseman, J. M., Soong, S-J., and Barger, B. O.: Immunogenetics of melanoma. In: Melanoma Antigens and Antibodies (Reisfeld, R. A. and Ferrone, S., eds.) Plenum Publishing Corporation, New York, pp. 1-21, 1982.

7. Reitnauer, P. J., Go, R. C. P., Acton R. T., Murphy, C. C., Budowle, B., Barger, B. O. and Roseman, J. M.: Evidence for genetic admixture as a determinant in the occurrence of IDDM in U.S. Blacks. Diabetes 31:532-537, 1982.

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11. Budowle, B., Go, R. C. P., Roseman, J. M., Barger, B. O. and Acton, R. T.: C4 phenotypes in Caucasians from the southeastern United States. In: Electrophoresis '82 (Stathakos, D., ed.), Walter de Gruyter, Berlin, pp. 715-723, 1982.

12. Budowle, B., Roseman, J. M., Go, R. C. P., Crist, W. and Dearth, J.: Complement phenotypes for prediction of risk and prognosis for acute lymphocytic leukemia (ALL). In: Cancer: Etiology and Prevention (Crispen, R. G., ed.) Elsevier Biomedical, New York, pp. 109-123, 1983.

13. Budowle, B., Roseman, J. M., Go, R. C. P., Louv, W. C., Barger, B. O. and Acton, R. T.: Phenotypes of the fourth complement component (C4) in black Americans from the southeastern United States. J. Immunogenetics 10 (3):199-204, 1983.

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627. Budowle, B. and Eisenberg, A.: A view of the new DNA technologies and their potential impact on the next generation forensic laboratory. American Society of Crime Laboratory Directors 40th Anniversary Meeting, Durham, North Carolina, 2013.

628. Budowle, B.: The genetic technology revolution. 3rd Annual Life Sciences Sympoium – Improving the Quality of Life Through DNA Technology, Ft. Worth, TX, 2013.

629. Martinez-Jarreta, B., Sosa, C., Casalod, Y., Baeta, M., Nunez, C., Lorenzo, J., Gimeno, B., Bolea, M., Laliena, C., Budowle, B., Hedges, R., and The “Reyes De Aragon” Team: Skeletal analyses allow to retrieve valuable data of early medieval ancestors Of the Spanish royal family: an interdisciplinary approach. Environmental and Archaeological Science Conference, AEA & UKAS 2013, Cardiff University, England, 2013.

630. Budowle, B.: Enhancing quality of extracted DNA. Technology Transition Workshop: a DNA Revolution – next generation technologies, Forensic Technology Center of excellence, National Institute of Justice, Ft. Worth, TX, 2013.

631. Budowle, B.: Single nucleotide polymorphisms – our future forensic markers. Technology Transition Workshop: a DNA Revolution – next generation technologies, Forensic Technology Center of excellence, National Institute of Justice, Ft. Worth, TX, 2013.

632. Budowle, B.: Comprehensive sequencing to address forensic needs. Technology Transition Workshop: a DNA Revolution – next generation technologies, Forensic Technology Center of excellence, National Institute of Justice, Ft. Worth, TX, 2013.

633. Budowle, B., Warshauer, D.H., Seo, S.B., King, J.L., Davis, C., and LaRue, B.: Next generation sequencing provides comprehensive multiplex capabilities, 25th Congress of the International society of Forensic Genetics, Melbourne, Australia, 2013.

634. Flores, S.K., Sun, J., King, J., Eisenberg, A.J., and Budowle, B.: Validation of the GlobalFiler™ Express PCR Amplification Kit for the direct amplification of reference DNA samples, 24th International Symposium on Human Identification, Atlanta, GA, 2013.

635. Sinha, S.K, Montgomery, A.H., Pineda, G., Thompson, R., LaRue, B.L., Ge, J., and Budowle, B.: Development of a novel multiplexed DNA analysis system for highly degraded DNA samples, 24th International Symposium on Human Identification, Atlanta, GA, 2013.

636. Seo, S.B., King, J., Warshauer, D., Ge, J., and Budowle, B.: Large panels of SNPs for human identity typing are feasible with current generation sequencing (CGS) technology, 24th International Symposium on Human Identification, Atlanta, GA, 2013.

637. Warshauer, D.H., Lin, D., Hari, K., Jain, R., Davis, C., LaRue, B., King, J.L., and Budowle, B: STRait Razor: a bioinformatic tool for length-based STR allele-calling in massively parallel sequencing data, 24th International Symposium on Human Identification, Atlanta, GA, 2013.

638. LaRue, B.L., King, J.L., and Budowle, B.: Highly reliable reference sample genotyping utilizing an automated rapid DNA typing platform, 24th International Symposium on Human Identification, Atlanta, GA, 2013.

639. Zeng, X., Seo, S.B., LaRue, B., King, J., and Budowle, B.: Whole mitochondrial genome typing on Ion Torrent™ PGM™ platform, 24th International Symposium on Human Identification, Atlanta, GA, 2013.

640. Ge, J. and Budowle, B.: One complete versus triplicate analyses in Low Template DNA typing, 24th International Symposium on Human Identification, Atlanta, GA, 2013.

641. Budowle, B.: Validation and reference materials for microbial forensics, Science Needs for Microbial Forensics: Developing an International Science Roadmap, National Academy of Sciences, Zagreb, Croatia, 2013.

642. Minot, S., Ternus, K., Allen, J., Budowle, B., and Kadavy, D.: Evaluating novel metagenomic classification algorithms for forensic microbial detection, Genome Informatics, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 2013.

643. Budowle, B.: Global trends in life sciences and genetics, Division on Engineering and Physical Sciences, National Academy of Sciences, Washington, DC, 2013.

644. Budowle, B., King, J., Moore, A., and Larue, B.: Rapid DNA and advancements in DNA extraction technology, 5th Asian Forensic Sciences Netwrok Annual Meeting & Symposium, Singapore, 2013.

645. Budowle, B.: Familial searching of DNA databases, 5th Asian Forensic Sciences Netwrok Annual Meeting & Symposium, Singapore, 2013.

646. Budowle, B., Ambers, a., and King, J.: DNA repair and whole genome amplification, Post-Symposium HSA DNA Workshop, Singapore, 2013.

647. Budowle, B., Marshall, P., Seo, S.B., and King, J.: Considerations and limitations of low template analysis, Post-Symposium HSA DNA Workshop, Singapore, 2013.

648. Budowle, B.: Validation and reference materials for microbial forensics, Science Needs for Microbial Forensics: Developing an Initial International Science Roadmap, Institute of Medicine, Board on Global Health, National Academy of Sciences, Zagreb, Croatia, 2013.

649. Seo, S.B., Zeng, X., Assidi, M., LaRue, B., King, J., Sajantila, A., and Budowle, B.: High throughput whole mitochondrial genome sequencing by two platforms of massively parallel sequencing, Second International Genomic Medicine Conference in November 2013 (24th-27th) by CEGMR at King Abdulaziz University, Jeddah, Saudi Arabia, 2013.

650. Budowle, B.: Principles and chemistries of next generation sequencing technologies, American Academy of Forensic Sciences, Seattle, WA, 2014.

651. Sinha, S.K., Montgomery, A.H., Pineda, G., Thompson, R., King, J., LaRue, B.L., Ge, J., Chakraborty, R., Budowle, B.: Development of a novel and sensitive DNA analysis multiplex based on INNUL markers for highly degraded forensic DNA samples, American Academy of Forensic Sciences, Seattle, WA, 2014.

652. Budowle, B.: Technologies of the future have arrived and communicating with the legal community, 2014 International Symposium on Forensic DNA in Law, Seoul, Korea, 2014.

653. Budowle, B.: Communicating scientific evidence in the legal system, Forensic Medical Science and Jurisprudence under Islamic Law, Riyadh, Saudi Arabia, 2014.

654. Budowle, B., Ambers, a., and King, J.: DNA repair and whole genome amplification - what they offer for forensic DNA typing, Bode Technology, 11th Annual DNA Technical Workshop – West, San Diego, CA, 2014.

655. Vuorio, A., Laukkala, T., Navathe, P., Budowle, B., Eyre, A., and Sajantila, A.: Aircraft-assisted pilot suicides, 15th European Symposium on Suicide and Suicidal Behaviour, Tallinn, Estonia, 2014.

656. Budowle, B., Ambers, a., and King, J.: DNA repair and whole genome amplification - what they offer for forensic DNA typing, Bode Technology, 13th Annual DNA Technical Workshop – East, Beuna Vista, FL, 2014.

657. Budowle, B.: Massively parallel sequencing and forensic identity testing, Fifth Annual Prescription for Criminal Justice Forensics, The ABA Criminal Justice Section and the Louis Stein Center for Law & Ethics, Fordham University, New York, New York, 2014.

658. Churchill, J.D., Chang, J., Ge, J., Rajagopalan, N., Lagacé, R., Liao, W., King, J.L., and Budowle, B.: Blinded genetic analysis of twelve genomic samples using the Ion Torrent PGM System, Green Mountain Conference, Burlington, VT, 2014.

659. Zeng, X., King, J.L., Stoljarova, M., Warshauer, D.H., LaRue, B.L., Sajantila, A., Patel, J., Storts, D.R., and Budowle, B.: High sensitivity multiplex short tandem repeat loci analyses with massively parallel sequencing, 25th International Symposium on Human Identification, Phoenix, AZ, 2014.

660. Stoljarova, M., King, J.L., Churchill, J.D., Budowle, B.: Massively parallel sequencing of multiplex short amplicons of mtDNA from challenged forensic samples, 25th International Symposium on Human Identification, Phoenix, AZ, 2014.

661. Novroski, N., Kindt, T., Schmedes, S., King, J., Marshall, P., and Budowle, B.: Diomics X-Swab™: a novel bio-specimen collection tool for increased trace material recovery and PCR enhancement, 25th International Symposium on Human Identification, Phoenix, AZ, 2014.

662. Churchill, J.D., Chang, J., Ge, J., Rajagopalan, N., Lagacé, R., Liao, W., King, J.L., and Budowle, B.: Evaluation of the Ion PGM™ System for use in human identity DNA typing, 25th International Symposium on Human Identification, Phoenix, AZ, 2014.

663. Schmedes, S.E., Churchill, J., King, J., and Budowle, B.: Genetic profiling using the Illumina® ForenSeq™ DNA Signature Prep Kit on the MiSeq Desktop Sequencer, 25th International Symposium on Human Identification, Phoenix, AZ, 2014.

664. Warshauer, D.H., King, J.L., and Budowle, B.: STRait Razor v2.0: the improved STR Allele Identification Tool – Razor, 25th International Symposium on Human Identification, Phoenix, AZ, 2014.

665. Zeng, X., King, J., Hermanson, S., Patel, J., Storts, D.R., and Budowle, B.: Evaluation of the PowerSeq™ Auto System by massively parallel sequencing, 25th International Symposium on Human Identification, Phoenix, AZ, 2014.

666. King, J.L., LaRue, B.L., Novroski, N.M., Stoljarova, M., Seo, S.B., Zeng, X., Warshauer, D.H., Davis, C.P., Parson, W., Sajantila, A., and Budowle, B.: The use of Massively Parallel Sequencing (MPS) to accurately and rapidly sequence the mtGenome of 283 individuals from 3 North American populations, 25th International Symposium on Human Identification, Phoenix, AZ, 2014.

667. Budowle, B. and Churchill, J.: Moving towards a validated high throughput sequencing solution for human identification: an evaluation of two SNP panels, autosomal STRs, and whole mitochondrial genomes, 25th International Symposium on Human Identification, Phoenix, AZ, 2014.

668. Thompson, L., King, J.L., Budowle, B. and LaRue, B.: Development of insertion-deletion (INDEL) marker panels for ancestral and individual identity genotyping, 25th International Symposium on Human Identification, Phoenix, AZ, 2014.

669. Budowle, B.: Bioterrorism and Microbial Forensics, Beto Lecture, Sam Houston State University, Huntsville, TX, 2014.

670. Budowle, B.: Development of DNA typing and forensic DNA databases in the US, Vietnam Forensic DNA Network 1st Annual Workshop, Hanoi, Vietnam, 2014.

671. Budowle, B.: Current approaches for the analysis of human remains, Vietnam Forensic DNA Network 1st Annual Workshop, Hanoi, Vietnam, 2014.

672. Budowle, B.: Methods to enhance the success of challenging samples, Vietnam Forensic DNA Network 1st Annual Workshop, Hanoi, Vietnam, 2014.

673. Budowle, B.: Bioterrorism and Microbial Forensics, Hjelt Lecture, University of Helsinki, Helsinki, Finland, 2014.

674. Budowle, B.: History of DNA databsing in the United States, Symposium on Human Identification DNA databasing in Peru, Lima, Peru, 2014.

675. Budowle, B.: Next generation sequencing for forensics, Symposium on Human Identification DNA databasing in Peru, Lima, Peru, 2014.

676. Budowle, B.: Current approaches for the analysis of human remains, Symposium on Human Identification DNA databasing in Peru, Lima, Peru, 2014.

677. Budowle, B.: The future of genetics and impact on forensic science, Symposium on Human Identification DNA databasing in Peru, Lima, Peru, 2014.

678. Budowle, B.: Perspectives on the Future of forensic genetics, Human Identification Solutions: Innovations and Perspectives, Madrid, Spain, 2015.

679. Budowle, B.: Ethics in the discipline of Forensic DNA, Bode Cellmark Forensics, 12th Annual DNA Technical Workshop – West, Coronado, CA, 2015.

680. Budowle, B.: Massively parallel sequencing: getting ready for forensic applications, Bode Cellmark Forensics, 12th Annual DNA Technical Workshop – West, Coronado, CA, 2015.

681. Budowle, B.: Forensic science – real world applied genomics, Genetics Graduate Student Association Spring Symposium on Applied Genomics, Texas A&M University, College Station, TX, 2015.

682. Budowle, B.: Overview of forensic genetics MPS, 26th Congress of the International society of Forensic Genetics, Krakow, Poland, 2015.

683. Budowle, B.: Forensic genetics MPS and Data Analysis Options, 26th Congress of the International society of Forensic Genetics, Krakow, Poland, 2015.

684. Schellberg, T. and Budowle, B.: Will NGS lead to significant expansion of the core loci? Identify benefits and policy/legal issues, 26th Congress of the International society of Forensic Genetics, Krakow, Poland, 2015.

685. Churchill, J.D., King, J.L., Chang, J., Wootten, S., Lagacé, R., and Budowle, B.: Chemistry and performance testing of forensically-relevant genetic marker systems within the Ion PGM™ System, 26th Congress of the International society of Forensic Genetics, Krakow, Poland, 2015.

686. Schellberg, T. and Budowle, B.: Implementation of NGS will ultimately lead to significant expansion of the core loci: An evaluation of the identity benefits and policy/legal issues? Identify benefits and policy/legal issues, 26th Congress of the International society of Forensic Genetics, Krakow, Poland, 2015.

687. Wiley, R., Sage, K., Sturm, S., King, J., Budowle, B., and LaRue, B.: An evaluation of a new Rapid DNA platform for field forward applications, 26th Congress of the International society of Forensic Genetics, Krakow, Poland, 2015.

688. Vanek, D., Budowle, B., Dubska, J.: Factors influencing the reliability of the bone sample DNA typing results, 26th Congress of the International society of Forensic Genetics, Krakow, Poland, 2015.

689. Stoljarova, S., King, J., Churchill, J., Aaspollu, A., and Budowle, B.: Massively parallel sequencing of multiplex short amplicons of mtDNA for analysis of challenged forensic samples, 26th Congress of the International society of Forensic Genetics, Krakow, Poland, 2015.

690. Bottino, C.G., Budowle, B., King, J., Churchill, J., Silva, R., and Moura-Neto, R.S..: STR genotyping with Ion Torrent PGM and STR 10-plex system: highlights on performance and data interpretation, 26th Congress of the International society of Forensic Genetics, Krakow, Poland, 2015.

691. Vanek, D., Budowle, B., and Votrubova, J..: The collaborative exercise concept on DNA typing of bone samples, 26th Congress of the International society of Forensic Genetics, Krakow, Poland, 2015.

692. Budowle, B.: Microbial forensics and its needs for standards and standardization, 2015 Rapid NGS Bioinformatic Pipelines for Enhanced Molecular Epidemiologic Investigation of Pathogens, American Society of Microbiology, Washington, D.C., 2015.

693. Harmon, R. and Budowle, B.: Recent developments: the illusion of quality through accreditation, 26th International Symposium on Human Identification, Grapevine, TX, 2015.

694. Zeng, X., King, J., Hermanson, S., Patel, J.,Storts, D.R., and Budowle, B.: An evaluation of the Powerseq™ Auto System: a multiplex short tandem repeat marker kit compatible with massively parallel sequencing, 26th International Symposium on Human Identification, Grapevine, TX, 2015.

695. Churchill, J., King, J.L., Schmedes, S.E., Novroski, N.M., Wendt, F., Ambers, A., and Budowle, B.: Power of the Illumina® Forenseq™ DNA Signature Preparation Kit in human identity DNA typing, 26th International Symposium on Human Identification, Grapevine, TX, 2015.

696. Novroski, N.M., Warshauer, D.H., King, J.L., Zeng, X., Churchill, J.D., and Budowle, B.: Detection of intra-allelic sequence variants in autosomal and X chromosome short tandem repeats using massively parallel sequencing, 26th International Symposium on Human Identification, Grapevine, TX, 2015.

697. Wendt, F., Zeng, X., Churchill, J., King, J., and Budowle, B.: Analysis of single-source short tandem repeat (STR) and single nucleotide polymorphism (SNP) loci using a custom HaloPlex Target Enrichment System panel, 26th International Symposium on Human Identification, Grapevine, TX, 2015.

698. Takahashi, M., King, J.L., Zeng, X., Churchill, J.D., and Budowle, B.: One amplification – two analyses: a combined CE and MPS workflow, 26th International Symposium on Human Identification, Grapevine, TX, 2015.

699. Churchill, J., King, J.L., Chang, J., Wootton, S.C., Chang, C-W., Lagacé, R., and Budowle, B.: Evaluation of a short-amplicon multiplex for the mitochondrial genome on the Ion PGM™, 26th International Symposium on Human Identification, Grapevine, TX, 2015.

700. Ambers, A., Gill-King, H., Dirkmaat, D., Benjamin, R., King, J., and Budowle, B.: Autosomal and Y-STR analysis of degraded DNA from the 120-year-old skeletal remains of Ezekiel Harper,, 26th International Symposium on Human Identification, Grapevine, TX, 2015.

701. Ambers, A., Churchill, J.D., King, J.L., Stoljarova, M., Gill-King, H., and Budowle, B.: Characterization of unidentified 140-year-old human skeletal remains using massively parallel DNA sequencing, 26th International Symposium on Human Identification, Grapevine, TX, 2015.

702. Wiley, R., Sage, K., Budowle, B., LaRue, B.: An evaluation of a new rapid DNA platform for field forward applications, 26th International Symposium on Human Identification, Grapevine, TX, 2015.

703. Budowle, B.: Maturation of the field of microbial forensics, Ecology of Soil Microorganisms, Prague, Czech Republic, 20015.

704. Budowle, B.: Current approaches for the analysis of human remains, Bone Workshop and Conference 2015, Prague, Czech Republic, 2015.

705. Budowle, B.: Technology advances to enhance analysis of human remains: MPS, Bone Workshop and Conference 2015, Prague, Czech Republic, 2015.

706. Budowle, B.: How did DNA mixture interpretation become corrupted? Actual Innocence: Establishing Innocence or Guilt, Causes of and Solutions to Wrongful Convictions, The Center for American and International Law, Plano, TX, 2016.

707. Churchill, J.D., King, J., Chang, J.P., Wootton, S.C., Chang, C-W., Lagace, R., and Budowle, B.: Forensic application of massively parallel sequencing (MPS) with the Ion Torrent™ Multiplex Mitochondrial Genome Panel and Hi-Q™ sequencing chemistry, American Academy of Forensic Sciences, Las Vegas, NV, 2016.

708. Wiley, R.E., Sage K., Budowle, B., and LaRue, B.L.: An evaluation of a new Rapid DNA platform for field-forward applications, American Academy of Forensic Sciences, Las Vegas, NV, 2016.

709. Budowle, B., Churchill, J.D., King, J.L., Chang, J., Wootton, S.C., Chang, C-W., and Lagacé, R.: Forensic applications using the AmpliSeq™ mtDNA whole genome panel and massively parallel sequencing, Human Identification Solutions Conference, Barcelona, Spain, 2016.

710. Budowle, B.: Massively parallel sequencing for human identification evaluating results of a mito panel for challenged samples. Innovations & Perspectives, Human Identification Solutions, Gurgaon, India, 2016.

711. Budowle, B.: Massively parallel sequencing for human identification evaluating results of Mixture ID panel and comparison with CE data. Innovations & Perspectives, Human Identification Solutions, Gurgaon, India, 2016.

712. Budowle, B.: Overview of CE data interpretation and mixture analysis. Innovations & Perspectives, Human Identification Solutions, Gurgaon, India, 2016.

FUNDING

PI; Improved Tools and Interpretation Guidelines for Examining Limited Low Copy Number DNA Obtained from Degraded Single Source Samples: Bones, Teeth, and Hairs; Awarded by the National Institute of Justice; Award Number: 2009-DN-BX-K188; 10/01/2009 – 9/30/2011; Total: $935,992.00.

Co-PI; Development of an Expert System for Automated Forensic mtDNA Data Analysis; Awarded by the National Institute of Justice; Award Number: 2009-DN-BX-K171; 10/01/2009 - 03/31/2011; Total: $353,857.00.

Co-PI; Establishing the quantitative basis for sufficiency: threshold and metrics for friction ridge pattern detail quality and foundation for a standard; Awarded by Virginia Tech subcontract; the National Institute of Justice; Award Number: 2009-DN-BX-K229; 10/01/2009 - 09/30/2011; Total: $854,907.00; Subcontract: $123,120.00.

PI; Addressing Quality and Quantity; the Role of DNA Repair and Whole Genome Amplification in Forensically Relevant Samples; Awarded by the National Institute of Justice; Award Number: 2010-DN-BX-K227; 10/01/2010 – 09/30/2012. Total: $363,613.00

PI; Identity, Lineage, and Phenotypic SNP Identification, Assay Development, and Data Interpretation; Awarded by the 2010 Intelligence Community Postdoctoral Research Fellowship Program; Award Number: 2010*0937130*000; 09/01/2010 – 08/31/2010; Total: $239,076.00.

PI; Indel Study; Awarded by Life Technologies; Project ID RP0060; 10/18/2010 –04/01/2011; Total: $30,000.00.

PI; Research Collaboration; Awarded by Promega Corporation; 10/01/2010 – 09/30/2012; Total: $142,006.28

Co-PI; Comprehensive Training Program in Forensic DNA Interpretation and Statistics; Awarded by National Institute of Justice; Award number: NIJ-2010-93494, 2010-DN-BX-K239; 10/01/10-09/30/12; Total: $999,481.00.

PI; Microbial Forensics Technical and Scientific Process; Awarded by Signature Science; Award number: 2012-030-0002; 02/01/2012-01/31/2013; Total: $131,164.98.

Co-PI; Testing, Evaluation and Demonstration of New Technologies; Awarded by RTI International subcontract; Awarded by the National Institute of Justice; Award number: 2011-DN-BX-K564; 10/01/2011 – 09/30/2012; Total: $375,000.00.

PI; Development of Reference Sample DNA Profiling for Databases Using Next Generation Sequencing Technologies; Awarded by the National Institute of Justice; Award Number: 2012-DN-BX-K033; 10/01/2012 – 6/30/2014; Total: $747,797.00.

Co-PI; NIJ Ph.D. Graduate Research Fellowship Program FY 2012; Awarded by the National Institute of Justice; Award Number: Award 2012-IJ-CX-0016; 10/01/2012 – 09/30/2013; Total: $24,988.00.

PI; Validation of Rapid DNA Typing System; Awarded by Department of Defense; Contract Number: HQ0034-13-P-0002; 1/28/2013 – 01/27/2014; Total: $32,659.80.

PI; Microbial Forensics Technical and Scientific Process; Awarded by Signature Science; Renewal of Award number: 2012-030-0002; 02/01/2013-01/31/2014; Total: $131,164.98.

Co-PI; Testing, Development of Improved Insertion-Deletion Assaysfor Human and Ancestral Identifications from Degraded Samples; Awarded by the National Institute of Justice; Award number: 2013-DN-BX-K036; 10/01/2013 – 09/30/2015; Total: $336,282.96.

PI; Microbial Forensics Technical and Scientific Process; Awarded by Signature Science; Renewal of Award number: 2012-030-0002; 02/01/2014-01/31/2015; Total: $131,164.98.

PI; Deadwood Project, Historic Preservation Archives Department Deadwood, South Dakota; 09/01/2014-12/31/2014; Total: $3000.00.

PI; Familial Searching; Awarded by RTI International subcontract; 09/05/2014-12/31/2014; Total: $ $71,550.77.

PI; Novel Collection Device for Enhanced DNA Recovery and Release from Biological Stain Samples; Awarded by the National Institute of Justice; Award Number: 2014-DN-llX-K031; 01/01/2015 – 12/31/2016; Total: $487,884.00.

PI; Human Microbiome Species and Genes for Human Identification; Awarded by the National Institute of Justice; Award Number: 2015-NE-BX-K006; 01/01/2016 – 12/31/2017; Total: $589,701.00.

PI; Enhancing Mixture Interpretation with Highly Informative STRs; Awarded by the National Institute of Justice; Award Number: 2015-DN-BX-K067; 01/01/2016 – 12/31/2017; Total: $585,415.00.

Co-PI; Enhanced Sample Preparation and Data Interpretation Strategies for Massively Parallel Sequencing for Human Identification in Missing Persons and DVI Casework; Awarded by the National Institute of Justice; Award Number: 2015-DN-BX-K067; 01/01/2016 – 12/31/2017; $294,805.59

GRADUATED STUDENTS

Masters

Shamika Kelley, Masters, Thesis Practicum: Assessment of DNA transfer events involving routine human behavior, May 2010.

David Warshauer, Masters, Thesis Practicum: An evaluation of saliva-based DNA transfer, August 2011.

Alyssa Koehn, Masters Thesis: Identification of unknown PCR products generated during STR analysis of bone samples, May 2013.

Andrea Moore, Masters Thesis: STR typing of reference samples with rapid DNA technology, May 2014.

Lisa Skandalis, Masters Thesis: Population variances in the whole mitochondrial genome impacting capture for human identification, May 2015.

Doctoral

Pamela Marshall, Doctoral Dissertation: Improved tools for the robust analysis of low copy number and challenged DNA samples, May 2014.

David Warshauer, Doctoral Dissertation: Development of a comprehensive massively parallel sequencing panel of single nucleotide polymorphism and short tandem repeat markers for human identification, August 2015.

Xiangpei Zeng, Doctoral Dissertation: Selection of Highly Informative Markers for Apportionment of Ancestry and Population Affiliation, May 2016.

POST-DOCTORAL FELLOWS

Meredith Turnbough 2010-2011

Bobby Larue 2010-2012

Seung Bum Seo 2012-2014

Jennifer Churchill 2014-present

Angela Ambers 2015-present

Maiko Takahashi 2015-present

Xiangpei Zeng 2016-present

EXHIBIT B

D. JODY KOEHLER
5800 Guadalupe Street, Austin, TX 78765 | 512-424-2997 | jody.koehler@dps.texas.gov

OBJECTIVE: Lead in a manner that my agency may continue to produce timely, high quality results to our clients, and continue being a frontrunner in the forensic community in technology. To meet these objectives I will identify ways to continuously improve the workflow, procedures and practices, and implement strategies to ensure that resources are used effectively and that personnel receive the support they need, thereby allowing for a greater service to the citizens of Texas.

EXPERIENCE SUMMARY:

- 11+ years progressive management experience in a forensic laboratory
- 16+ years forensic science experience
- 10+ years auditing experience
- Quality driven, goal oriented

EXPERIENCE:

2006 to Present **DNA Section Manager, TXDPS, Austin Laboratory**

- Directed and managed a team of 20 members that exceeded legislatively mandated performance measures, while driving change in DNA methods utilized, improving efficiency and optimizing processes in the Austin DNA Section and Regional DNA laboratories
  Result: lower case turn-around times and more cases being reported to clients with uncompromised quality standards
- Developed a logistically sound and fiscally responsible strategy to become paperless utilizing the new LIMs system implemented in 2012
  Result: More efficient storage of case files and lower overhead for our retention program.
- First manager to implement quarterly 1 on 1 meetings and quarterly performance evaluation meetings with team members.
  Result: Better forum within the DNA team that cultivates open and honest vertical communication, as well as more accountability given to team members to meet and exceed goals
- First manager to implement a quality rubric for technical reviews performed by team members that objectively quantitates member's skills or deficiencies
  Result: Better understanding of employee's strengths and weaknesses in this critical function with added clarity on where individual or systemic issues are formed
- Champion of the technical review project between private laboratories and the Texas Department of Public Safety Crime Laboratory
  Result: More DNA profiles being entered into the CODIS which generates more investigative leads for Texas law enforcement agencies, without the need for increases to the number of Department employees or additional equipment. This was recently expanded to include Innocence Project cases nationwide.
- Served as the DNA Technical Leader for the Weslaco Regional Laboratory
  Result: Implementation of automated processes utilized in the Austin laboratory to improve efficiency and case turn-around time
- Implemented group-based training scheme
  Result: More efficient use of resources for Regional laboratories as up to 5 untrained personnel can be trained simultaneously by one senior team member
- Served as a Department representative during the Cold Case Investigations meetings
  Result: Fostered relationships between agencies, aided in identifying evidence that may benefit from re-analysis, potentially leading to new developments in inactive investigations

2004-2006 **Assistant DNA Section Supervisor/DNA Technical Leader, TXDPS, Austin Laboratory**

- Second line manager responsible for providing oversight for the technical operations of the DNA section, training new analysts, organizing and maintaining accreditation standards for the DNA Section, overseeing proficiency testing, troubleshooting instrumentation, evaluating employee abilities, recommending remedial training, validating new instrumentation, serving as a team member of the DNA advisory board, and investigating crime scenes.
- One of the 1st individuals within the Department to be qualified as a technical assessor for ASCLD-LAB International
  Result: Able to assist the Crime Laboratory System in becoming accredited under the new ISO-17025 standards

1996-2001 **Criminalist I-IV/DNA Technical Leader, TXDPS, Austin Laboratory**

- Team member responsible for providing oversight for the technical operations of the DNA section, training new analysts, organizing and maintaining accreditation for the DNA section, overseeing proficiency testing, troubleshooting instrumentation, evaluating employee abilities, recommending remedial training, validating new instrumentation, serving as a team member of the DNA advisory board, and investigating crime scenes.

2002-2003 **Teacher, Austin Independent School District**

- Responsible for teaching 7th grade Magnet Science, Medical Technology and Marine Biology.
- Supervised the work of 28 students. Maintained accurate records of attendance, grades, and conversations with students and parents.
- Successfully met the needs of students and parents on a daily basis in a professional manner.
- Planned lessons to ensure TEKS guidelines were satisfied and students were engaged in learning.

1994-1996 **Microbiologist, Texas Parks and Wildlife Department, San Marcos, Texas**

- Established 2 DNA laboratories within the Inland Fisheries Division.
- Trained new staff on DNA technologies.
- Wrote research proposals and conducted genetic analyses on fish populations within Texas.

2002-2005 **Adjunct Instructor, Austin Community College**

- Taught lecture and laboratory sections of Introductory Biology and Microbiology to approximately 28 students each semester. Graded all written work and developed course curriculum.

2005-2009 **Adjunct Instructor, Concordia University**

- Taught lecture and laboratory sections of Introductory Biology.
- Taught Forensic Science.
- Team member of a Curriculum Development Team to improve the Forensic Biology course and add a laboratory component to the course.

EDUCATION:

1994-1996 **Southwest Texas State University, San Marcos, TX**
Master of Science, Biology, Minor-Biochemistry

1992-1993 **Southwest Texas State University, San Marcos, TX**
Bachelor of Science in Aquatic Biology, Minor-Chemistry, Magna Cum Laude

MEMBERSHIPS:

American Society of Crime Laboratory Directors
Association of Forensic DNA Analysts and Administrators

AUDITOR QUALIFICATIONS

American Society of Crime Laboratory Directors-LAB-*International* Assessor (since 2006)
American Society of Crime Laboratory Directors-LAB-Legacy Inspector (since 2005)
The FBI Quality Assurance Standards for Forensic DNA Testing Laboratories Auditor (since 2005)

AWARDS:

Unit Citation, Texas Department of Public Safety
Phyiscal Fitness Star, Texas Department of Public Safety
Graduate Stipend, Southwest Texas State University
Academic Excellence Award, Southwest Texas State University
Fred and Yetta Richan Aquatic Biology Award, Southwest Texas State University

PUBLICATIONS:

Kathryn Oostdik, Kristy Lenz, Jeffrey Nye, Kristin Schelling, Donald Yet, Scott Bruski, Joshua Strong, Clint Buchanan, Joel Sutton, Jessica Linner, Nicole Frazier, Hays Young, Learden Matthies, Amber Sage, Jeff Hahn, Regina Wells, Natasha Williams, Monica Price, D. Jody Koehler, Melisa Staples, Katie L. Swango, et al. 2014. Developmental validation of the PowerPlex® Fusion System for analysis of casework and reference samples: A 24-locus multiplex for new database standards. FSI: Genetics, Vol. 12: 69-76

Jonelle M. Thompson, Margaret M. Ewing, William E. Frank, Jill J. Pogemiller, Craig A. Nolde, D. Jody Koehler, Alyssandra M. Shaffer, Dawn R. Rabbach, Patricia M. Fulmer, Cynthia J. Sprecher, Douglas R. Storts. 2013. Developmental validation of the PowerPlex® Y23 System: A single multiplex Y-STR analysis system for casework and database samples. FSI: Genetics Vol 7 (2): 240-250.

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Available upon request.

EXHIBIT C

SUGGESTED READING (ARRANGED IN CHRON ORDER FROM 1991-2015)

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**The Commission is eagerly awaiting the publication of an informative and highly relevant article on evaluation of forensic DNA evidence, including a protocol for interpretation and statistical calculations using the combined probability of inclusion. Staff will include a link to the article at www.fsc.texas.gov upon release. The article's authors are Drs. Frederick Bieber, John Buckleton, Bruce Budowle, John Butler and Michael Coble.*

EXHIBIT D

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June 30, 2015

The Texas Department of Public Safety Crime Laboratory system was informed by the Federal Bureau of Investigation in May 2015 of errors in the FBI-developed population database. This database has been used by the Texas DPS Crime Laboratory system as well as many other crime laboratories across the country for calculating match statistics in criminal investigations and other types of human identification applications since 1999.

Upon notification, the forensic DNA community immediately began corrective action. During implementation of corrective measures, minor discrepancies were discovered in additional data used exclusively by the Texas Department of Public Safety. All of the errors have been corrected and the changes have empirically demonstrated minimal impact on the calculations used to determine the significance of an association. **Further, the database corrections have no impact on the inclusion or exclusion of victims or defendants in any result.**

If requested in writing, the Texas DPS Crime Laboratory System will recalculate and report statistics previously reported in individual cases.

If you have any questions, please contact your local crime laboratory.

Brady W Mills
Deputy Assistant Director
Law Enforcement Support
Crime Laboratory Service

EXHIBIT E

SWGDAM Interpretation Guidelines for Autosomal STR Typing by Forensic DNA Testing Laboratories

Scientific Working Group on DNA Analysis Methods (SWGDAM)

The Scientific Working Group on DNA Analysis Methods, better known by its acronym of SWGDAM, is a group of approximately 50 scientists representing federal, state, and local forensic DNA laboratories in the United States and Canada. During meetings, which are held twice a year, subcommittees discuss topics of interest to the forensic DNA community and often develop documents to provide direction and guidance for the community. A mixture interpretation subcommittee was formed in January 2007 and worked for several years to provide a guidance document on autosomal short tandem repeat (STR). This document was presented to the full SWGDAM group and received approval in January 2010.

This document provides guidelines for the interpretation of DNA typing results from short tandem repeats (STR) and supersedes the Scientific Working Group on DNA Analysis Methods (SWGDAM) Short Tandem Repeat (STR) Interpretation Guidelines (2000). The revised guidelines are not intended to be applied retroactively. Guidance is provided for forensic casework analyses on the identification and application of thresholds for allele detection and interpretation, and appropriate statistical approaches to the interpretation of autosomal STRs with further guidance on mixture interpretation. Laboratories are encouraged to review their standard operating procedures and validation data in light of these guidelines and to update their procedures as needed. It is anticipated that these guidelines will evolve further as future technologies emerge. Some aspects of these guidelines may be applicable to low level DNA samples. However, this document is not intended to address the interpretation of analytical results from enhanced low template DNA techniques.

Introduction

The interpretation of DNA typing results for human identification purposes requires professional judgment and expertise. Additionally, laboratories that analyze DNA samples for forensic casework purposes are required by the Quality Assurance Standards for Forensic DNA Testing Laboratories (effective July 1, 2009) to establish and follow documented procedures for the interpretation of DNA typing results and reporting. Due to the multiplicity of forensic sample types and the potential complexity of DNA typing results, it is impractical and infeasible to cover every aspect of DNA interpretation by a preset rule. However, the laboratory should utilize written procedures for interpretation of analytical results with the understanding that specificity in the standard operating protocols will enable greater consistency and accuracy among analysts within a laboratory. It is recommended that standard operating procedures for the interpretation of DNA typing results be sufficiently detailed that other forensic

DNA analysts can review, understand in full, and assess the laboratory's policies and practices. The laboratory's interpretation guidelines should be based upon validation studies, scientific literature, and experience.

Background

Upon completion of the technical aspects of DNA analysis, DNA typing results must be verified and interpreted. The verification of the accuracy of the DNA typing results involves a review of peak designations and other software-generated information, as well as an evaluation of quality controls. Based on this assessment, the DNA analyst performs interpretations, makes comparisons among samples (where appropriate) and draws conclusions. These data and conclusions are technically reviewed and the conclusions are typically captured for documentation and communication purposes within a laboratory report.

Using current technologies for human identification, DNA typing results are derived through application of analytical software during and after electrophoresis of fluorescently-labeled amplification products that are generated for each sample using an amplification kit. For each sample, the software translates fluorescence intensity data into electropherograms and then labels any detected peaks with such descriptors as size (in base-pairs, or bp) and peak height (in relative fluorescence units, or RFU). Using allelic ladders for reference, the software then labels peaks that meet certain criteria with allelic designations.

To ensure the accuracy of these computer-generated allele designations, the DNA analyst must verify that appropriate genotyping parameters (i.e., internal size standard and allelic ladder) were used and that the correct genotyping results were obtained for a known positive control. Additionally, if a sample is amplified using multiple kits that contain redundant loci, the DNA analyst must address the concordance of the genotyping results at the loci that are common to both kits. As an example, a given sample amplified using both the Profiler Plus™ and COfiler™ Amplification Kits exhibits concordance when identical alleles for the genetic loci amelogenin, D3S1358, and D7S820 are obtained. After verification of the allelic designations, the alleles are classified based on their peak height relative to an established minimum peak height threshold for comparison purposes.

The results of the analysis controls [i.e., reagent blank(s), positive amplification control(s), and negative amplification control(s)] are evaluated. If the reagent blank(s), positive amplification control(s), and negative amplification control(s) yield results that are within their prescribed specifications, the DNA analyst interprets the DNA typing results from each sample to determine if the DNA typing results originated from a single donor or multiple donors. If the expected results are not obtained from a control sample(s), the DNA analyst must determine if the control(s) and/or sample(s) should be re-processed or proceed within the prescribed limitations of interpretation.

Based on the interpretation of the forensic samples and a comparison of the DNA typing results obtained from the questioned sample(s) to those of any known sample(s), or a comparison between multiple questioned samples, a DNA analyst can reach one of three primary conclusions: cannot exclude, can exclude, or inconclusive/uninterpretable.

Statistical interpretation for reported inclusionary results provides weight to the inclusionary statement. Statistical analysis is not required for exclusionary conclusions, comparisons between multiple questioned samples without a comparison to a known sample, nor applicable to inconclusive/uninterpretable results. The conclusions reached as part of the DNA interpretation process are compiled into a written draft by the DNA analyst and are subjected to technical and administrative reviews prior to issuing a final case report.

This document addresses definitions, data evaluation, interpretation of results and conclusions/reporting for autosomal STR typing, including guidance on mixture interpretation. Approaches to statistical interpretation are presented. A list of relevant literature is also included to provide further source material.

1. Preliminary Evaluation of Data

The laboratory should develop criteria to determine whether an instrumental response represents the detection of DNA fragment(s) rather than instrument noise. An analytical threshold defines the minimum height requirement at and above which detected peaks can be reliably distinguished from background noise. Because the analytical threshold is based upon a distribution of noise values, it is expected that occasional, non-reproducible noise peaks may be detected above the analytical threshold. An analytical threshold should be sufficiently high to filter out noise peaks. Usage of an exceedingly high analytical threshold increases the risk of allelic data loss which is of potential exclusionary value.

1.1. Analytical threshold: The Laboratory should establish an analytical threshold based on signal-to-noise analyses of internally derived empirical data. As an example, an analytical threshold may be based on two times the intensity difference between the highest peak and lowest trough within the instrumental noise data. Other scientific methods may be used. The usage of an analytical threshold value that differs substantially from manufacturer's recommendations should be supported by internal signal-to-noise assessments.

1.2. The laboratory must develop criteria to evaluate internal standards and/or allelic ladders.

1.3. Controls are required to assess analytical procedures.

1.3.1. The laboratory must establish criteria for evaluation of the following controls, including but not limited to: reagent blank and positive and negative amplification controls.

1.3.2. The laboratory must develop criteria for the interpretation and documentation of results in the event that the controls do not perform as expected.

1.4. A laboratory using STR multiplexes that contain redundant loci must establish criteria regarding the concordance of such data.

2. Allele Designation

2.1. The laboratory establishes criteria to assign allele designations to appropriate peaks.

2.1.1. Locus Designation: The laboratory establishes criteria to address locus assignment for alleles. The criteria should address alleles that fall above the largest or below the smallest allele (or virtual bin) of the allelic ladder.

2.1.2. Allele Designation: The laboratory designates alleles as numerical values in accordance with recommendations of the International Society of Forensic Genetics.

2.1.2.1. Allele designation is based operationally on the number of repeat sequences contained within the allele and by comparison to an allelic ladder.

2.1.2.2. The laboratory establishes guidelines for the designation of alleles containing an incomplete repeat motif (i.e., an off-ladder allele falling within the range spanned by the ladder alleles). This designation includes the number of complete repeats and, separated by a decimal point, the number of base pairs in the incomplete repeat (e.g., FGA 18.2 allele).

2.1.2.3. The laboratory establishes criteria for designating alleles that fall above the largest or below the smallest allele of the allelic ladder (or virtual bin). Extrapolation of an above/below ladder allele to a specific designation (e.g., generally to no more than one repeat unit) should also be supported by precision studies, validation and determination of measurement variance. Above/below ladder alleles should be designated as either greater than (>) or less than (<) the respective ladder allele (or virtual bin), or designated numerically when appropriate extrapolation can be used. When the “>” or “<” designation is used, the laboratory should establish criteria, based on relative sizes, for the comparison of such alleles among samples.

3. Interpretation of DNA Typing Results

3.1. Non-Allelic Peaks

Because forensic DNA typing characterizes STR loci using PCR and electrophoretic technologies, some data that result from this analytical scheme may not represent actual alleles that originate in the sample. It is therefore necessary, before the STR typing results can be used for comparison purposes, to identify any potential non-allelic peaks. Non-allelic peaks may be PCR products (e.g., stutter, non-template dependent nucleotide addition, and non-specific amplification product), analytical artifacts (e.g., spikes and raised baseline), instrumental limitations (e.g., incomplete spectral separation resulting in pull-up or bleed-through), or may be introduced into the process (e.g., disassociated primer dye). Generally, non-allelic data such as stutter, non-template dependent nucleotide addition, disassociated dye, and incomplete spectral separation are reproducible; spikes and raised baseline are generally non-reproducible.

3.1.1. The laboratory establishes criteria based on empirical data (obtained internally or externally), and specific to the amplification and detection systems used, to address the interpretation of non-allelic peaks. The guidelines address identification of non-allelic peaks and the uniform application, across all loci of a DNA profile, of the criteria used to identify non-allelic peaks.

3.1.1.1. In general, the empirical criteria are based on qualitative and/or quantitative characteristics of peaks. As an example, dye artifacts and spikes may be distinguished from allelic peaks based on morphology and/or reproducibility. Stutter and non-template dependent nucleotide addition peaks may be characterized based on size relative to an allelic peak and amplitude.

3.1.1.2. While the application of an analytical threshold may serve to filter out some non-allelic peaks, the analytical threshold should be established based on signal-to-noise considerations (i.e., distinguishing potential allelic peaks from background). The analytical threshold should not be established for purposes of avoiding artifact labeling as such may result in the potential loss of allelic data.

3.1.1.3. The laboratory establishes guidelines addressing off-scale data. Fluorescence detection instruments have a limited linear range of detection, and signal saturation can result in off-scale peaks. Following peak detection, such peaks in the analyzed data are assigned an artificial height value which is not representative of the true amplitude. Peak

height values for off-scale peaks should not be used in quantitative aspects of interpretation (e.g., stutter and peak height ratio assessments).

3.2. Application of Peak Height Thresholds to Allelic Peaks

Amplification of low-level DNA samples may be subject to stochastic effects, where two alleles at a heterozygous locus exhibit considerably different peak heights (i.e., peak height ratio generally <60%) or an allele fails to amplify to a detectable level (i.e., allelic dropout). Stochastic effects within an amplification may affect one or more loci irrespective of allele size. Such low-level samples exhibit peak heights within a given range which is dependent on quantitation system, amplification kit and detection instrumentation. A threshold value can be applied to alert the DNA analyst that all of the DNA typing information may not have been detected for a given sample. This threshold, referred to as a stochastic threshold, is defined as the value above which it is reasonable to assume that allelic dropout has not occurred within a single-source sample. The application of a stochastic threshold to the interpretation of mixtures should take into account the additive effects of potential allele sharing.

3.2.1. The laboratory establishes a stochastic threshold based on empirical data derived within the laboratory and specific to the quantitation and amplification systems (e.g., kits) and the detection instrumentation used. It is noted that a stochastic threshold may be established by assessing peak height ratios across multiple loci in dilution series of DNA amplified in replicate. The RFU value above which it is reasonable to assume that, at a given locus, allelic dropout of a sister allele has not occurred constitutes a stochastic threshold.

3.2.1.1. If measures are used to enhance detection sensitivity (i.e., allelic height), the laboratory should perform additional studies to establish independent criteria for application of a separate stochastic threshold(s). Such measures may include but not be limited to increased amplification cycle number, increased injection time, and post-amplification purification/concentration of amplified products.

3.2.1.2. For samples for which an assumption can be made as to the number of contributors, the laboratory should establish criteria for comparison of allelic peaks which fall below the stochastic threshold. As an example, if a locus in an assumed single-source sample exhibits two peaks, one or both of which are below the stochastic threshold, the laboratory may use that locus for comparison purposes. Also, the presence of male DNA may be established based on a Y-allele at amelogenin that is below the stochastic threshold.

3.2.2. If a stochastic threshold based on peak height is not used in the evaluation of DNA typing results, the laboratory must establish alternative

criteria (e.g., quantitation values or use of a probabilistic genotype approach) for addressing potential stochastic amplification. The criteria must be supported by empirical data and internal validation and must be documented in the standard operating procedures.

3.3. Peak Height Ratio

Intra-locus peak height ratios (PHR) are calculated for a given locus by dividing the peak height of an allele with a lower RFU value by the peak height of an allele with a higher RFU value, and then multiplying this value by 100 to express the PHR as a percentage.

3.3.1. The laboratory should establish PHR requirements based on empirical data for interpretation of DNA typing results from single-source samples. Different PHR expectations can be applied to individual loci (e.g., 70% for D3S1358, 65% for vWA, etc.); alternatively, a single PHR expectation can be applied to multiple loci (e.g., 60%).

3.3.1.1. The laboratory may evaluate PHRs at various DNA template levels (e.g., dilution series of DNA). It is noted that different PHR expectations at different peak height ranges may be established.

3.3.2. PHR requirements are only applicable to allelic peaks that meet or exceed the stochastic threshold.

3.4. Number of Contributors to a DNA Profile

Generally, a sample is considered to have originated from a single individual if one or two alleles are present at all loci for which typing results were obtained (although tri-allelic loci may occur), and the peak height ratios for all heterozygous loci are within the empirically determined values. It is noted that peak height imbalances may be seen in the typing results from, for example, a primer binding site variant that results in attenuated amplification of one allele of a heterozygous pair.

A sample is generally considered to have originated from more than one individual if three or more alleles are present at one or more loci (excepting tri-allelic loci) and/or the peak height ratios between a single pair of allelic peaks for one or more loci are below the empirically determined heterozygous peak height ratio expectation. Generally, the minimum number of contributors to a mixed sample can be determined based on the locus that exhibits the greatest number of allelic peaks. As an example, if at most five alleles are detected per locus, then the DNA typing results are consistent with having arisen from at least three individuals.

3.4.1. For DNA mixtures, the laboratory should establish guidelines for determination of the minimum number of contributors to a sample. Alleles need not meet the stochastic threshold to be used in this assessment.

3.4.2. The laboratory should define the number of alleles per locus and the relative intra-locus peak height requirements for assessing whether a DNA typing result is consistent with originating from one or more sources. The minimum number of loci should be defined for determination of whether a sample is a mixture.

3.4.3. Where multiple amplifications and/or injections are generated for a given sample extract, the laboratory should establish guidelines for determining which results are used for comparisons and statistical calculations.

3.4.3.1. If composite profiles (i.e., generated by combining typing results obtained from multiple amplifications and/or injections) are used, the laboratory should establish guidelines for the generation of the composite result. When separate extracts from different locations on a given evidentiary item are combined prior to amplification, the resultant DNA profile is not considered a composite profile. Unless there is a reasonable expectation of sample(s) originating from a common source (e.g., duplicate vaginal swabs or a bone), allelic data from separate extractions from different locations on a given evidentiary item should not be combined into a composite profile. The laboratory should establish guidelines for determining the suitability of developing composite profiles from such samples.

3.5. Interpretation of DNA Typing Results for Mixed Samples

An individual's contribution to a mixed biological sample is generally proportional to their quantitative representation within the DNA typing results. Accordingly, depending on the relative contribution of the various contributors to a mixture, the DNA typing results may potentially be further refined.

As an example, if a sample contains a predominance of one individual's DNA, that individual's DNA profile may be determined. This state results in a distinguishable mixture, whereby there is a distinct contrast in signal intensities (e.g., peak heights) among the different contributors' alleles. In such instances, major and/or minor contributors may be determined. Discernment of the STR typing results for the major or minor contributors to a mixture may be limited to only some loci (with the remaining loci yielding multiple potential genotypes for the major or minor contributor). The major (and possibly the minor) contributor may effectively constitute a deduced single-source profile.

Alternatively, if the amounts of biological material from multiple donors are similar, it may not be possible to further refine the mixture profile. When major or minor contributors cannot be distinguished because of similarity in signal intensities, the sample is considered to be an indistinguishable mixture. The classification as indistinguishable may be limited to some, not all, of the loci for which DNA typing results are obtained and does not imply that the profile is uninterpretable. Individuals may still be included or excluded as possible contributors to an indistinguishable mixture.

Evidence items taken directly from an intimate sample, as determined by the laboratory, are generally expected to yield DNA from the individual from whom the sample was taken. If another source of DNA is present in sufficient quantity in such a sample, a mixture of DNA is likely to be detected. Based on this expectation, any DNA typing results from such a mixture that match a conditional known sample (e.g., from the victim) may be separated from the other mixture results to facilitate identification of the foreign alleles. The obligate alleles may effectively constitute a single-source profile (i.e., if there is one DNA contributor in addition to the individual from whom the sample was taken) or a mixture profile (i.e., if there are multiple additional DNA contributors). A similar state can exist when another known individual (i.e., consensual partner) is expected to have contributed biological material to the mixed sample.

3.5.1. The laboratory should establish guidelines based on peak height ratio assessments for evaluating potential sharing of allelic peaks among contributors and for determining whether contributors to a mixed DNA typing result are distinguishable. When assessing peak height ratios, pair-wise comparison of all potential genotypic combinations should be evaluated.

3.5.2. The laboratory should define and document what, if any, assumptions are used in a particular mixture deconvolution.

3.5.2.1. If no assumptions are made as to the number of contributors, at a minimum, the laboratory should assign to a major contributor an allele (e.g., homozygous) or pair of alleles (e.g., heterozygous) of greater amplitude at a given locus that do not meet peak height ratio expectations with any other allelic peak(s).

3.5.2.2. If assumptions are made as to the number of contributors, additional information such as the number of alleles at a given locus and the relative peak heights can be used to distinguish major and minor contributors.

3.5.3. A laboratory may define other quantitative characteristics of mixtures (e.g., mixture ratios) to aid in further refining the contributors.

3.5.3.1. Differential degradation of the contributors to a mixture may impact the mixture ratio across the entire profile.

3.5.4. Mixtures with a Single Major Contributor and One or More Minor Contributors:

3.5.4.1. In general, heterozygous alleles attributed to a major contributor should meet the laboratory's established peak height ratio expectations for single-source samples. Due to the potential for overlapping peaks to cause imbalance of major heterozygous alleles, the laboratory may establish a quantitative means of evaluating the distinction in peak heights of the major and minor contributors (i.e., mixture ratio).

3.5.4.2. After deconvolution, the DNA typing results attributed to an individual minor contributor should also meet PHR expectations. The PHR expectations of a minor contributor may be reduced due to stochastic peak height variation and the additive effects of peak sharing (e.g., minor peak and stutter peaks).

3.5.4.3. Due to the possibility that the minor contributor's alleles may be shared by the major contributor (and thus masked), determination of a single genotype for a minor contributor may be possible at only some loci (while multiple allelic combinations, or allelic drop out, are possible at other loci).

3.5.5. Mixtures with Multiple Major Contributors and One or More Minor Contributors: The laboratory should establish guidelines based on peak height ratio assessments and/or mixture ratios for determining whether multiple major contributors are present in a mixed sample.

3.5.6. Mixtures with Indistinguishable Contributors: The laboratory should establish guidelines based on peak height ratio assessments for identifying mixtures for which no major or minor contributors can be discerned.

3.5.7. Mixtures with a Known Contributor(s): The laboratory should establish guidelines for determining whether separation of a known contributor's profile is applicable (e.g., based on the types of evidentiary items).

3.5.7.1. At a minimum, where there is no indication of sharing of the known and obligate alleles, the laboratory should separate out those alleles attributable to the known sample (e.g., victim, consensual partner, etc.).

3.5.7.2. To further refine the obligate alleles in a profile, the laboratory may establish guidelines for addressing potential sharing of alleles among

the individual known to have contributed to a sample and the additional contributor(s).

3.5.8. Interpretation of Potential Stutter Peaks in a Mixed Sample

3.5.8.1. For mixtures in which minor contributors are determined to be present, a peak in stutter position (generally n-4) may be determined to be 1) a stutter peak, 2) an allelic peak, or 3) indistinguishable as being either an allelic or stutter peak. This determination is based principally on the height of the peak in the stutter position and its relationship to the stutter percentage expectations established by the laboratory.

3.5.8.2. Generally, when the height of a peak in the stutter position exceeds the laboratory's stutter expectation for a given locus, that peak is consistent with being of allelic origin and should be designated as an allele.

3.5.8.3. If a peak is at or below this expectation, it is generally designated as a stutter peak. However, it should also be considered as a possible allelic peak, particularly if the peak height of the potential stutter peak(s) is consistent with (or greater than) the heights observed for any allelic peaks that are conclusively attributed (i.e., peaks in non-stutter positions) to the minor contributor(s).

3.6 Comparison of DNA Typing Results

The following determinations can be made upon comparison of evidentiary and known DNA typing results (and between evidentiary samples):

- The known individual cannot be excluded (i.e., is included) as a possible contributor to the DNA obtained from an evidentiary item.
- The known individual is excluded as a possible contributor.
- The DNA typing results are inconclusive/uninterpretable.
- The DNA typing results from multiple evidentiary items are consistent or inconsistent with originating from a common source(s).

3.6.1. The laboratory must establish guidelines to ensure that, to the extent possible, DNA typing results from evidentiary samples are interpreted before comparison with any known samples, other than those of assumed contributors.

3.6.2. DNA typing results may not be obtained at all loci for a given evidentiary sample (e.g., due to DNA degradation, inhibition of amplification and/or low-template quantity); a partial profile thus results.

3.6.2.1. For partial profiles, the determination of which alleles/loci are suitable for comparison and statistical analysis should be made prior to comparison to the known profiles.

3.6.2.2. The laboratory should establish guidelines for inclusions and exclusions when a known individual's DNA profile is not fully observed in the evidentiary profile.

3.6.3. The laboratory must establish guidelines for inclusionary, exclusionary and inconclusive/uninterpretable conclusions based on comparisons of DNA typing results from known samples and both single-source and mixed evidentiary samples.

3.6.4. For mixtures for which two or more individuals cannot be excluded as potential contributors, the laboratory may establish guidelines for assessing whether all of the DNA typing results obtained from the mixed sample are accounted for by the multiple known samples.

3.6.5. Because assumptions regarding the origin of evidence or the number of contributors to a mixture can impact comparisons, the laboratory should establish guidelines for documenting any assumptions that are made when formulating conclusions.

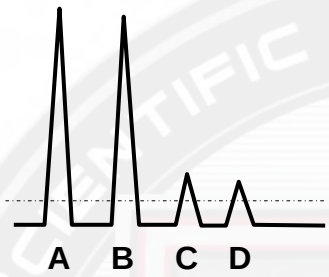
3.6.6. The laboratory should establish guidelines for identifying DNA typing results for which comparisons of evidentiary and known samples are not made (at a minimum, to include inconclusive/uninterpretable results).

4. Statistical Analysis of DNA Typing Results

In forensic DNA testing, calculations are performed on evidentiary DNA profiles that are established as relevant in the context of the case to aid in the assessment of the significance of an inclusion. These calculations are based on the random match probability (RMP), the likelihood ratio (LR), or the combined probability of exclusion/inclusion (CPE/CPI).

While the RMP is commonly thought of in terms of single-source profiles, the application of this formula to evidentiary profiles inherently includes an assumption of the number of contributors to the DNA sample. As such, this document also applies the term RMP to mixture calculations where the number of contributors is assumed (this has sometimes been referred to as a "modified RMP"). By using the RMP nomenclature, these calculations are distinguished from the CPI nomenclature which is commonly thought of in terms of a mixture calculation that makes no assumption as to the number of contributors.

In addition to assumptions of the number of contributors, quantitative peak height information and mixture ratio assessments may or may not be included in the interpretation of an evidentiary profile. Calculations performed using interpretations incorporating this information are termed “restricted.” When this quantitative peak height information is not included, the resultant calculation is termed “unrestricted” (Figure 1).



Unrestricted

All combinations of alleles are deemed possible (relative peak height differences are not utilized)

AB + AC + AD + BC + BD + CD

Restricted

Based on relative peak heights, alleles are paired only where specific combinations of alleles are deemed possible

AB + ~~AC~~ + ~~AD~~ + ~~BC~~ + ~~BD~~ + CD

Figure 1. Illustration of “restricted” versus “unrestricted” approaches based on relative peak heights (using an assumption of two donors with all peaks above the stochastic threshold).

The genetic loci and assumptions used for statistical calculations must be documented, at a minimum, in the case notes.

4.1. The laboratory must perform statistical analysis in support of any inclusion that is determined to be relevant in the context of a case, irrespective of the number of alleles detected and the quantitative value of the statistical analysis.

4.1.1. The laboratory should establish guidelines where multiple stains from the same or separate items have provided genetic information that is consistent with originating from a common source(s) but having various levels of discrimination. In general, the statistics for the typing results that provide the most genetic information and/or the highest discrimination potential are reported.

4.2. For calculating the CPE or RMP, any DNA typing results used for statistical analysis must be derived from evidentiary items and not known samples. This precludes combining multiple CPE or RMP results for the same mixture component of an evidentiary sample. However, different calculations may be made for the same mixture component if different assumptions as to the number of contributors are made and clearly stated in the case notes and/or report.

4.3. The laboratory must not use inconclusive/uninterpretable data (e.g., at individual loci or an entire multi-locus profile) in statistical analysis.

4.3.1. For a distinguishable mixture, a major contributor(s) profile may be suitable for statistical analysis even in the presence of inconclusive minor contributor results.

4.4. Exclusionary conclusions do not require statistical analysis.

4.5. The laboratory must document the source of the population database(s) used in any statistical analysis.

4.6. The formulae used in any statistical analysis must be documented and must address both homozygous and heterozygous typing results, multiple locus profiles, mixtures, minimum allele frequencies, and, where appropriate, biological relationships.

4.6.1. Given a profile for which multiple formulae are applicable, the laboratory must have guidelines for the selection of the formula(e) suitable for statistical application (see Table 1).

4.6.2. It is not appropriate to calculate a composite statistic using multiple formulae for a multi-locus profile. For example, the CPI and RMP cannot be multiplied across loci in the statistical analysis of an individual DNA profile because they rely upon different fundamental assumptions about the number of contributors to the mixture.

4.6.3. When using CPE/CPI (with no assumptions of number of contributors) to calculate the probability that a randomly selected person would be excluded/included as a contributor to the mixture, loci with alleles below the stochastic threshold may not be used for statistical purposes to support an inclusion. In these instances, the potential for allelic dropout raises the possibility of contributors having genotypes not encompassed by the interpreted alleles.

4.6.3.1. Alleles below the stochastic threshold may be used for comparisons and/or to establish the presence of a mixture or male DNA (e.g., Y allele at amelogenin).

4.6.3.2. A restricted CPE/CPI may be applied to multiple major contributors despite the presence of minor contributor(s) alleles below the stochastic threshold; a description of how to calculate can be found in Section 5.3.5.

4.7. If a laboratory uses source attribution statements, then it must establish guidelines for the criteria on which such a declaration is based.

5. Statistical Formulae

5.1. Whenever the statistical analysis at a locus is meant to represent all possible contributors to a mixture, if there is a reasonable possibility that locus dropout could have led to the loss of an entire genotype, then a statistical calculation should not be performed for that locus. Similarly, the product rule should not be applied when the resultant set of combined profiles would not include all individuals who would not be excluded as possible contributors to the mixture.

5.2. Random Match Probability (RMP)

5.2.1. When the interpretation is based upon the assumption of a single contributor (or a single major contributor to a mixture), the RMP formulae are those described in NRCII recommendations 4.1, 4.2, 4.3, and 4.4. The most commonly used formulae are listed below:

5.2.1.1. For heterozygote genotypes, the formula is 2pq. This is NRCII formula 4.1b.

5.2.1.2. For homozygote genotypes, the formula is $p^2 + p(1-p)\theta$, where $\theta = 0.01$ or 0.03 in accordance with NRCII. This is NRCII formula 4.4a.

5.2.1.3. For single-allele profiles where the zygosity is in question (e.g., it falls below the stochastic threshold):

5.2.1.3.1. The formula 2p, as described in recommendation 4.1 of NRCII, may be applied to this result.

5.2.1.3.2. Instead of using 2p, the algebraically identical formulae $2p - p^2$ and $p^2 + 2p(1-p)$ may be used to address this situation without double-counting the proportion of homozygotes in the population.

5.2.1.3.3. Laboratories may choose to assign the value of 1 to the scenario described in 5.2.1.3., i.e. not use the locus for statistical weight.

5.2.1.4. Conditional subpopulation calculations may also be performed in accordance with NRCII formulae 4.10a and 4.10b.

5.2.2. When the interpretation is conditioned upon the assumption of a particular number of contributors greater than one, the RMP is the sum of the individual frequencies for the genotypes included following a mixture deconvolution. Examples are provided below.

5.2.2.1. In a sperm fraction mixture (at a locus having alleles P, Q, and R) assumed to be from two contributors, one of whom is the victim (having genotype QR), the sperm contributor genotypes included post-deconvolution might be PP, PQ, and PR. In this case, the RMP for the sperm DNA contributor could be calculated as $[p^2 + p(1-p)\theta] + 2pq + 2pr$.

5.2.2.2. In a sperm fraction mixture (at a locus having alleles P, Q, and R) assumed to be from two contributors, where the major contributor is the victim (having genotype QR), there remains an obligate minor contributor P allele above the stochastic threshold. Also present in the results are two peaks filtered as possible stutter (S* and T*). If both filtered peaks are within an RFU range that could reasonably be paired with the P allele as heterozygous genotypes, the sperm contributor genotypes included post-deconvolution might be PP, PQ, PR, PS* and PT*. In this case, the RMP for the sperm DNA contributor could be calculated as $[p^2 + p(1-p)\theta] + 2pq + 2pr + 2ps + 2pt$. Some laboratories might instead choose to apply a single-allele formula as discussed in section 5.2.1.3, e.g., 2p.

5.2.2.3. In a mixture having at a locus alleles P, Q, and R, assumed to be from two contributors, where all three alleles are below the stochastic threshold, the interpretation may be that the two contributors could be a heterozygote-homozygote pairing where all alleles were detected, a heterozygote-heterozygote pairing where all alleles were detected, or a heterozygote-heterozygote pairing where a fourth allele might have dropped out. In this case, the RMP must account for all heterozygotes and homozygotes represented by these three alleles, but also all heterozygotes that include one of the detected alleles. The RMP for this interpretation could be calculated as $(2p - p^2) + (2q - q^2) + (2r - r^2) - 2pq - 2pr - 2qr$.

5.2.2.3.1. Since 2p includes 2pq and 2pr, 2q includes 2pq and 2qr, and 2r includes 2pr and 2rq, the formula in 5.2.2.3 subtracts 2pq, 2pr, and 2qr to avoid double-counting these genotype frequencies.

5.2.2.3.2. Laboratories may choose to use the formula 2p + 2q + 2r for the scenario described in 5.2.2.3.

5.2.2.3.3. Laboratories may choose to assign the value of 1 to the scenario described in 5.2.2.3, i.e. not use the locus for statistical weight.

5.2.2.4. Care should be taken to not report a calculated RMP greater than 1.0. This can occur when using the calculations discussed in 5.2.2.1 and 5.2.2.2 (due to the application of θ in the standard homozygote formula but not in the heterozygote formula) and in 5.2.2.3.1 (due to the double counting of the PP, QQ, RR, PQ, PR, and QR genotype frequencies).

5.2.2.5. In a sperm fraction assumed to be from two contributors, one of whom is the victim, the sperm contributor genotypes included post-deconvolution might include only a single genotype (PQ) at locus 1, but multiple possible genotypes (UU or UV) at locus 2. In this case, the two-locus RMP for the sperm DNA contributor could be calculated as $2pq * [u^2 + u(1-u)\theta + 2uv]$.

5.2.2.6. The unrestricted RMP might be calculated for mixtures that display no indications of allelic dropout. The formulae include an assumption of the number of contributors, but relative peak height information is not utilized. For two-person mixtures, the formulae for loci displaying one, two, or three alleles are identical to the CPI calculation discussed in section 5.3. For loci displaying four alleles (P, Q, R, and S), homozygous genotypes would not typically be included. The unrestricted RMP in this case would require the subtraction for homozygote genotype frequencies, e.g., $(p + q + r + s)^2 – p^2 – q^2 – r^2 – s^2$.

5.2.3. When a suspect's profile has been determined to match the unknown profile, if the alternate hypothesis is that a relative of the suspect is in fact the source of the unknown profile, then all efforts should be undertaken to obtain a sample directly from the relative in question so that there is no need to rely on a probability-based estimate of a coincidental match.

In the absence of a direct comparison, conditional match probabilities for various relatives can be calculated in accordance with NRCII formulae 4.8 and 4.9.

5.2.3.1. Full Siblings (NRCII formulae 4.9a and 4.9b)

Genotype of suspect	Probability of the same genotype in a sibling
PP	$(1 + 2p + p^2) / 4$
PQ	$(1 + p + q + 2pq) / 4$

5.2.3.2. Other Relatives (NRCII formulae 4.8a and 4.8b)

Genotype of suspect	Probability of the same genotype in a relative
PP	$p^2 + 4p(1 – p)F$
PQ	$2pq + 2(p + q – 4pq)F$

where F = 1/4 for parent and offspring
1/8 for half-siblings
1/8 for uncle and nephew
1/8 for grandparent and grandchild
1/16 for first cousins

5.2.3.3. Conditional subpopulation corrections could also be applied to these formulae following the methods of Ayres (2000) as described in Fung and Hu (2008).

5.3. Combined Probability of Inclusion (CPI) and Exclusion (CPE)

5.3.1. PI is calculated as (sum of allele frequencies)^2 for each locus.

5.3.2. The CPI is the product of the individual locus PIs:
$CPI = PI_1 * PI_2 * ... * PI_N$

5.3.3. The PE has been commonly presented two ways

5.3.3.1. PE = 1 – PI

5.3.3.2. PE = $q^2 + 2pq$, where p is the sum of allele frequencies and q represents all other alleles (1 – p). This is analogous to the single allele formula described in 5.2.1.3.2.

5.3.3.3. Population substructure corrections can also be applied using $PE = 1 – [p^2 – p(1 – p)\theta]$, where p is the sum of allele frequencies observed at that locus.

5.3.4. The CPE has been commonly presented two ways

5.3.4.1. CPE = 1 – CPI

5.3.4.2. $CPE = 1 – [(1 – PE_1) * [(1 – PE_2) * ... * (1 – PE_N)]$

5.3.5. The CPI and CPE are typically applied to all alleles detected in a mixture, subject to the limitations described in section 4.6.3. This section also allowed for a restricted CPI and CPE. Examples of both scenarios are provided below.

5.3.5.1. Unrestricted CPI and CPE. In a mixture at a locus having alleles P, Q, and R, all above the laboratory's stochastic threshold, the interpretation might be that all potential contributors to this mixture have genotypes consisting of some combination of the detected alleles (PP, QQ, RR, PQ, PR, and QR). In this case, the probability of inclusion for the mixture could be calculated as $(p + q + r)^2$.

5.3.5.2. Unrestricted CPI and CPE. In a mixture at a locus having alleles P, Q, R, and S where alleles P, Q, and R are above the stochastic threshold, but allele S is below that threshold, in the standard application of the CPI and CPE, no calculation would be performed at this locus.

5.3.5.3. Restricted CPI and CPE. Given (a) a mixture at a locus having alleles P, Q, R, and S, (b) alleles P, Q, and R significantly (as defined by the laboratory) above the stochastic threshold, and (c) allele S is below the stochastic threshold, the interpretation might be that the higher RFU alleles are a distinct group, separate from the contributor(s) of the low-RFU S allele. The lab might choose to calculate a restricted probability of inclusion utilizing just the P, Q, and R alleles, $(p + q + r)^2$.

5.3.5.3.1. Based on the above example, had the S allele been greater than the stochastic threshold, but still identified as distinct from the higher-RFU alleles, a second general CPI or CPE could have been calculated using all four alleles.

5.4. Likelihood Ratio (LR)

5.4.1. When the evidence profile is determined to be single source, and the reference and evidence profiles are identical at all loci, LR = 1/RMP.

5.4.1.1. The numerator of the LR calculation would assume the suspect's contribution, meaning that the probability of observing results consistent with his profile would be 1.0.

5.4.1.2. The denominator would assume that the suspect is not the contributor. The probability of a randomly selected person having the evidence profile is represented by the RMP.

5.4.2. The calculation of the LR in a mixture is dependent upon the evidence profile, the comparison reference profile(s), and the individual hypotheses. Given the myriad possible combinations, any list would be necessarily incomplete. A limited set of examples is provided below.

5.4.2.1. An “unrestricted” LR is the LR calculated without taking peak heights into consideration, especially in the denominator.

5.4.2.1.1. At a locus, a mixture with alleles P and Q, is assumed to be from two contributors, and displays no indications of allelic dropout. No further considerations of peak heights are undertaken. The suspect in question is PP, and no other reference standards are being considered for inclusion.

The numerator of the LR calculation would assume the suspect’s contribution, meaning that the probability of observing results consistent with his genotype would be 1.0. The second, unknown contributor must complete the mixture by having allele Q and nothing other than P or Q. Therefore the numerator to the calculation would be the sum of the frequencies for the second contributor’s possible genotypes (QQ and PQ)

LR numerator = $[q^2 + q(1-q)\theta] + 2pq$

The denominator of the LR calculation might assume that the mixture is a combination of two unknown contributors. (Alternate hypotheses are possible as long as the numerator and denominator hypotheses are mutually exclusive.) The unknown contributors must have no alleles other than P or Q, and the combination of their genotypes must complete the detected mixture of P and Q.

Contrib. # 1	Contrib. # 2	Combined Probability
PP	QQ	$[p^2 + p(1-p)\theta] * [q^2 + q(1-q)\theta]$
QQ	PP	$[q^2 + q(1-q)\theta] * [p^2 + p(1-p)\theta]$
PQ	PP	$2pq * [p^2 + p(1-p)\theta]$
PP	PQ	$[p^2 + p(1-p)\theta] * 2pq$
PQ	QQ	$2pq * [q^2 + q(1-q)\theta]$
QQ	PQ	$[q^2 + q(1-q)\theta] * 2pq$
PQ	PQ	$2pq * 2pq$

LR denominator = the sum of the possible combinations of genotypes (i.e., summing the seven combined probabilities).

5.4.2.2. A “restricted” LR is the LR calculated once relative peak heights are taken into consideration. Note: Within an STR profile, some loci may have results that give identical restricted and unrestricted LRs.

5.4.2.2.1. At a locus, a mixture with alleles P and Q, is assumed to be from two contributors, and displays no indications of allelic dropout. The peak height ratio is 50% (P allele taller). Across the entire profile, the mixture appears to be 2:1. The suspect in question is PP, and no other reference standards are being considered for inclusion.

The numerator of the LR calculation would assume the suspect's contribution, meaning that the probability of observing results consistent with his genotype would be 1.0.

The second, unknown contributor must complete the mixture by having allele Q and nothing other than P or Q. If the assumed contributor (the suspect) is the minor contributor to the mixture, the possible second contributor genotypes included post-deconvolution might be PQ.

LR numerator = $2pq$

Conversely, if the second contributor is the minor contributor, the possible second contributor genotypes included post-deconvolution might be QQ.

LR numerator = $q^2 + q(1-q)\theta$

The denominator of the LR calculation might assume that the mixture is a combination of two unknown contributors. The unknown contributors must have no alleles other than P or Q, and the combination of their genotypes must complete the detected mixture of P and Q. Based upon the relative peak height ratios and the overall mixture ratio, the restricted LR denominator might be limited to the following pairs of genotypes:

Major Contrib.	Minor. Contrib.	Combined Probability
PP	QQ	$[p^2 + p(1-p)\theta] * [q^2 + q(1-q)\theta]$
PQ	PP	$2pq * [p^2 + p(1-p)\theta]$

LR denominator = the sum of the possible combinations of genotypes (i.e., summing the two combined probabilities).

5.4.2.3 Additional formulae for restricted and unrestricted LRs can be found in Fung and Hu (2008).

Table 1 – Suitable Statistical Analyses for DNA Typing Results
The statistical methods listed in the table cannot be combined into one calculation. For example, combining RMP at one locus with a CPI calculation at a second locus is not appropriate. However, an RMP may be calculated for the major component of a mixture and a CPE/CPI for the entire mixture (as referred to in section 4.6.2).

Category of DNA Typing Result	RMP	CPE/CPI	LR (1)
Single Source	✔		✔
Single Major Contributor to a Mixture	✔		✔
Multiple Major Contributors to a Mixture	✔ (2)	✔ (2)	✔
Single Minor Contributor to a Mixture	✔	✔ (3)	✔
Multiple Minor Contributors to a Mixture	✔ (2)	✔ (3)	✔
Indistinguishable Mixture	✔ (1)	✔	✔

(1) Restricted or unrestricted
(2) Restricted
(3) All potential alleles identified during interpretation are included in the statistical calculation

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7. Additional Suggested Readings

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Glossary for this document

Allelic dropout: failure to detect an allele within a sample or failure to amplify an allele during PCR.

Analytical threshold: the minimum height requirement at and above which detected peaks can be reliably distinguished from background noise; peaks above this threshold are generally not considered noise and are either artifacts or true alleles.

Artifact: a non-allelic product of the amplification process (e.g., stutter, non-templated nucleotide addition, or other non-specific product), an anomaly of the detection process (e.g., pull-up or spike), or a by-product of primer synthesis (e.g., “dye blob”).

Coincidental match: a match which occurs by chance.

Composite profile: a DNA profile generated by combining typing results from different loci obtained from multiple injections of the same amplified sample and/or multiple amplifications of the same DNA extract. When separate extracts from different locations on a given evidentiary item are combined prior to amplification, the resultant DNA profile is not considered a composite profile.

Conditional: an interpretation category that incorporates assumption(s) as to the number of contributors.

CPE: combined probability of exclusion; produced by multiplying the probabilities of inclusion from each locus and subtract the product from 1; (i.e., 1-CPI).

CPI: combined probability of inclusion; produced by multiplying the probabilities of inclusion from each locus; (i.e., 1-CPE).

Deconvolution: separation of contributors to a mixed DNA profile based on quantitative peak height information and any underlying assumptions.

Deduced: inference of an unknown contributor’s DNA profile after taking into consideration the contribution of a known/assumed contributor’s DNA profile based on quantitative peak height information.

Differential Degradation: a DNA typing result in which contributors to a DNA mixture are subject to different levels of degradation (e.g., due to time of deposition), thereby impacting the mixture ratios across the entire profile.

Distinguishable Mixture: a DNA mixture in which relative peak height ratios allow deconvolution of the profiles of major/minor contributor(s).

Evidence sample: also known as Questioned sample.

Exclusion: a conclusion that eliminates an individual as a potential contributor of DNA obtained from an evidentiary item based on the comparison of known and questioned DNA profiles (or multiple questioned DNA profiles to each other).

Guidelines: a set of general principles used to provide directions and parameters for decision making.

Heterozygote: an individual having different alleles at a particular locus; usually manifested as two distinct peaks for a locus in an electropherogram.

Homozygote: an individual having the same (or indistinguishable) alleles at a particular locus; manifested as a single peak for a locus in an electropherogram.

Inclusion: a conclusion for which an individual cannot be excluded as a potential contributor of DNA obtained from an evidentiary item based on the comparison of known and questioned DNA profiles (or multiple questioned DNA profiles to each other).

Inconclusive/uninterpretable: an interpretation or conclusion in which the DNA typing results are insufficient, as defined by the laboratory, for comparison purposes.

Indistinguishable mixture: a DNA mixture in which relative peak height ratios are insufficient to attribute alleles to individual contributor(s).

Intimate sample: a biological sample from an evidence item that is obtained directly from an individual's body; it is not unexpected to detect that individual's allele(s) in the DNA typing results.

Known sample: biological material for which the identity of the donor is established and used for comparison purposes (referred to as a "K").

Likelihood ratio (LR): the ratio of two probabilities of the same event under different hypotheses; typically the numerator contains the prosecution's hypothesis and the denominator the defense's hypothesis.

Major contributor(s): an individual(s) who can account for the predominance of the DNA in a mixed profile.

Masked allele: an allele of the minor contributor that may not be readily distinguishable from the alleles of the major contributor or an artifact.

Minor contributor(s): an individual(s) who can account for the lesser portion of the DNA in a mixed profile.

Mixture: a DNA typing result originating from two or more individuals.

Mixture ratio: the relative ratio of the DNA contributions of multiple individuals to a mixed DNA typing result, as determined by the use of quantitative peak height information; may also be expressed as a percentage.

Noise: background signal detected by a data collection instrument.

No results: no allelic peaks detected above the analytical threshold.

Obligate allele: an allele in a mixed DNA typing result that is (a) foreign to an assumed contributor, or (b) based on quantitative peak height information, determined to be shared with the assumed contributor.

Partial profile: a DNA profile for which typing results are not obtained at all tested loci due, for example, to DNA degradation, inhibition of amplification and/or low- quantity template.

Peak height ratio (PHR): the relative ratio of two alleles at a given locus, as determined by dividing the peak height of an allele with a lower relative fluorescence unit (RFU) value by the peak height of an allele with a higher RFU value, and then multiplying this value by 100 to express the PHR as a percentage; used as an indication of which alleles may be heterozygous pairs and also in mixture deconvolution.

Probability of exclusion (PE): the percentage of the population that can be excluded as potential contributors to a DNA mixture.

Probability of inclusion (PI): the percentage of the population that can be included as potential contributors to a DNA mixture; also known as Random Man Not Excluded.
.
Questioned sample: biological sample recovered from a crime scene or collected from persons or objects associated with a crime (referred to as a “Q”).

Random Match Probability (RMP): the probability of randomly selecting an unrelated individual from the population who could be a potential contributor to an evidentiary profile.

Reference sample: also known as Known sample.

Restricted: referring to a statistical approach conditioned on the number of contributors and with consideration of quantitative peak height information and inference of contributor mixture ratios; used to limit the genotypic combinations of possible contributors.

Signal-to-noise ratio: an assessment used to establish an analytical threshold to distinguish allelic peaks (signal) from background/instrumental noise.

Single-source profile: DNA typing results determined to originate from one individual based on peak height ratio assessments and the number of alleles at given loci.

Source attribution: a declaration which identifies an individual as the source of an evidentiary profile to a reasonable degree of scientific certainty based on a single-source or major contributor profile.

Stochastic effects: the observation of intra-locus peak imbalance and/or allele drop-out resulting from random, disproportionate amplification of alleles in low-quantity template samples.

Stochastic threshold: the peak height value above which it is reasonable to assume that, at a given locus, allelic dropout of a sister allele has not occurred.

Stutter: a minor peak typically observed one repeat unit smaller than a primary STR allele resulting from strand slippage during amplification.

Unrestricted: referring to a statistical approach performed without consideration of quantitative peak height information and inference of contributor mixture ratios; for CPE/CPI this may or may not be conditioned on the number of contributors.

EXHIBIT F

Sampling Limitations with Sperm Cells

New sampling techniques such as laser capture microdissection (see Chapter 2) enable collection of specific cells. Keep in mind that individual sperm cells contain only half of the genomic material from the donor. Therefore, multiple sperm cells will need to be collected in order to represent the complete DNA profile (D.N.A. Box 11.5).

Whole Genome Amplification

A DNA enrichment technology known as whole genome amplification (WGA) has been explored as a possible method for recovery of limited quantities of DNA from evidentiary samples (Bergen et al. 2005, Hanson & Ballantyne 2005, Ballantyne et al. 2007). WGA involves a different DNA polymerase (phi29) than the TaqGold enzyme commonly used in forensic DNA analysis and amplifies the entire genome using random hexamers as priming points. The WGA enzymes work by multiple displacement amplification (MDA), which is sometimes referred to as rolling circle amplification. MDA is isothermal with an incubation temperature of 30°C and requires no heating and cooling like PCR.

QIAGEN (Valencia, CA) and Sigma-Aldrich (St. Louis, MO) both offer phi29 DNA polymerase cocktails for performing WGA. The kit sold by QIAGEN is called REPLI-g while Sigma-Aldrich's kit is GenomePlex. Yields of 4 μg to 7 μg of amplified genomic DNA are possible from as little as 1 ng of starting material. The phi29 enzyme has a high processivity and can amplify fragments of up to 100 kb because it displaces downstream product strands enabling multiple concurrent and overlapping rounds of amplification. In addition, phi29 has a higher replication fidelity compared to Taq polymerase due to 3′–5′ proofreading activity.

While all of these characteristics make WGA seem like a possible solution to the forensic problem of limited DNA starting material, studies have found that stochastic effects at low levels of DNA template prevent WGA from working reliably (Schneider et al. 2004). Allele drop-outs from STR loci were observed at 50 pg and 5 pg levels of starting material (Schneider et al. 2004) just as are seen with current LT-DNA testing. Work with "molecular crowding" materials such as polyethylene glycol, where the amount of DNA is enriched in localized areas of a sample, has shown improved success with STR typing from low amounts of DNA (Ballantyne et al. 2006).

It is possible that WGA may play a limited role in enriching samples for archiving purposes that are in the low ng range (Lasken & Egholm 2003), but it will probably not be the end-all solution to LT-DNA samples in the low picogram range. Thus, it appears, as with regular PCR techniques, stochastic selection of alleles present in low-level DNA samples limits the effectiveness of WGA to several hundred picograms in order to recover a full profile with a single amplification attempt.

Caution in Relying on DNA Quantitation Values

Is it possible to ascertain that sufficient DNA material exists to obtain reliable results where the DNA profile obtained is expected to accurately reflect the source of the biological sample? There are two primary points in the DNA testing process where potential DNA reliability may be assessed: (1) at the DNA quantitation stage prior to performing PCR

D.N.A. BOX 11.5

PROBABILITY OF ACHIEVING FULL ALLELIC REPRESENTATION FOR LOW NUMBERS OF HAPLOID CELLS

Sexual assault evidence is composed of sperm cells, which contain only half of the full complement of genomic DNA from the donor male because of meiosis. Thus, to obtain a representative *diploid* DNA profile containing both possible alleles from the original sample source, multiple sperm will need to be analyzed. Techniques such as laser capture microdissection enable collection of individual sperm cells (Elliott et al. 2003, see Chapter 2). When working with limited amounts of evidentiary material, the question becomes how many individual sperm cells are needed to obtain a fully representative DNA profile when multiple loci are being examined.

In a 2007 paper published in the journal *Science & Justice*, David Lucy and colleagues found that 19 haploid cells would be theoretically required to obtain a full 15-locus DNA profile with a 99.99% probability (Lucy et al. 2007). Since each sperm cell contains just over 3pg of DNA (see D.N.A. Box 3.1), approximately 60pg of sperm DNA extract would therefore be required to obtain a full profile containing both alleles at all 15 tested loci.

The number of haploid cells required to obtain complete representation of a full profile at a specified number of heterozygous loci with a given probability (ranging from 90% to 99.99%) is provided in the table to the right (Lucy et al. 2007).

Number of Loci	0.90	0.95	0.99	0.999	0.9999
1	5	6	8	11	15
2	6	7	9	12	16
3	6	7	10	13	16
6	7	8	11	14	17
10	8	9	11	15	18
15	9	10	12	15	19

This information is theoretical in nature and may not necessarily reflect the probabilities of what is observed after DNA extraction, PCR amplification, and capillary electrophoresis injection. The efficiency of these processes will govern the actual numbers of cells required. Research scientists from the Forensic Science Service found that several hundred sperm cells can be required in order to have a high probability of obtaining a complete STR profile (Elliott et al. 2003). Poor extraction efficiency and less than 100% amplification efficiency due to DNA degradation or PCR inhibition play a role in this discrepancy between the theoretical predictions and empirical observations.

Sources:

Elliott, K., et al. (2003). Use of laser microdissection greatly improves the recovery of DNA from sperm on microscope slides. Forensic Science International, *137, 28–36.*

Lucy, D., et al. (2007). The probability of achieving full allelic representation for LCN-STR profiling of haploid cells. Science & Justice, *47, 168–171.*

amplification of the STR markers of interest or (2) by examining the peak heights—and peak height ratios in heterozygous loci—in the STR profile obtained.

An empirically determined threshold (usually termed a "stochastic threshold") may be used at either the DNA quantitation or data interpretation stage to assess samples in the potential danger zone of unreliable results. For example, if the total amount of measured DNA is below 150 pg, a laboratory may decide not to proceed with PCR amplification assuming that allelic drop-out due to stochastic effects is a very real possibility. Alternatively, a laboratory may proceed with testing a low-level DNA sample and then evaluate the peak height signals and peak ratios at heterozygous loci. When peak height ratios for heterozygous loci in single-source samples dip below 60%, there is an indication that stochastic effects are significant which would make it challenging to reliably pair alleles into major and minor genotypes with mixtures. This topic will be covered more extensively in the forthcoming volume *Advanced Topics in Forensic DNA Typing: Interpretation*.

Since the advent of quantitative PCR (qPCR) assays, DNA quantitation tests have become more sensitive—enabling quantities as small as a few genomic copies to be detected (see Chapter 3). However, it is important to keep in mind that qPCR is also subject to stochastic variation especially on the low end of DNA quantity measurement. Thus, numbers in the low picogram range may not be reliable and results with little or no "detectable" DNA may still amplify with STR kits (Cupples et al. 2009; see also D.N.A. Box 3.3).

In an early paper discussing stochastic effects and the limitations of PCR assays, Walsh et al. (1992) proposed avoiding stochastic effects by adjusting the number of PCR cycles in an assay so that the sensitivity limit is around 20 or more copies of target DNA. In other words, their goal was to enable a full DNA profile to be reliably obtained with approximately 125 pg of DNA. Below roughly that amount, allele and locus drop-out would be expected and partial DNA profiles would result (Walsh et al. 1992). Obtaining a partial DNA profile is an indication that a low-level DNA amplification has occurred.

Depending on the STR typing kit primer and DNA polymerase concentrations and the fluorescent dye sensitivities, the number of PCR cycles is typically set by manufacturers in the range of 28 cycles to 32 cycles. However, as noted previously, STR kits certainly work beyond manufacturer recommended cycle numbers. If laboratories choose to increase cycle numbers beyond what is recommended by manufacturers, validation studies are needed to help set appropriate interpretation guidelines.

Thresholds Are Difficult to Set with Enhanced Detection Methods

Stochastic thresholds, such as 150 RFU or even 500 RFU, may not apply for enhanced detection methods that include increasing the number of PCR cycles. Instead, independent replicate amplifications and concensus profile development are necessary to compensate for allele drop-out and drop-in. Computer software that involves probabilistic modeling to data, such as LoComatioN (Gill et al. 2007), will be important to future advances with low-level DNA analysis.

STRBase Website on Low Template DNA

A low template DNA section of the NIST STRBase website was launched in October 2009 following the International Symposium on Human Identification LCN Panel. This website,

EXHIBIT G

AUSTIN POLICE DEPARTMENT
FORENSIC SCIENCE SERVICES DIVISION

812 Springdale Road
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LABORATORY REPORT
Forensic Biology Section
04/21/2016
Information Only Report

Laboratory Number: [redacted]
LRN: [redacted]

Agency: Austin Police Department
Offense Number: [redacted]

Offense Date
04/15/2015

Offense
SEXUAL ASSAULT

Suspect(s)
[redacted]

Victim(s)
[redacted]

Elimination
[redacted]

On March 21, 2016, Dr. Bruce Budowle issued a report regarding his analysis of the data provided to him by the APD DNA laboratory regarding case [redacted]. I have reviewed this report and am providing the following summary of my comments and observations regarding Dr. Budowle's analysis.

Dr. Budowle agrees with the interpretation provided by APD regarding items 11.1 (Stain A sperm fraction), 11.1 (Stain A epithelial fraction), 11.2 (Stain B sperm fraction), 11.2 (Stain B epithelial fraction), item 11.3 (stain C sperm fraction), item 11.3 (stain C epithelial fraction), item 11.4 (stain D sperm fraction), and 11.4 (stain D epithelial fraction).

1. <u>APD's Quantification-Based Stochastic Threshold</u> – In Dr. Budowle's report, in relation to the use of a quantification-based, rather than RFU-based, stochastic threshold (ST) he states "…APDL applied a stochastic threshold based on specified amount of input DNA. The amount of input DNA is not defensible, especially when the amount of input DNA is relatively low (total or of some contributors of the mixture)."
    a. While future developments at APD may utilize a RFU-based ST or probabilistic genotyping for accounting for dropout, both RFU-based and quantification-based STs are approved methods by SWGDAM and the protocols used by the APD DNA laboratory were deemed acceptable during external audits by ASCLD/LAB and other external auditing entities at the time this report was issued.
        i. REFERENCE: SWGDAM Interpretation Guidelines for Autosomal STR Typing SWGDAM (APPROVED 1/14/10) "3.2.2. If a stochastic threshold based on peak height is not used in the evaluation of DNA typing results, the laboratory must establish alternative criteria

(e.g., quantitation values or use of a probabilistic genotype approach) for addressing potential stochastic amplification.

2. Inclusion with no CPI Calculation (Item 3.5.6 neck swab): In Dr. Budowle's report regarding item 3.5.6, he posits one method of using CPI with locus D1S1656 and states "The use of only one locus is a very conservative approach, but does not reflect well the strength of the evidence." He does not specifically state whether [redacted] would be included or not in this scenario.
    a. The APD DNA laboratory is bound by ASCLD/LAB guidelines which do not allow us to make statements of association, such as inclusion, without providing a statistic to estimate the significance of the association in non-intimate samples. Therefore, an opinion involving a statement of inclusion of any individual with Item 3.5.6 with no associated statistic cannot be rendered by the APD DNA laboratory while still maintaining accreditation status with ASCLD/LAB.
        i. REFERENCE: ASCLD/LAB Board of Directors Interpretations, Clearly and Properly Qualifying DNA Associations in Test Reports. "Each DNA association must be clearly and properly qualified in a test report by either 1) a statistic; or 2) a qualitative statement….A qualitative statement must provide sufficient information to clearly express the significance of the association….A qualitative statement not based on a statistical calculation should be limited to situations in which the presence of an individual's DNA on an item is reasonably expected."

3. Statistics Provided for the Victim Deduction Method (mRMP) [Item 3.5.6 neck swab]: In Dr. Budowle's report regarding his second option of interpreting item 3.5.6, the option he provides for this sample is a method that involves subtraction of the victim from the profile, which he assumes to be a two person mixture. He then proceeds to calculate a modified random match probability statistic on some of the remaining profile. While I don't disagree that this is one possible method he could choose to approach the sample, I am unable to issue a similar report in the APD laboratory at this time as a new protocol development process would need to occur at APD since we must comply with all TFSC and ASCLD/LAB requirements for new protocol development.

4. This case utilized the Promega Fusion chemistry and a 3130 Genetic Analyzer. In order for APD to issue a new report with a RFU based ST according to currently recognized practices, APD would need extensive time to perform validations of this methodology on this particular chemistry and instrument combination. After a RFU-based ST is established, but before APD could issue any new reports, we would need to train analysts on the new system, write new protocols that follow all of the appropriate guidelines, and perform competency testing for our analysts. This is possible, but is not an insignificant task for an accredited laboratory. In order to establish new RFU-based ST and CPI protocols according to current methods available today, APD would be expected to comply with the following, some of which have only become available recently:
    a. The TFSC "Criteria for Evaluation of DNA Mixture Interpretation Protocols" (published 10/15/15)
    b. CPI related principles published in "Advanced Topics in Forensic DNA Typing: Interpretation" by John Butler (published October, 2014)

c. Current ASCLD/LAB accreditation requirements
d. "Quality Assurance Standards for Forensic DNA Testing Laboratories", Federal Bureau of Investigation (published 9/1/11)
e. "SWGDAM Interpretation Guidelines for Autosomal STR Typing By Forensic DNA Testing Laboratories", Scientific Working Group on DNA Analysis Methods (SWGDAM) [1/14/10]

Elizabeth L. Morris
Senior Forensic Scientist

Subject: Re: List of Criteria for Protocol Review

Date: Friday, October 16, 2015 at 3:41:03 PM Central Daylight Time

From: Lynn Garcia

To: Sailus, Jeff, jody.koehler@dps.texas.gov, allison.heard@dps.texas.gov, joshua.stewart@dps.texas.gov, nicolas.ronquillo@dps.texas.gov, melissa.haas@dps.texas.gov, andrew.mcwhorter@dps.texas.gov, nicole.hahn@dps.texas.gov, david.young@dps.texas.gov, starla.copeland@dps.texas.gov, vanessa.nelson@dps.texas.gov, christina.capt@unthsc.edu, gfoster@bexar.org, Timothy.Sliter@dallascounty.org, katie.welch@ifs.hctx.net, clpatton@tarrantcounty.com, cassie.johnson@fortworthtexas.gov, rguidry@houstonforensicscience.org, Kahn, Roger (IFS)

CC: Vincent Di maio, Arthur Eisenberg, Sheree Hughes-Stamm, Jeffrey Barnard, Bobby Lerma, Richard B. Alpert, Mills, Brady, Leigh Tomlin, Nick Vilbas, Kathryn Adams, Tom - LGL Allen, Butler, John M., Budowle, Bruce, Bieber, Frederick R.,Ph.D., John.Buckleton@esr.cri.nz, Carson Guy, Coble, Michael D.

Jeff,

From what I understand in speaking with our experts, quantitation values provide only a general level of protection against stochastic effects, and are primarily beneficial for good quality single-source samples. CE peak heights are the best way to assess stochastic effects.

Hope this information is helpful.

Thanks,
Lynn Robitaille Garcia
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Texas Forensic Science Commission
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From: "Sailus, Jeff" <Jeff.Sailus@austintexas.gov>
Date: Friday, October 16, 2015 at 3:01 PM
To: Lynn Robitaille Garcia <lynn.garcia@fsc.texas.gov>, "jody.koehler@dps.texas.gov" <jody.koehler@dps.texas.gov>, "allison.heard@dps.texas.gov" <allison.heard@dps.texas.gov>, "joshua.stewart@dps.texas.gov" <joshua.stewart@dps.texas.gov>, "nicolas.ronquillo@dps.texas.gov" <nicolas.ronquillo@dps.texas.gov>, "melissa.haas@dps.texas.gov" <melissa.haas@dps.texas.gov>, "andrew.mcwhorter@dps.texas.gov" <andrew.mcwhorter@dps.texas.gov>, "nicole.hahn@dps.texas.gov" <nicole.hahn@dps.texas.gov>, "david.young@dps.texas.gov" <david.young@dps.texas.gov>, "starla.copeland@dps.texas.gov" <starla.copeland@dps.texas.gov>, "vanessa.nelson@dps.texas.gov" <vanessa.nelson@dps.texas.gov>, "christina.capt@unthsc.edu" <christina.capt@unthsc.edu>, "gfoster@bexar.org" <gfoster@bexar.org>, "Timothy.Sliter@dallascounty.org" <Timothy.Sliter@dallascounty.org>, "katie.welch@ifs.hctx.net" <katie.welch@ifs.hctx.net>, "clpatton@tarrantcounty.com" <clpatton@tarrantcounty.com>, "cassie.johnson@fortworthtexas.gov" <cassie.johnson@fortworthtexas.gov>, "rguidry@houstonforensicscience.org" <rguidry@houstonforensicscience.org>, "Kahn, Roger (IFS)" <Roger.Kahn@ifs.hctx.net>

Cc: Vincent Di maio <vincent_dimaio@yahoo.com>, Arthur Eisenberg <arthur.eisenberg@unthsc.edu>, Sheree Hughes-Stamm <sxh039@shsu.edu>, Jeffrey Barnard <Jeffrey.Barnard@dallascounty.org>, Bobby Lerma <bobby@bobbylerma.com>, "Richard B. Alpert" <RAlpert@tarrantcountytx.gov>, "Mills, Brady" <Brady.Mills@dps.texas.gov>, Leigh Tomlin <leigh@fsc.texas.gov>, Nick Vilbas <nick.vilbas@fsc.texas.gov>, Kathryn Adams <kathryn.adams@fsc.texas.gov>, Tom - LGL Allen <Tom.Allen@houstontx.gov>, "Butler, John M." <john.butler@nist.gov>, "Budowle, Bruce" <Bruce.Budowle@unthsc.edu>, "Bieber, Frederick R.,Ph.D." <FBIEBER@PARTNERS.ORG>, "John.Buckleton@esr.cri.nz" <John.Buckleton@esr.cri.nz>, Carson Guy <Carson.Guy@txcourts.gov>, "Coble, Michael D." <michael.coble@nist.gov>, "Sailus, Jeff" <Jeff.Sailus@austintexas.gov>
Subject: RE: List of Criteria for Protocol Review

Hello,

One question. The language of this seems to focus only on a RFU based ST. SWGDAM allows for a quant based ST. Is it the position of the TFSC that only RFU based STs are acceptable and not quant based STs?

3.2.2. If a stochastic threshold based on peak height is not used in the evaluation of DNA typing results, the laboratory must establish alternative criteria (e.g., **quantitation values** or use of a probabilistic genotype approach) for addressing potential stochastic amplification. The criteria must be supported by empirical data and internal validation and must be documented in the standard operating procedures.

Thank you,

Jeff Sailus
Austin, TX

From: Lynn Garcia [lynn.garcia@fsc.texas.gov]
Sent: Thursday, October 15, 2015 2:58 PM
To: jody.koehler@dps.texas.gov; allison.heard@dps.texas.gov; joshua.stewart@dps.texas.gov; nicolas.ronquillo@dps.texas.gov; melissa.haas@dps.texas.gov; andrew.mcwhorter@dps.texas.gov; nicole.hahn@dps.texas.gov; david.young@dps.texas.gov; starla.copeland@dps.texas.gov; vanessa.nelson@dps.texas.gov; christina.capt@unthsc.edu; gfoster@bexar.org; Timothy.Sliter@dallascounty.org; katie.welch@ifs.hctx.net; Sailus, Jeff; clpatton@tarrantcounty.com; cassie.johnson@fortworthtexas.gov; rguidry@houstonforensicscience.org; Kahn, Roger (IFS)
Cc: Vincent Di maio; Arthur Eisenberg; Sheree Hughes-Stamm; Jeffrey Barnard; Bobby Lerma; Richard B. Alpert; Mills, Brady; Leigh Tomlin; Nick Vilbas; Kathryn Adams; Tom - LGL Allen; Butler, John M.; Budowle, Bruce; Bieber, Frederick R.,Ph.D.; John.Buckleton@esr.cri.nz; Carson Guy; Coble, Michael D.
Subject: List of Criteria for Protocol Review

All,

We have received a few requests for the criteria that will be used in reviewing the laboratory protocols. Attached is a list of 7 items developed by Drs. Bieber, Buckleton, Budowle, Butler and Coble. They are intended to provide guidance so you know the types of issues they consider important. As the criteria state at the outset, the absence of an item is not an indication the protocol is flawed, and there may be approaches that have the same practical effect even if not phrased precisely as indicated in the attached list.

Thanks to everyone for your participation in this initiative so far. We are very grateful for the proactive and collaborative spirit we are witnessing in Texas on a daily basis. If you have any questions, please let me know.

Best,
Lynn Robitaille Garcia
General Counsel
Texas Forensic Science Commission
1700 North Congress, Suite 445

Austin, Texas 78701
(512) 936-0649 (direct)
(512) 936-7986 (fax)

www.fsc.texas.gov

From: Lynn Robitaille Garcia <lynn.garcia@fsc.texas.gov>
Date: Wednesday, October 7, 2015 at 3:06 PM
To: "jody.koehler@dps.texas.gov" <jody.koehler@dps.texas.gov>, "allison.heard@dps.texas.gov" <allison.heard@dps.texas.gov>, "joshua.stewart@dps.texas.gov" <joshua.stewart@dps.texas.gov>, "nicolas.ronquillo@dps.texas.gov" <nicolas.ronquillo@dps.texas.gov>, "melissa.haas@dps.texas.gov" <melissa.haas@dps.texas.gov>, "andrew.mcwhorter@dps.texas.gov" <andrew.mcwhorter@dps.texas.gov>, "nicole.hahn@dps.texas.gov" <nicole.hahn@dps.texas.gov>, "david.young@dps.texas.gov" <david.young@dps.texas.gov>, "starla.copeland@dps.texas.gov" <starla.copeland@dps.texas.gov>, "vanessa.nelson@dps.texas.gov" <vanessa.nelson@dps.texas.gov>, "christina.capt@unthsc.edu" <christina.capt@unthsc.edu>, "gfoster@bexar.org" <gfoster@bexar.org>, "Timothy.Sliter@dallascounty.org" <Timothy.Sliter@dallascounty.org>, "katie.welch@ifs.hctx.net" <katie.welch@ifs.hctx.net>, "Jeff.sailus@austintexas.gov" <Jeff.sailus@austintexas.gov>, "clpatton@tarrantcounty.com" <clpatton@tarrantcounty.com>, "cassie.johnson@fortworthtexas.gov" <cassie.johnson@fortworthtexas.gov>, "rguidry@houstonforensicscience.org" <rguidry@houstonforensicscience.org>, "Kahn, Roger (IFS)" <Roger.Kahn@ifs.hctx.net>
Cc: Vincent Di maio <vincent_dimaio@yahoo.com>, Arthur Eisenberg <arthur.eisenberg@unthsc.edu>, Sheree Hughes-Stamm <sxh039@shsu.edu>, Jeffrey Barnard <Jeffrey.Barnard@dallascounty.org>, Bobby Lerma <bobby@bobbylerma.com>, "Richard B. Alpert" <RAlpert@tarrantcountytx.gov>, "Mills, Brady" <Brady.Mills@dps.texas.gov>, Leigh Tomlin <leigh@fsc.texas.gov>, Nick Vilbas <nick.vilbas@fsc.texas.gov>, Kathryn Adams <kathryn.adams@fsc.texas.gov>, Tom - LGL Allen <Tom.Allen@houstontx.gov>, "Butler, John M." <john.butler@nist.gov>, "Budowle, Bruce" <Bruce.Budowle@unthsc.edu>, "Bieber, Frederick R.,Ph.D." <FBIEBER@PARTNERS.ORG>, "John.Buckleton@esr.cri.nz" <John.Buckleton@esr.cri.nz>, Carson Guy <Carson.Guy@txcourts.gov>
Subject: DNA Mixture Case Lists

Dear Texas DNA lab representatives:

On October 4, 2015, the Texas Forensic Science Commission voted to seek input from the laboratories regarding their assessment of what would be involved in generating a list of DNA mixture interpretation cases, similar to the list of cases DPS released to the public on September 10, 2015. Even better would be a list of mixture cases where statistics were calculated using CPI/CPE, but we understand drilling down to that level of detail may not be realistic. If you would kindly respond to the following questions regarding case identification, we would appreciate it:

1. Is it possible for your laboratory to generate a list of cases? If so, are you willing to generate a list of cases to assist the Commission in determining potential volume of cases statewide?
2. Would your case list be all DNA cases, or could you restrict your search to just DNA mixtures, or (even better) DNA mixtures using CPI/CPE?
3. What resources do you believe it would take to generate the list?
4. What potential obstacles do you anticipate?
5. How long do you believe it will take to generate the list?

DPS is working on a project internally to cross reference its mixture case list against the TCIC/NCIC database. This will allow prosecutors to reduce the size of the list by those cases for which no conviction was secured. In that process, we have discovered there are certain key data fields that may become useful in conducting the cross-reference against TCIC and NCIC, such as suspect date of birth and SID (State Identification Number). For those laboratories in the process of generating case lists, we respectfully request that you provide suspect names, DOBs and SIDs if you have them in your files, as these are the data fields against which we may be able to run automated searches of TCIC/NCIC.

The Commission also decided it would request mixture interpretation protocols from the laboratories from the point at which the laboratory began conducting Short Tandem Repeat (STR) testing to present, along with a representative sample of 10 current cases for review by a panel of experts and DNA section leaders. The purpose of the exercise is to confirm that current protocols fall within the range of accepted scientific practices, identify historical changes and trends, and to offer suggestions for improvement where appropriate. The individual case review is designed to assess whether current protocols are being applied appropriately and consistently. We encourage laboratories to offer complex cases, so the experts may assist the laboratories in addressing challenging issues. We also encourage the laboratories to work collaboratively with local prosecutors so they understand how the cases are being selected and can provide input where appropriate.

Please let us know if you are able to comply with the Commission's requests set forth in this email, and by when you expect to be able to have the material collected.

We appreciate your assistance in what we hope will be a collaborative and educational process for all involved.

If you have any questions, please let me know.

Best regards,
Lynn Robitaille Garcia
General Counsel
Texas Forensic Science Commission
1700 North Congress, Suite 445
Austin, Texas 78701
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Subject: RE: List of Criteria for Protocol Review
Date: Friday, October 16, 2015 at 3:35:42 PM Central Daylight Time
From: Butler, John M.
To: Budowle, Bruce, Lynn Garcia, Bieber, Frederick R.,Ph.D., Butler, John M., Coble, Michael D., John.Buckleton@esr.cri.nz

Quant values provide only a general level of protection against stochastic effects, and are primarily beneficial for single-source samples. CE peak heights are the best way to assess stochastic effects.

John

Sent from my Verizon Wireless 4G LTE smartphone

-------- Original message --------
From: "Budowle, Bruce" <Bruce.Budowle@unthsc.edu>
Date: 10/16/2015 4:13 PM (GMT-05:00)
To: Lynn Garcia <lynn.garcia@fsc.texas.gov>, "Bieber, Frederick R.,Ph.D." <FBIEBER@PARTNERS.ORG>, "Butler, John M." <john.butler@nist.gov>, "Coble, Michael D." <michael.coble@nist.gov>, John.Buckleton@esr.cri.nz
Subject: RE: List of Criteria for Protocol Review

I am adamantly opposed to quant values. The other one –probabilistic- does not apply to the CPI issue.

From: Lynn Garcia [mailto:lynn.garcia@fsc.texas.gov]
Sent: Friday, October 16, 2015 3:08 PM
To: Budowle, Bruce; Bieber, Frederick R.,Ph.D.; Butler, John M.; Coble, Michael D.; John.Buckleton@esr.cri.nz
Subject: FW: List of Criteria for Protocol Review

Any thoughts on Jeff's question?

Lynn Robitaille Garcia
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EXHIBIT H

MEMORANDUM

Austin Police Department
Forensic Science Services

TO: Bill Gibbens, Laboratory Manager
FROM: Jeff Sailus, DNA Supervisor/DNA Technical Leader
DATE: 11/24/14
SUBJECT: DNA Section Update – Past and Future

Now that I have had time to assess the laboratory, identify needs and look to the future, here is a summary of the state of the DNA laboratory as of today.

Accomplishments - June 2014 to November 2014

- Meet and understand the work habits of the staff and the laboratory workflow
- Update all the user manuals to correct gaps so I could relieve Cassie of her duties regarding case escalations, and I could feel comfortable handling technical questions from analysts
- Prepare for the NFSTC external DNA audit that occurred last week, where we performed very well given all the changes
- Assist with proper management of the grant monies and requests from NIJ, and identify where resources should be best spent to close out the 2013 DNA Backlog grant
- Perform R&D studies to correct problems in the current analysis that the analysts and myself are aware of since switching to a different chemistry last year
- Perform R&D studies to identify the next generation chemistries and software to improve the lab workflow and identify software needed to meet the Windows 7 requirement of the department.
- Become proficient on the administrative review process of DNA cases to help decrease the amount of time cases sit in review after analysis is complete

Goals for 2015 (listed by priority)

1. Improve the Chemistry and Implement New Software

a. There are too many cases now that have inconclusive results or are too complicated because of mixtures of multiple individuals, because the chemistry needs optimization. During my first week here, analysts expressed their concern with the chemistry and my review of the situation confirmed their comments.
b. Replace old quantitation chemistry with new updated chemistry and software that will allow us to stop some samples half way through the process if there is not enough DNA to continue. This will improve efficiency.
c. Validating this new chemistry and software is dependent on the city council approving our software purchase which is delayed now until at least February.

2. Control the Backlog (currently >700 cases)
   a. Streamline the case review process to try to find areas where we can minimize the amount of time in technical or administrative review.
   b. Identify areas where outsourcing will be beneficial to close the gap in the short term
   c. Train the new individuals. There will be a period of time in early 2015 where Senior analysts will need to be partially removed from casework to train new individuals on DNA testing. Nationally, training a new DNA analyst takes 3-6 months from the time they start training to completion, in order to meet all of the national accreditation guidelines around training. If the analyst has experience, it would be closer to 3 months.
   d. Implement a "batching" workflow for burglaries/property crimes. Of the 700 cases, about 300 are burglaries so this work is important. Some laboratories have had success treating these types of crimes as more of a group analysis workflow which, with the new chemistry when validated, will improve the throughput for property crimes.
   e. Work with DAs to obtain a larger advance notice on which cases are going to trial. The current process notifies us of Rush cases way too late in the process.

3. Comply with National recommendations on Y-STR Testing
   a. National recommendations were issued that expect forensic laboratories perform Y-STR DNA typing. We currently do not do this and Texas DPS and the majority of forensic labs do Y-STR testing. I plan on adding Y-STR typing to our chemistry options in 2015 to comply with this recommendation and help give investigators better tools to identify individuals. It is a very powerful chemistry in cases where the evidence is a mixture of male and female DNA.
      i. National Recommendation: *"Given that under certain conditions a male minor contributor in a mixture of female:male DNA may only be detectable by Y-STR typing, laboratories should pursue Y-STR analysis as the most appropriate means of detecting a male contributor(s) in some forensic samples."*

4. Software to Improve Testing of Mixture Samples
   a. A large percentage of our DNA samples contain mixed samples of DNA from multiple individuals. Interpretation on these samples is extremely difficult and our

current method is out dated and results in inconclusive results and/or DNA statistics that are not as accurate as they could be.

b. A NIST Study last year found that people using our method incorrectly interpreted a mixture of four individuals. There is software available that will help automate DNA mixture interpretation and help make mixture interpretation more consistent among analysts. I would like to purchase and validate this software in 2015. http://strmix.esr.cri.nz/

Goals for 2016

1. Implement New Investigative Lead Technology (NGS)
    a. Within 5-10 years, labs nationally will be converting wholesale their entire laboratories over to NGS for all analysis where you can do all DNA markers needed in one sample, rather than multiple different tests as is now done.
    b. The first phase of this is a technology, available now, that will allow us to inform investigators of certain visible traits like hair color, eye color, ancestry (genetic background), facial features, etc for samples where there is no suspect or DNA match in CODIS.

What about "Rapid DNA"?

There are two forms of "Rapid" DNA that are talked about. They generally are not used much in current law enforcement crime laboratories.

1. Company Based Rapid Turnaround Times (i.e. 12-48 hours from submission)
    a. Some companies offer an expedited service to perform Rapid DNA testing of forensic cases. They employ extra staff that are dedicated to only this analysis and charge a premium, often thousands of dollars per case to support the cost of the program. The staff is basically "on call" waiting for an urgent case to arrive. Virtually no government lab has this ability due to funding. Some government labs have their turnaround time down to 30-60 days which is our goal as well. An example is the Bode Technology Group and the process is outlined here: (http://www.bodetech.com/rapid-dna/)
        i. Send the forensic evidence and reference samples to Bode overnight
        ii. Bode will process and deliver DNA profiles by 1100 hours the next day using Rapid DNA Service.
        iii. The law enforcement agency can enter in the forensic unknown sample and compare to a local DNA database in the event the samples do not match.

2. Rapid DNA Machines (Portable)

a. There are two main devices beginning to hit the market. The most advanced one is from Integen X (http://integenx.com/rapidhit-system/)
b. This is a portable device which has some uses but is of minimal use in today's government forensic laboratories. Here is the current state of the technology:
    i. The FBI has not approved this for use on DNA casework from crime scenes, therefore no data generated can be used to search CODIS. The marketing material is misleading to be kind. It is only NDIS approved for reference samples, and with major restriction on that approval.
        1. It has been NDIS approved by the FBI for running reference (suspect, victim) samples, but the data still has to go through full review in an accredited laboratory, so this has minimal use for us in processing cases quicker because this is not where our backup is coming from. However, if the department management would like us to get this instrument for this limited use (analyzing suspect and victim samples), I would be able to implement this technology for this purpose.
    ii. The technology is very crude and no forensic scientist in the community at this time approves this technology on precious samples from crime scenes. We would be risking our access to CODIS and our ASCLD/LAB accreditation if we tried to implement this technology in any way not approved by the FBI as described above.

EXHIBIT I

Calculation of Stochastic Threshold

The Austin Police Department DNA laboratory uses the QIAgen Investigator Kit and QIAcube extraction robot for evidence sample extraction. This is a silica column based technology that is a very clean extraction with rare indications of inhibitors remaining. We also use the Corbett CAS-1200 liquid handling robot for quantification set-up. These robotic platforms have resulted in consistent and reliable results.

In order to calculate the stochastic threshold for the Austin Police Department DNA Laboratory, the following samples were processed using the RFU threshold of 90 with no smoothing and with the 10 sec injection time option (the parameters that would typically be used for low level samples):

MP- purple top blood tube from 2005 draw
JM- purple top blood tube from 2001 draw
CH- purple top blood tube from 2009 draw
LM- purple top blood tube from 2001 draw

Profiler Plus
310
Single Source blood sample -MP

TOTAL DNA	# ALLELES ABOVE THRESHOLD-310	Heterozygote allele not above threshold	Would call profile inc. by standard practice	Lowest peak height ratio
0.75 ng	15	N	N	67
0.375 ng	15	N	N	80
0.1875 ng	13	Y	N	
0.09375 ng	12	Y	N	
0.046875 ng	8	Y	N	
0.0234375 ng	2		Y	
0.0117187 ng	1		Y	
0.0058593 ng	1		Y	
0.0029296 ng	1		Y	

Single Source blood sample - JM

TOTAL DNA	# ALLELES ABOVE THRESHOLD-310	Heterozygote allele not above threshold	Would call profile inc. by standard practice	Lowest peak height ratio
0.75 ng	16	N	N	84
0.375 ng	16	N	N	67
0.1875 ng	16	N	N	65
0.09375 ng	11	Y	N	
0.046875 ng	4		Y	
0.0234375 ng	0		Y	
0.0117187 ng	0		Y	
0.0058593 ng	0		Y	
0.0029296 ng	0		Y	

Single Source blood sample - CH

TOTAL DNA	# ALLELES ABOVE THRESHOLD-310	Heterozygote allele not above threshold	Would call entire profile inc. by standard practice	Lowest peak height ratio
0.75 ng	16	N	N	74
0.375 ng	16	N	N	73
0.1875 ng	16	N	N	42
0.09375 ng	14	Y	N	
0.046875 ng	7	Y	N	
0.0234375 ng	3		Y	
0.0117187 ng	0		Y	
0.0058593 ng	0		Y	
0.0029296 ng	0		Y	

Single Source blood sample - LM

TOTAL DNA	# ALLELES ABOVE THRESHOLD-310	Heterozygote allele not above threshold	Would call profile inc. by standard practice	Lowest peak height ratio
0.75 ng	18	N	N	83
0.375 ng	18	N	N	49
0.1875 ng	18	N	N	68
0.09375 ng	13	Y	N	
0.046875 ng	7		Y	
0.0234375 ng	1		Y	
0.0117187 ng	0		Y	
0.0058593 ng	1		Y	
0.0029296 ng	1		Y	

310

TOTAL DNA	# ALLELES ABOVE THRESHOLD-310	Heterozygote allele not above threshold	Would call profile inc. by standard practice
0.07 ng MP	10	Y	N
0.07 ng JM	8	Y	N
0.07 ng CH	12	Y	N
0.07 ng LM	13	Y	N

Profiler Plus
3130
Single Source blood sample -MP

TOTAL DNA	# ALLELES ABOVE THRESHOLD-~~310~~ 60	Heterozygote allele not above threshold	Would call profile inc. by standard practice	Lowest peak height ratio
0.75 ng	15	N	N	70
0.375 ng	15	N	N	86
0.1875 ng	15	N	N	35
0.09375 ng	13	Y	N	
0.046875 ng	9	Y	N	
0.0234375 ng	8	Y	N	
0.0117187 ng	5		Y	
0.0058593 ng	5		Y	
0.0029296 ng	4		Y	

Single Source blood sample - JM

TOTAL DNA	# ALLELES ABOVE THRESHOLD-~~310~~ 60	Heterozygote allele not above threshold	Would call profile inc. by standard practice	Lowest peak height ratio
0.75 ng	16	N	N	77
0.375 ng	16	N	N	65
0.1875 ng	16	N	N	62
0.09375 ng	13	Y	N	
0.046875 ng	7	Y	N	
0.0234375 ng	5		Y	
0.0117187 ng	1		Y	
0.0058593 ng	0		Y	
0.0029296 ng	0		Y	

Single Source blood sample - CH

TOTAL DNA	# ALLELES ABOVE THRESHOLD-~~310~~ 60	Heterozygote allele not above threshold	Would call entire profile inc. by standard practice	Lowest peak height ratio
0.75 ng	16	N	N	75
0.375 ng	16	N	N	65
0.1875 ng	16	N	N	38
0.09375 ng	15	Y	N	
0.046875 ng	13	Y	N	
0.0234375 ng	6		Y	
0.0117187 ng	0		Y	
0.0058593 ng	0		Y	
0.0029296 ng	0		Y	

Single Source blood sample - LM

TOTAL DNA	# ALLELES ABOVE THRESHOLD-~~310~~ CC	Heterozygote allele not above threshold	Would call profile inc. by standard practice	Lowest peak height ratio
0.75 ng	18	N	N	87
0.375 ng	18	N	N	52
0.1875 ng	18	N	N	66
0.09375 ng	16	Y	N	
0.046875 ng	13	Y	N	
0.0234375 ng	11	Y	N	
0.0117187 ng	7	Y	N	
0.0058593 ng	1		Y	
0.0029296 ng	1		Y	

Cofiler

310

Single Source blood sample - CH

TOTAL DNA	# ALLELES ABOVE THRESHOLD-~~310~~ 310 CC	Heterozygote allele not above threshold	Would call entire profile inc. by standard practice	Lowest peak height ratio
0.75 ng	10	N	N	83
0.375 ng	10	N	N	74
0.1875 ng	10	N	N	56
0.09375 ng	10	N	N	55
0.046875 ng	8	Y	N	
0.0234375 ng	2		Y	
0.0117187 ng	1		Y	
0.0058593 ng	0		Y	
0.0029296 ng	0		Y	

3130

Single Source blood sample - CH

TOTAL DNA	# ALLELES ABOVE THRESHOLD-310	Heterozygote allele not above threshold	Would call entire profile inc. by standard practice	Lowest peak height ratio
0.75 ng	10	N	N	85
0.375 ng	10	N	N	72
0.1875 ng	10	N	N	54
0.09375 ng	10	N	N	50
0.046875 ng	8	Y	N	
0.0234375 ng	0		Y	
0.0117187 ng	0		Y	
0.0058593 ng	0		Y	
0.0029296 ng	0		Y	

The following observations were made:

- No allelic drop-in was observed
- No instances of increased stutter (where a stutter peak was called) were observed
- Peak height imbalance can be expected but, in general, the peak height balance was above 70% (174 of 200/87%).
- Allelic drop-out (one allele of heterozygous pair below threshold) was observed and was noted in the tables. This information was used to help establish the stochastic threshold.

Based on the observations of this study, the stochastic thresholds for Profiler Plus and Cofiler amplifications (310 and 3130) will be 0.1875 total ng DNA in a sample with a minimum threshold of 90 RFU with no smoothing. The assumption is that any sample with a total DNA amount below this would be concentrated with the speed vac and the entire volume would be amplified at once.

Any profile from a sample below the stochastic threshold will not be CODIS eligible. Interpretation of profiles from samples below the stochastic threshold shall be made with caution; especially in the case of mixtures. Samples that are assumed to be single source and are below the stochastic threshold (minimum quantitation value) but have two alleles called above the RFU threshold at a locus can be interpreted at that locus. Loci from mixture samples with alleles below the stochastic threshold may not be used for statistical purposes. Calling samples inconclusive or uninterpretable may be necessary.

This policy will take effect 04/26/10. This memo will serve as an addendum to the SOP until the next revision.

Cassie C. Carradine CCC
DNA Supervisor/DNA Technical Leader

QA MGR
4.22.10

AUSTIN POLICE DEPARTMENT
SEROLOGY/ DNA SECTION
STANDARD OPERATING PROCEDURES

DNA EXTRACT LOG

DATE/TIME: 12/10/09/0930 CASE #: ST

ANALYST: CCC

QIAGEN ✓ MAXWELL DIFFERENTIAL

KIT LOT 13202088 KIT EXP 08.13.11 DTT

PRO K ETHANOL 014916BK

	CASE #/ITEM	RNA	ul ELUTION vol.	[ ] TO ~ 10UL	Cells /field	ul Quant	ng/ul Quant	ul PP	ul CO	Comments
1	ST MP	Y	~20ul			2	.624	See setup sheet		0.5ul 2005 Draw
2	ST JM	Y	~20ul			2	.251			0.5ul 2001 Draw
3	ST CH	Y	~20ul			2	.292		See setup sheet	0.5ul 2009 Draw
4	ST LM	Y	~20ul			2	.392			0.5ul 2001 Draw
5	BBQ	Y	~20ul			ND	ND		See setup sheet	
6										
7										
8										
9										
10										
11										
12										

AUSTIN POLICE DEPARTMENT
SEROLOGY/ DNA SECTION
STANDARD OPERATING PROCEDURES

QIACUBE LOADING CHART

CASE # ST

ANALYST CCC

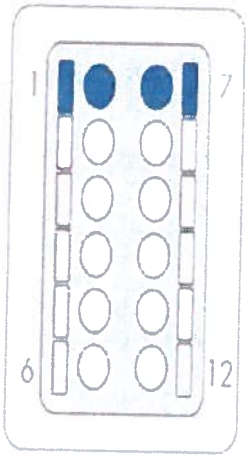
DATE/TIME: LYSIS 12/10/09/0930 PURIFICATION 12/10/09 /1045

QIACUBE # 4

Kit Lot # 1332020888

Ethanol Lot # 01496BK

Position	Sample	Position	Sample
1	ST MP	7	
2	ST JM	8	ST LM
3		9	RBQ
4		10	
5	ST CH	11	
6		12	



AUSTIN POLICE DEPARTMENT
SEROLOGY/ DNA SECTION
STANDARD OPERATING PROCEDURES

QUANTIFILER WORKSHEET

Date: 12.10.09 Case # ST Analyst: CCC
Kit Type: Human

Kit Lot # 0905111 7000 7500

Exp date: 060810 Disk 09-12

	1	2	3	4	5	6	7	8	9	10	11	12
A	STD1 50ng/ul	STD1 50ng/ul	STMP .024									
B	STD 2 16.7ng/ul	STD 2 16.7ng/ul	ST JM .251									
C	STD 3 5.56ng/ul	STD 3 5.56ng/ul	STCH .292									
D	STD 4 1.85ng/ul	STD 4 1.85ng/ul	STLM .392									
E	STD 5 .620ng/ul	STD 5 .620ng/ul										
F	STD 6 .210ng/ul	STD 6 .210ng/ul										
G	STD 7 .068ng/ul	STD 7 .068ng/ul										
H	~~STD 8 .023ng/ul~~ DMT	STD 8 .023ng/ul	TEMP CTM ①									

SLOPE: -3.317 CCC R2 VALUE: 0.99

Version: 111609

Corbett Robotics: Post-run

Results

CAS1200 Series

Robot serial number: **80805**

Username (Role): **D68MDQH1/CAS1200user (Administrators)**

Program file: C:\Documents and Settings\CAS1200user\Desktop\TEMPLATES\ST121009.CAS4
Report saved as: C:\Program Files\Robotics4\Data\Reports\ST121009 Report 12-10-2009 1.19.23 PM.htm

Duration

Event	Time
Start time	12/10/2009 1:04:06 PM
Stop time	12/10/2009 1:19:23 PM
Duration	00:15:17

Exceptions occurred during this run.

Exceptions

Date	Activity	Exception	User Response
12/10/2009 1:06:28 PM	Adding 252µL Primer Set to Master Mix Source: Reagent block (standard 200,flat,tapered) @ R1 A Destination: Mix plate (5 tube positions) @ M1 B	Couldn't find liquid (insufficient or false trigger)	User retried.

ST CCC

AUSTIN POLICE DEPARTMENT
SEROLOGY/DNA SECTION
STANDARD OPERATING PROCEDURES

PCR SETUP PROFILER PLUS

Date: 12/11/2009
Case #: ST
Analyst: CCC CCC

Profiler Plus™
LOT # 0905139
EXP DATE 5/26/2010

TE LOT # 013009

	CASE/ITEM	EXTRACT AMT (ul)	TE AMT (ul)	MASTER MIX	[ ] TO ~ 10ul
1	ST MP 0.75ng	1.2	8.8	15	
2	ST MP 0.375ng	0.6	9.4	15	
3	ST MP 0.1875ng	0.3	9.7	15	
4	ST MP 0.09375ng	0.15	9.85	15	
5	ST MP 0.046875ng	0.075	9.925	15	
6	ST MP 0.0234375ng	0.038	9.962	15	
7	ST MP 0.0117187ng	0.0188	9.9812	15	
8	ST MP 0.0058593ng	0.009	9.991	15	
9	ST MP 0.0029296ng	0.005	9.995	15	
10	RBQ	1.2	8.8	15	
11	POS CTRL	10	0	15	
12	NEG CTRL	0	10	15	
13					
14					
15					
16					
17					
18					

	# OF SAMPLES	REAGENT AMT (ul)	TOTAL (ul)
REACTION MIX	13	9.5	123.5
MER SET	13	5	65
TAQ	13	0.5	6.5

9700 HEAT BLOCK POSITION WORKSHEET

Date: 12/11/2009 **Case #:** ST **Analyst:** CC

Profiler Plus™ COfiler™ **Thermal Cycler #** 2

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B	ST MP 0.75ng	ST MP 0.375ng	ST MP 0.1875ng	ST MP 0.09375ng	ST MP 0.046875ng	ST MP 0.0234375ng	ST MP 0.0117187ng	ST MP 0.0058593ng	ST MP 0.0029296ng	RBQ	POS CTRL	NEG CTRL
C												
D												
E												
F												
G												
H												

Comments:

AUSTIN POLICE DEPARTMENT
SEROLOGY/DNA SECTION
STANDARD OPERATING PROCEDURES

PCR SETUP PROFILER PLUS

Date: 12/16/2009
Case #: ST
Analyst: CCC CCC

1012

Profiler Plus™
LOT # 0905139
EXP DATE 5/26/2010

TE LOT # 013009

	CASE/ITEM	EXTRACT AMT (ul)	TE AMT (ul)	MASTER MIX	[ ] TO ~ 10ul
1	ST JM 0.75ng	3	7	15	
2	ST JM 0.375ng	1.5	8.5	15	
3	ST JM 0.1875ng	0.75	9.25	15	
4	ST JM 0.09375ng	0.37	9.63	15	
5	ST JM 0.046875ng	0.19	9.81	15	
6	ST JM 0.0234375ng	0.095	9.905	15	
7	ST JM 0.0117187ng	0.0475	9.9525	15	
8	ST JM 0.0058593ng	0.0238	9.9762	15	
9	ST JM 0.0029296ng	0.012	9.988	15	
10	ST CH 0.75ng	2.6	7.4	15	
11	ST CH 0.375ng	1.3	8.7	15	
12	ST CH 0.1875ng	0.64	9.36	15	
13	ST CH 0.09375ng	0.32	9.68	15	
14	ST CH 0.046875ng	0.16	9.84	15	
15	ST CH 0.0234375ng	0.08	9.92	15	
16	ST CH 0.0117187ng	0.04	9.96	15	
17	ST CH 0.0058593ng	0.02	9.98	15	
18	ST CH 0.0029296ng	0.01	9.99	15	

	# OF SAMPLES	REAGENT AMT (ul)	TOTAL (ul)
REACTION MIX	~~13~~ CCC	9.5	~~123.5~~ CCC
PRIMER SET	~~13~~ CCC	5	~~65~~ CCC
TAQ	~~13~~ CCC	0.5	~~6.5~~ CCC

AUSTIN POLICE DEPARTMENT
SEROLOGY/DNA SECTION
STANDARD OPERATING PROCEDURES

PCR SETUP PROFILER PLUS

Date: 12/16/2009
Case #: ST
Analyst: CCC CCC

2012

Profiler Plus™
LOT # 0905139
EXP DATE 5/26/2010

TE LOT # 013009

	CASE/ITEM	EXTRACT AMT (ul)	TE AMT (ul)	MASTER MIX	[ ] TO ~ 10ul
19	ST LM 0.75ng	1.9	8.1	15	
20	ST LM 0.375ng	0.96	9.04	15	
21	ST LM 0.1875ng	0.48	9.52	15	
22	ST LM 0.09375ng	0.24	9.76	15	
23	ST LM 0.046875ng	0.12	9.88	15	
24	ST LM 0.0234375ng	0.06	9.94	15	
25	ST LM 0.0117187ng	0.03	9.97	15	
26	ST LM 0.0058593ng	0.015	9.985	15	
27	ST LM 0.0029296ng	0.007	9.993	15	
28	RBQ	3	7	15	
29	POS CTRL	10	0	15	
30	NEG CTRL	0	10	15	

	# OF SAMPLES	REAGENT AMT (ul)	TOTAL (ul)
REACTION MIX	31	9.5	294.5
MER SET	31	5	155
TAQ	31	0.5	15.5

9700 HEAT BLOCK POSITION WORKSHEET

Date: 12/16/2009 **Case #:** ST **Analyst:** CCC

Profiler Plus™ COfiler™ Thermal Cycler # 2

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B	ST JM 0.75ng	ST JM 0.375ng	ST JM 0.1875ng	ST JM 0.09375ng	ST JM 0.046875ng	ST JM 0.0234375ng	ST JM 0.0117187ng	ST JM 0.0058593ng	ST JM 0.0029296ng	ST CH 0.75ng	ST CH 0.375ng	ST CH 0.1875ng
C												
D	ST CH 0.09375ng	ST CH 0.046875ng	ST CH 0.0234375ng	ST CH 0.0117187ng	ST CH 0.0058593ng	ST CH 0.0029296ng	ST LM 0.75ng	ST LM 0.375ng	ST LM 0.1875ng	ST LM 0.09375ng	ST LM 0.046875ng	ST LM 0.0234375ng
E												
F	ST LM 0.0117187ng	ST LM 0.0058593ng	ST LM 0.0029296ng	RBQ	POS CTRL	NEG CTRL						
G												
H												

Comments:

AUSTIN POLICE DEPARTMENT
SEROLOGY/ DNA SECTION
STANDARD OPERATING PROCEDURES

STR RECORD SHEET

DATE OF RUN 12.11.09

CASE # (S) ST

ANALYST (S) CCC CCC

310/3130 INSTRUMENT #3

LOT RECORDS

CAPILLARY VDB09A

POLYMER 0908175

ROX 0906500

FORMAMIDE 0.902799

GA BUFFER 0905322

CASE RECORDS

KIT LOT #/ Expiration Date (PP/CO) 0905139 / 5.26.10

MATRIX/SPECTRAL 111709

SIZE STANDARD Rox 500

ANALYZED AT THRESHOLD OF: 90
(for evidence samples only)

90NS ALL

☐ Others at 150

Other

CASEWORK DISK # 09-12

Applied Systems

GeneMapper ID v3.2.1

	Status	Sample File	Sample Name	Sample Type	Analysis Method	Panel	Size Standard	OS	SQ
1		A11ST MP 0.0234375-1.fsa	ST MP 0.0234375	Sample	None	None	None	0.0	0.0
2		A11ST MP 0.0234375.fsa	ST MP 0.0234375	Sample	None	None	None	0.0	0.0
3		A1ST MP 0.75-1.fsa	ST MP 0.75	Sample	None	None	None	0.0	0.0
4		A1ST MP 0.75.fsa	ST MP 0.75	Sample	None	None	None	0.0	0.0
5		A3ST MP 0.375.fsa	ST MP 0.375	Sample	None	None	None	0.0	0.0
6		A5ST MP 0.1875-1.fsa	ST MP 0.1875	Sample	None	None	None	0.0	0.0
7		A5ST MP 0.1875.fsa	ST MP 0.1875	Sample	None	None	None	0.0	0.0
8		A7ST MP 0.09375-1.fsa	ST MP 0.09375	Sample	None	None	None	0.0	0.0
9		A7ST MP 0.09375.fsa	ST MP 0.09375	Sample	None	None	None	0.0	0.0
10		A9ST MP 0.046875-1.fsa	ST MP 0.046875	Sample	None	None	None	0.0	0.0
11		A9ST MP 0.046875.fsa	ST MP 0.046875	Sample	None	None	None	0.0	0.0
12		B10RBQ.fsa	RBQ	Sample	None	None	None	0.0	0.0
13		B10RBQ1.fsa	RBQ	Sample	None	None	None	0.0	0.0
14		B12POS CTRL.fsa	POS CTRL	Sample	None	None	None	0.0	0.0
15		B12POS CTRL1.fsa	POS CTRL	Sample	None	None	None	0.0	0.0
16		B2ST MP 0.0117187-1.fsa	ST MP 0.0117187	Sample	None	None	None	0.0	0.0
17		B2ST MP 0.0117187.fsa	ST MP 0.0117187	Sample	None	None	None	0.0	0.0
18		B4ST MP 0.0058593-1.fsa	ST MP 0.0058593	Sample	None	None	None	0.0	0.0
19		B4ST MP 0.0058593.fsa	ST MP 0.0058593	Sample	None	None	None	0.0	0.0
20		B6ST MP 0.0029296-1.fsa	ST MP 0.0029296	Sample	None	None	None	0.0	0.0
21		B6ST MP 0.0029296.fsa	ST MP 0.0029296	Sample	None	None	None	0.0	0.0
22		B8ST MP 0.0014648-1.fsa	ST MP 0.0014648	Sample	None	None	None	0.0	0.0
23		B8ST MP 0.0014648.fsa	ST MP 0.0014648	Sample	None	None	None	0.0	0.0
24		C1NEG CTRL.fsa	NEG CTRL	Sample	None	None	None	0.0	0.0
25		C1NEG CTRL1.fsa	NEG CTRL	Sample	None	None	None	0.0	0.0
26		C3LADDER.fsa	LADDER	Allelic Ladder	None	None	None	0.0	0.0
27		C3LADDER1.fsa	LADDER	Allelic Ladder	None	None	None	0.0	0.0

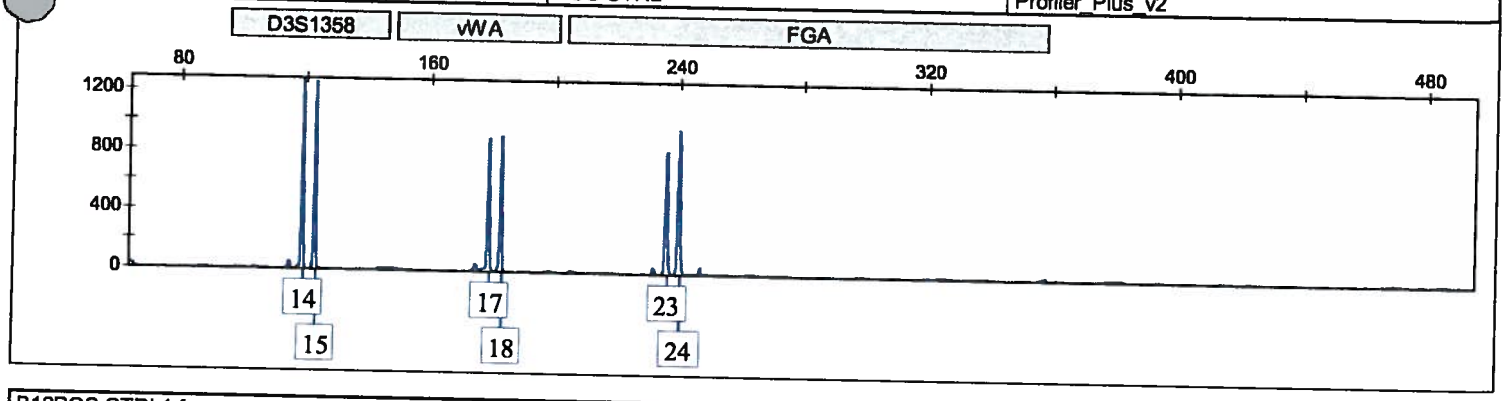
ST CCC
Run 12-11-09

Applied Biosystems

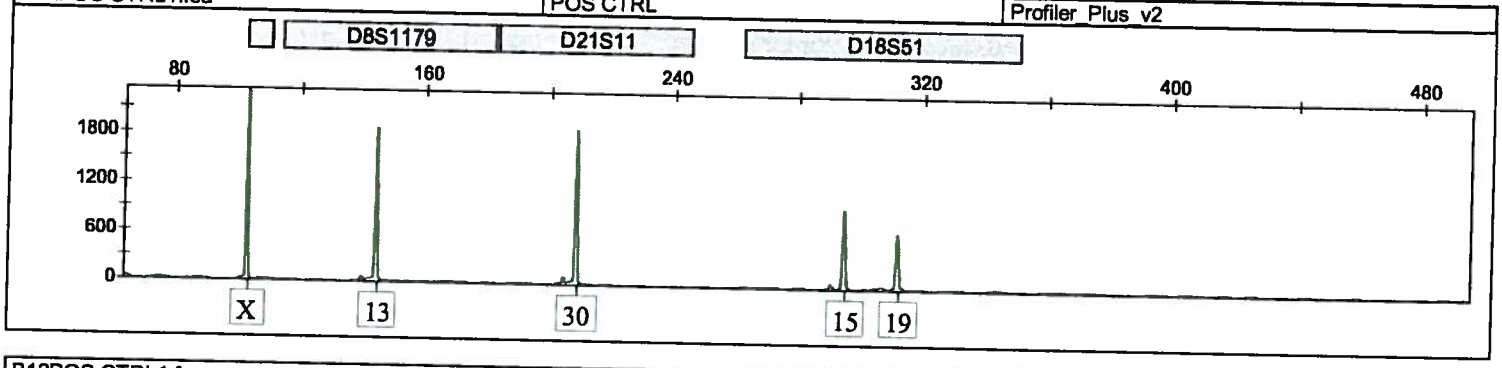
GeneMapper ID v3.2.1

ST 121109

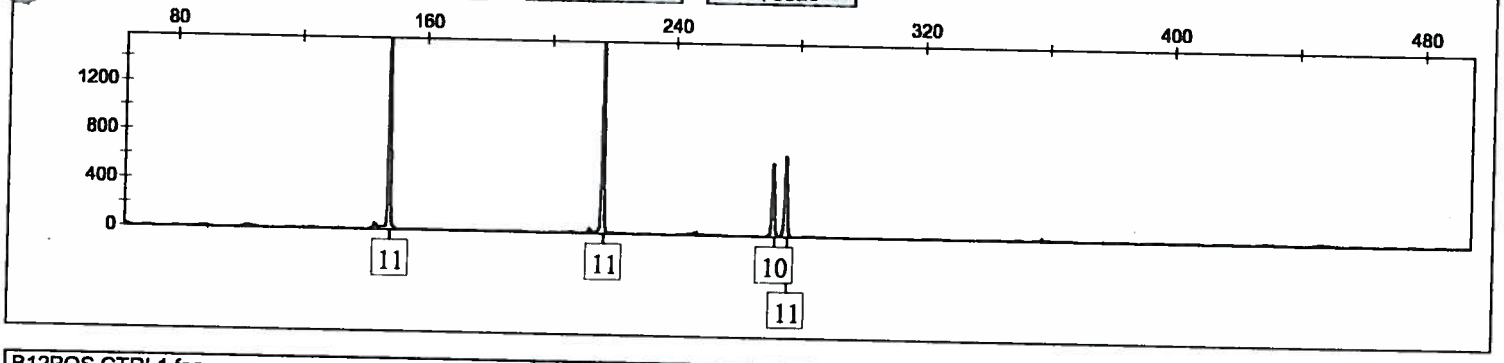
Sample File	Sample Name	Panel
2POS CTRL1.fsa	POS CTRL	Profiler_Plus_v2



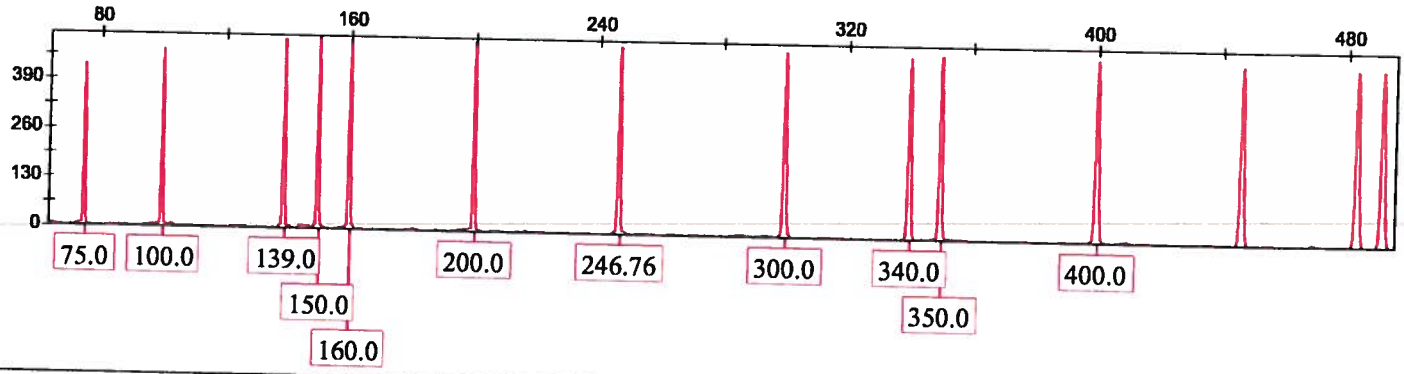
B12POS CTRL1.fsa	POS CTRL	Profiler_Plus_v2



B12POS CTRL1.fsa	POS CTRL	Profiler_Plus_v2



B12POS CTRL1.fsa	POS CTRL	Profiler_Plus_v2



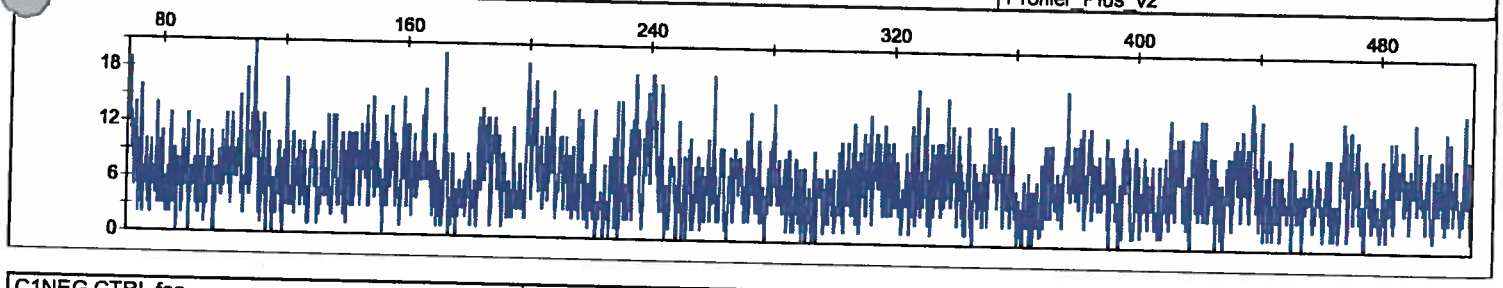
ST CCC

Applied Biosystems

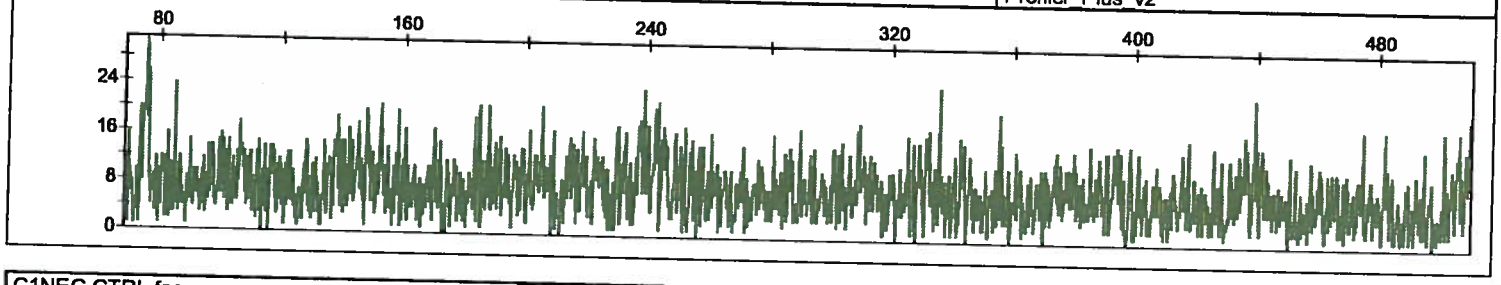
GeneMapper ID v3.2.1

ST 121109

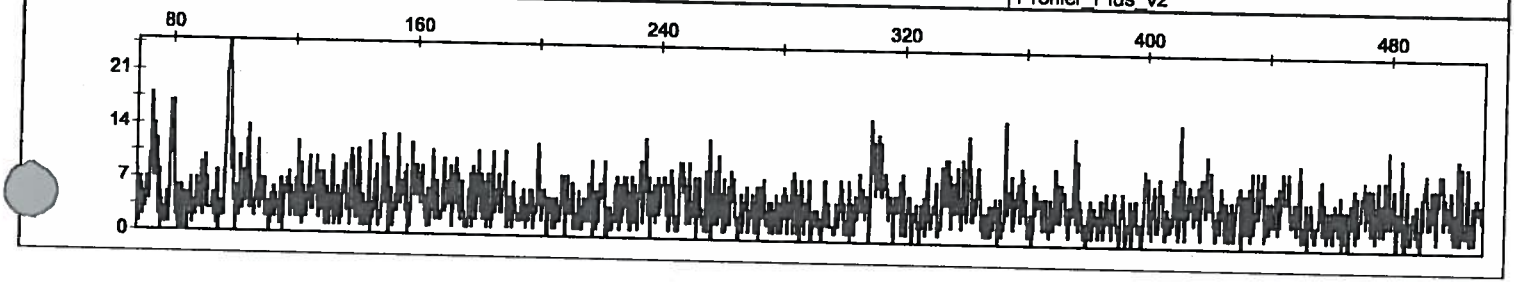
Sample File	Sample Name	Panel
NEG CTRL.fsa	NEG CTRL	Profiler_Plus_v2



C1NEG CTRL.fsa	NEG CTRL	Profiler_Plus_v2



C1NEG CTRL.fsa	NEG CTRL	Profiler_Plus_v2



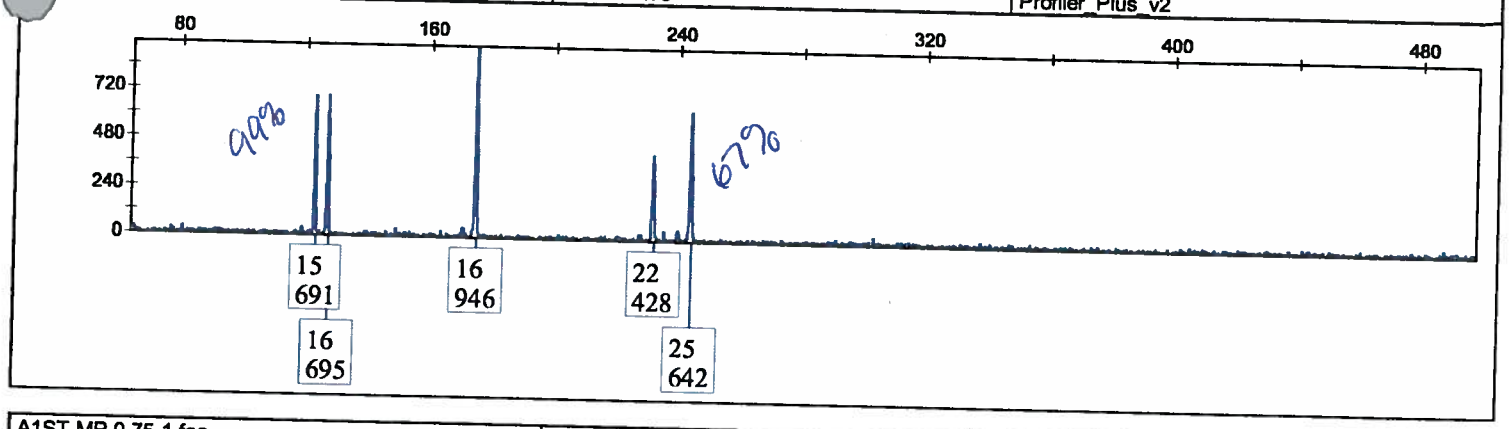
ST CCC

Applied Biosystems

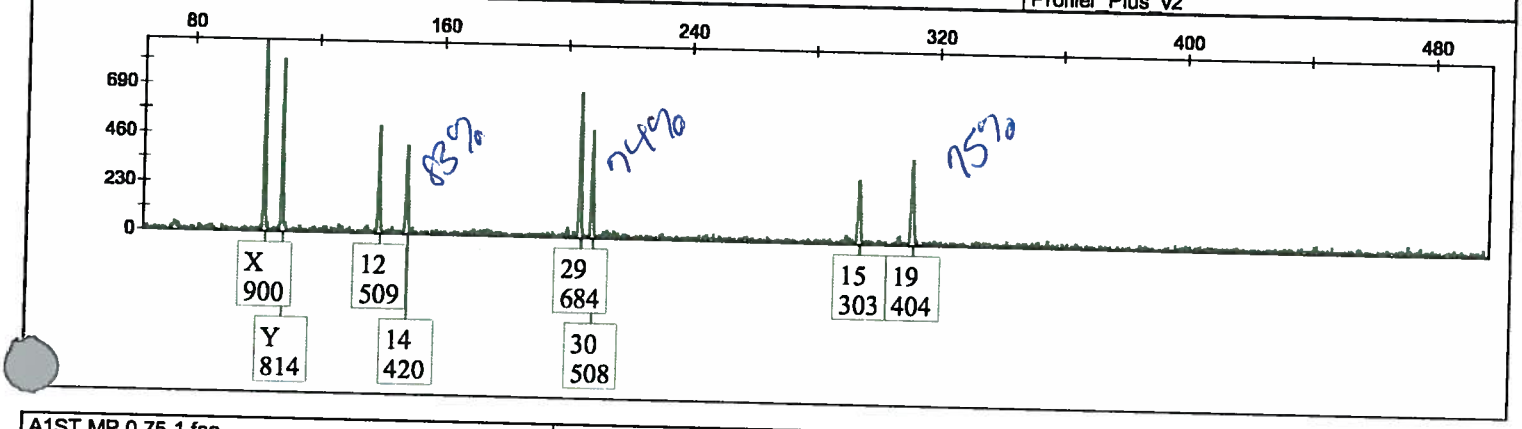
GeneMapper ID v3.2.1

ST 121109

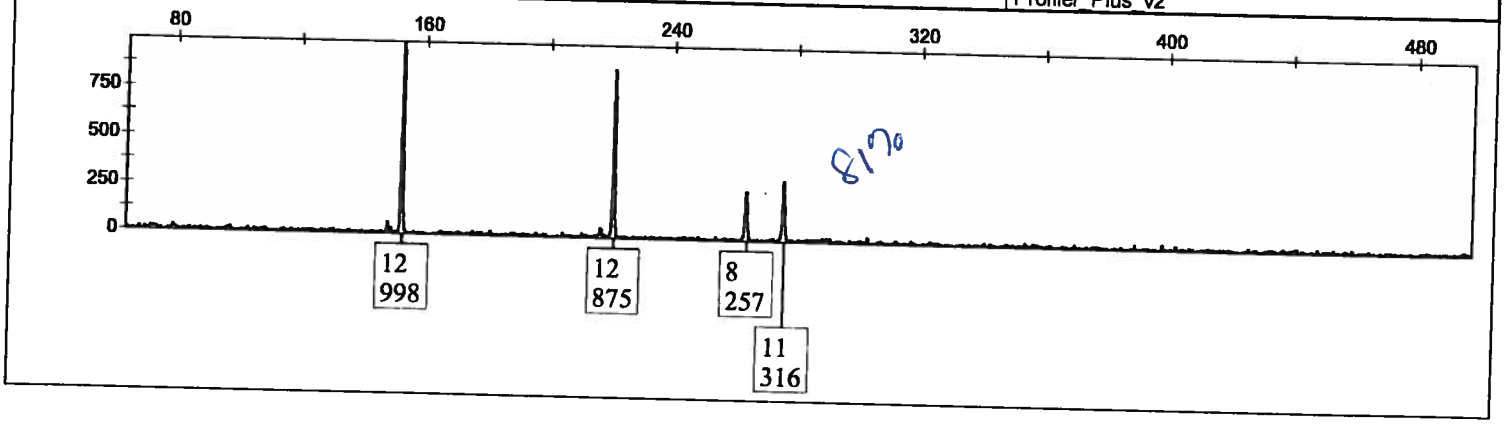
Sample File	Sample Name	Panel
ST MP 0.75-1.fsa	ST MP 0.75	Profiler_Plus_v2



A1ST MP 0.75-1.fsa	ST MP 0.75	Profiler_Plus_v2



A1ST MP 0.75-1.fsa	ST MP 0.75	Profiler_Plus_v2



ST CCC
5 sec.

Tue Dec 15,2009 08:40AM, CST

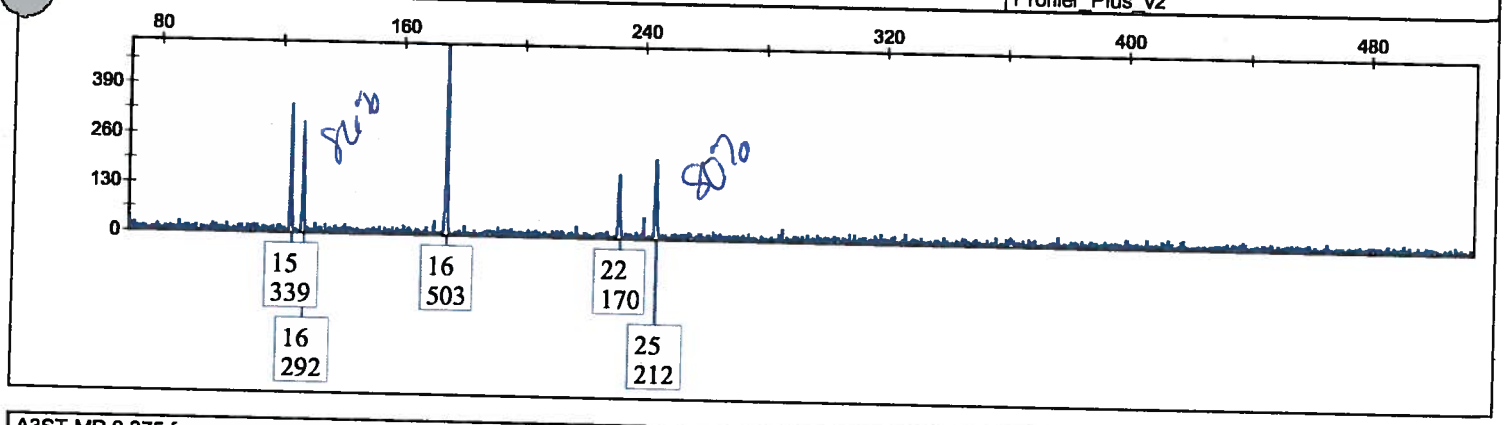
Printed by: gmid

Applied Biosystems

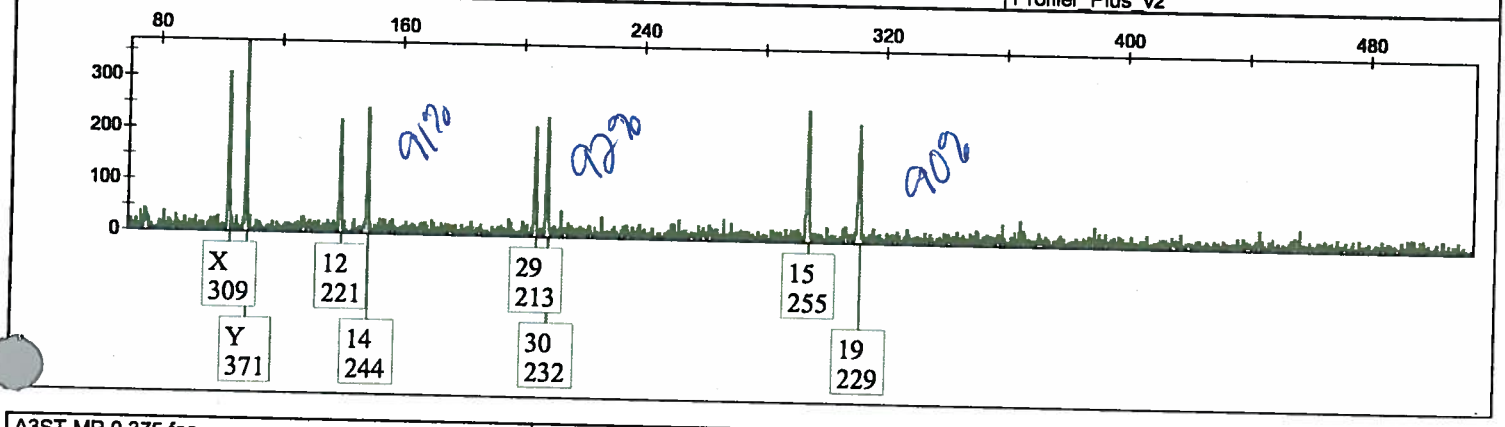
GeneMapper ID v3.2.1

ST 121109

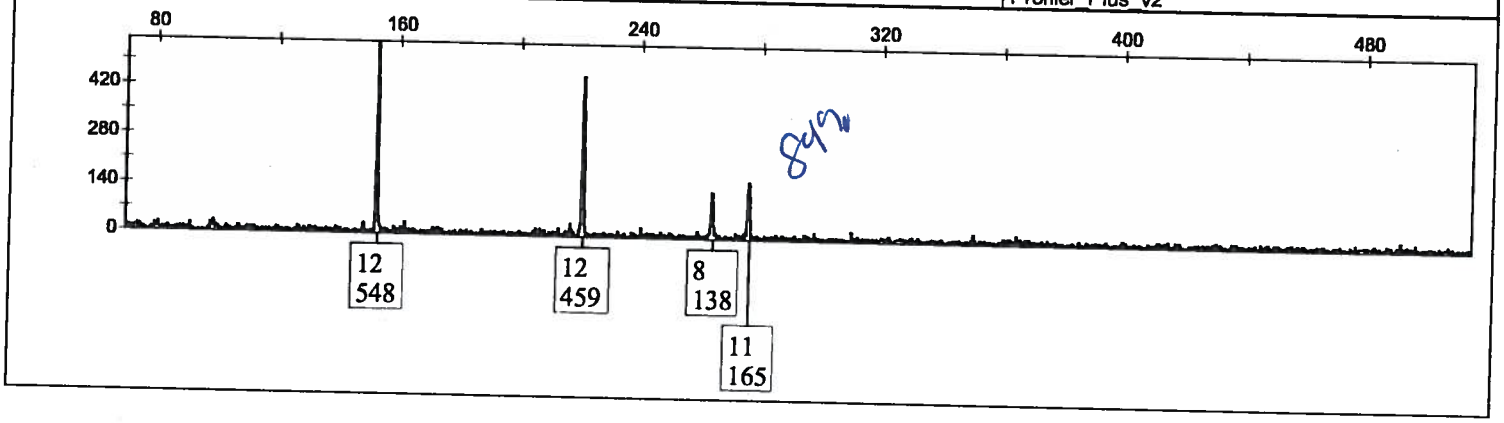
Sample File	Sample Name	Panel
ST MP 0.375.fsa	ST MP 0.375	Profiler_Plus_v2



Sample File	Sample Name	Panel
A3ST MP 0.375.fsa	ST MP 0.375	Profiler_Plus_v2



Sample File	Sample Name	Panel
A3ST MP 0.375.fsa	ST MP 0.375	Profiler_Plus_v2



ST CCC
5 sec

Tue Dec 15,2009 08:41AM, CST

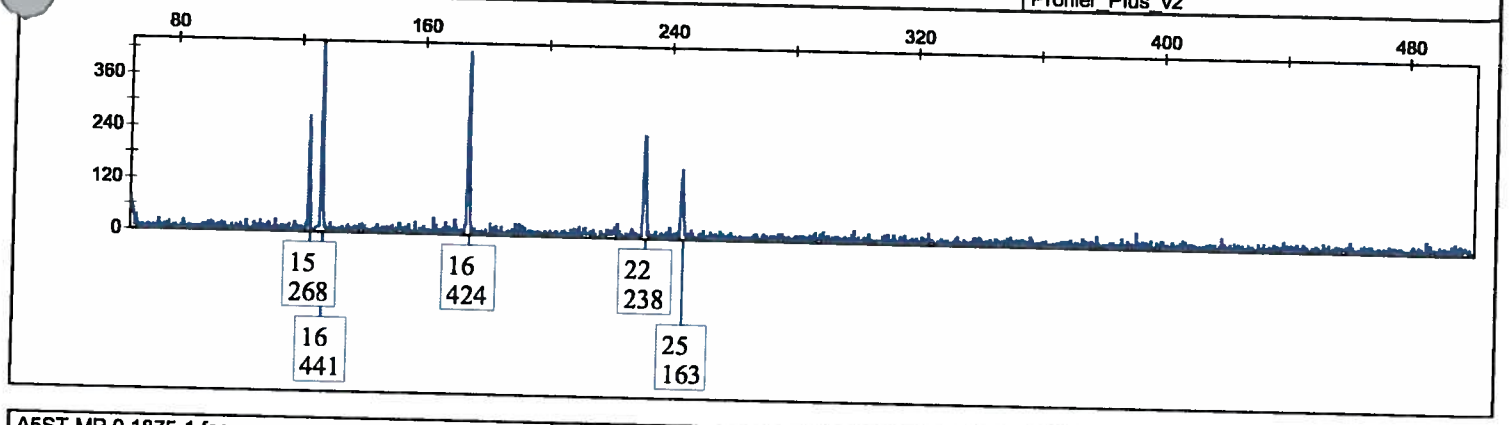
Printed by: gmid

Applied Biosystems

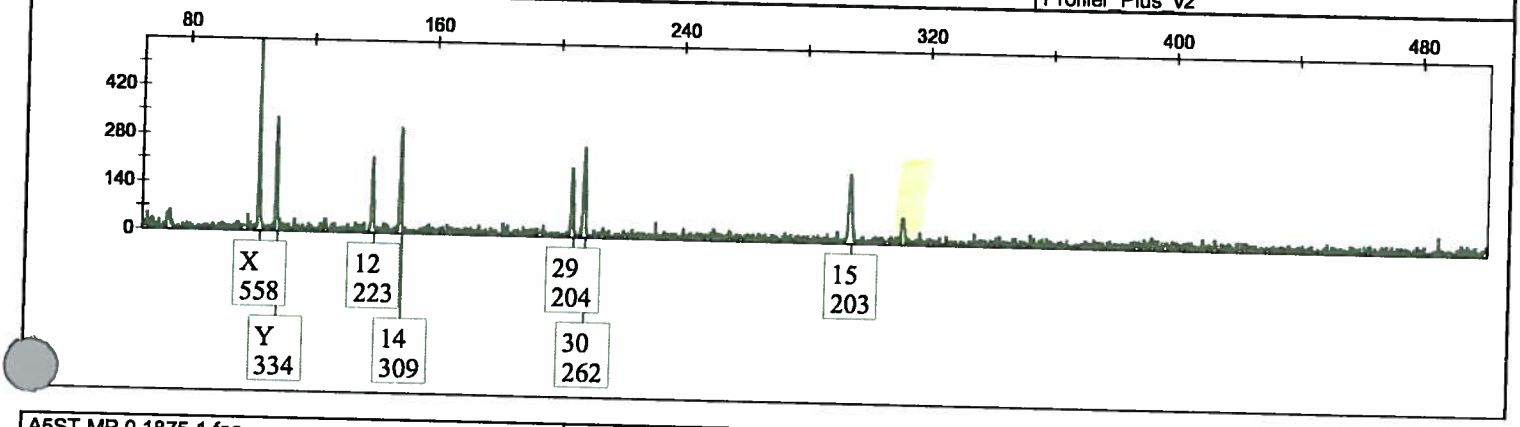
GeneMapper ID v3.2.1

ST 121109

Sample File	Sample Name	Panel
A5ST MP 0.1875-1.fsa	ST MP 0.1875	Profiler_Plus_v2



A5ST MP 0.1875-1.fsa	ST MP 0.1875	Profiler_Plus_v2



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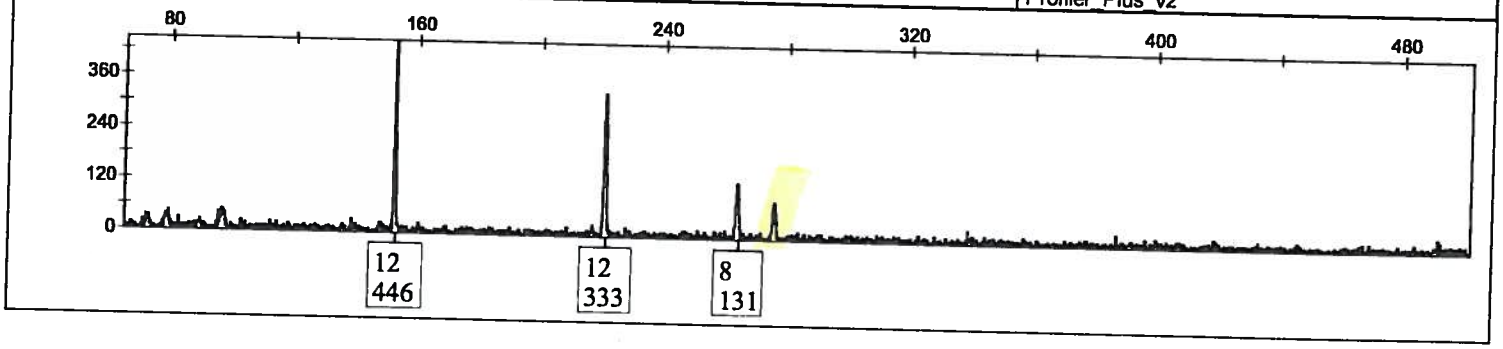


10 sec

ST CCC

Tue Dec 15,2009 08:41AM, CST

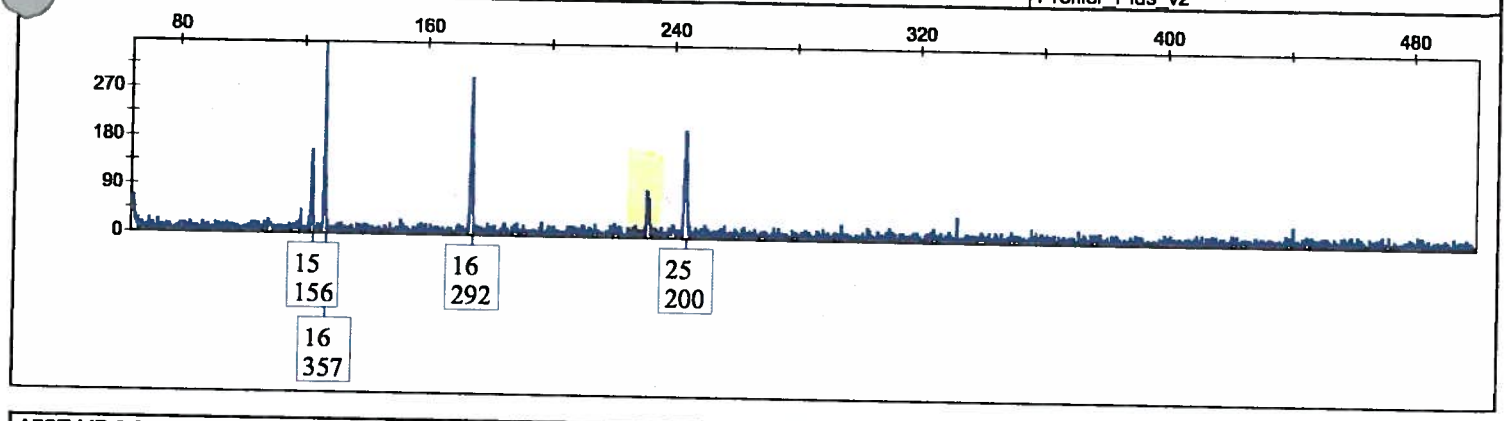
Printed by: gmid

Applied Biosystems

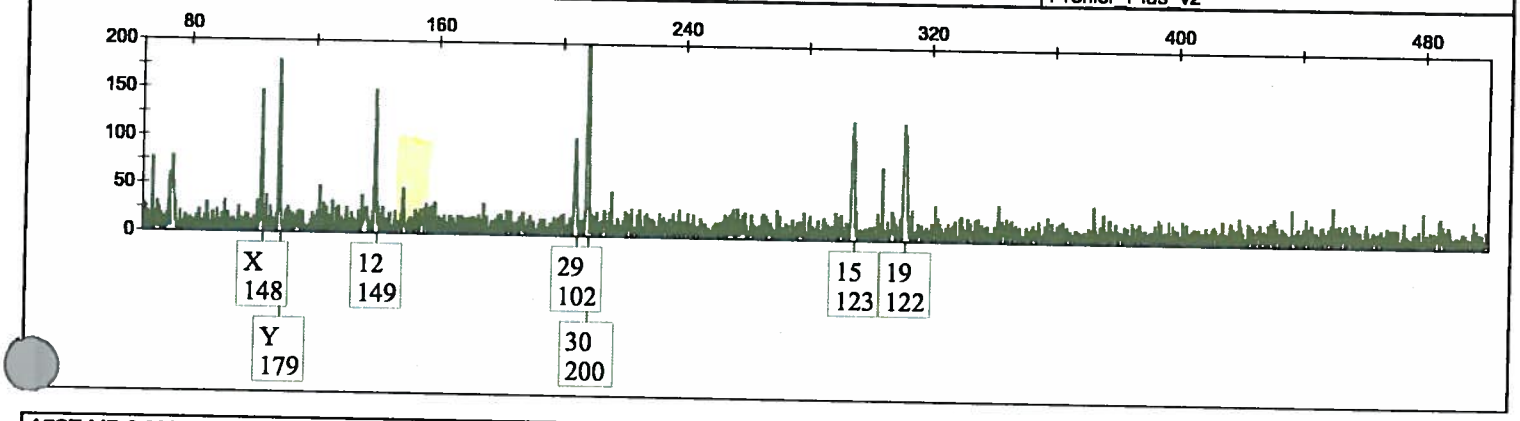
GeneMapper ID v3.2.1

ST 121109

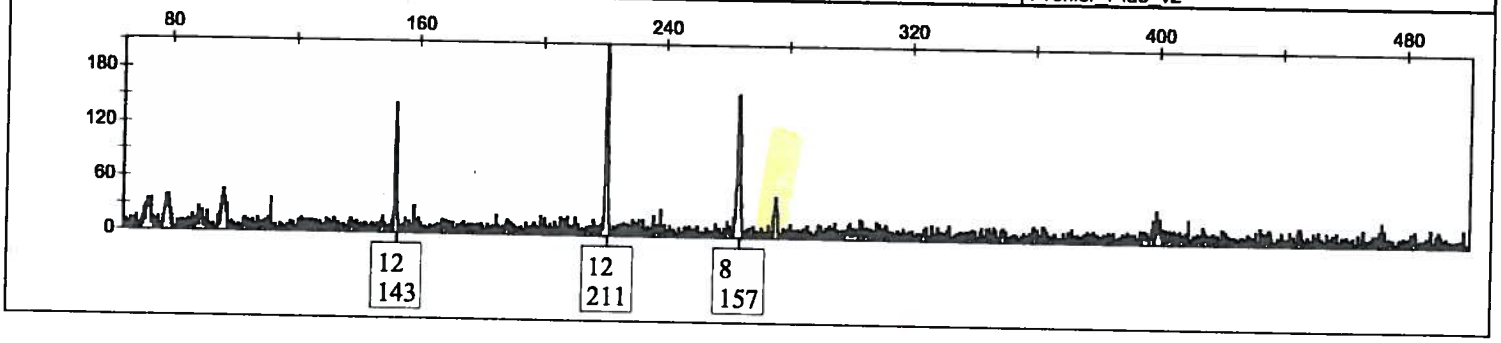
Sample File	Sample Name	Panel
ST MP 0.09375-1.fsa	ST MP 0.09375	Profiler_Plus_v2



A7ST MP 0.09375-1.fsa	ST MP 0.09375	Profiler_Plus_v2



A7ST MP 0.09375-1.fsa	ST MP 0.09375	Profiler_Plus_v2



10 sec

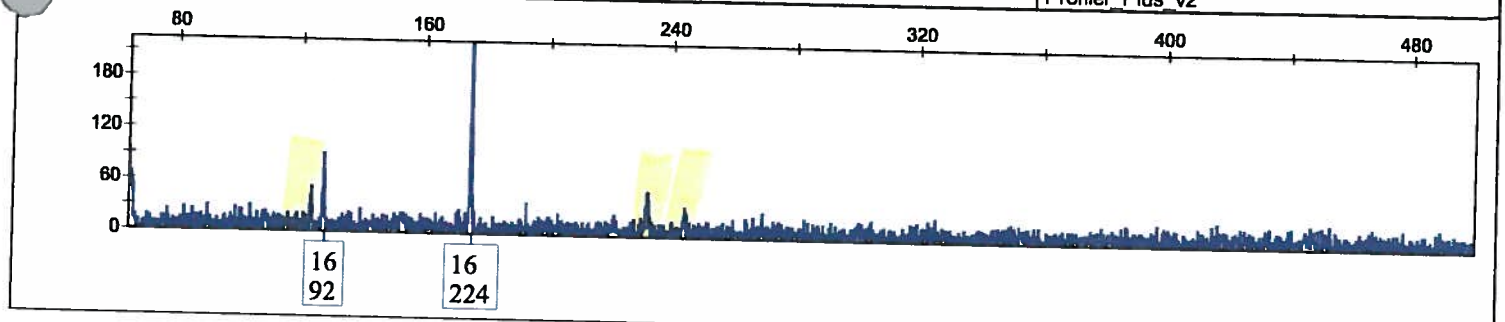
ST CCC

Applied Biosystems

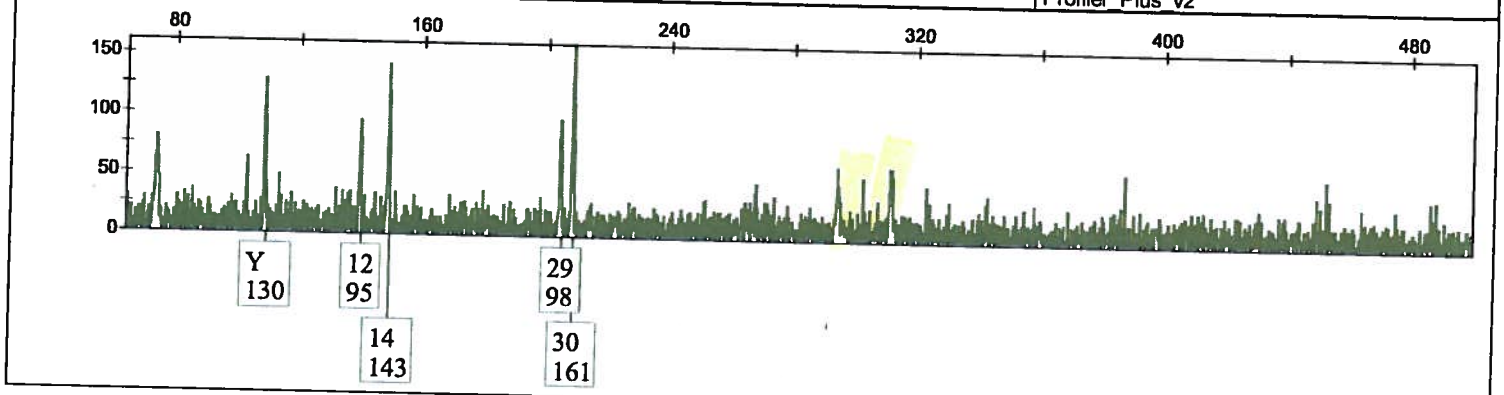
GeneMapper ID v3.2.1

ST 121109

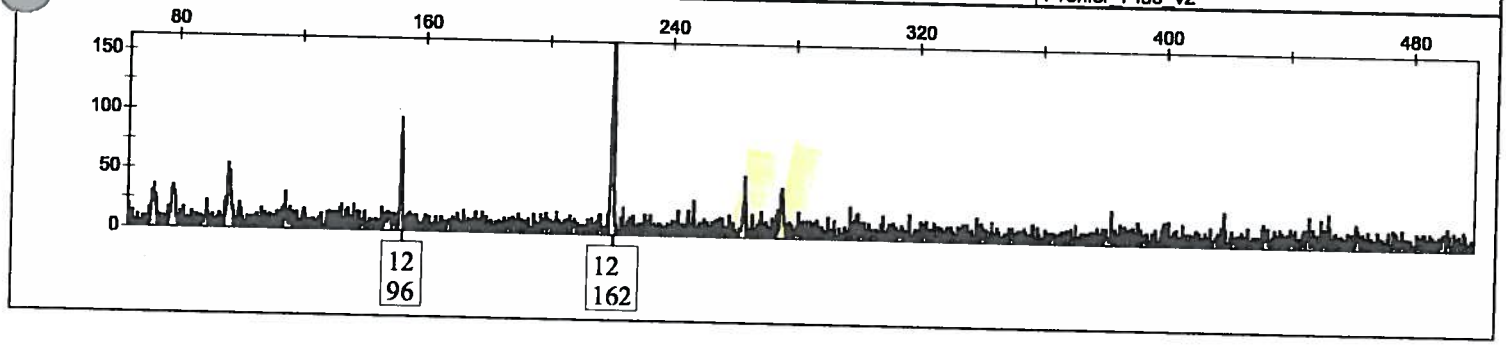
Sample File	Sample Name	Panel
ST MP 0.046875-1.fsa	ST MP 0.046875	Profiler_Plus_v2



A9ST MP 0.046875-1.fsa	ST MP 0.046875	Profiler_Plus_v2



ST MP 0.046875-1.fsa	ST MP 0.046875	Profiler_Plus_v2



10 sec

ST ccc

Applied Biosystems

GeneMapper ID v3.2.1

ST 121109

Sample File	Sample Name	Panel
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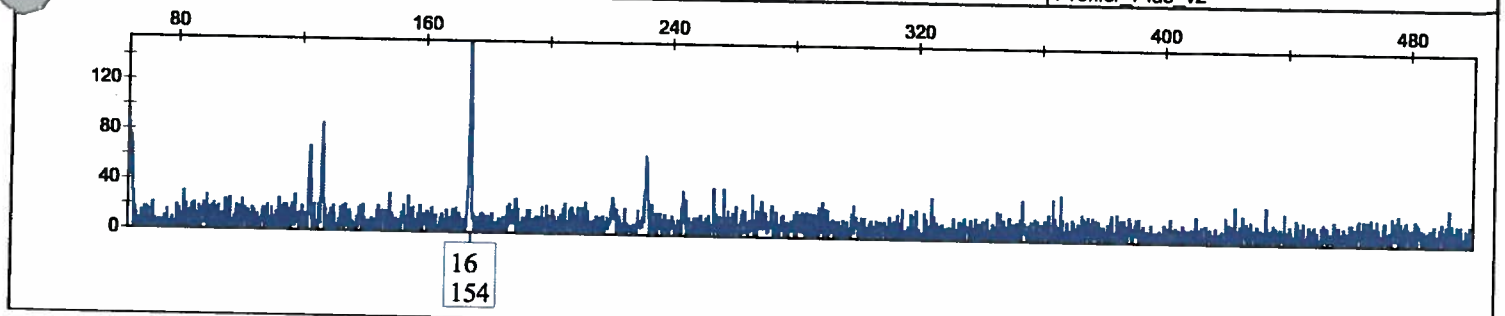
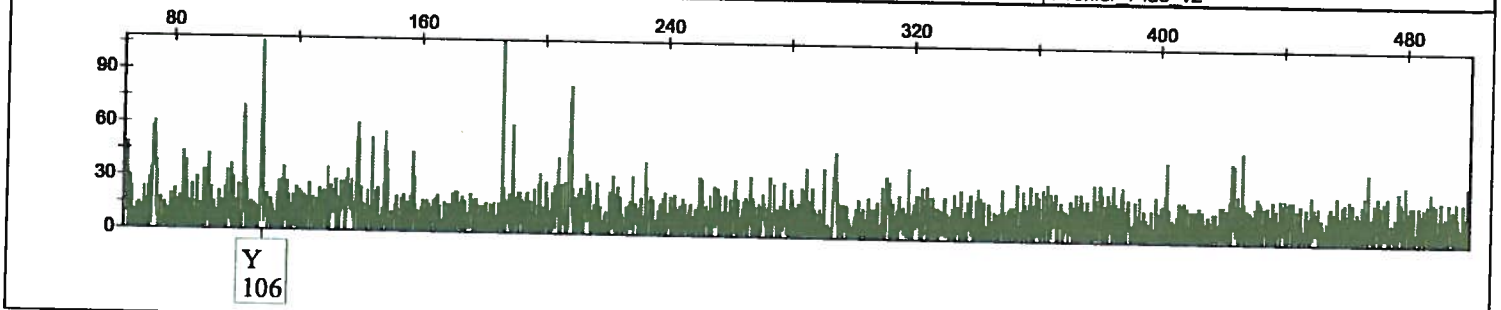
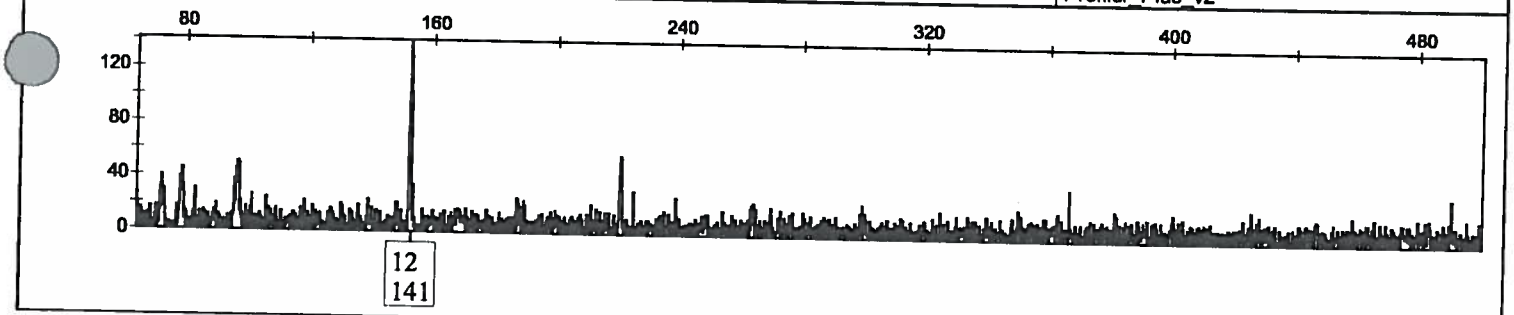


A11ST MP 0.0234375-1.fsa	ST MP 0.0234375	Profiler_Plus_v2



A11ST MP 0.0234375-1.fsa	ST MP 0.0234375	Profiler_Plus_v2



1 DSeC

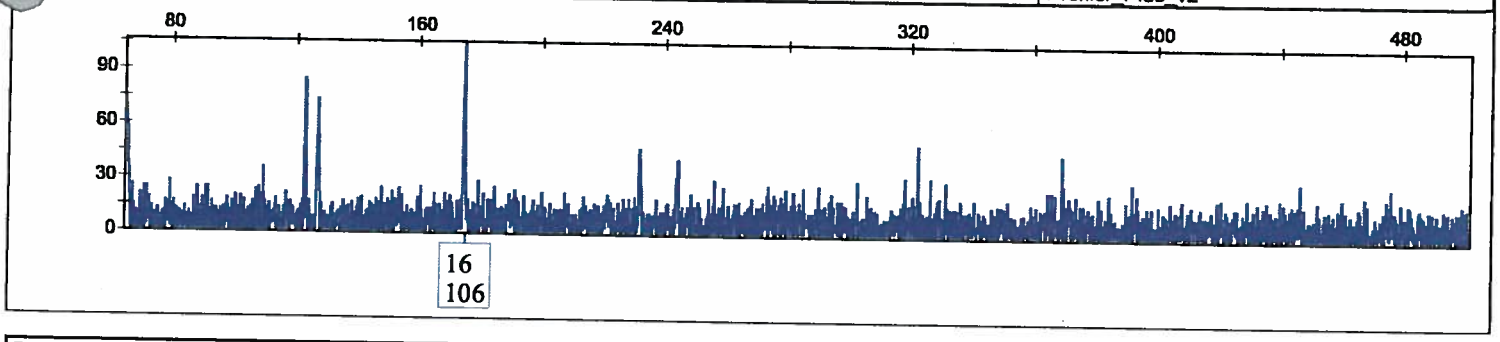
ST CCC

Applied Biosystems

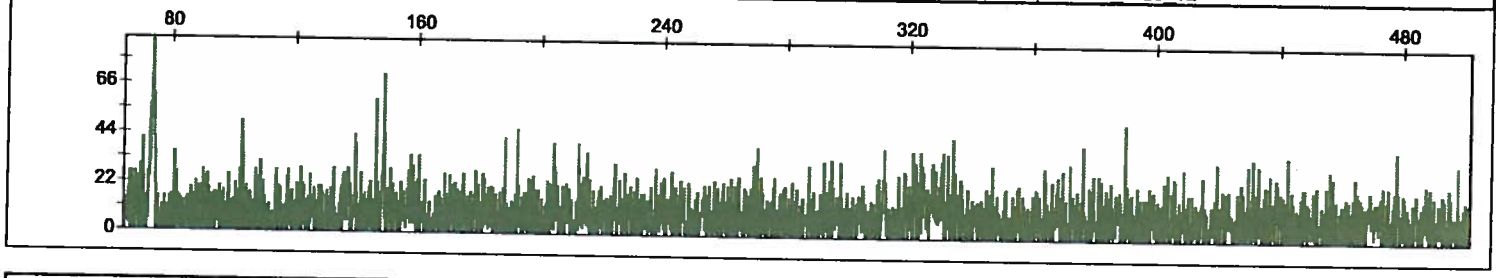
GeneMapper ID v3.2.1

ST 121109

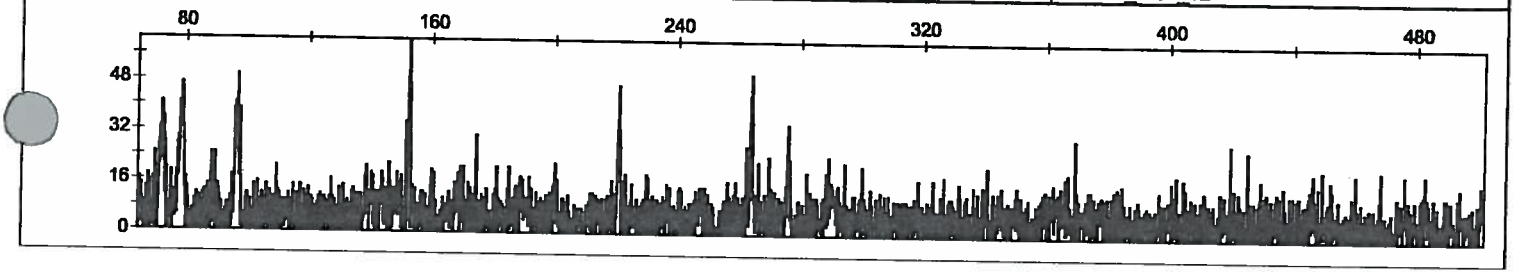
Sample File	Sample Name	Panel
ST MP 0.0117187-1.fsa	ST MP 0.0117187	Profiler_Plus_v2



B2ST MP 0.0117187-1.fsa	ST MP 0.0117187	Profiler_Plus_v2



B2ST MP 0.0117187-1.fsa	ST MP 0.0117187	Profiler_Plus_v2



10 sec

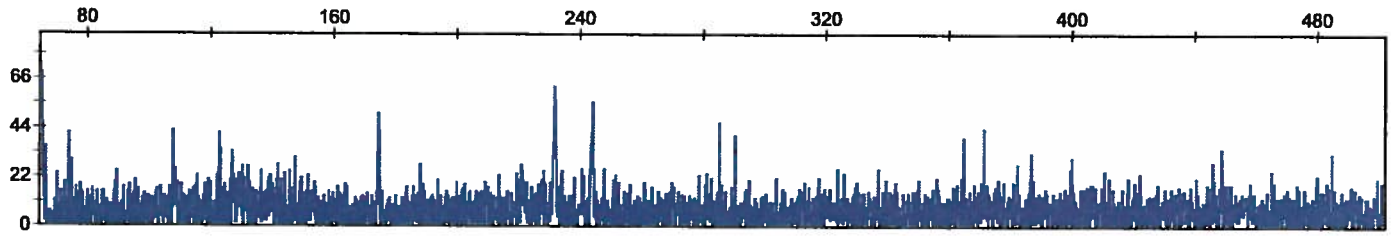
ST CCC

Applied Biosystems

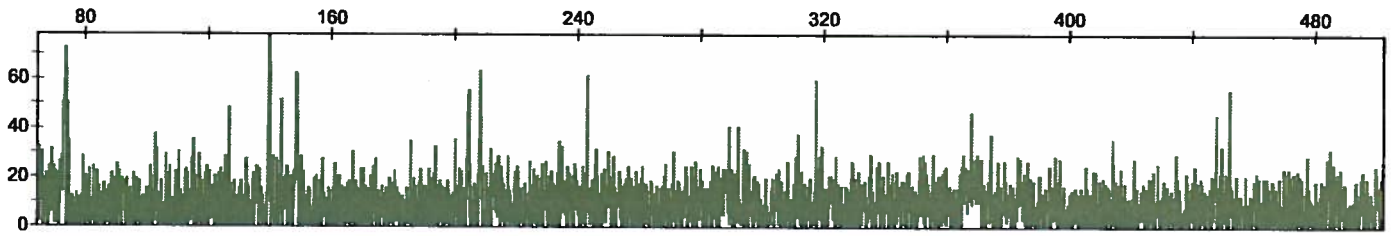
GeneMapper ID v3.2.1

ST 121109

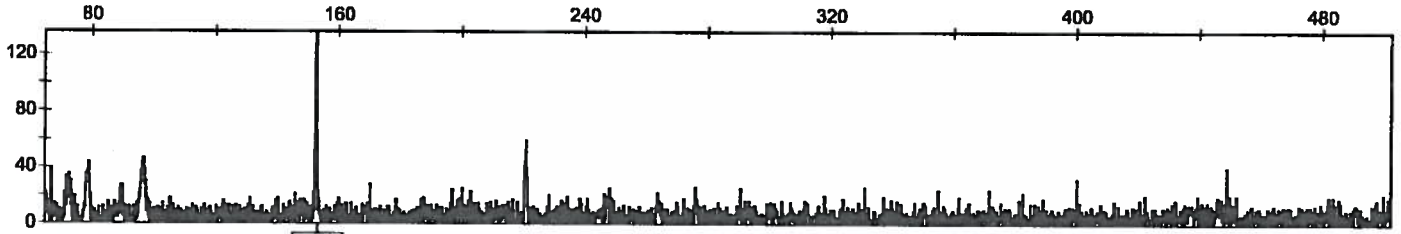
Sample File	Sample Name	Panel
ST MP 0.0058593-1.fsa	ST MP 0.0058593	Profiler_Plus_v2



B4ST MP 0.0058593-1.fsa	ST MP 0.0058593	Profiler_Plus_v2



B4ST MP 0.0058593-1.fsa	ST MP 0.0058593	Profiler_Plus_v2



10sec

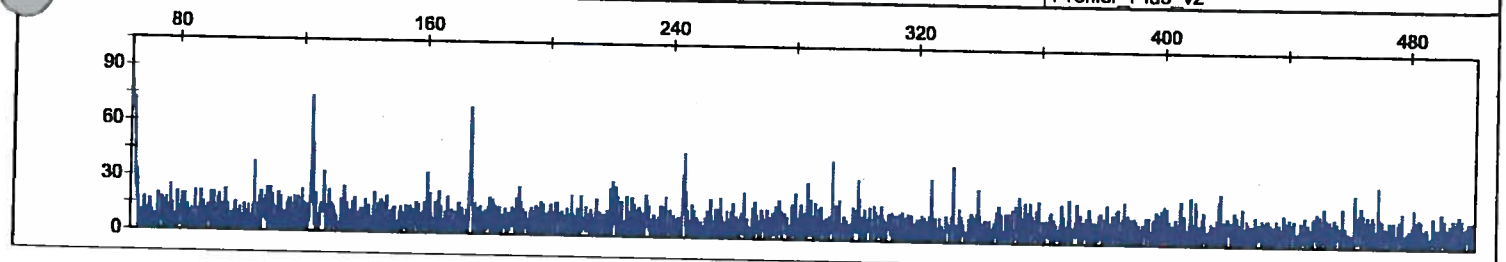
STCCC

Applied Biosystems

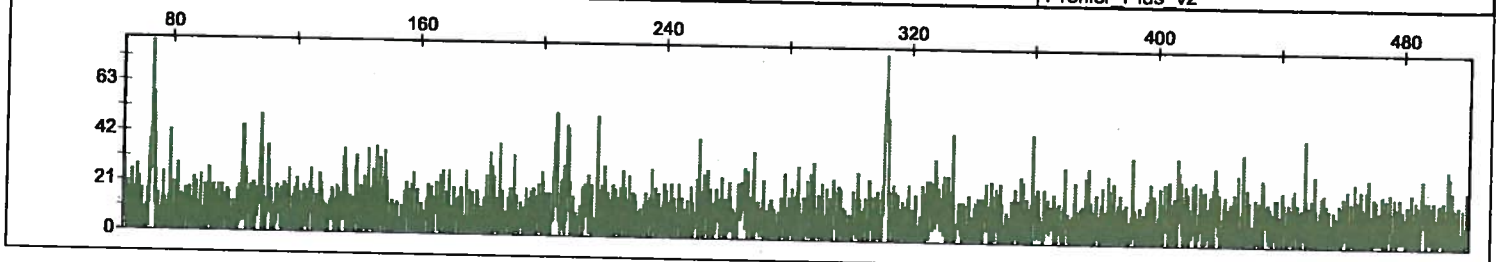
GeneMapper ID v3.2.1

ST 121109

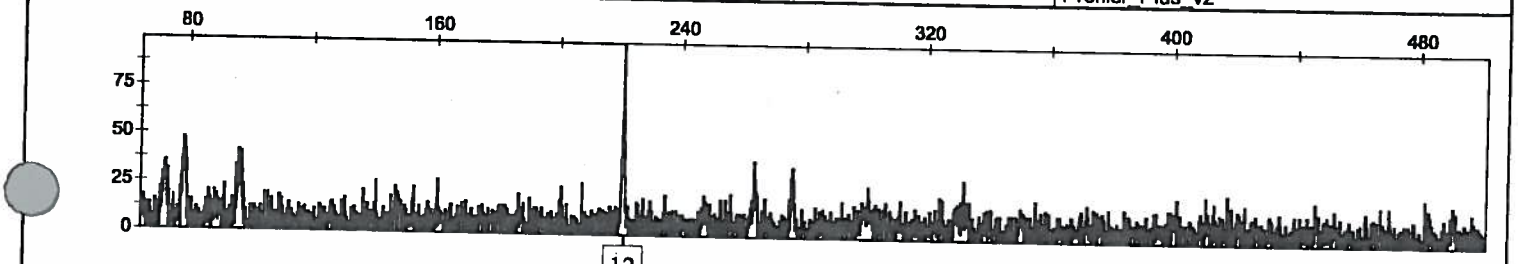
Sample File	Sample Name	Panel
ST MP 0.0029296-1.fsa	ST MP 0.0029296	Profiler_Plus_v2



B6ST MP 0.0029296-1.fsa	ST MP 0.0029296	Profiler_Plus_v2



B6ST MP 0.0029296-1.fsa	ST MP 0.0029296	Profiler_Plus_v2



10Sec

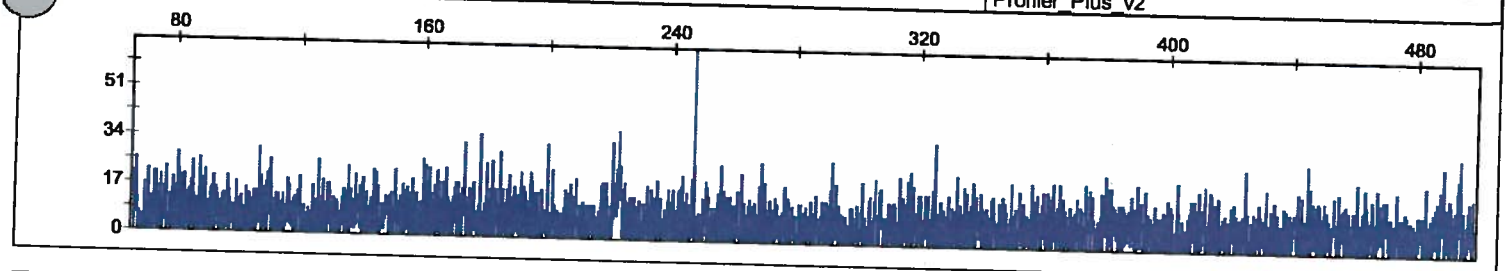
ST CCC

Applied Biosystems

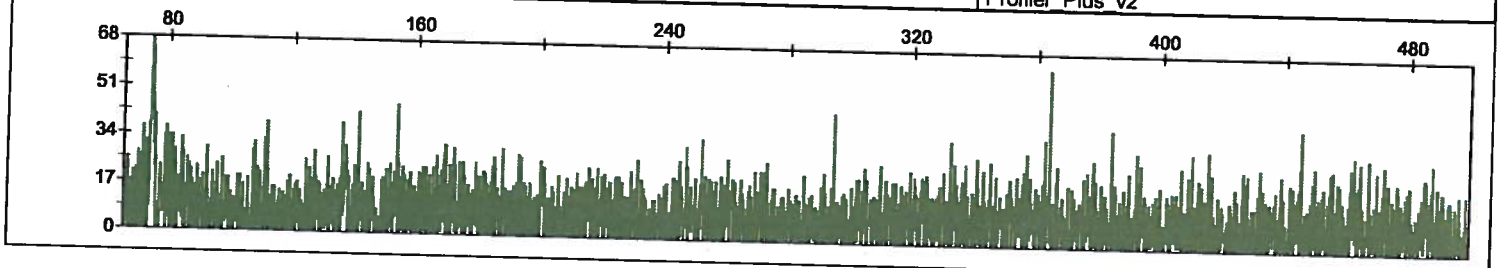
GeneMapper ID v3.2.1

ST 121109

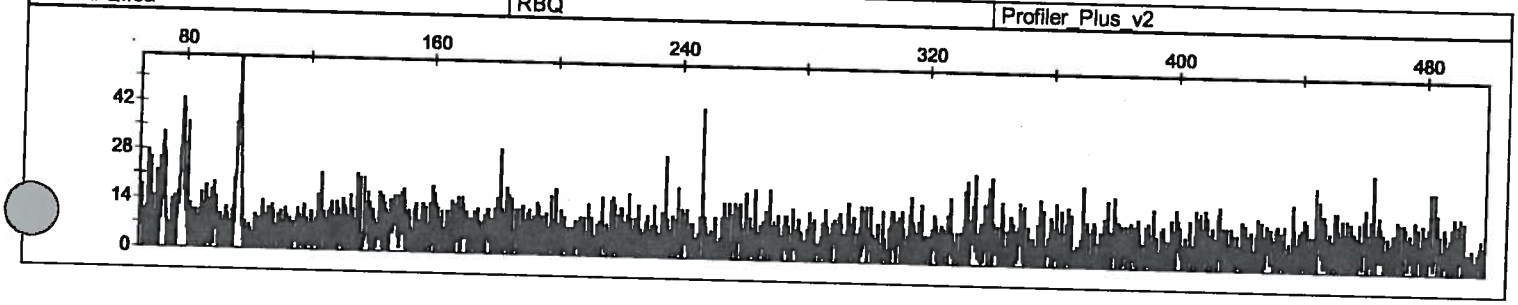
Sample File	Sample Name	Panel
0RBQ.fsa	RBQ	Profiler_Plus_v2



B10RBQ.fsa	RBQ	Profiler_Plus_v2



B10RBQ.fsa	RBQ	Profiler_Plus_v2



AUSTIN POLICE DEPARTMENT
SEROLOGY/ DNA SECTION
STANDARD OPERATING PROCEDURES

STR RECORD SHEET

DATE OF RUN 12-16-09

CASE # (S) ST

ANALYST (S) CCC-CCC

310/3130 INSTRUMENT 3

LOT RECORDS

CAPILLARY V0B09A

POLYMER 0908175

ROX 0906500

FORMAMIDE 0902799

GA BUFFER 0905322

CASE RECORDS

KIT LOT #/ Expiration Date (PP/CO) 0905139 / 5-26-10

MATRIX/SPECTRAL 111709

SIZE STANDARD ROX 500

ANALYZED AT THRESHOLD OF: 90
(for evidence samples only)

90NS All

☐ Others at 150

Other

CASEWORK DISK # 09-12-

Applied Systems

GeneMapper ID v3.2.1

	Status	Sample File	Sample Name	Sample Type	Analysis Method	Panel	Size Standard	OS	SQ
1		A11ST JM 0.0234375-1.fsa	ST JM 0.0234375	Sample	None	None	None	0.0	0.0
2		A11ST JM 0.0234375.fsa	ST JM 0.0234375	Sample	None	None	None	0.0	0.0
3		A1ST JM 0.75-1.fsa	ST JM 0.75	Sample	None	None	None	0.0	0.0
4		A1ST JM 0.75.fsa	ST JM 0.75	Sample	None	None	None	0.0	0.0
5		A3ST JM 0.375.fsa	ST JM 0.375	Sample	None	None	None	0.0	0.0
6		A5ST JM 0.1875.fsa	ST JM 0.1875	Sample	None	None	None	0.0	0.0
7		A7ST JM 0.09375.fsa	ST JM 0.09375	Sample	None	None	None	0.0	0.0
8		A9ST JM 0.046875.fsa	ST JM 0.046875	Sample	None	None	None	0.0	0.0
9		B10ST CH 0.375.fsa	ST CH 0.375	Sample	None	None	None	0.0	0.0
10		B12ST CH 0.1875.fsa	ST CH 0.1875	Sample	None	None	None	0.0	0.0
11		B2ST JM 0.0117187.fsa	ST JM 0.0117187	Sample	None	None	None	0.0	0.0
12		B4ST JM 0.0058593.fsa	ST JM 0.0058593	Sample	None	None	None	0.0	0.0
13		B6ST JM 0.0029296.fsa	ST JM 0.0029296	Sample	None	None	None	0.0	0.0
14		B8STCH 0.75.fsa	STCH 0.75	Sample	None	None	None	0.0	0.0
15		C11ST CH 0.0029296.fsa	ST CH 0.0029296	Sample	None	None	None	0.0	0.0
16		C1ST CH 0.09375.fsa	ST CH 0.09375	Sample	None	None	None	0.0	0.0
17		C3ST CH 0.046875.fsa	ST CH 0.046875	Sample	None	None	None	0.0	0.0
18		C5ST CH 0.0234375.fsa	ST CH 0.0234375	Sample	None	None	None	0.0	0.0
19		C7ST CH 0.0117187.fsa	ST CH 0.0117187	Sample	None	None	None	0.0	0.0
20		C9ST CH 0.0058593.fsa	ST CH 0.0058593	Sample	None	None	None	0.0	0.0
21		D10ST LM 0.046875.fsa	ST LM 0.046875	Sample	None	None	None	0.0	0.0
22		D12ST LM 0.0234375.fsa	ST LM 0.0234375	Sample	None	None	None	0.0	0.0
23		D2ST LM 0.75.fsa	ST LM 0.75	Sample	None	None	None	0.0	0.0
24		D4ST LM 0.375.fsa	ST LM 0.375	Sample	None	None	None	0.0	0.0
25		D6ST LM 0.1875.fsa	ST LM 0.1875	Sample	None	None	None	0.0	0.0
26		D8ST LM 0.09375-1.fsa	ST LM 0.09375	Sample	None	None	None	0.0	0.0
27		D8ST LM 0.09375.fsa	ST LM 0.09375	Sample	None	None	None	0.0	0.0
28		E11NEG CTRL.fsa	NEG CTRL	Sample	None	None	None	0.0	0.0
29		E11NEG CTRL1.fsa	NEG CTRL	Sample	None	None	None	0.0	0.0
30		E1ST LM 0.0117187.fsa	ST LM 0.0117187	Sample	None	None	None	0.0	0.0
31		E3ST LM 0.0058593.fsa	ST LM 0.0058593	Sample	None	None	None	0.0	0.0
32		E5ST LM 0.0029296.fsa	ST LM 0.0029296	Sample	None	None	None	0.0	0.0

Applied Systems

GeneMapper ID v3.2.1

	Status	Sample File	Sample Name	Sample Type	Analysis Method	Panel	Size Standard	OS	SQ
33		E7RBQ.fsa	RBQ	Sample	None	None	None	0.0	0.0
34		E7RBQ1.fsa	RBQ	Sample	None	None	None	0.0	0.0
35		E9POS CTRL.fsa	POS CTRL	Sample	None	None	None	0.0	0.0
36		E9POS CTRL1.fsa	POS CTRL	Sample	None	None	None	0.0	0.0
37		F2LADDER.fsa	LADDER	Allelic Ladder	None	None	None	0.0	0.0
38		F2LADDER1.fsa	LADDER	Allelic Ladder	None	None	None	0.0	0.0
39		F2LADDER2.fsa	LADDER	Allelic Ladder	None	None	None	0.0	0.0

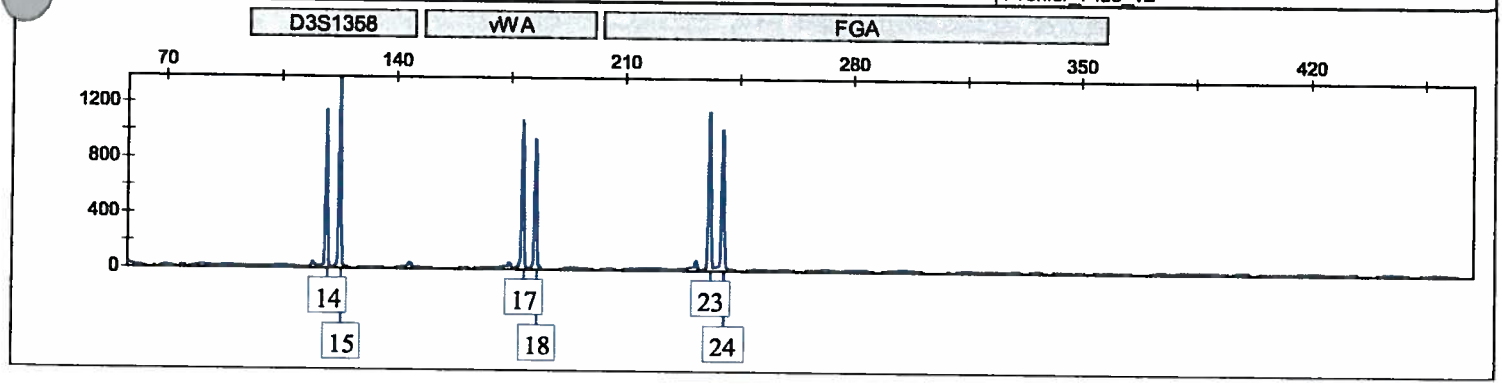
ST CCR
Run 12-16-09

Applied Biosystems

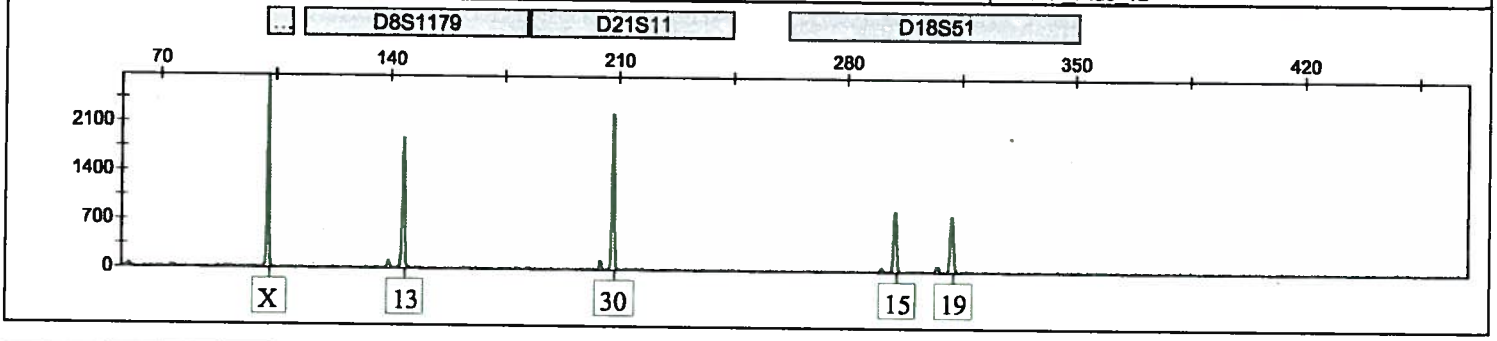
GeneMapper ID v3.2.1

ST121609

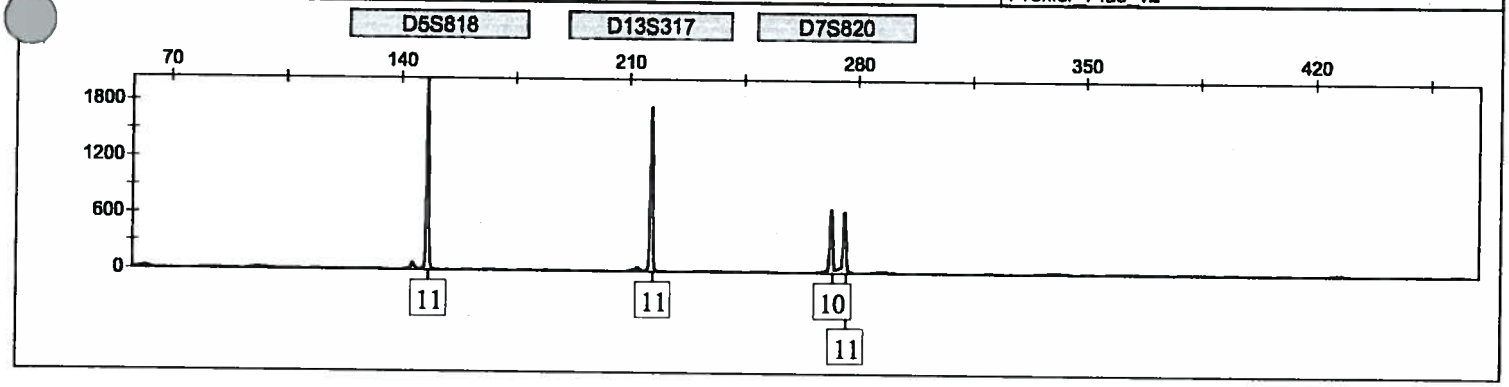
Sample File	Sample Name	Panel
POS CTRL.fsa	POS CTRL	Profiler_Plus_v2



E9POS CTRL.fsa	POS CTRL	Profiler_Plus_v2



E9POS CTRL.fsa	POS CTRL	Profiler_Plus_v2



E9POS CTRL.fsa	POS CTRL	Profiler_Plus_v2

Thu Dec 17,2009 08:46AM, CST

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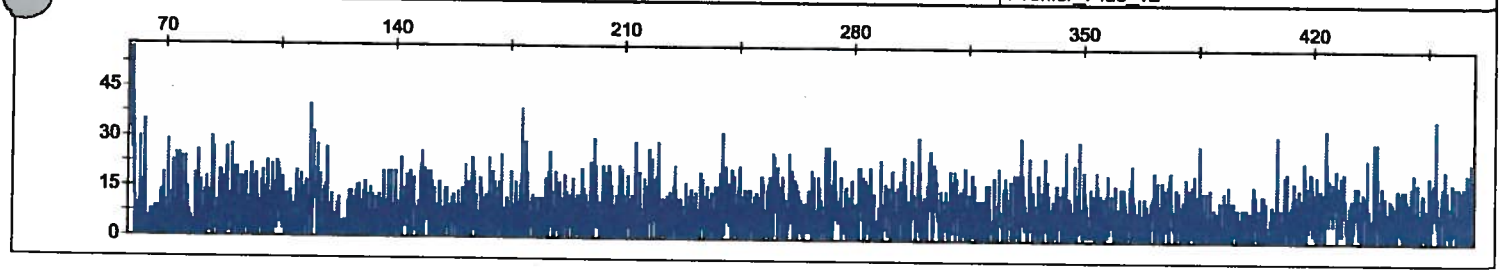
ST CCC

Applied Biosystems

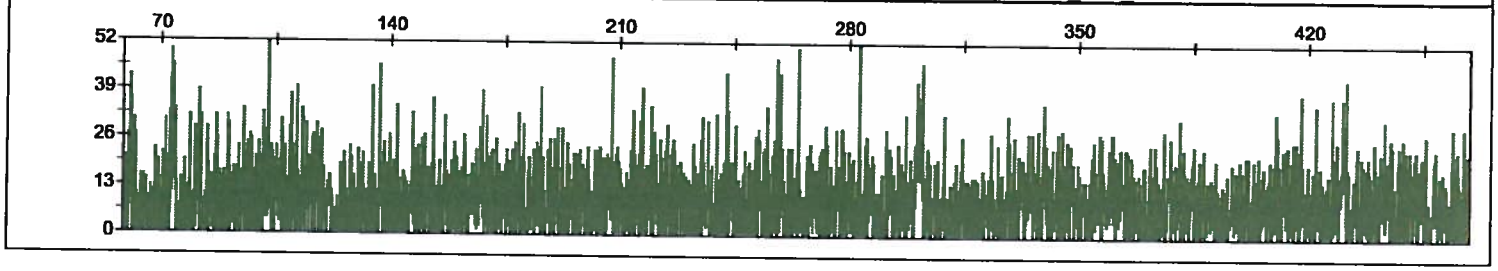
GeneMapper ID v3.2.1

ST121609

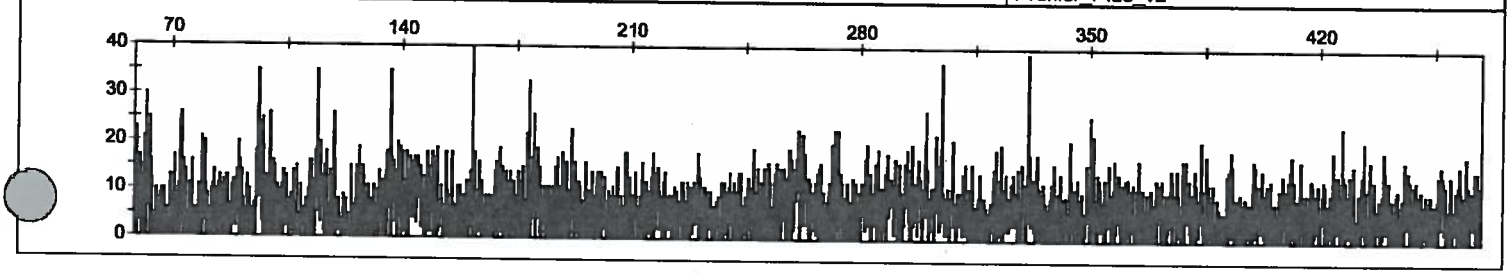
Sample File	Sample Name	Panel
1NEG CTRL.fsa	NEG CTRL	Profiler_Plus_v2



E11NEG CTRL.fsa	NEG CTRL	Profiler_Plus_v2



E11NEG CTRL.fsa	NEG CTRL	Profiler_Plus_v2

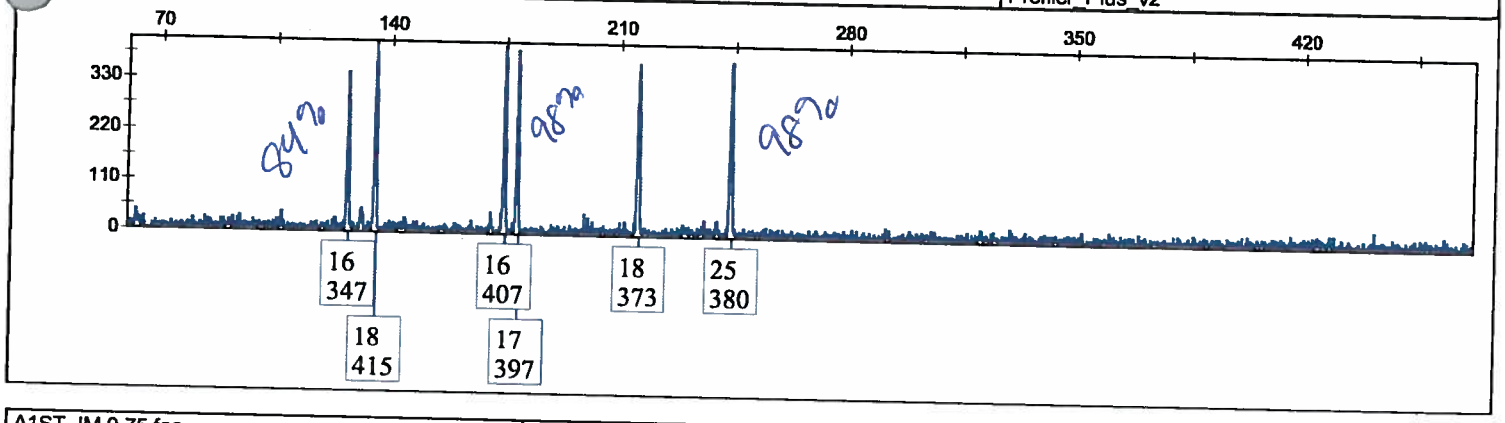


Applied Biosystems

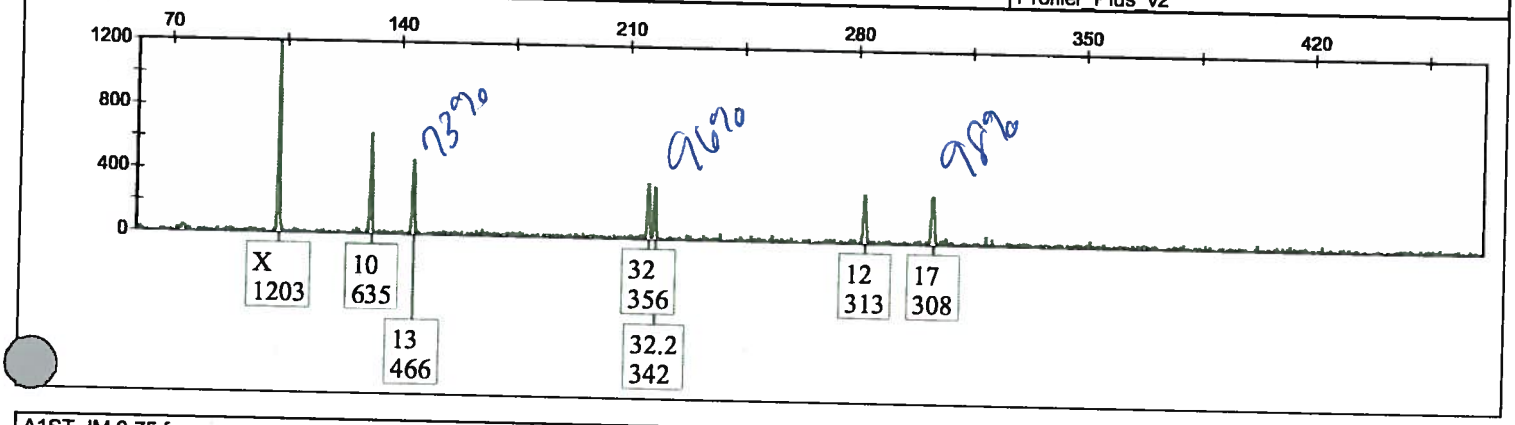
GeneMapper ID v3.2.1

ST121609

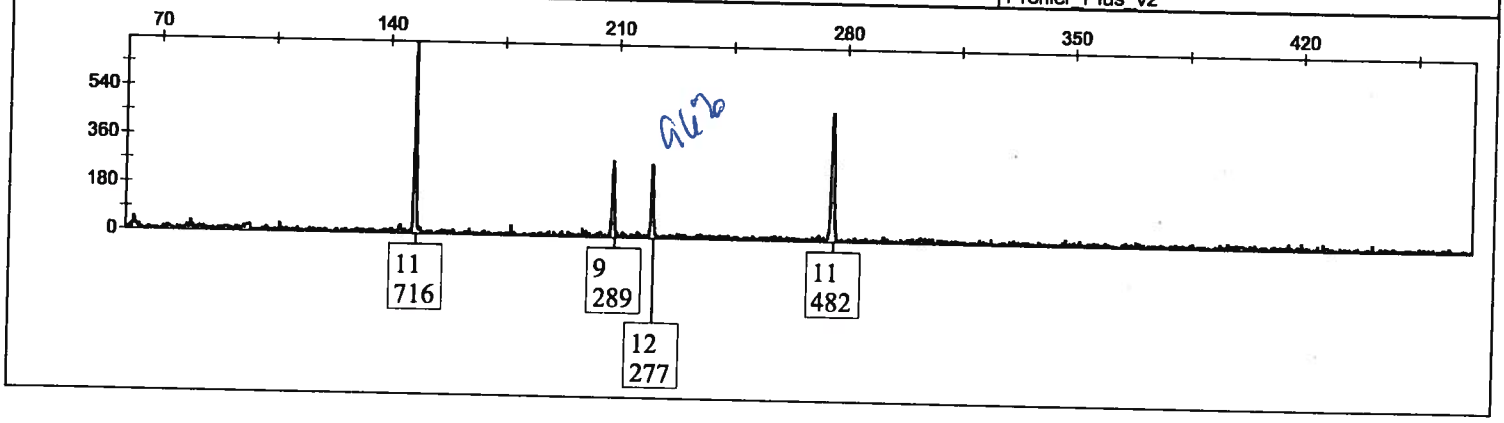
Sample File	Sample Name	Panel
ST JM 0.75.fsa	ST JM 0.75	Profiler_Plus_v2



A1ST JM 0.75.fsa	ST JM 0.75	Profiler_Plus_v2



A1ST JM 0.75.fsa	ST JM 0.75	Profiler_Plus_v2



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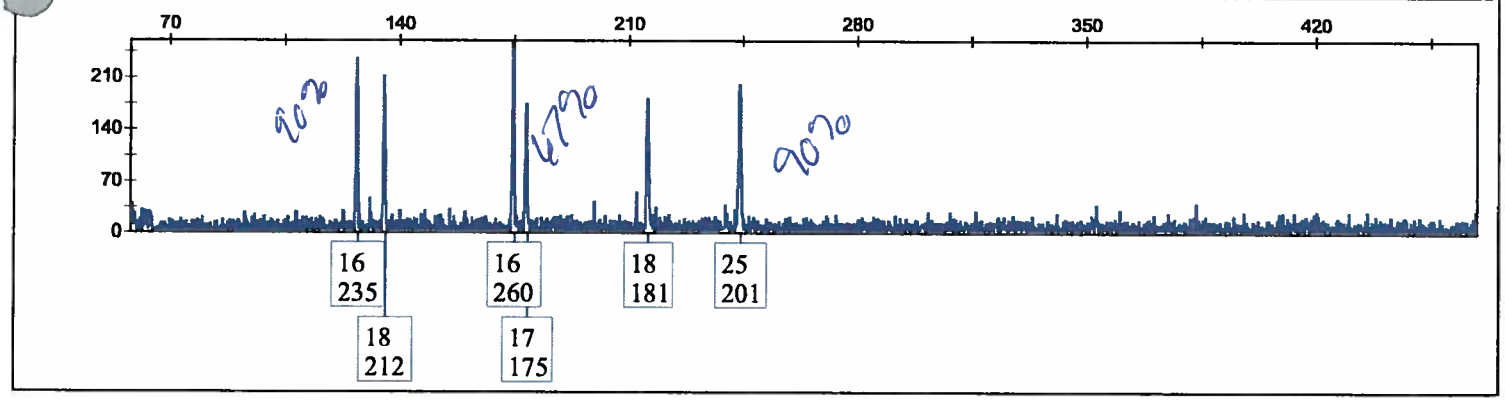
STCCC

Applied Biosystems

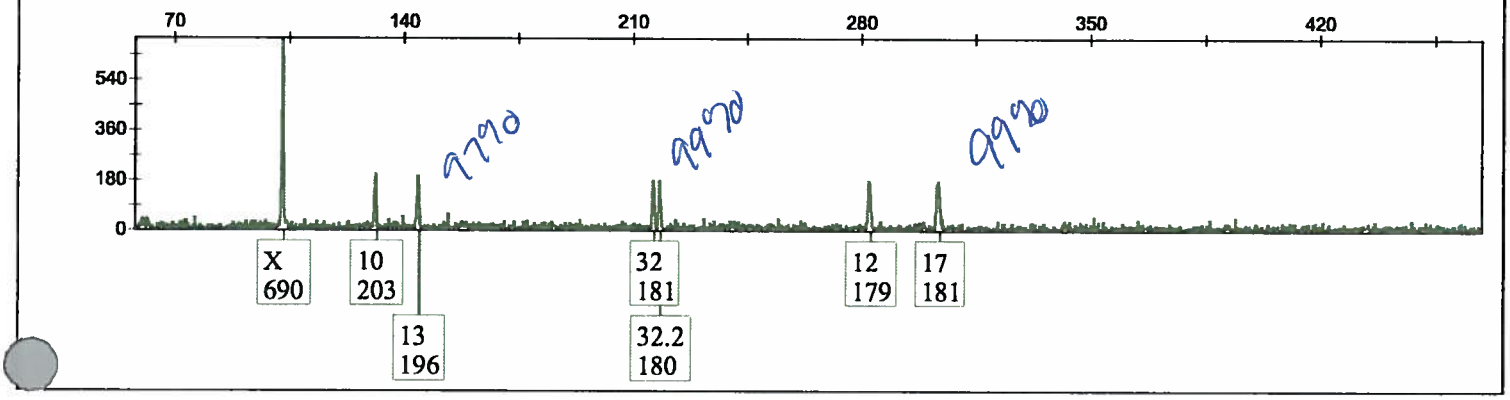
GeneMapper ID v3.2.1

ST121609

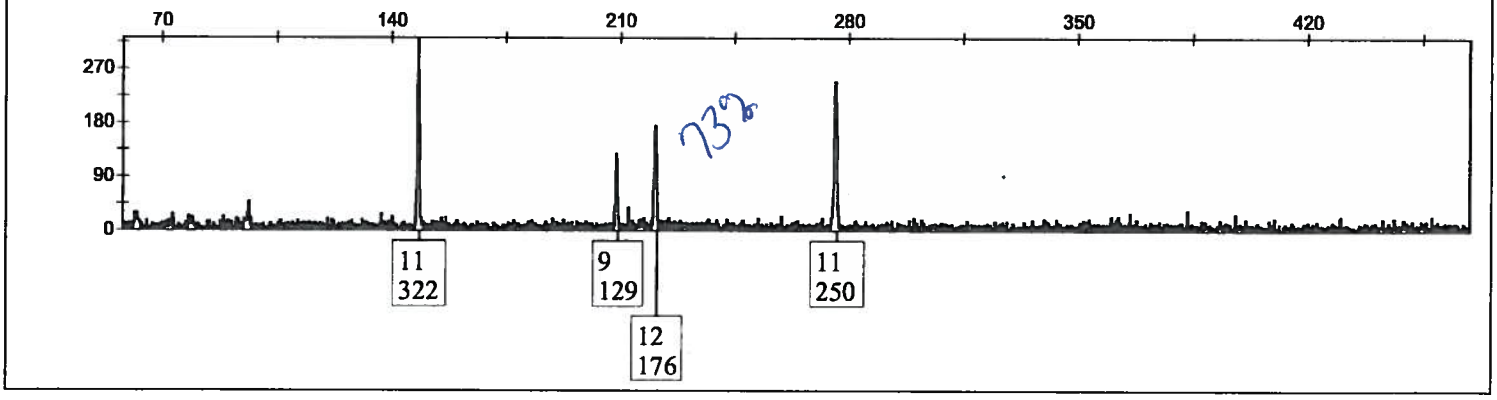
Sample File	Sample Name	Panel
ST JM 0.375.fsa	ST JM 0.375	Profiler_Plus_v2



A3ST JM 0.375.fsa	ST JM 0.375	Profiler_Plus_v2



A3ST JM 0.375.fsa	ST JM 0.375	Profiler_Plus_v2



Thu Dec 17,2009 08:41AM, CST

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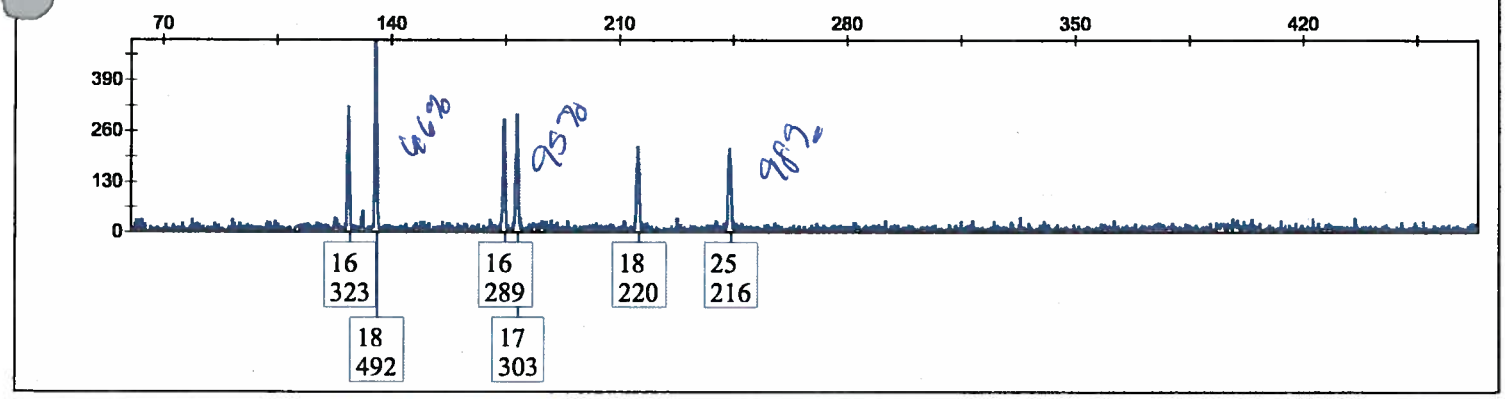
STCC

Applied Biosystems

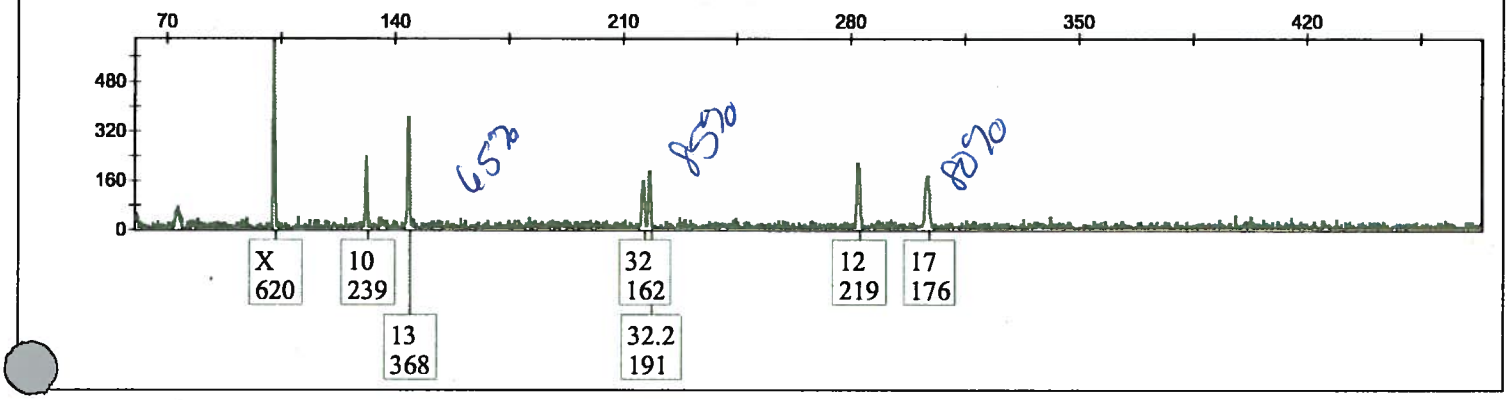
GeneMapper ID v3.2.1

ST121609

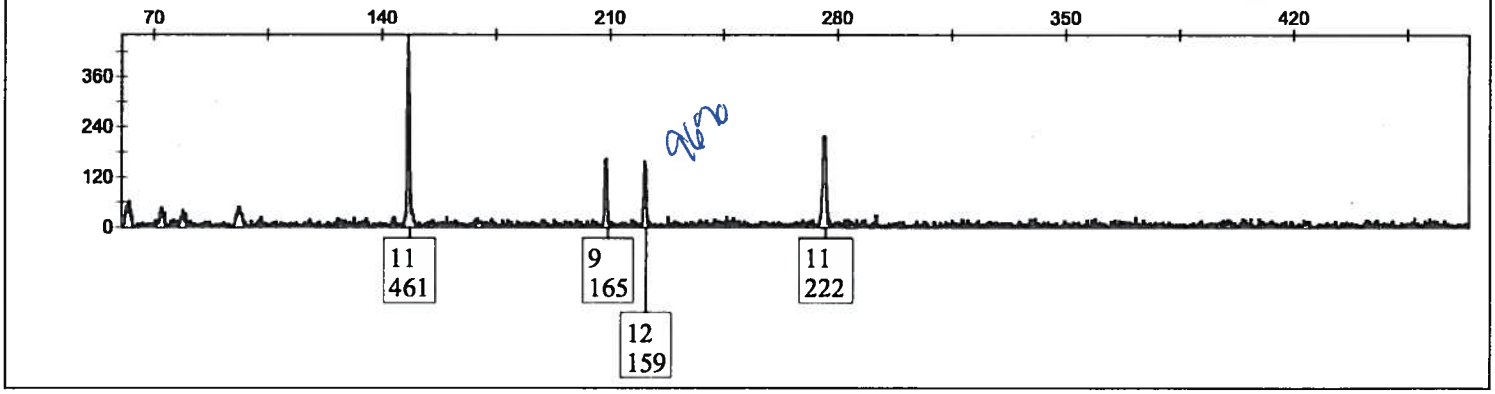
Sample File	Sample Name	Panel
ST JM 0.1875.fsa	ST JM 0.1875	Profiler_Plus_v2



A5ST JM 0.1875.fsa	ST JM 0.1875	Profiler_Plus_v2



A5ST JM 0.1875.fsa	ST JM 0.1875	Profiler_Plus_v2

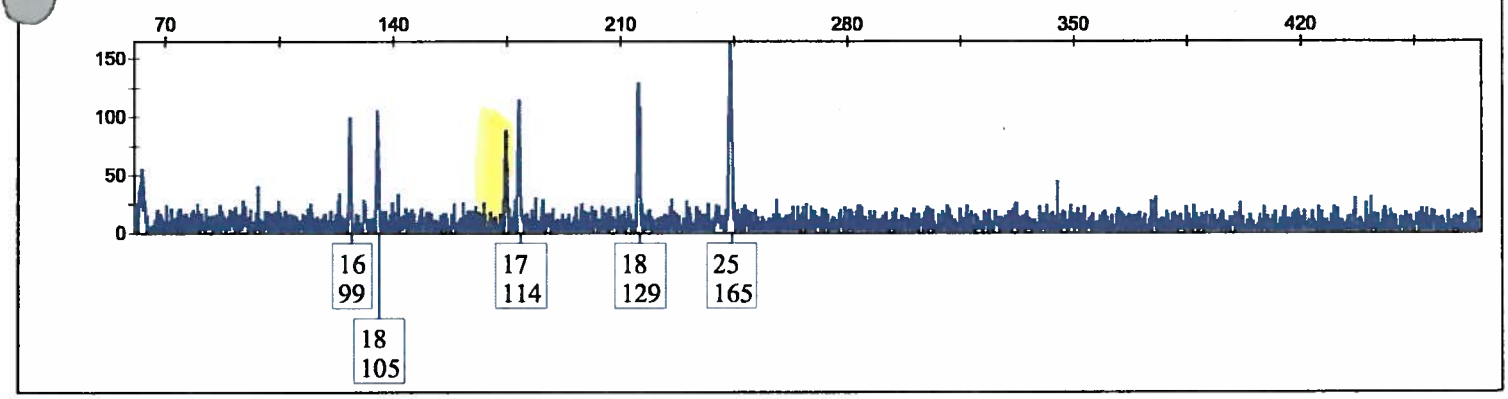


Applied Biosystems

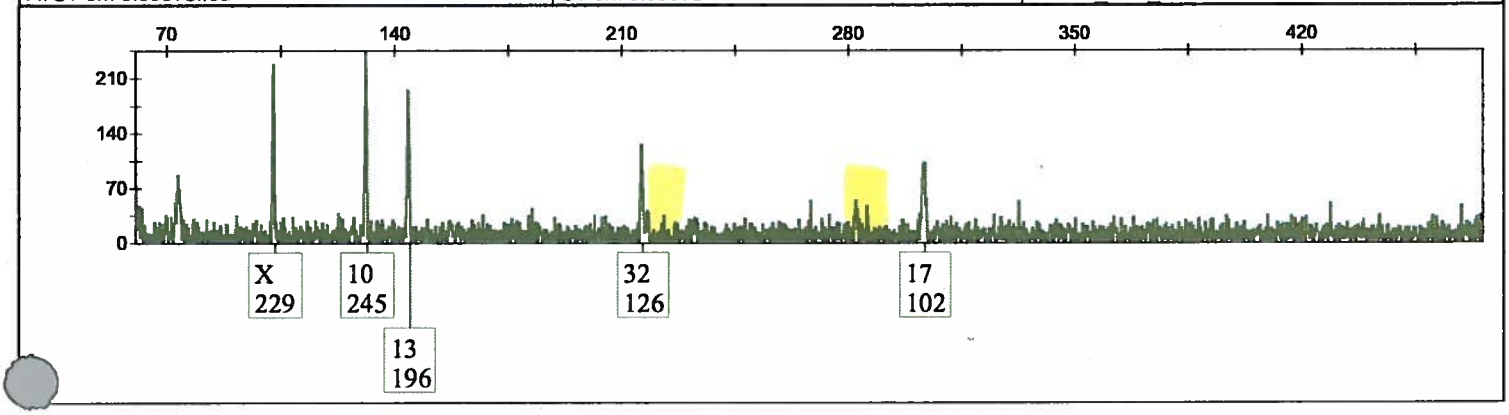
GeneMapper ID v3.2.1

ST121609

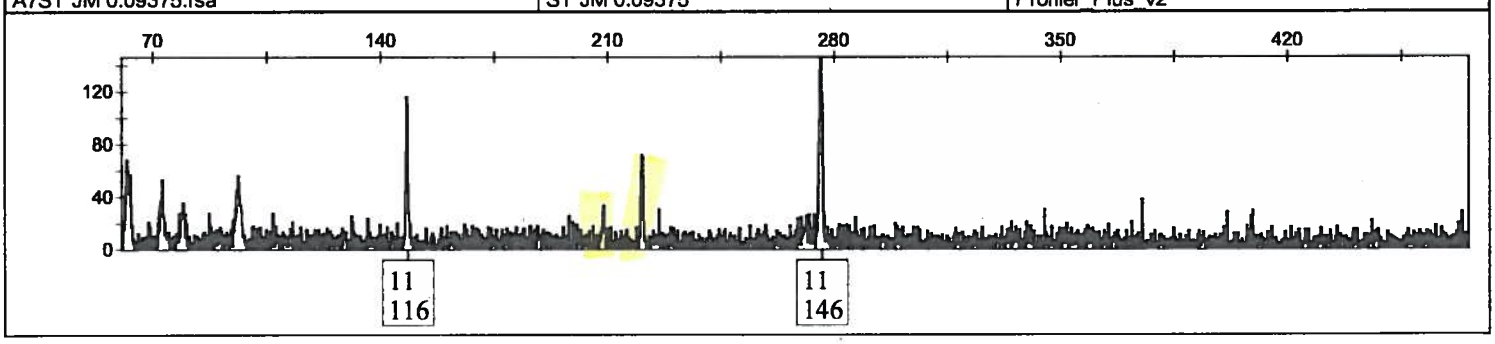
Sample File	Sample Name	Panel
ST JM 0.09375.fsa	ST JM 0.09375	Profiler_Plus_v2



A7ST JM 0.09375.fsa	ST JM 0.09375	Profiler_Plus_v2



A7ST JM 0.09375.fsa	ST JM 0.09375	Profiler_Plus_v2

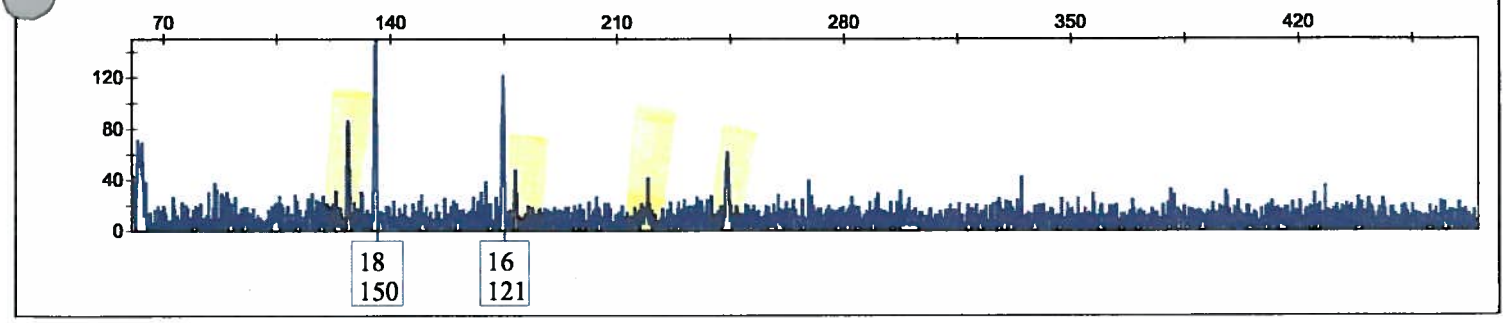


Applied Biosystems

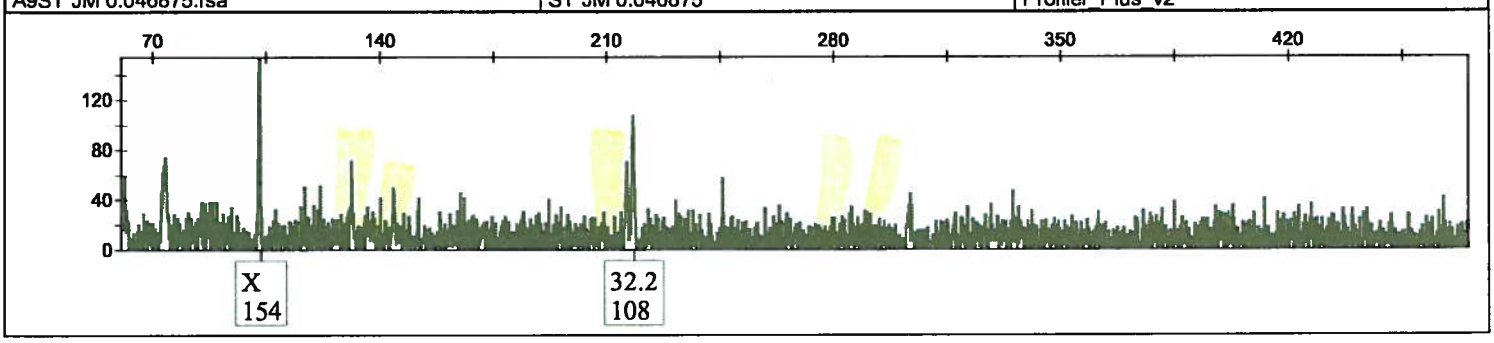
GeneMapper ID v3.2.1

ST121609

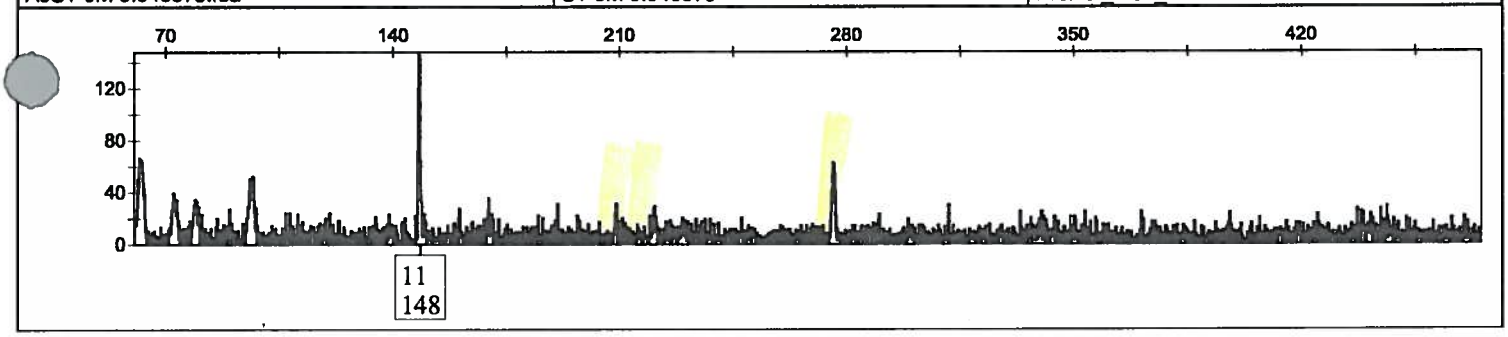
Sample File	Sample Name	Panel
ST JM 0.046875.fsa	ST JM 0.046875	Profiler_Plus_v2



A9ST JM 0.046875.fsa	ST JM 0.046875	Profiler_Plus_v2



A9ST JM 0.046875.fsa	ST JM 0.046875	Profiler_Plus_v2

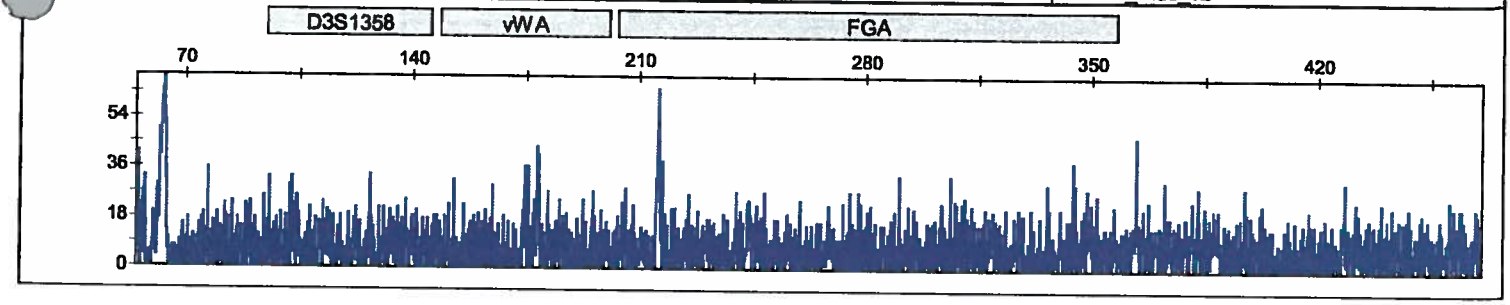


Applied Biosystems

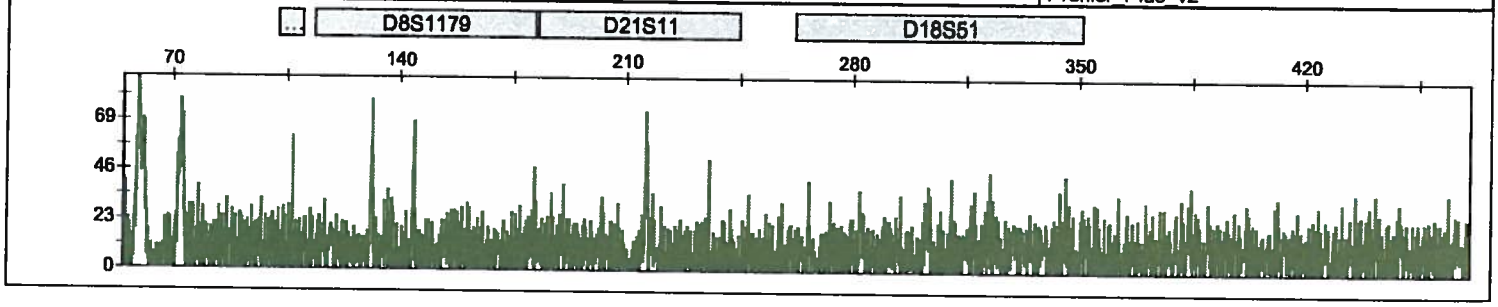
GeneMapper ID v3.2.1

ST121609CCC

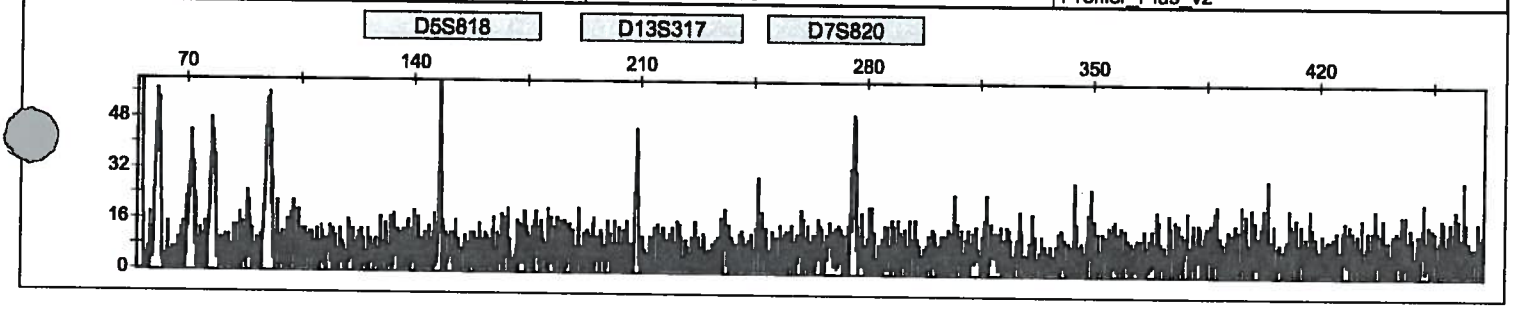
Sample File	Sample Name	Panel
1ST JM 0.0234375-1.fsa	ST JM 0.0234375	Profiler_Plus_v2



A11ST JM 0.0234375-1.fsa	ST JM 0.0234375	Profiler_Plus_v2



A11ST JM 0.0234375-1.fsa	ST JM 0.0234375	Profiler_Plus_v2



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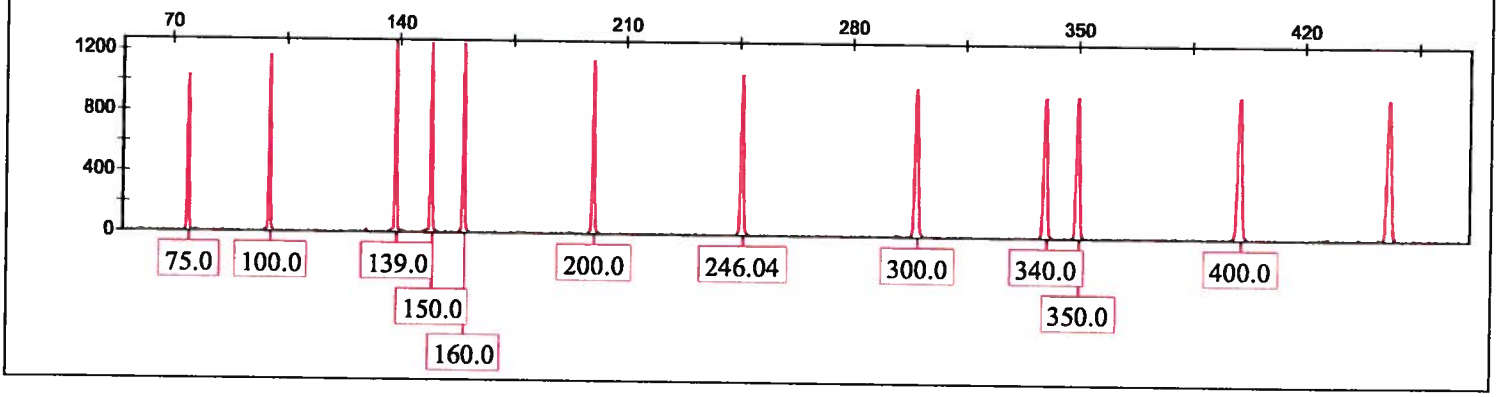
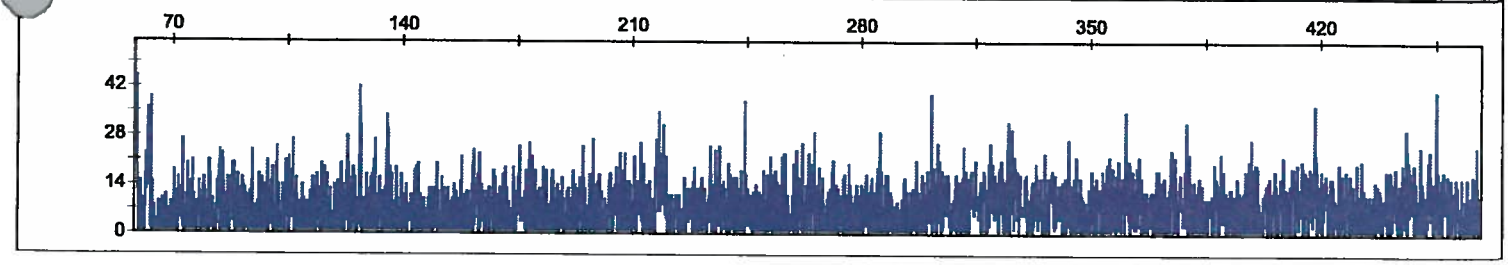


Applied Biosystems

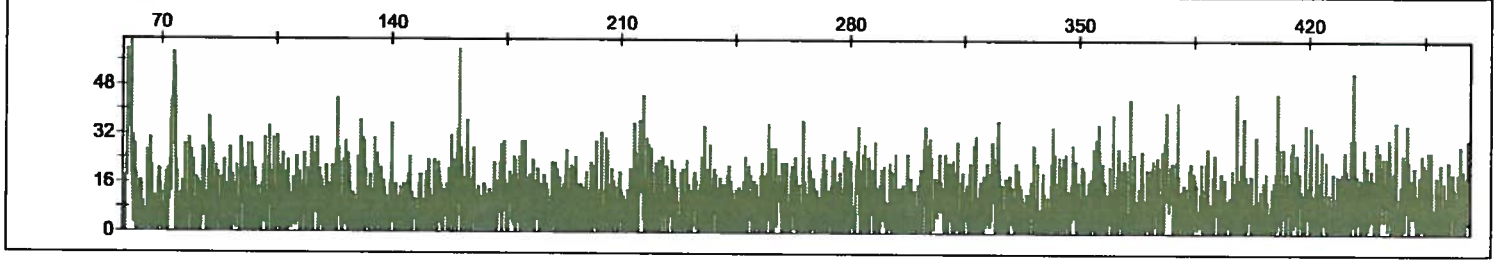
GeneMapper ID v3.2.1

ST121609

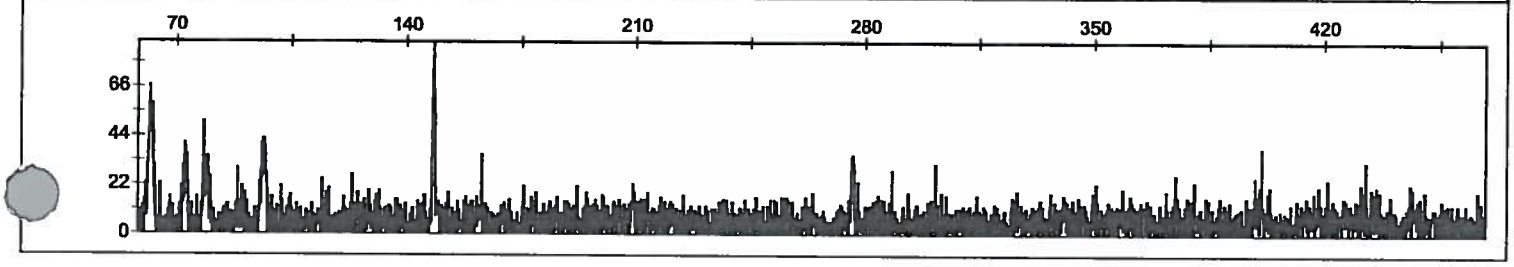
Sample File	Sample Name	Panel
ST JM 0.0117187.fsa	ST JM 0.0117187	Profiler_Plus_v2



B2ST JM 0.0117187.fsa	ST JM 0.0117187	Profiler_Plus_v2



B2ST JM 0.0117187.fsa	ST JM 0.0117187	Profiler_Plus_v2

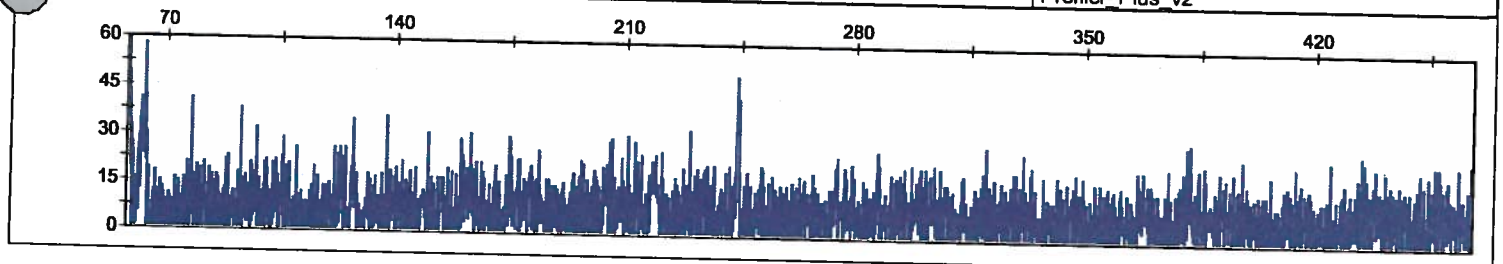


Applied Biosystems

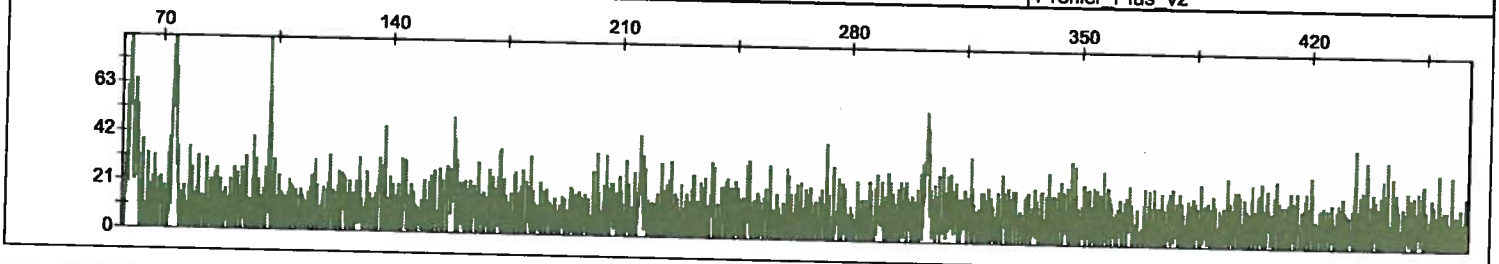
GeneMapper ID v3.2.1

ST121609

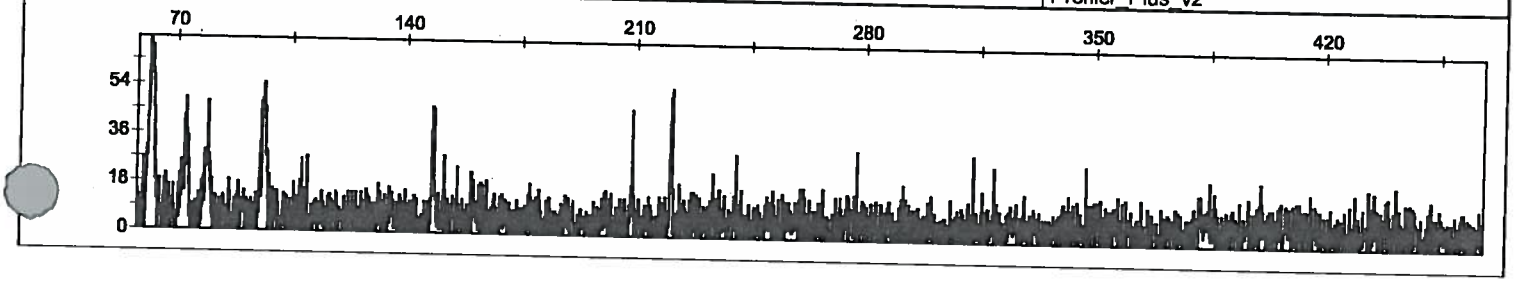
Sample File	Sample Name	Panel
ST JM 0.0058593.fsa	ST JM 0.0058593	Profiler_Plus_v2



B4ST JM 0.0058593.fsa	ST JM 0.0058593	Profiler_Plus_v2



B4ST JM 0.0058593.fsa	ST JM 0.0058593	Profiler_Plus_v2

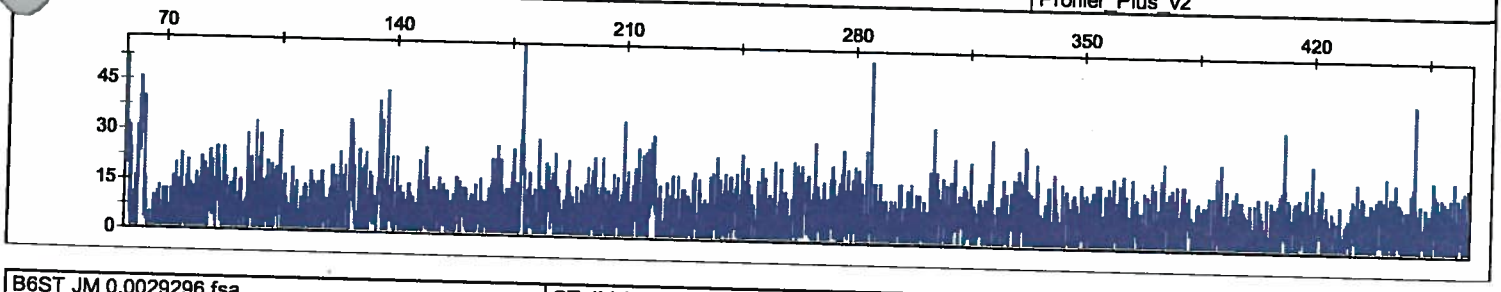


Applied Biosystems

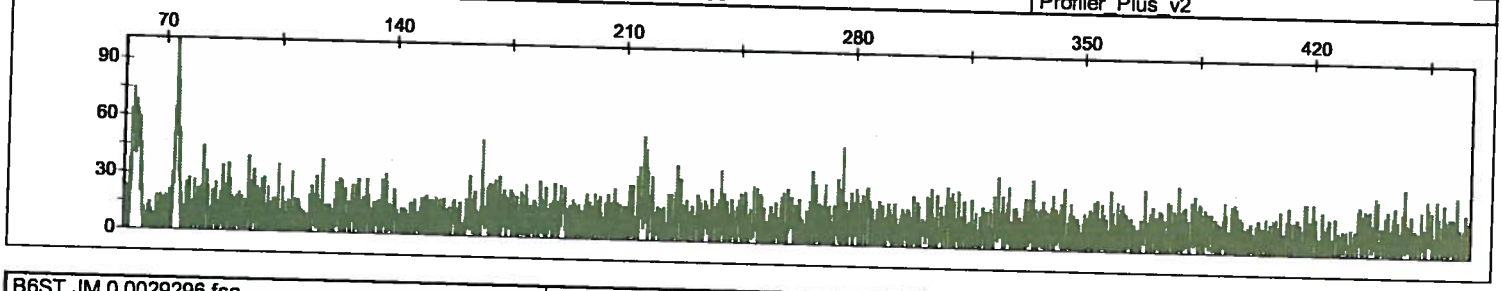
GeneMapper ID v3.2.1

ST121609

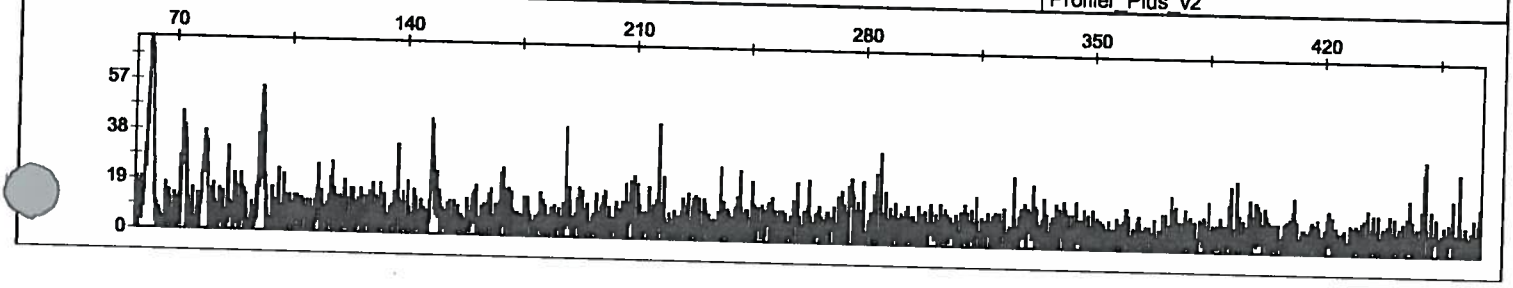
Sample File	Sample Name	Panel
ST JM 0.0029296.fsa	ST JM 0.0029296	Profiler_Plus_v2



B6ST JM 0.0029296.fsa	ST JM 0.0029296	Profiler_Plus_v2



B6ST JM 0.0029296.fsa	ST JM 0.0029296	Profiler_Plus_v2

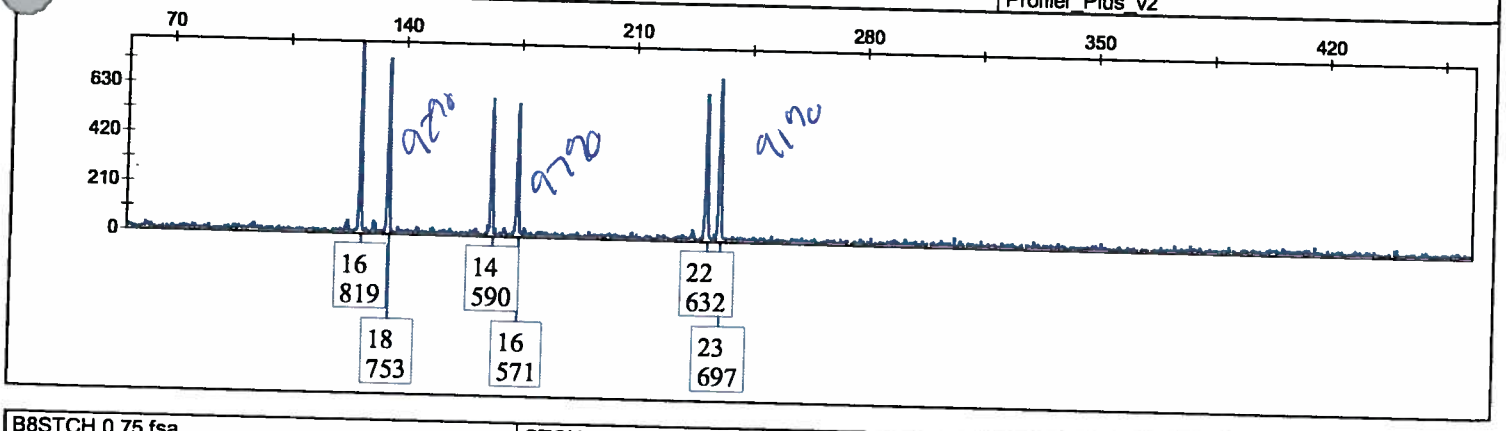


Applied Biosystems

GeneMapper ID v3.2.1

ST121609

Sample File	Sample Name	Panel
STCH 0.75.fsa	STCH 0.75	Profiler_Plus_v2



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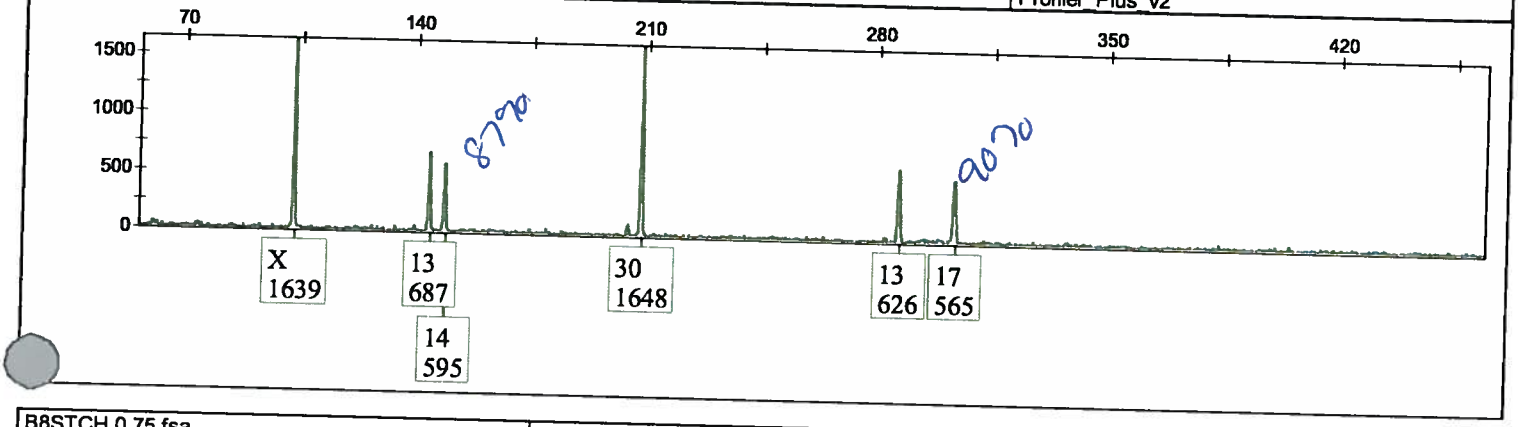
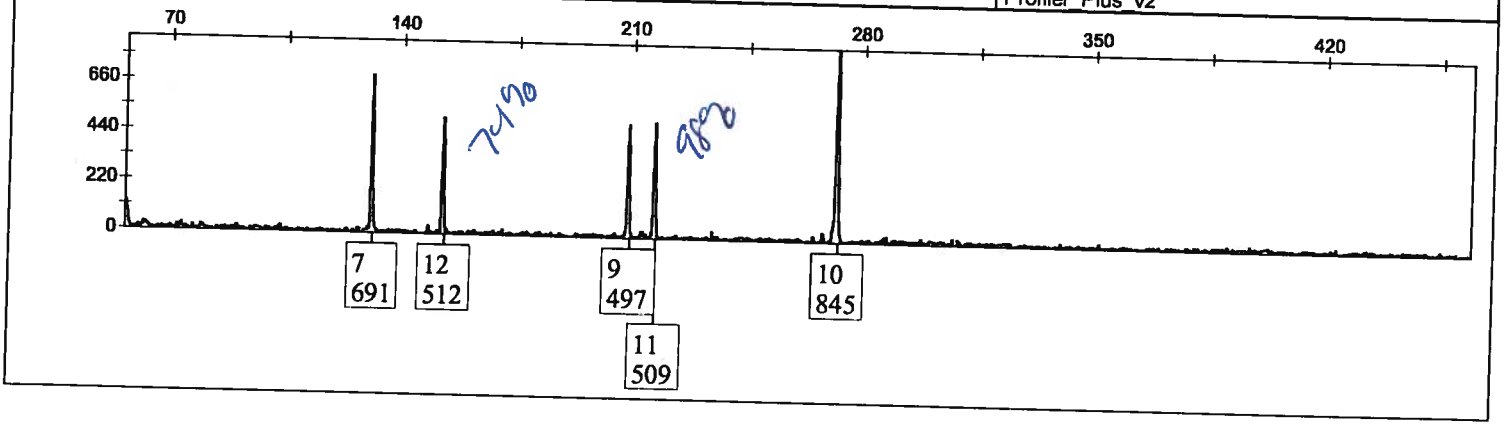


B8STCH 0.75.fsa	STCH 0.75	Profiler_Plus_v2

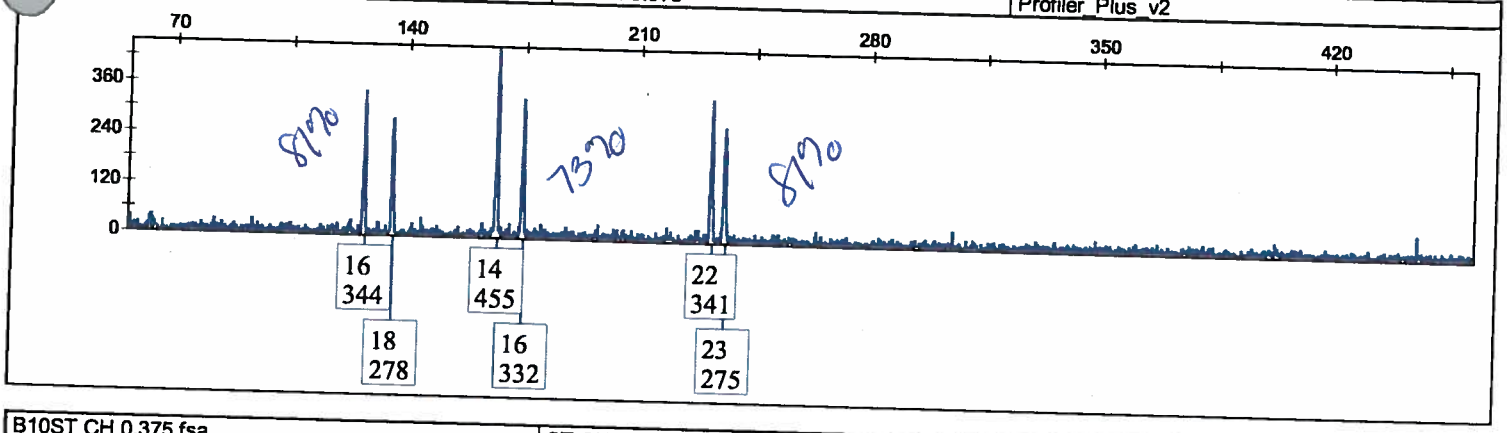


Applied Biosystems

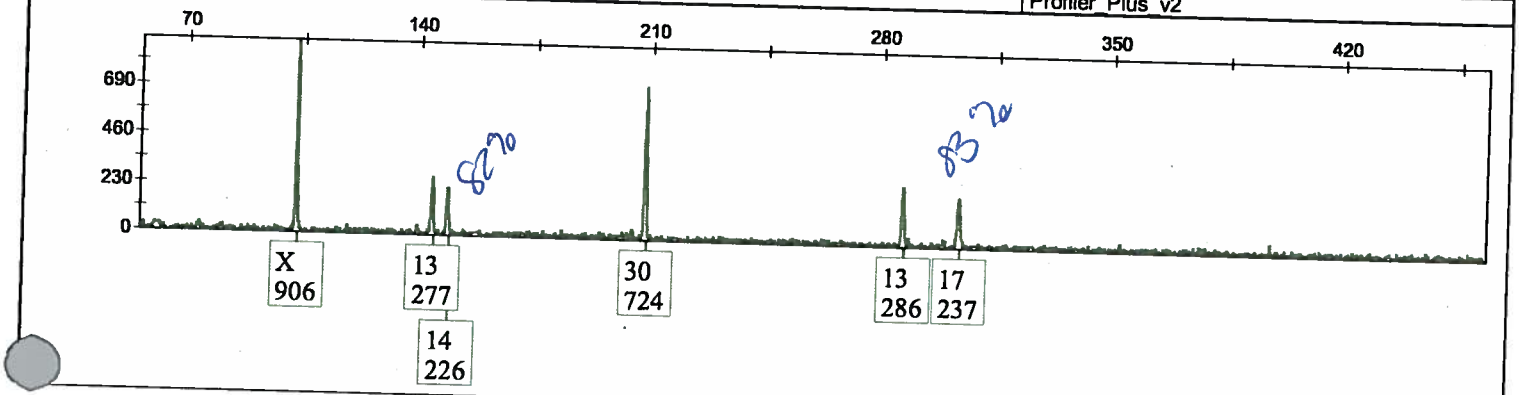
GeneMapper ID v3.2.1

ST121609

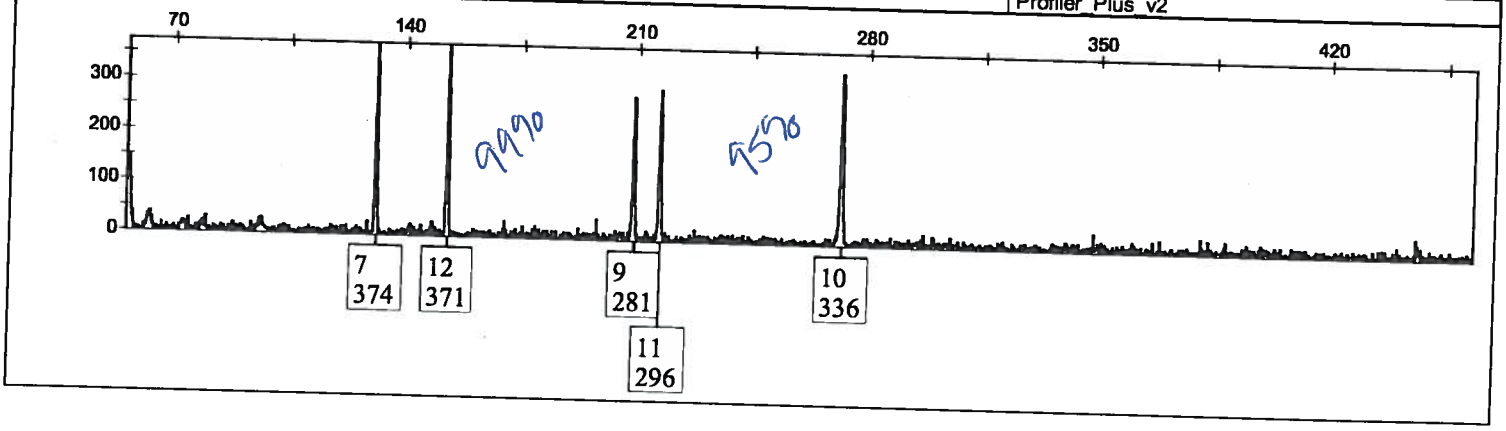
Sample File	Sample Name	Panel
0ST CH 0.375.fsa	ST CH 0.375	Profiler_Plus_v2



B10ST CH 0.375.fsa	ST CH 0.375	Profiler_Plus_v2



B10ST CH 0.375.fsa	ST CH 0.375	Profiler_Plus_v2

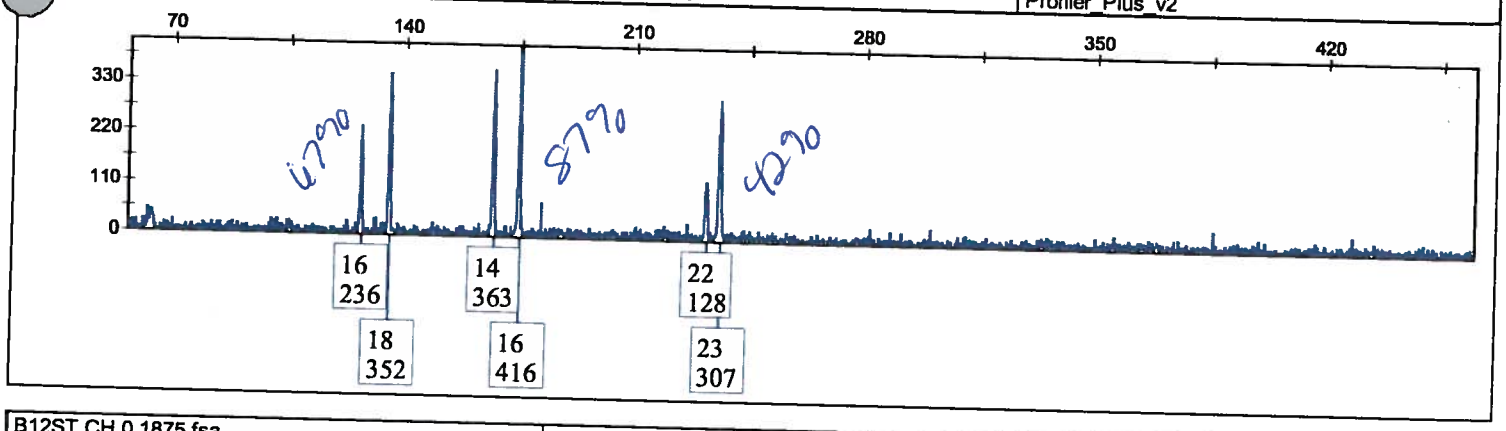


Applied Biosystems

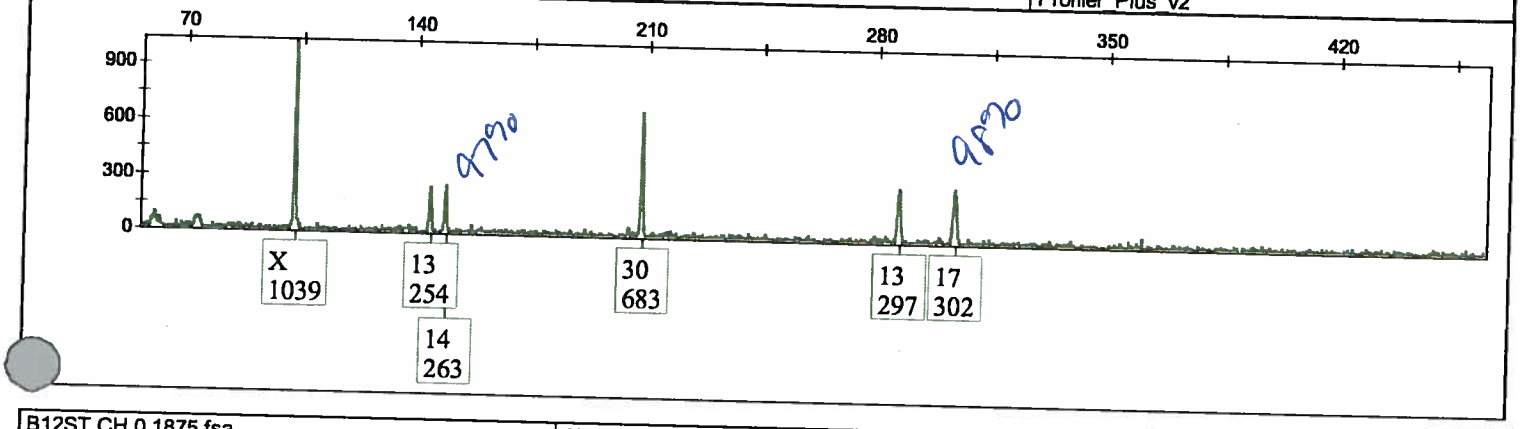
GeneMapper ID v3.2.1

ST121609

Sample File	Sample Name	Panel
B12ST CH 0.1875.fsa	ST CH 0.1875	Profiler_Plus_v2



B12ST CH 0.1875.fsa	ST CH 0.1875	Profiler_Plus_v2



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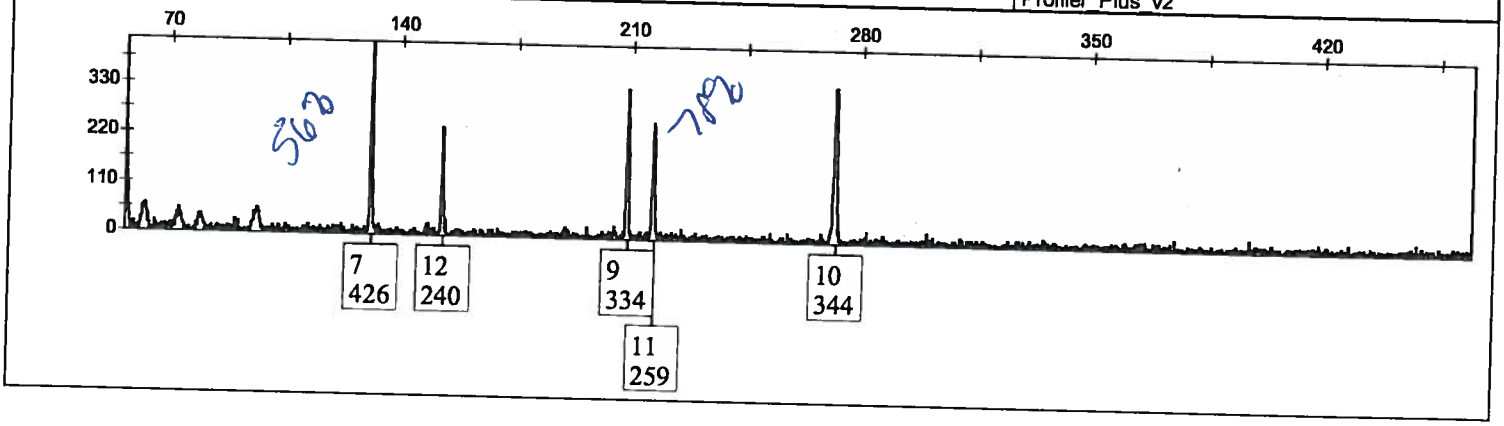

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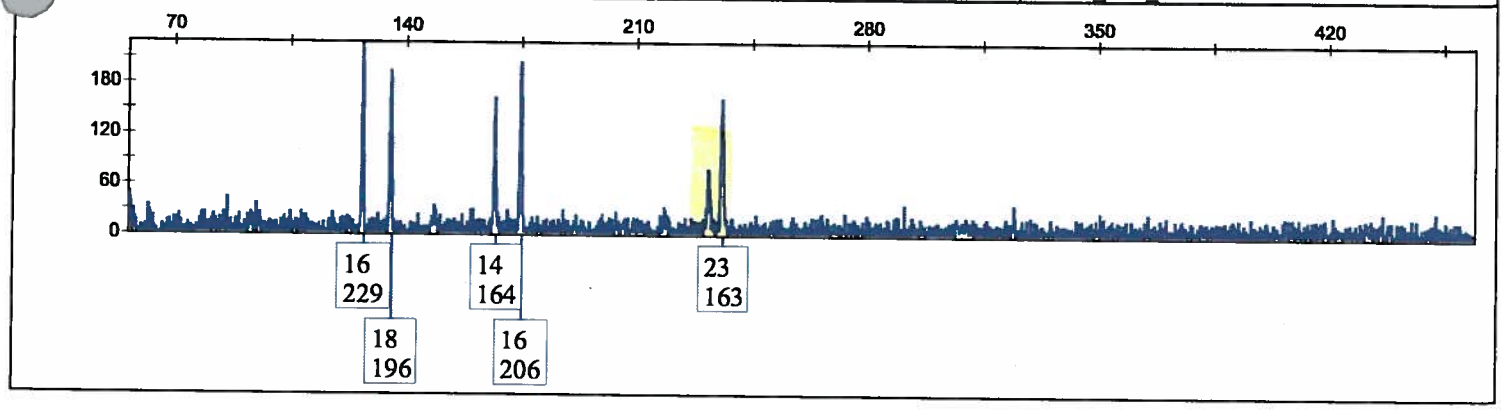
Applied Biosystems

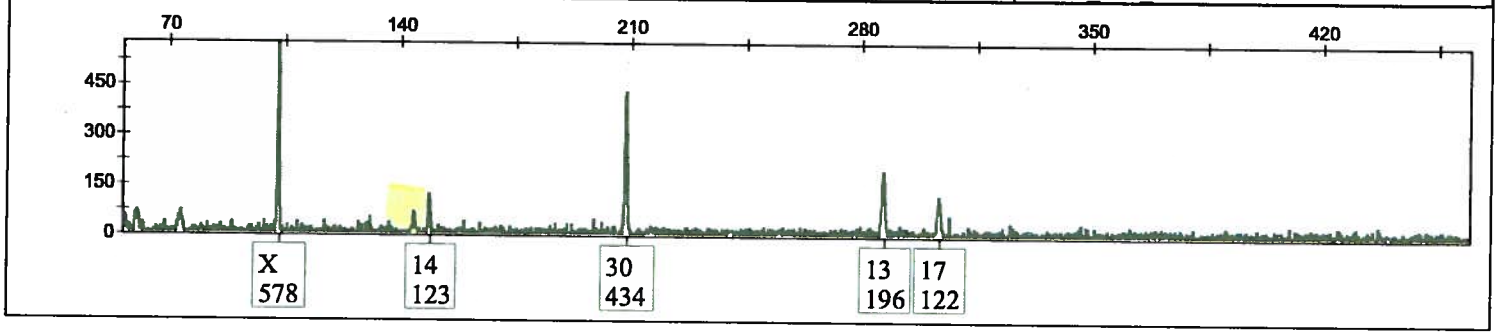
GeneMapper ID v3.2.1

ST121609

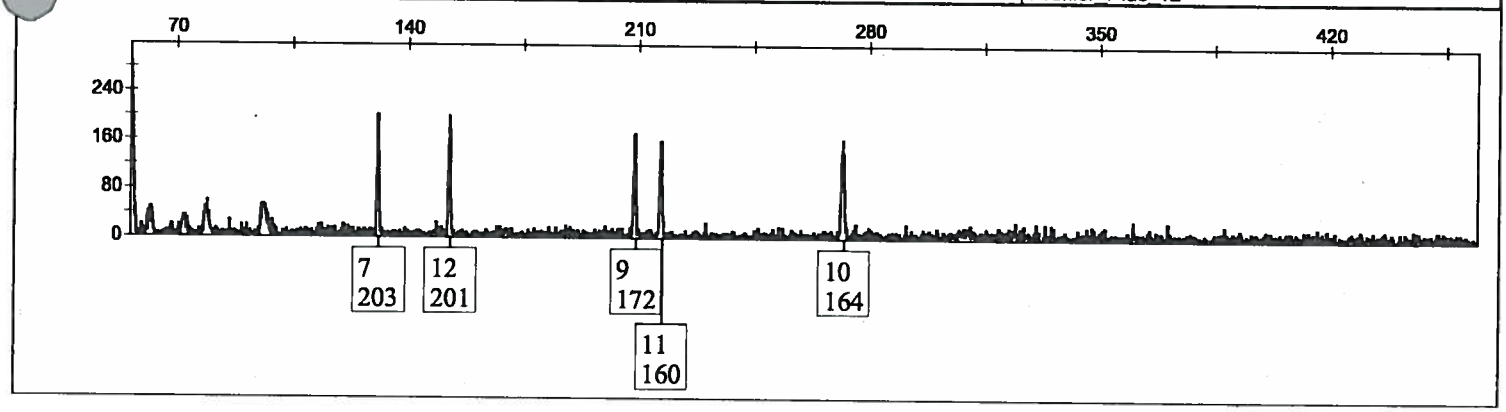
Sample File	Sample Name	Panel
ST CH 0.09375.fsa	ST CH 0.09375	Profiler_Plus_v2



C1ST CH 0.09375.fsa	ST CH 0.09375	Profiler_Plus_v2



ST CH 0.09375.fsa	ST CH 0.09375	Profiler_Plus_v2

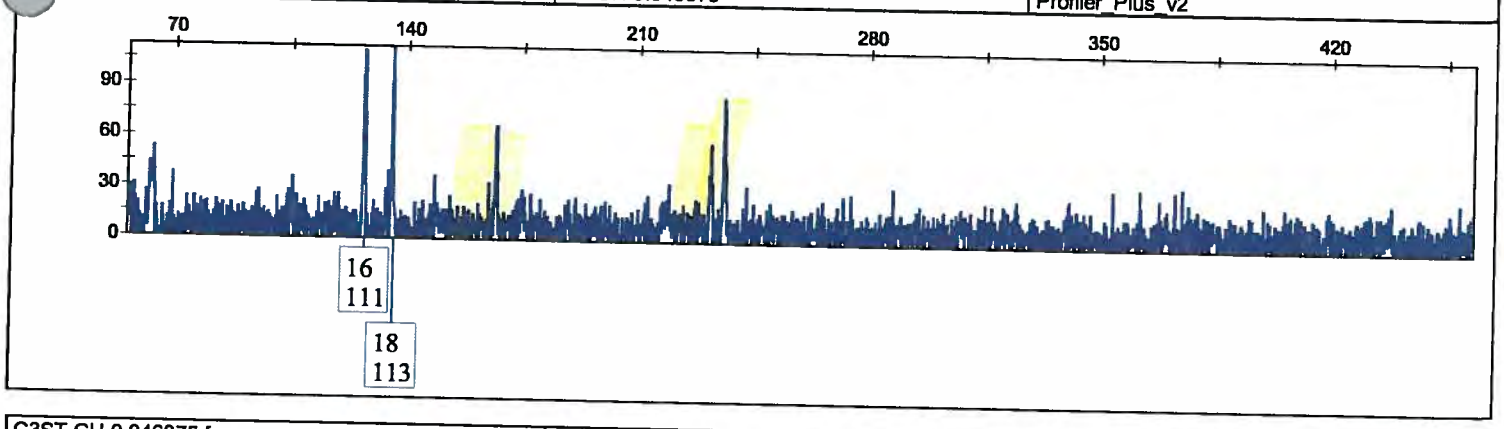


Applied Biosystems

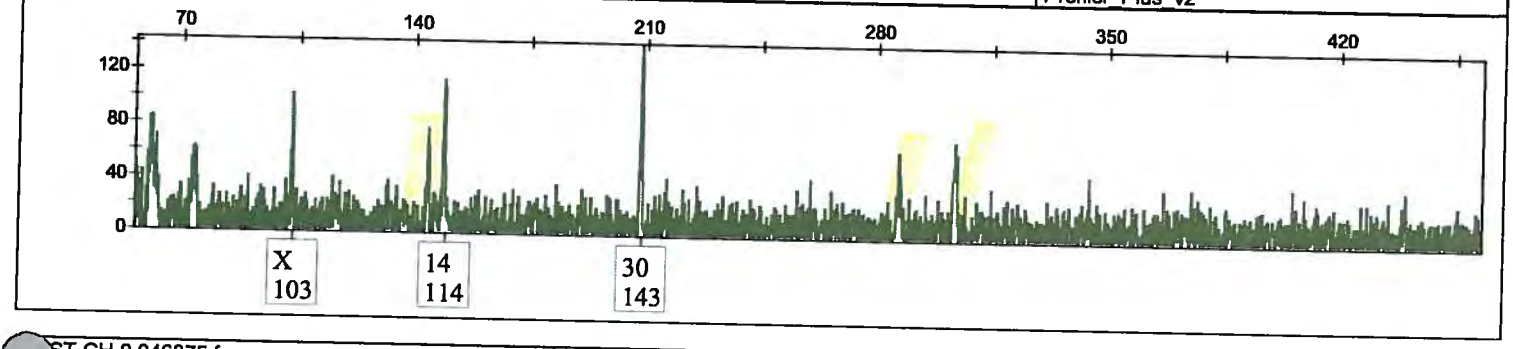
GeneMapper ID v3.2.1

ST121609

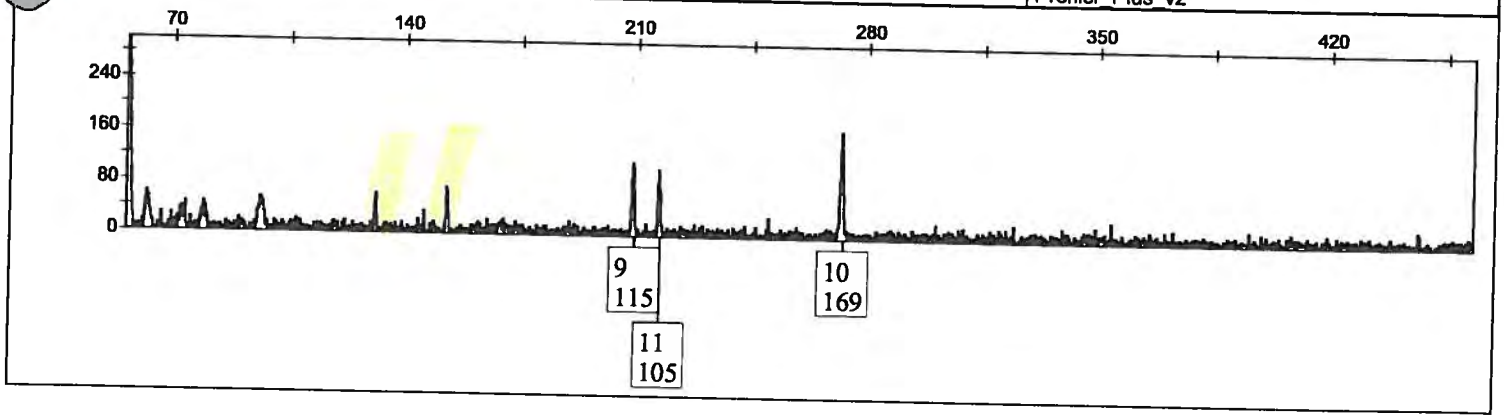
Sample File	Sample Name	Panel
ST CH 0.046875.fsa	ST CH 0.046875	Profiler_Plus_v2



C3ST CH 0.046875.fsa	ST CH 0.046875	Profiler_Plus_v2



ST CH 0.046875.fsa	ST CH 0.046875	Profiler_Plus_v2

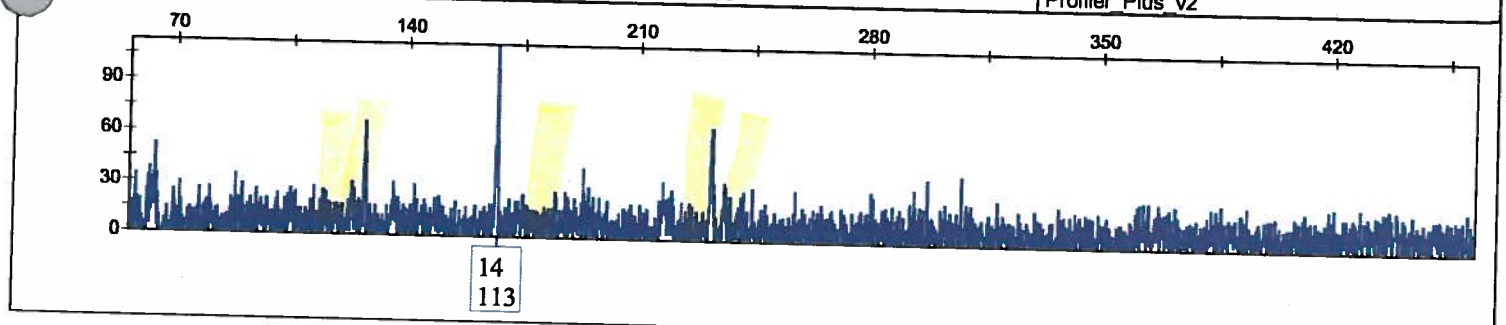


Applied Biosystems

GeneMapper ID v3.2.1

ST121609

Sample File	Sample Name	Panel
ST CH 0.0234375.fsa	ST CH 0.0234375	Profiler_Plus_v2



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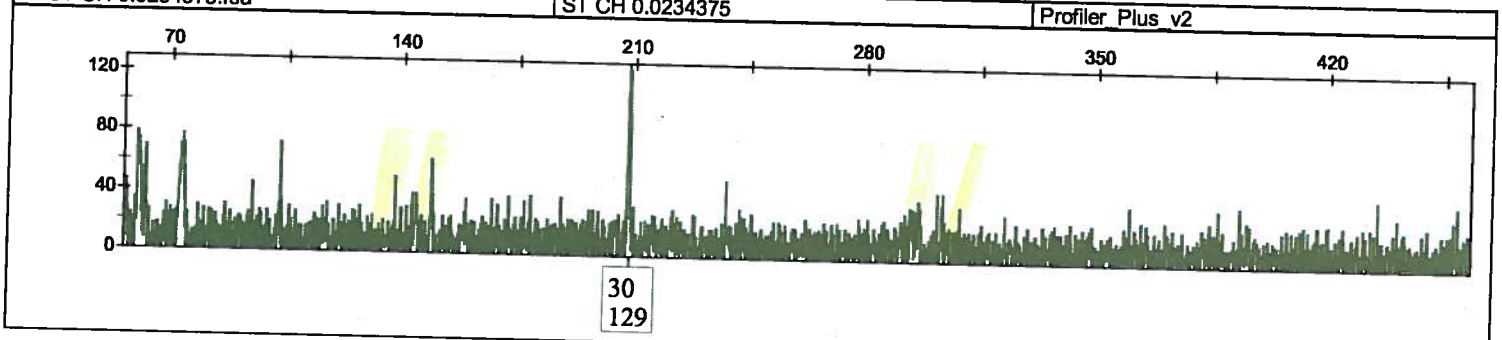
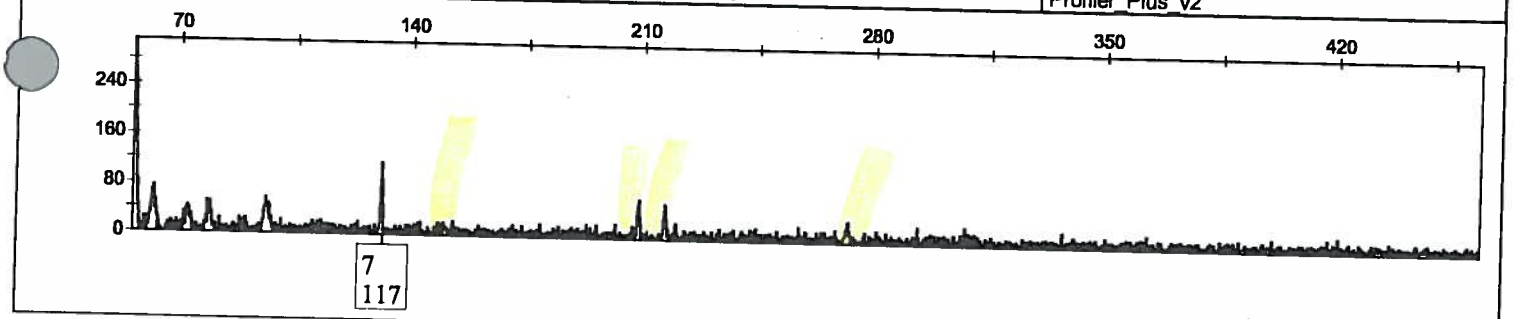


C5ST CH 0.0234375.fsa	ST CH 0.0234375	Profiler_Plus_v2

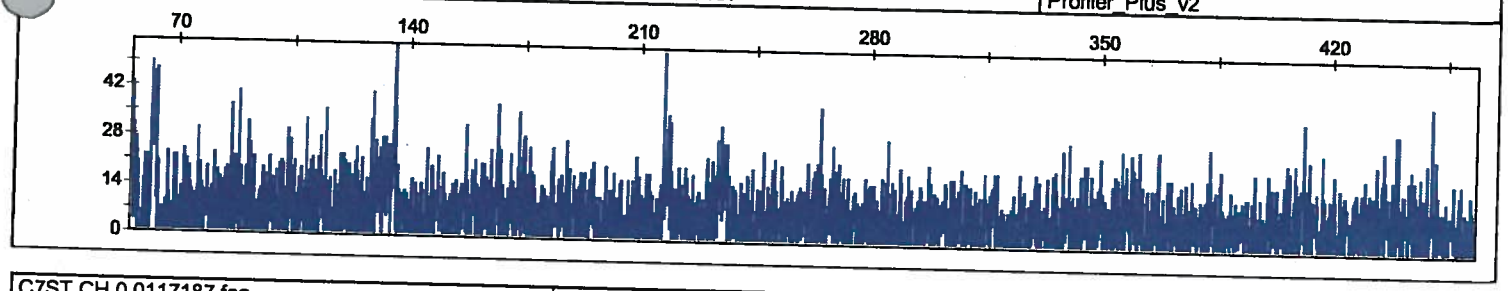


Applied Biosystems

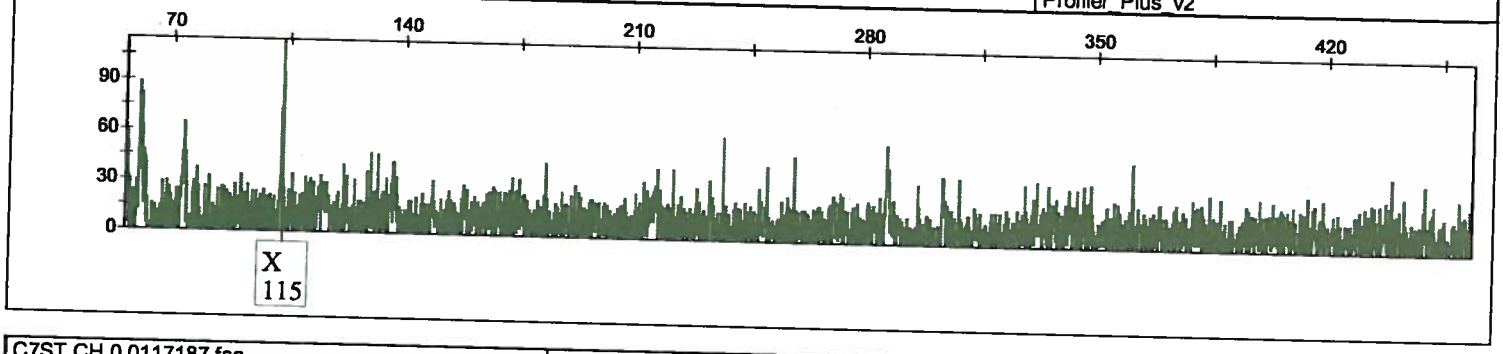
GeneMapper ID v3.2.1

ST121609

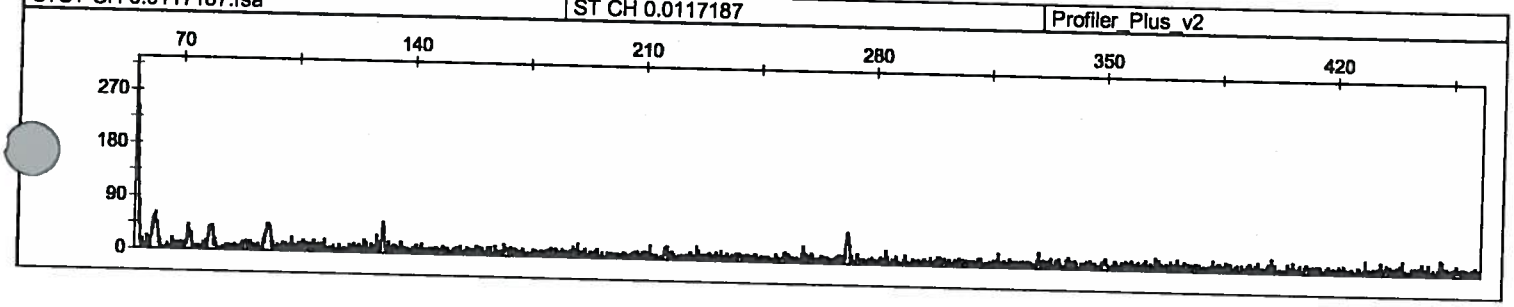
Sample File	Sample Name	Panel
ST CH 0.0117187.fsa	ST CH 0.0117187	Profiler_Plus_v2



C7ST CH 0.0117187.fsa	ST CH 0.0117187	Profiler_Plus_v2



C7ST CH 0.0117187.fsa	ST CH 0.0117187	Profiler_Plus_v2

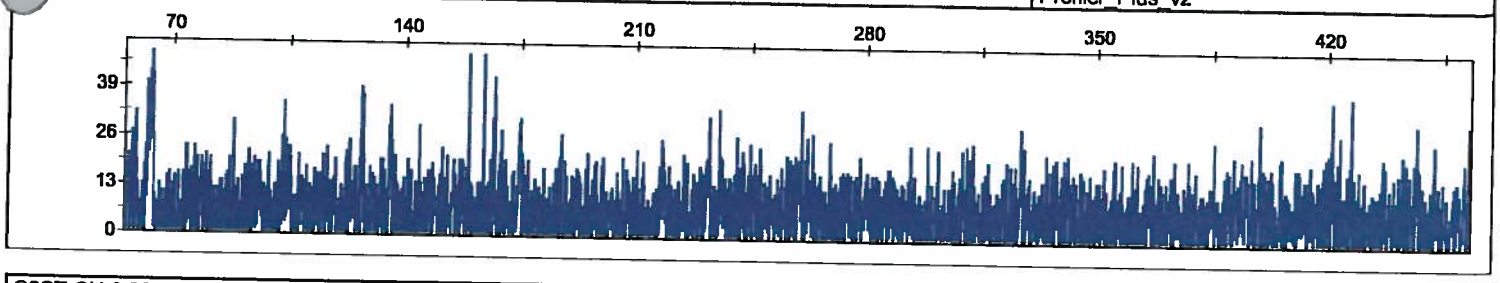


Applied Biosystems

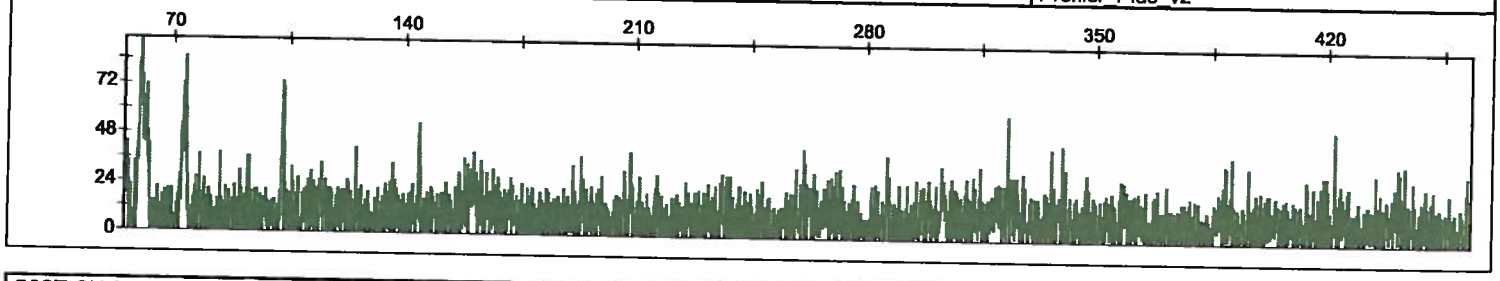
GeneMapper ID v3.2.1

ST121609

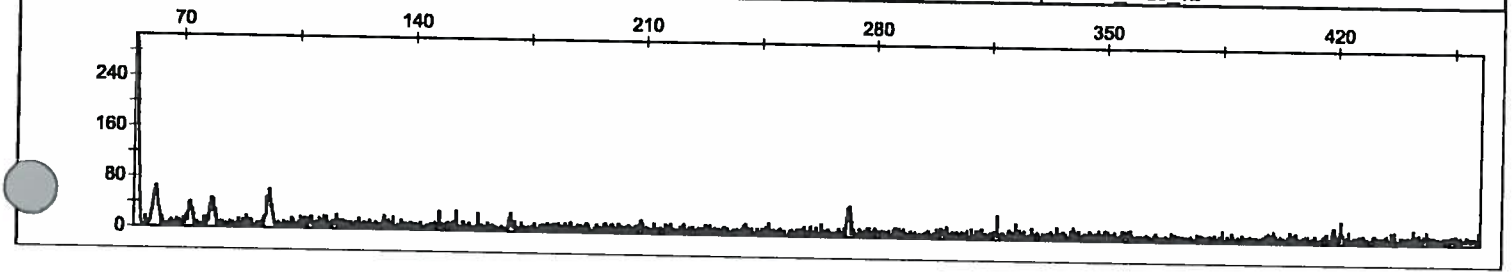
Sample File	Sample Name	Panel
ST CH 0.0058593.fsa	ST CH 0.0058593	Profiler_Plus_v2



C9ST CH 0.0058593.fsa	ST CH 0.0058593	Profiler_Plus_v2



C9ST CH 0.0058593.fsa	ST CH 0.0058593	Profiler_Plus_v2

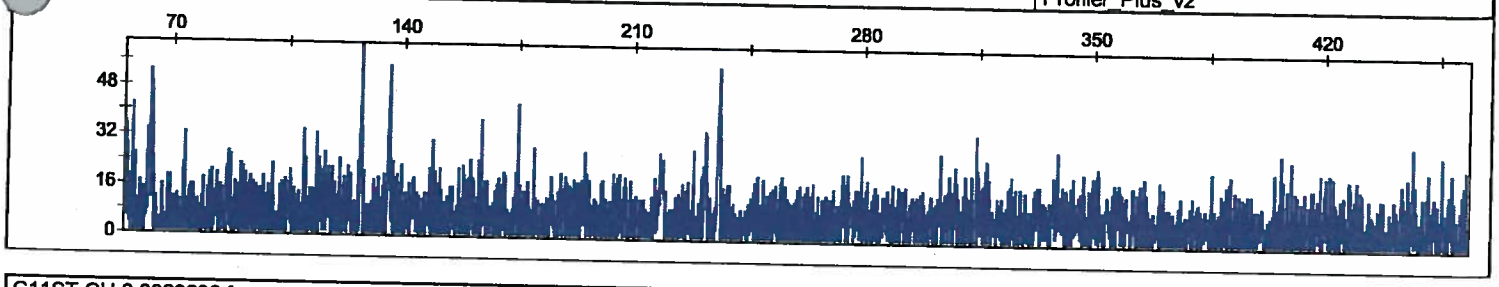


Applied Biosystems

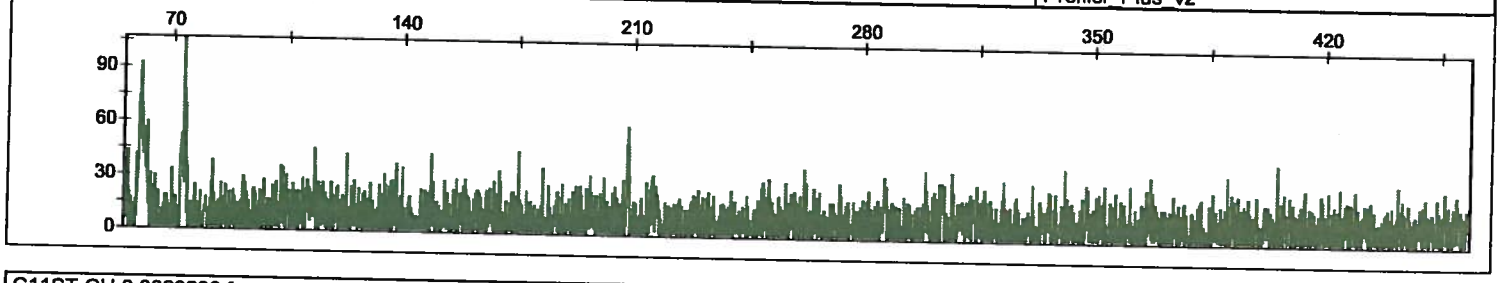
GeneMapper ID v3.2.1

ST121609

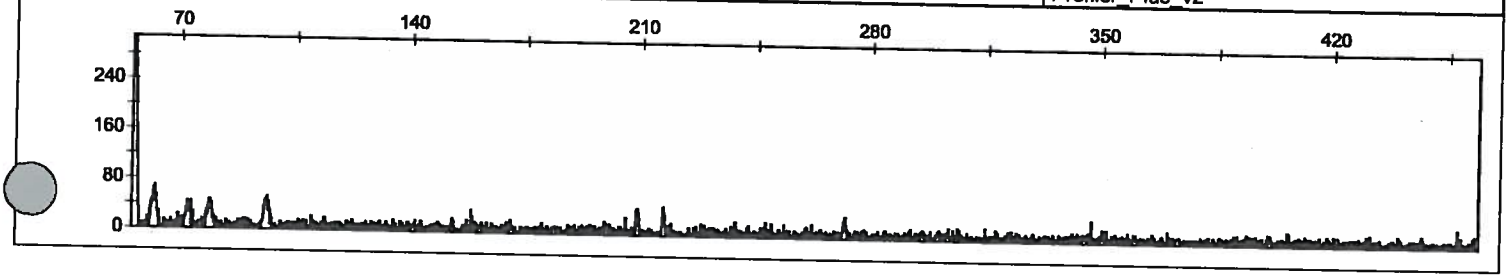
Sample File	Sample Name	Panel
1ST CH 0.0029296.fsa	ST CH 0.0029296	Profiler_Plus_v2



C11ST CH 0.0029296.fsa	ST CH 0.0029296	Profiler_Plus_v2



C11ST CH 0.0029296.fsa	ST CH 0.0029296	Profiler_Plus_v2

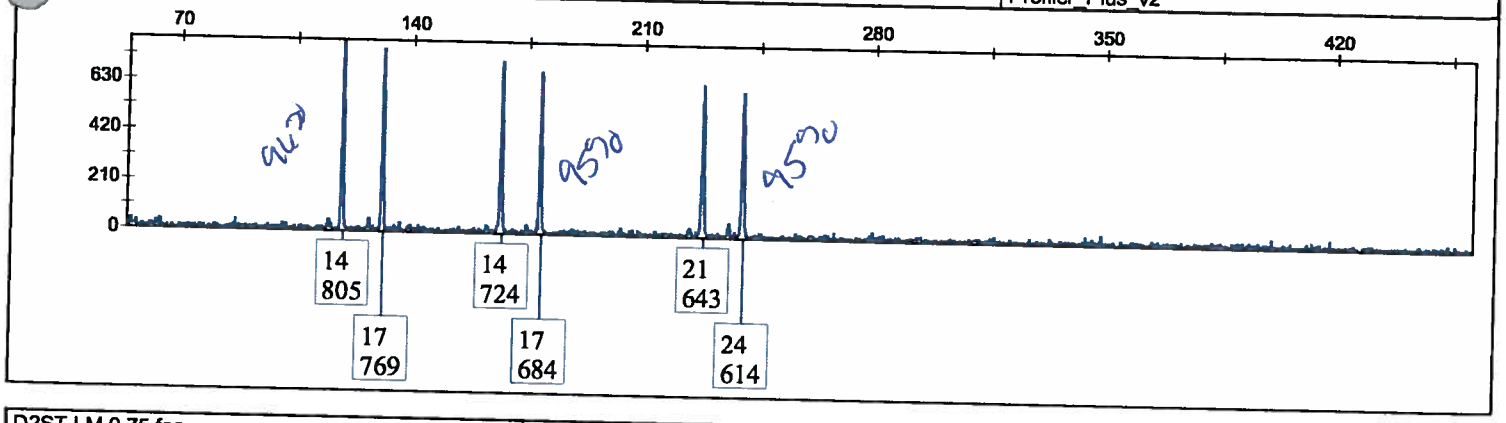


Applied Biosystems

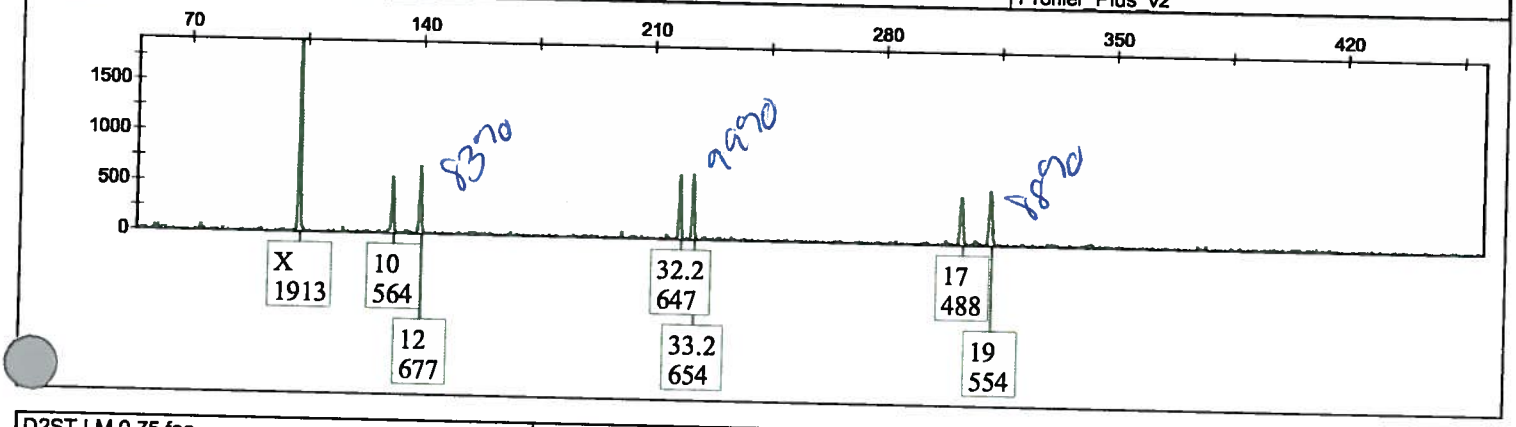
GeneMapper ID v3.2.1

ST121609

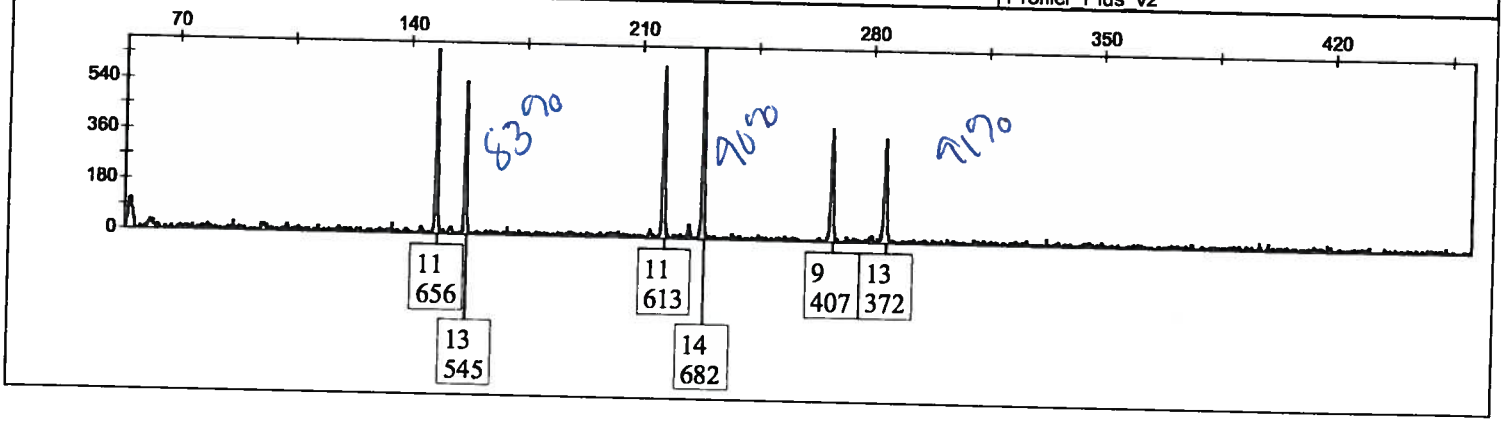
Sample File	Sample Name	Panel
ST LM 0.75.fsa	ST LM 0.75	Profiler_Plus_v2



D2ST LM 0.75.fsa	ST LM 0.75	Profiler_Plus_v2



D2ST LM 0.75.fsa	ST LM 0.75	Profiler_Plus_v2

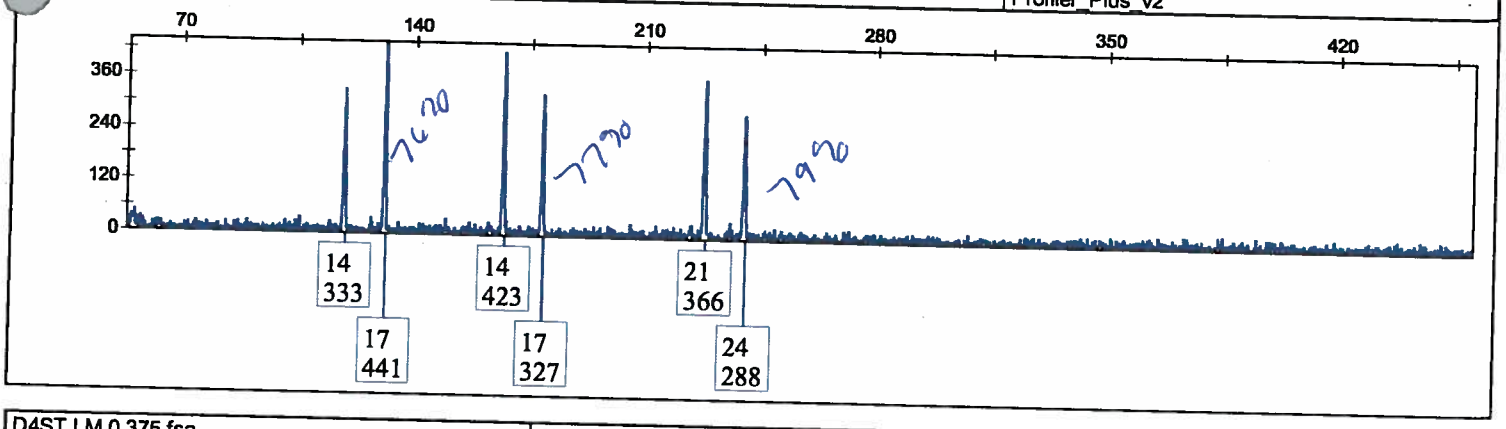


Applied Biosystems

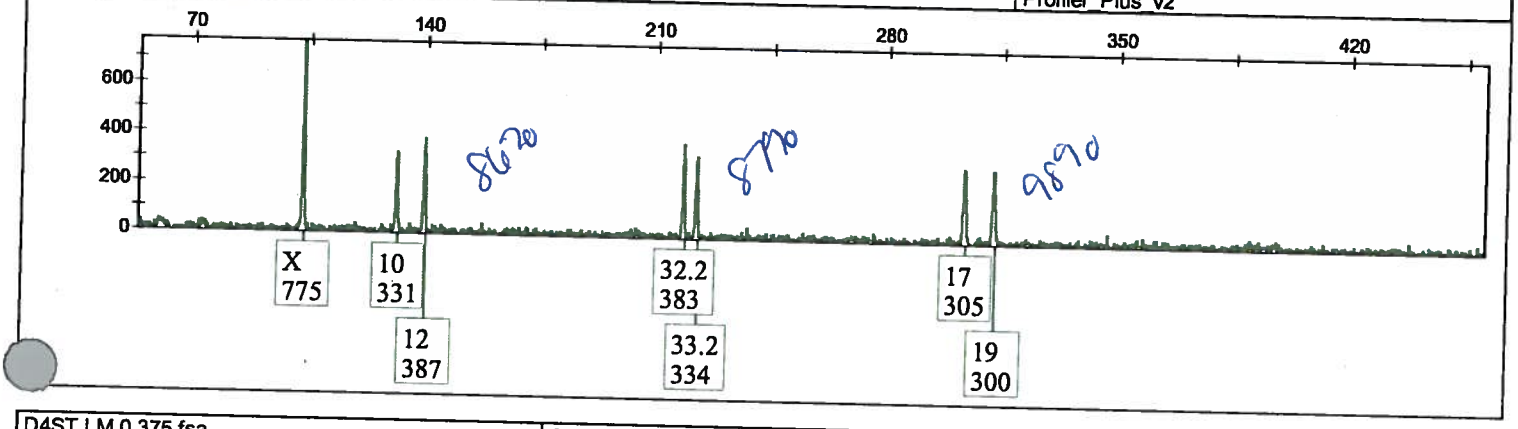
GeneMapper ID v3.2.1

ST121609

Sample File	Sample Name	Panel
ST LM 0.375.fsa	ST LM 0.375	Profiler_Plus_v2



D4ST LM 0.375.fsa	ST LM 0.375	Profiler_Plus_v2



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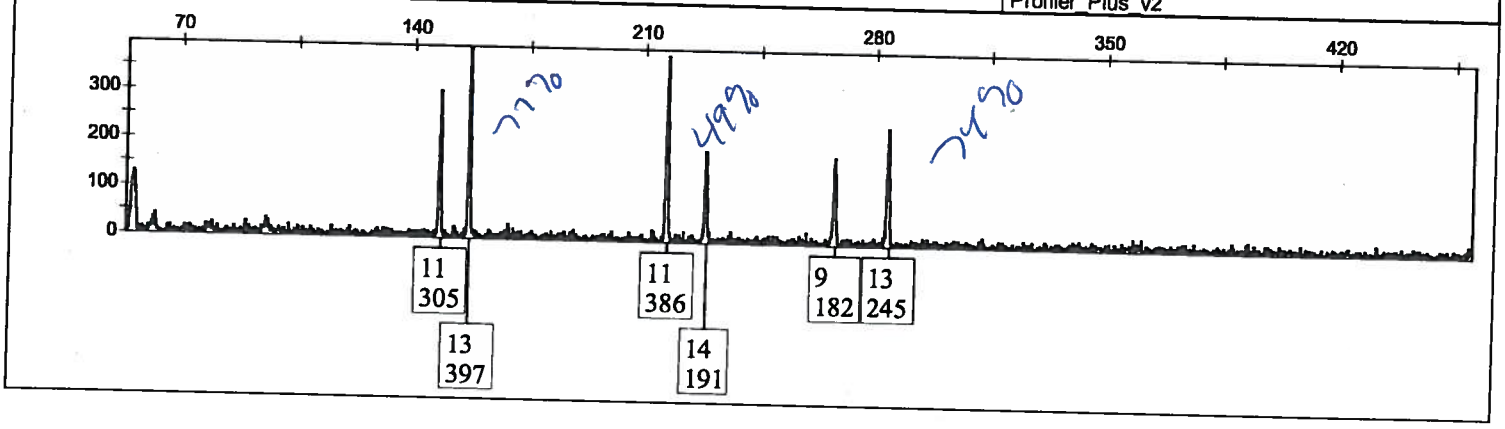
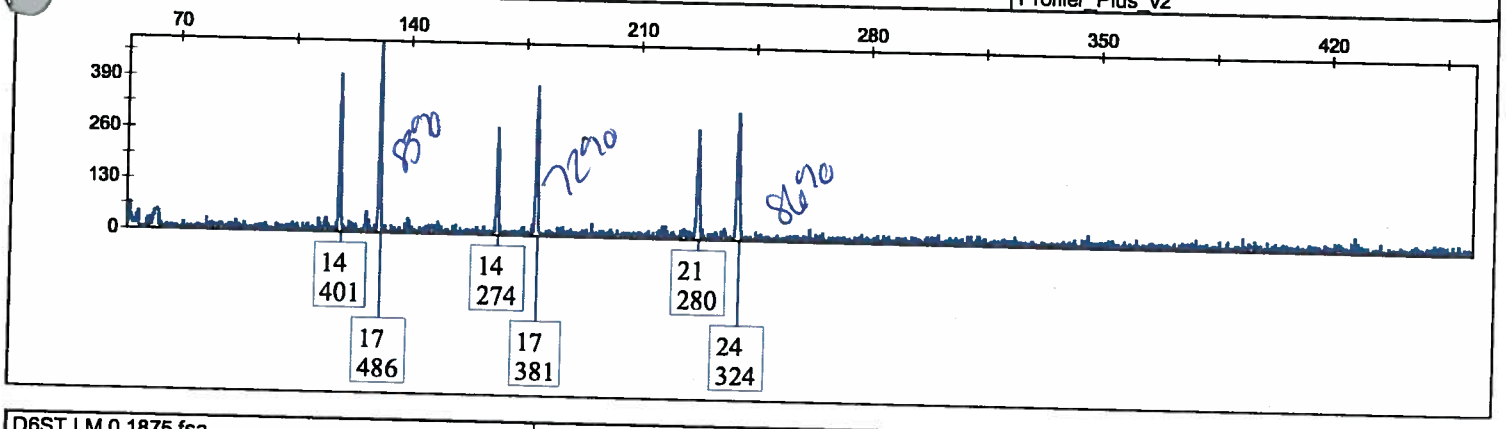


Applied Biosystems

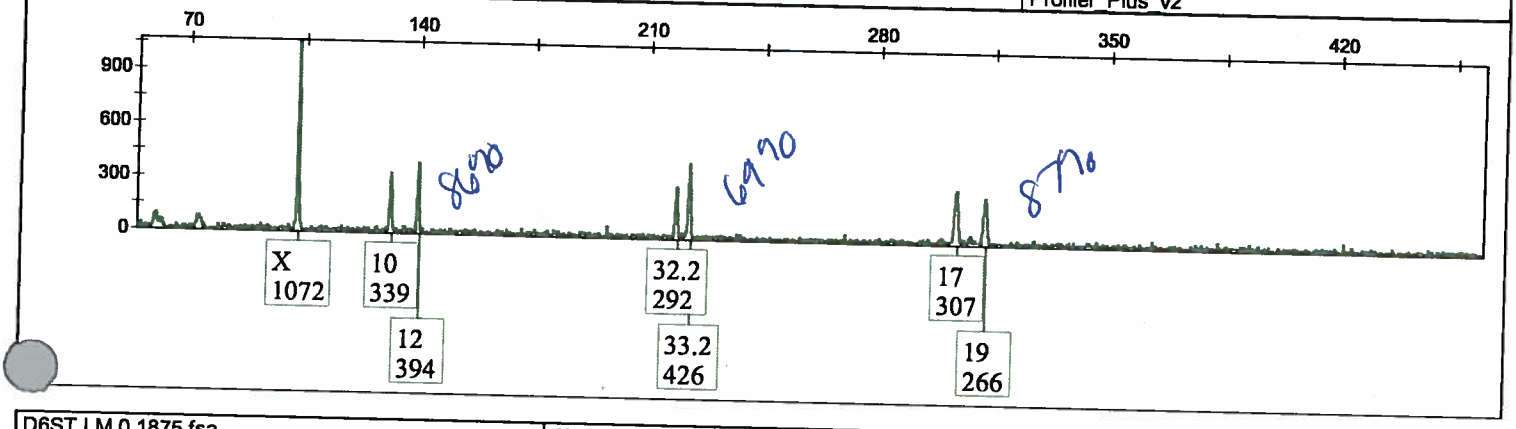
GeneMapper ID v3.2.1

ST121609

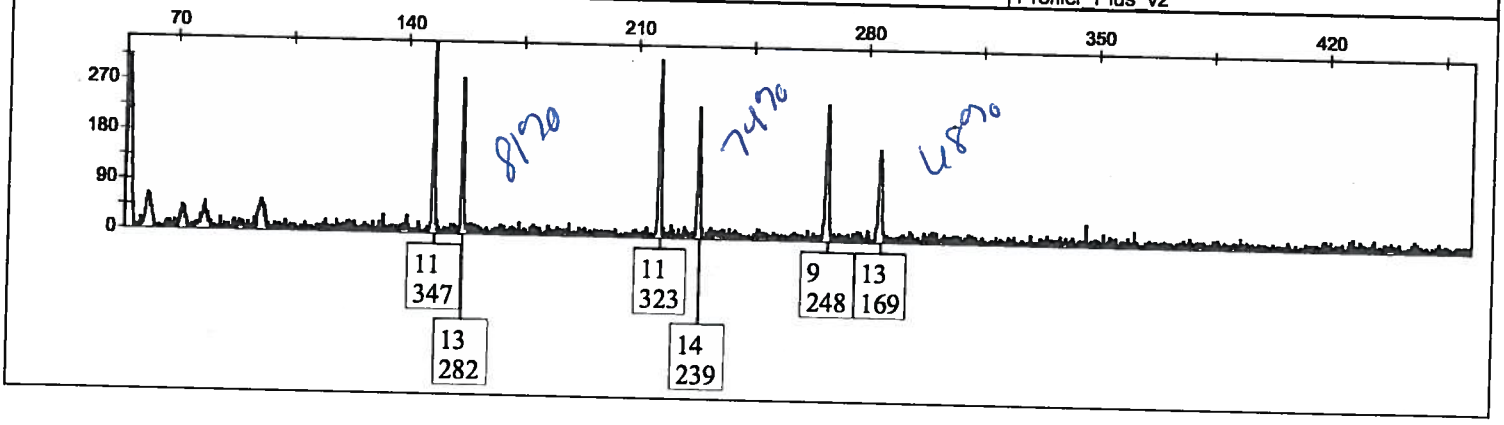
Sample File	Sample Name	Panel
ST LM 0.1875.fsa	ST LM 0.1875	Profiler_Plus_v2



D6ST LM 0.1875.fsa	ST LM 0.1875	Profiler_Plus_v2



D6ST LM 0.1875.fsa	ST LM 0.1875	Profiler_Plus_v2



Thu Dec 17,2009 08:44AM, CST

Printed by: gmid

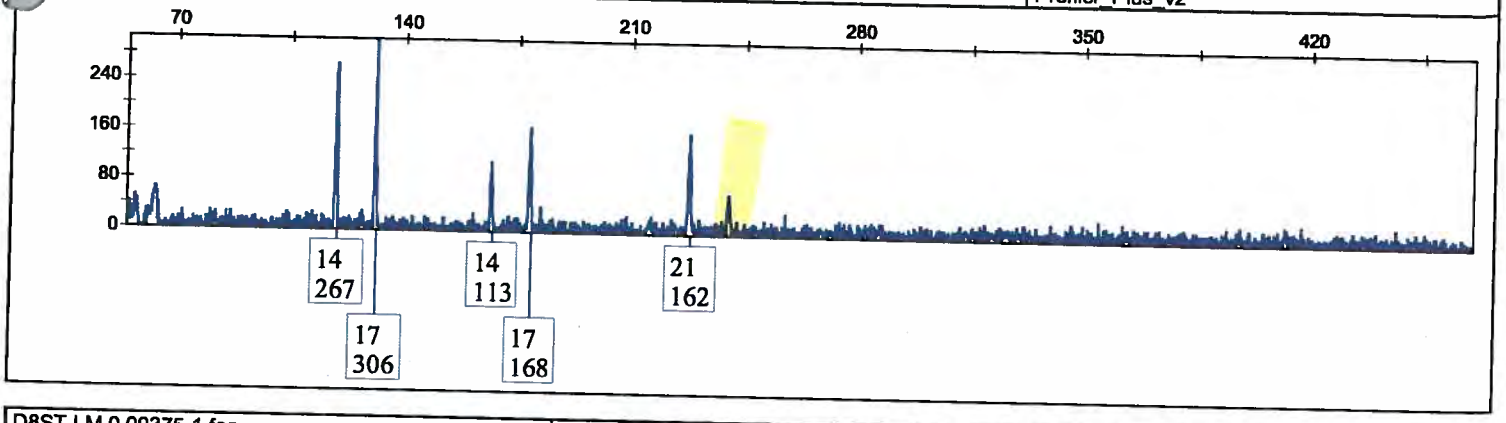
ST CCC

Applied Biosystems

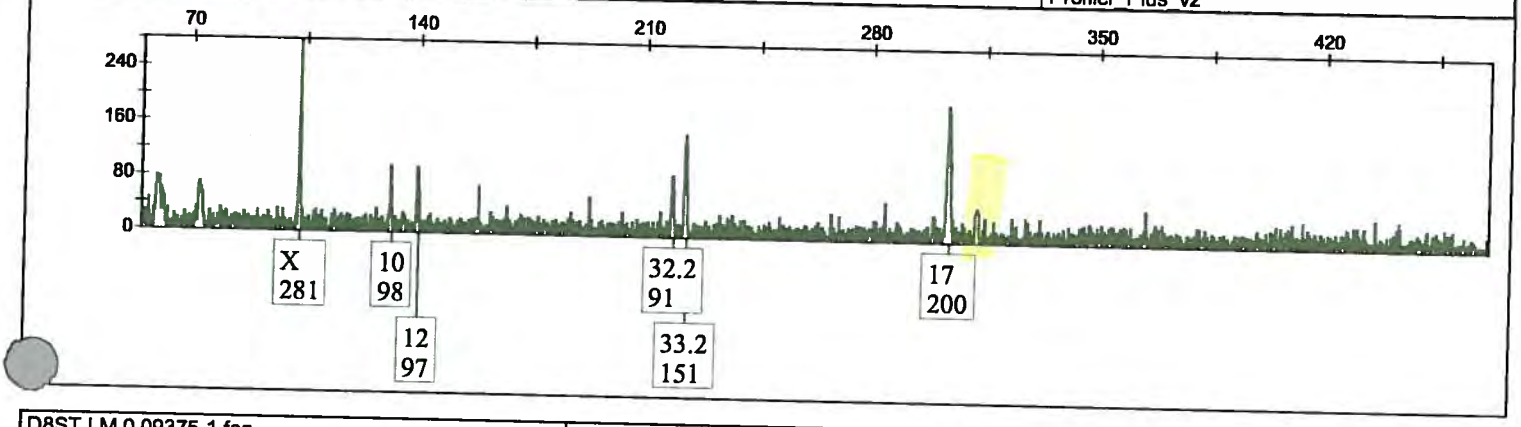
GeneMapper ID v3.2.1

ST121609CCC

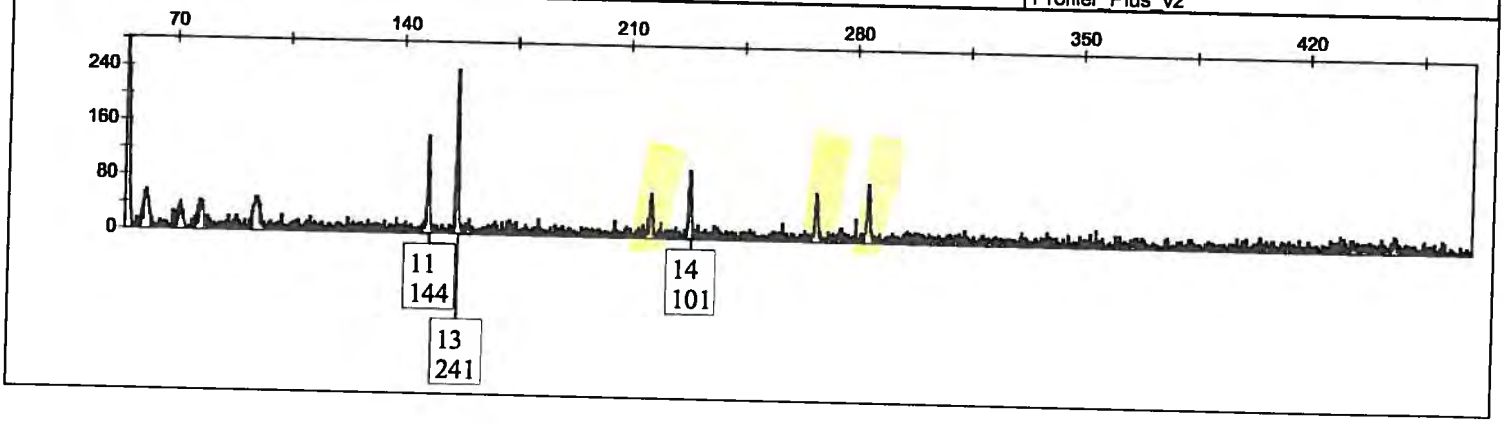
Sample File	Sample Name	Panel
D8ST LM 0.09375-1.fsa	ST LM 0.09375	Profiler_Plus_v2



D8ST LM 0.09375-1.fsa	ST LM 0.09375	Profiler_Plus_v2



D8ST LM 0.09375-1.fsa	ST LM 0.09375	Profiler_Plus_v2

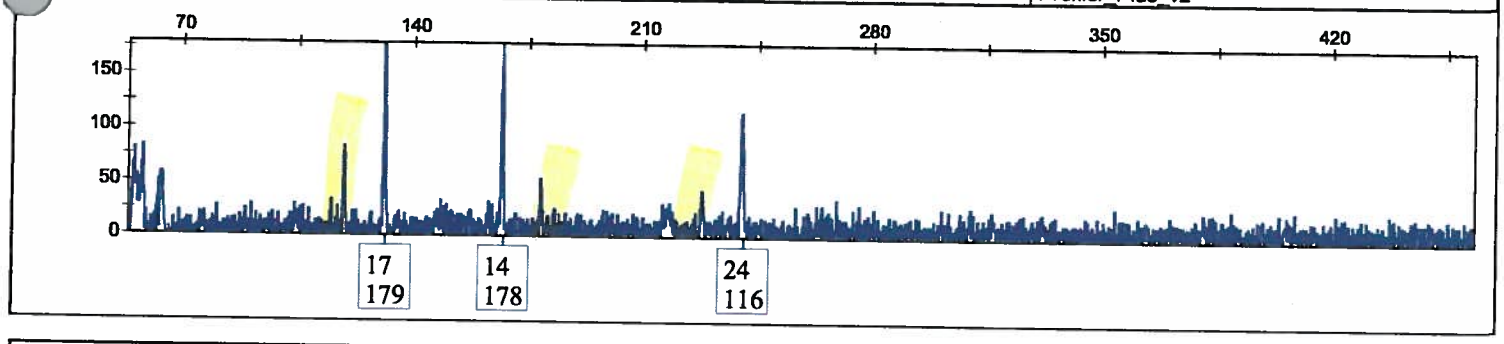


Applied Biosystems

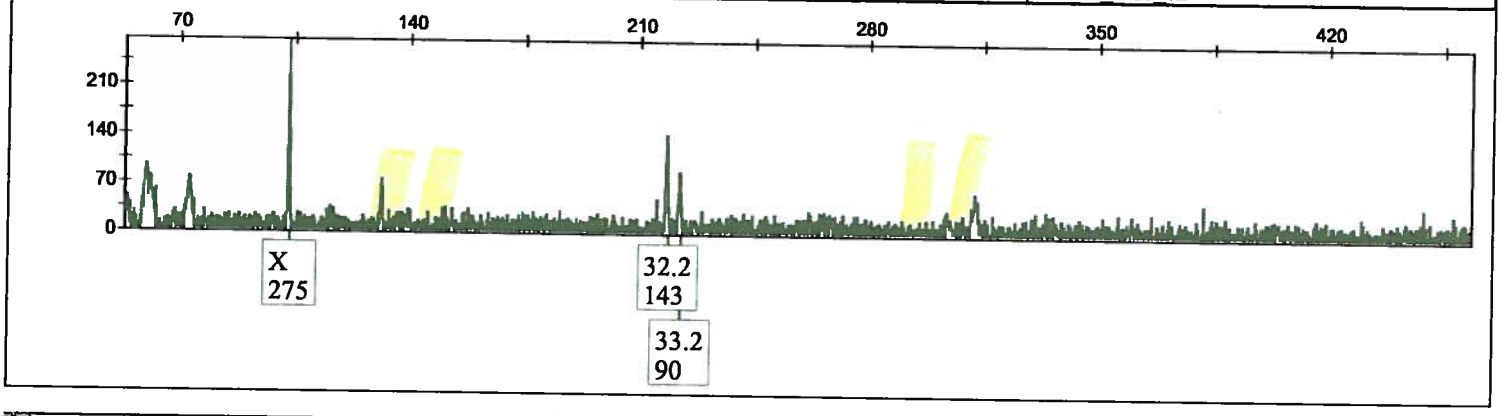
GeneMapper ID v3.2.1

ST121609

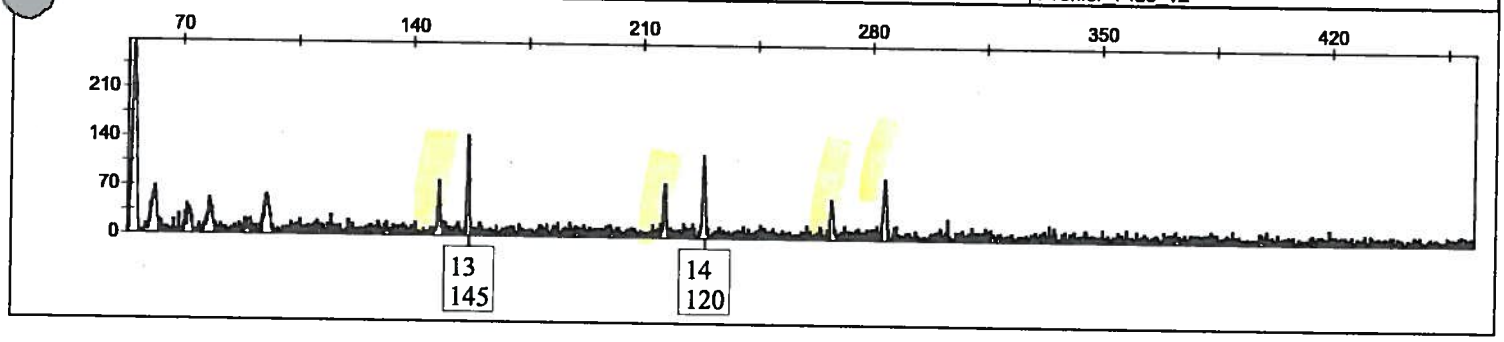
Sample File	Sample Name	Panel
0ST LM 0.046875.fsa	ST LM 0.046875	Profiler_Plus_v2



D10ST LM 0.046875.fsa	ST LM 0.046875	Profiler_Plus_v2



0ST LM 0.046875.fsa	ST LM 0.046875	Profiler_Plus_v2

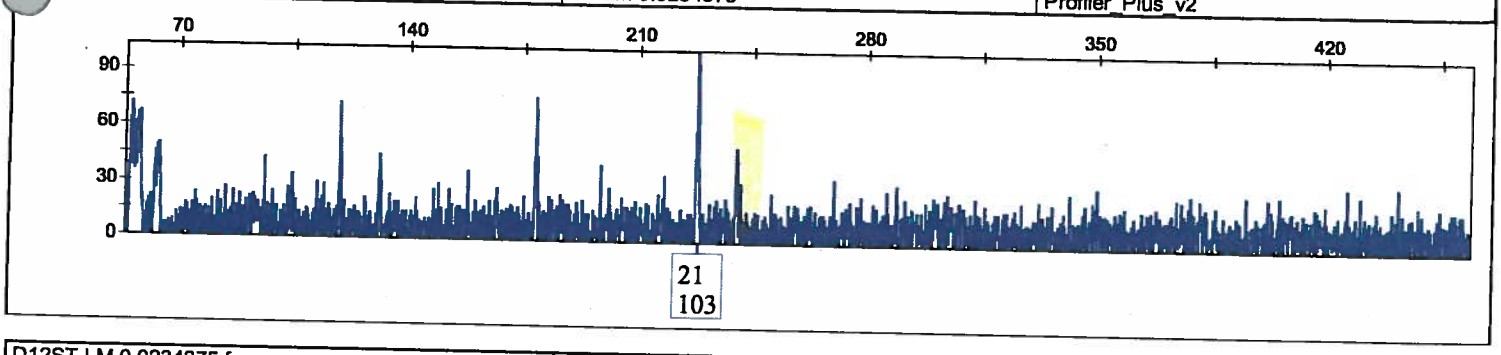


Applied Biosystems

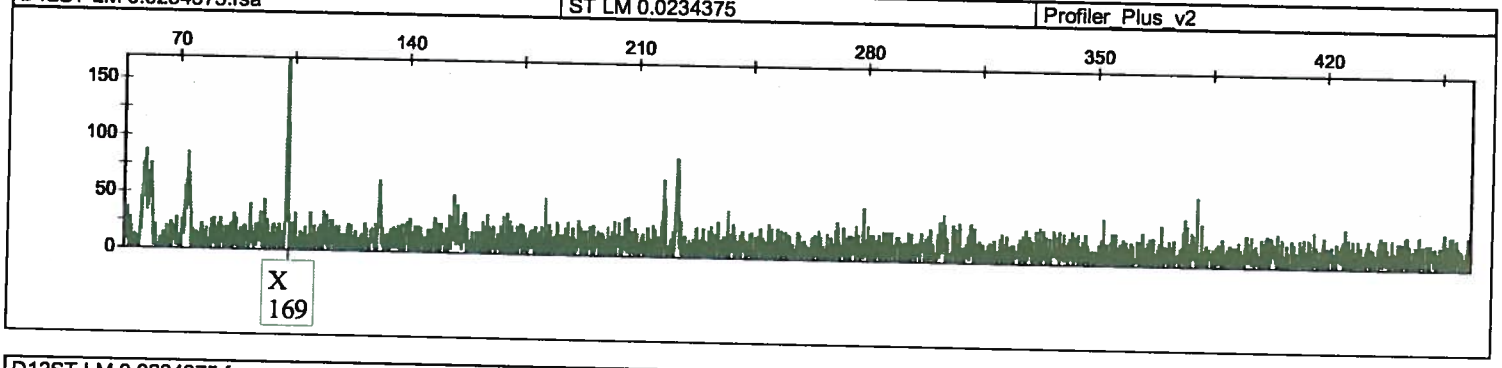
GeneMapper ID v3.2.1

ST121609

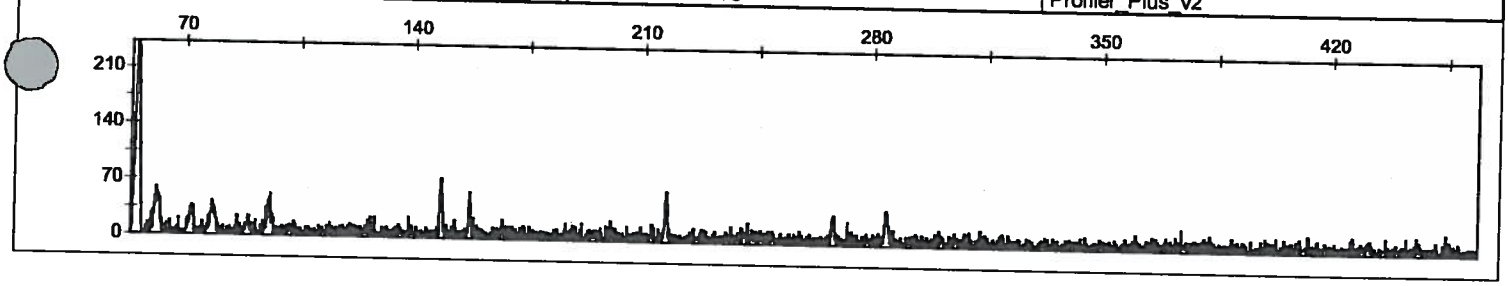
Sample File	Sample Name	Panel
2ST LM 0.0234375.fsa	ST LM 0.0234375	Profiler_Plus_v2



D12ST LM 0.0234375.fsa	ST LM 0.0234375	Profiler_Plus_v2



D12ST LM 0.0234375.fsa	ST LM 0.0234375	Profiler_Plus_v2

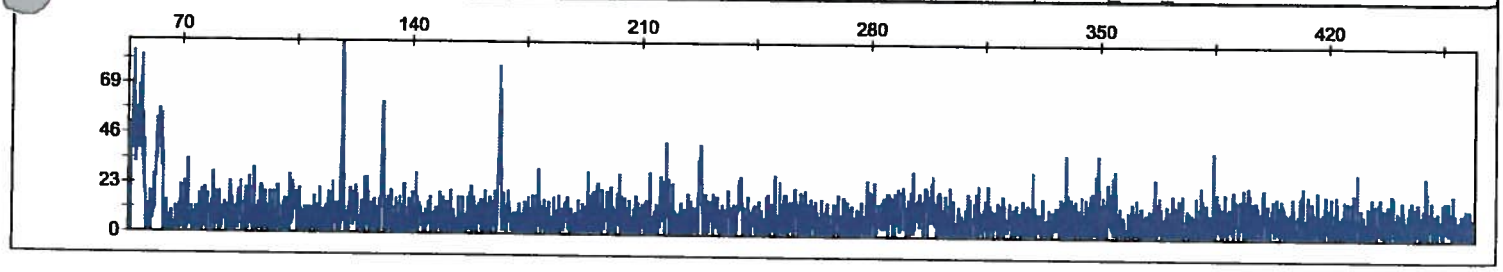


Applied Biosystems

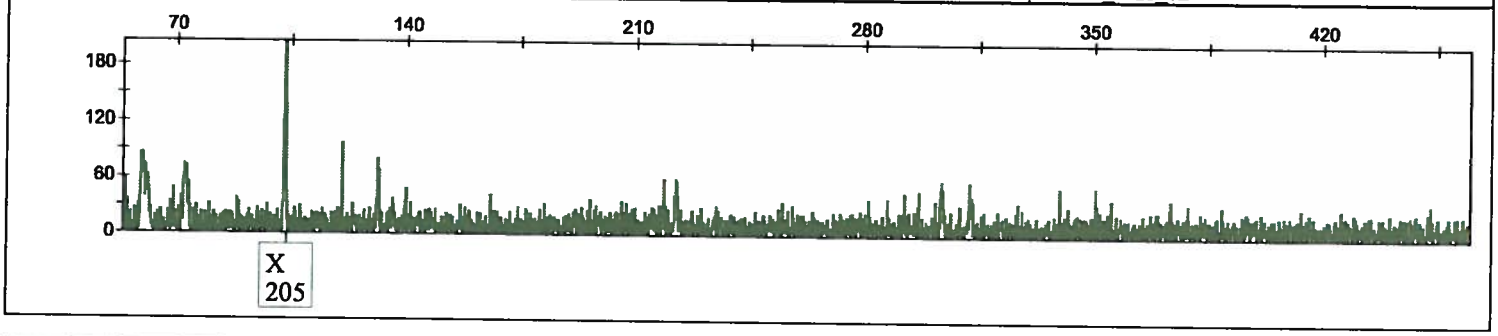
GeneMapper ID v3.2.1

ST121609

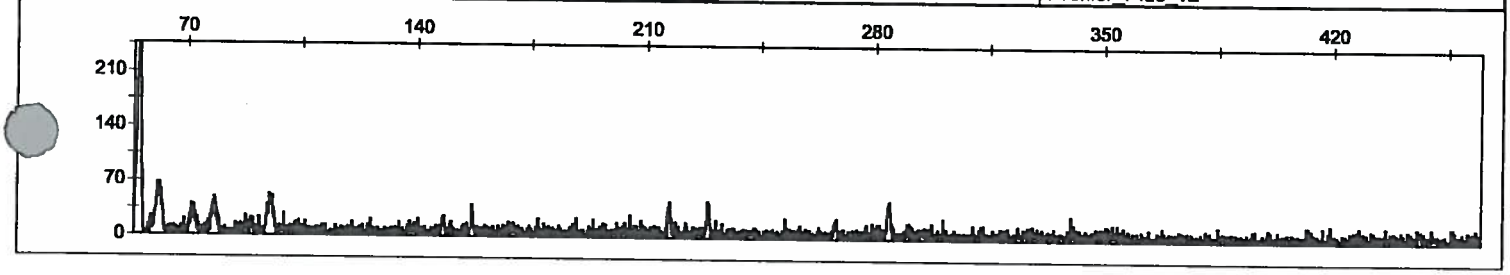
Sample File	Sample Name	Panel
ST LM 0.0117187.fsa	ST LM 0.0117187	Profiler_Plus_v2



E1ST LM 0.0117187.fsa	ST LM 0.0117187	Profiler_Plus_v2



E1ST LM 0.0117187.fsa	ST LM 0.0117187	Profiler_Plus_v2

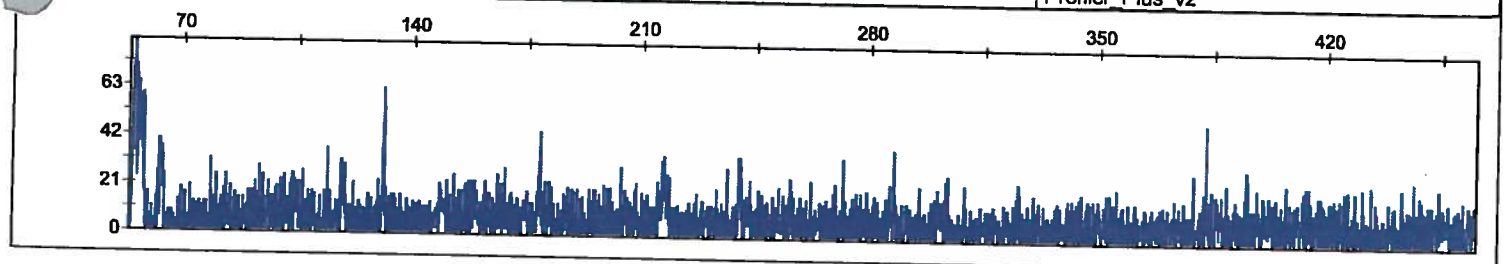


Applied Biosystems

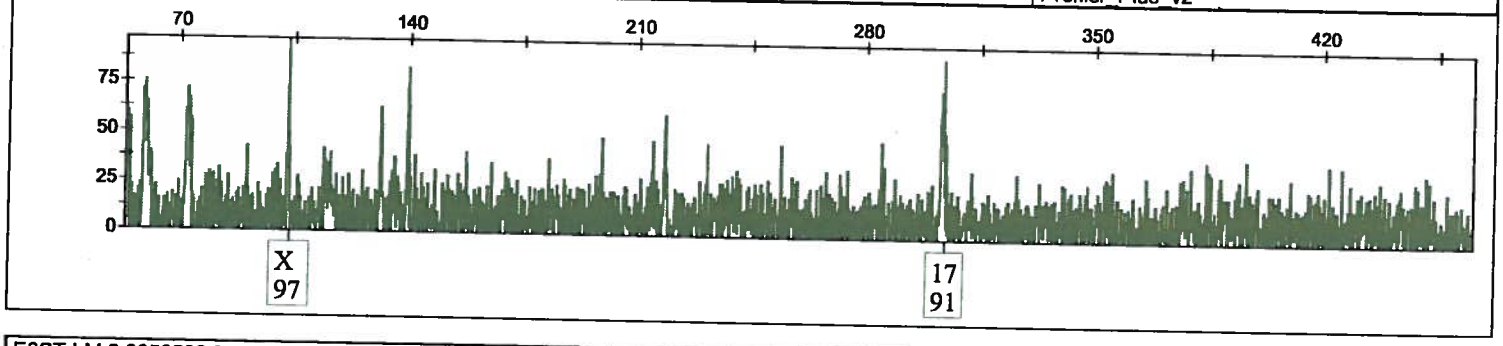
GeneMapper ID v3.2.1

ST121609

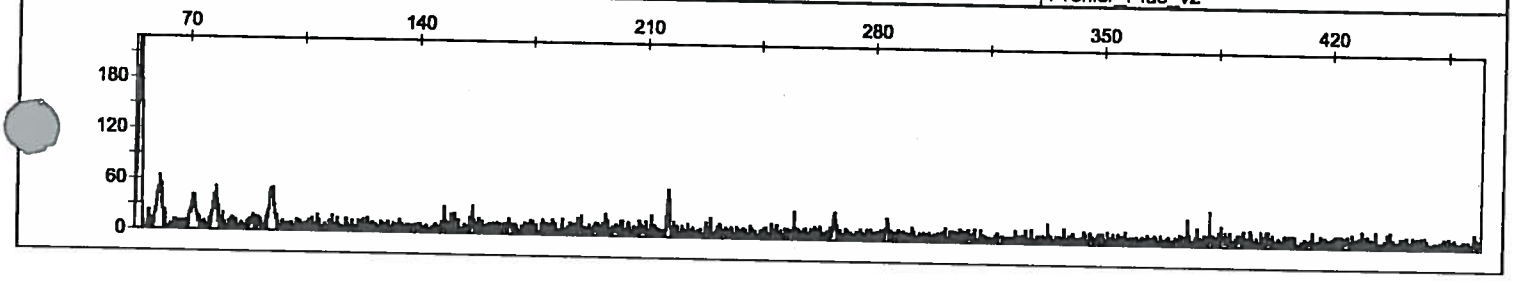
Sample File	Sample Name	Panel
ST LM 0.0058593.fsa	ST LM 0.0058593	Profiler_Plus_v2



E3ST LM 0.0058593.fsa	ST LM 0.0058593	Profiler_Plus_v2



E3ST LM 0.0058593.fsa	ST LM 0.0058593	Profiler_Plus_v2

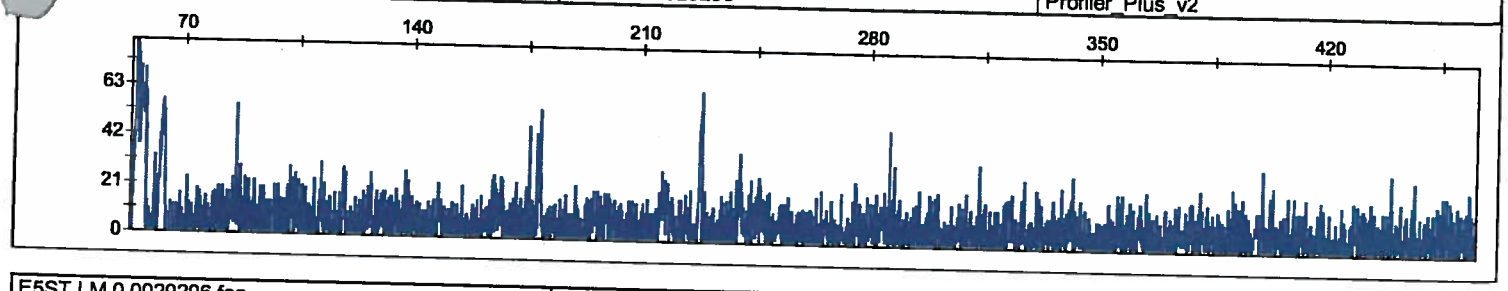


Applied Biosystems

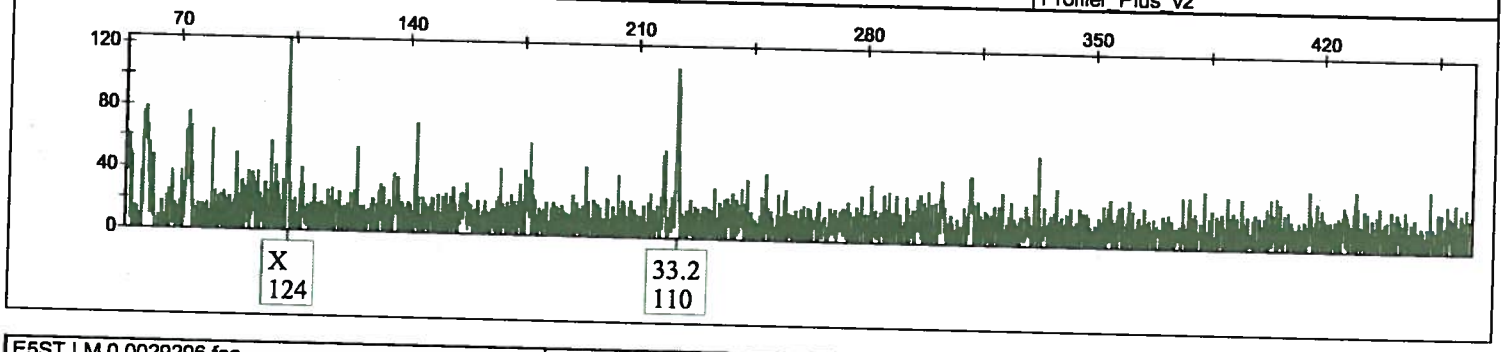
GeneMapper ID v3.2.1

ST121609

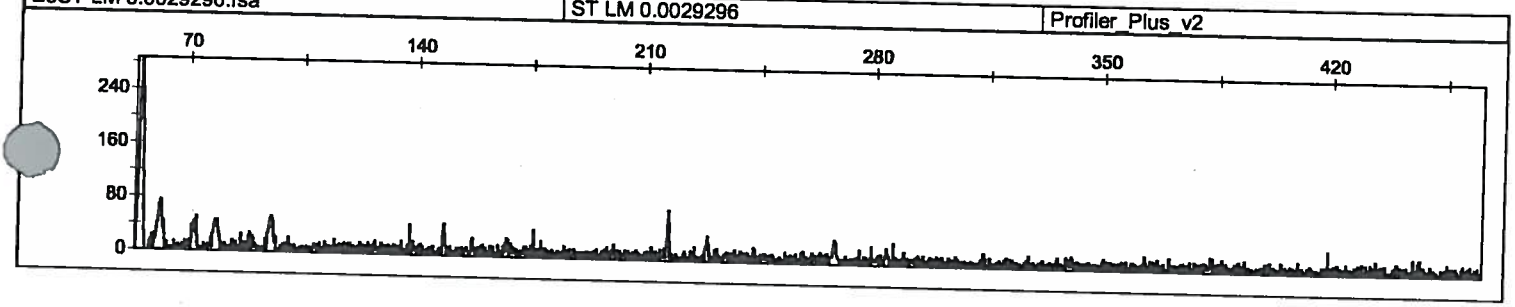
Sample File	Sample Name	Panel
ST LM 0.0029296.fsa	ST LM 0.0029296	Profiler_Plus_v2



E5ST LM 0.0029296.fsa	ST LM 0.0029296	Profiler_Plus_v2



E5ST LM 0.0029296.fsa	ST LM 0.0029296	Profiler_Plus_v2

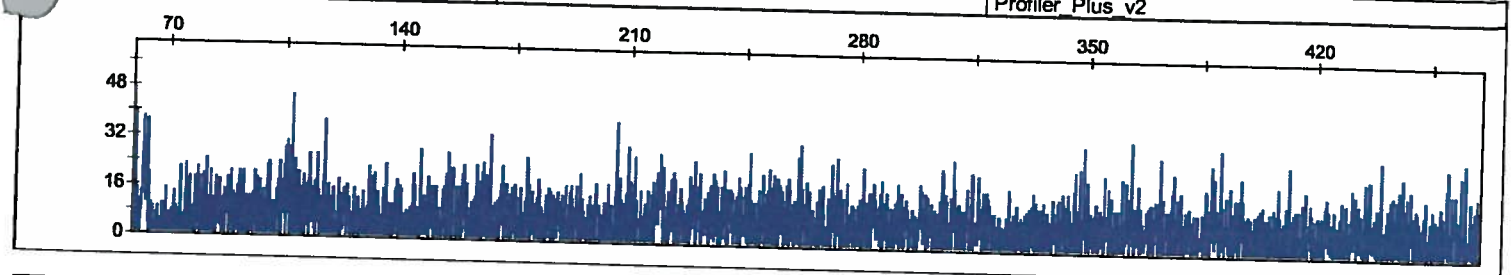


Applied Biosystems

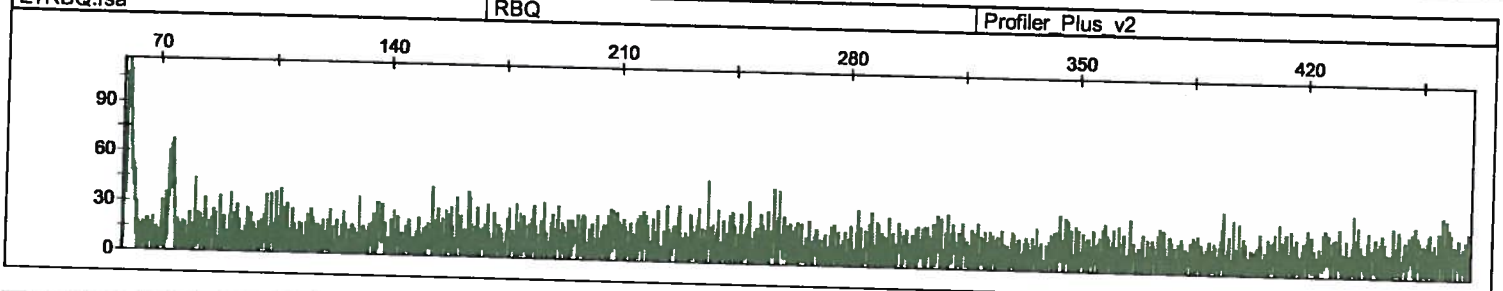
GeneMapper ID v3.2.1

ST121609

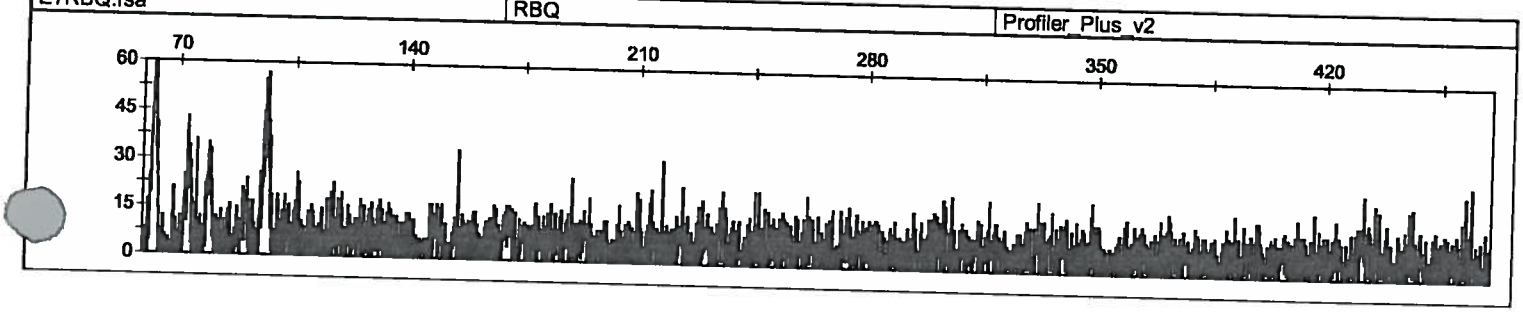
Sample File	Sample Name	Panel
RBQ.fsa	RBQ	Profiler_Plus_v2



E7RBQ.fsa	RBQ	Profiler_Plus_v2



E7RBQ.fsa	RBQ	Profiler_Plus_v2



AUSTIN POLICE DEPARTMENT
SEROLOGY/ DNA SECTION
STANDARD OPERATING PROCEDURES

STR RECORD SHEET

DATE OF RUN 01 DEC 10

CASE # (S) ST

ANALYST (S) DM/CCC

310/3130 INSTRUMENT #2

LOT RECORDS

CAPILLARY 39CA0187

POLYMER 0911072

ROX 0906500

FORMAMIDE 0907799

GA BUFFER 0905322

CASE RECORDS

KIT LOT #/ Expiration Date (PP/CO) 0905139 | 5.26.10

MATRIX/SPECTRAL 111909

SIZE STANDARD ROX 500

ANALYZED AT THRESHOLD OF: 90
(for evidence samples only)

90NS ✓

☐ Others at 150

Other

CASEWORK DISK # 10 – 01

Applied Systems

GeneMapper ID v3.2.1

	Status	Sample File	Sample Name	Sample Type	Analysis Method	Panel	Size Standard	OS	SQ
1		BLANK_C05_DGM.fsa	BLANK	Sample	None	None	None	0.0	0.0
2		BLANK_C07_DGM.fsa	BLANK	Sample	None	None	None	0.0	0.0
3		BLANK_C10_DGM.fsa	BLANK	Sample	None	None	None	0.0	0.0
4		BLANK_D05_DGM.fsa	BLANK	Sample	None	None	None	0.0	0.0
5		BLANK_D07_DGM.fsa	BLANK	Sample	None	None	None	0.0	0.0
6		BLANK_D08_DGM.fsa	BLANK	Sample	None	None	None	0.0	0.0
7		BLANK_D10_DGM.fsa	BLANK	Sample	None	None	None	0.0	0.0
8		BLANK_G06_DGM.fsa	BLANK	Sample	None	None	None	0.0	0.0
9		BLANK_G08_DGM.fsa	BLANK	Sample	None	None	None	0.0	0.0
10		BLANK_H06_DGM.fsa	BLANK	Sample	None	None	None	0.0	0.0
11		BLANK_H08_DGM.fsa	BLANK	Sample	None	None	None	0.0	0.0
12		BLANK_H09_DGM.fsa	BLANK	Sample	None	None	None	0.0	0.0
13		BLANK_H11_DGM.fsa	BLANK	Sample	None	None	None	0.0	0.0
14		CH1_E08_DGM.fsa	CH1	Sample	None	None	None	0.0	0.0
15		CH2_F08_DGM.fsa	CH2	Sample	None	None	None	0.0	0.0
16		CH3_A09_DGM.fsa	CH3	Sample	None	None	None	0.0	0.0
17		CH4_B09_DGM.fsa	CH4	Sample	None	None	None	0.0	0.0
18		CH5_C09_DGM.fsa	CH5	Sample	None	None	None	0.0	0.0
19		CH6_D09_DGM.fsa	CH6	Sample	None	None	None	0.0	0.0
20		CH7_E09_DGM.fsa	CH7	Sample	None	None	None	0.0	0.0
21		CH8_F09_DGM.fsa	CH8	Sample	None	None	None	0.0	0.0
22		CH9_G09_DGM.fsa	CH9	Sample	None	None	None	0.0	0.0
23		JM1_A07_DGM.fsa	JM1	Sample	None	None	None	0.0	0.0
24		JM2_B07_DGM.fsa	JM2	Sample	None	None	None	0.0	0.0
25		JM3_E07_DGM.fsa	JM3	Sample	None	None	None	0.0	0.0
26		JM4_F07_DGM.fsa	JM4	Sample	None	None	None	0.0	0.0
27		JM5_G07_DGM.fsa	JM5	Sample	None	None	None	0.0	0.0
28		JM6_H07_DGM.fsa	JM6	Sample	None	None	None	0.0	0.0
29		JM7_A08_DGM.fsa	JM7	Sample	None	None	None	0.0	0.0
30		JM8_B08_DGM.fsa	JM8	Sample	None	None	None	0.0	0.0
31		JM9_C08_DGM.fsa	JM9	Sample	None	None	None	0.0	0.0
32		LADDER_G11_DGM.2.fsa	LADDER	Sample	None	None	None	0.0	0.0

Applied Systems

GeneMapper ID v3.2.1

	Status	Sample File	Sample Name	Sample Type	Analysis Method	Panel	Size Standard	OS	SQ
33		LADDER_G11_DGM.fsa	LADDER	Sample	None	None	None	0.0	0.0
34		LM1_A10_DGM.fsa	LM1	Sample	None	None	None	0.0	0.0
35		LM2_B10_DGM.fsa	LM2	Sample	None	None	None	0.0	0.0
36		LM3_E10_DGM.fsa	LM3	Sample	None	None	None	0.0	0.0
37		LM4_F10_DGM.fsa	LM4	Sample	None	None	None	0.0	0.0
38		LM5_G10_DGM.fsa	LM5	Sample	None	None	None	0.0	0.0
39		LM6_H10_DGM.fsa	LM6	Sample	None	None	None	0.0	0.0
40		LM7_A11_DGM.fsa	LM7	Sample	None	None	None	0.0	0.0
41		LM8_B11_DGM.fsa	LM8	Sample	None	None	None	0.0	0.0
42		LM9_C11_DGM.fsa	LM9	Sample	None	None	None	0.0	0.0
43		MP1_A05_DGM.fsa	MP1	Sample	None	None	None	0.0	0.0
44		MP2_B05_DGM.fsa	MP2	Sample	None	None	None	0.0	0.0
45		MP3_E05_DGM.fsa	MP3	Sample	None	None	None	0.0	0.0
46		MP4_F05_DGM.fsa	MP4	Sample	None	None	None	0.0	0.0
47		MP5_G05_DGM.fsa	MP5	Sample	None	None	None	0.0	0.0
48		MP6_H05_DGM.fsa	MP6	Sample	None	None	None	0.0	0.0
49		MP7_A06_DGM.fsa	MP7	Sample	None	None	None	0.0	0.0
50		MP8_B06_DGM.fsa	MP8	Sample	None	None	None	0.0	0.0
51		MP9_C06_DGM.fsa	MP9	Sample	None	None	None	0.0	0.0
52		NEG_F11_DGM.fsa	NEG	Sample	None	None	None	0.0	0.0
53		NEGMP_F06_DGM.2.fsa	NEGMP	Sample	None	None	None	0.0	0.0
54		NEGMP_F06_DGM.fsa	NEGMP	Sample	None	None	None	0.0	0.0
55		POS_E11_DGM.fsa	POS	Sample	None	None	None	0.0	0.0
56		POSMP_E06_DGM.2.fsa	POSMP	Sample	None	None	None	0.0	0.0
57		POSMP_E06_DGM.fsa	POSMP	Sample	None	None	None	0.0	0.0
58		RBQ_D11_DGM.fsa	RBQ	Sample	None	None	None	0.0	0.0
59		RBQMP_D06_DGM.fsa	RBQMP	Sample	None	None	None	0.0	0.0

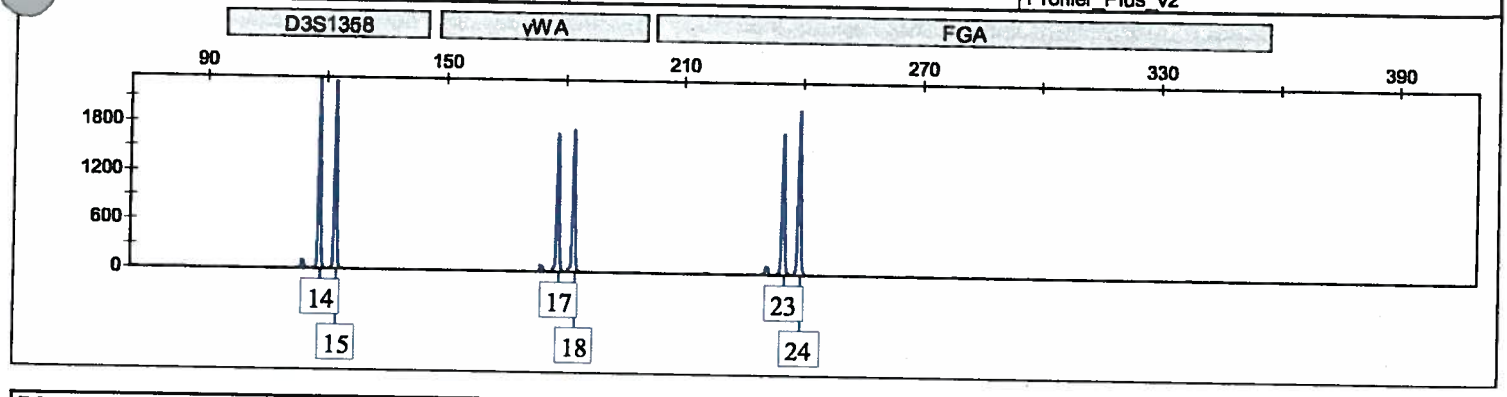
ST Run 010610

Applied Biosystems

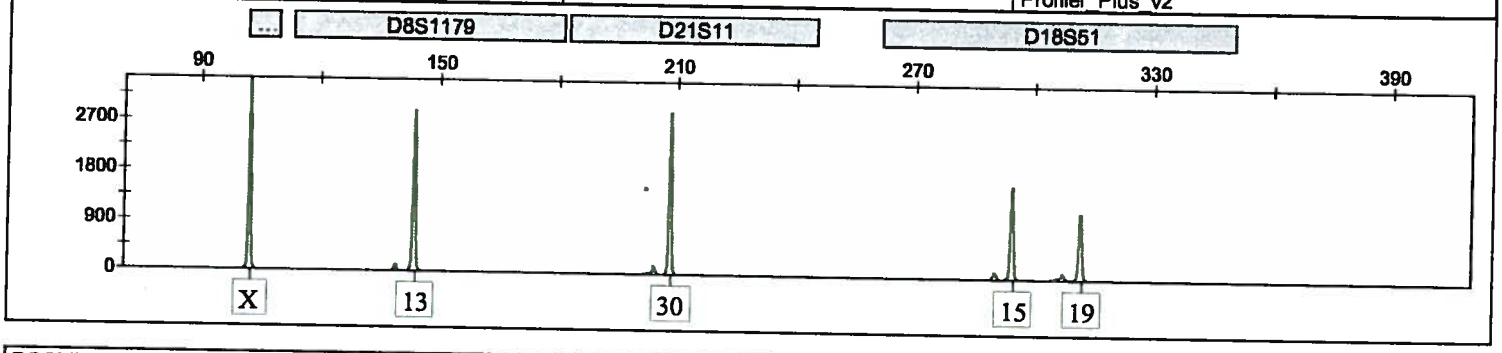
GeneMapper ID v3.2.1

ST010410DM

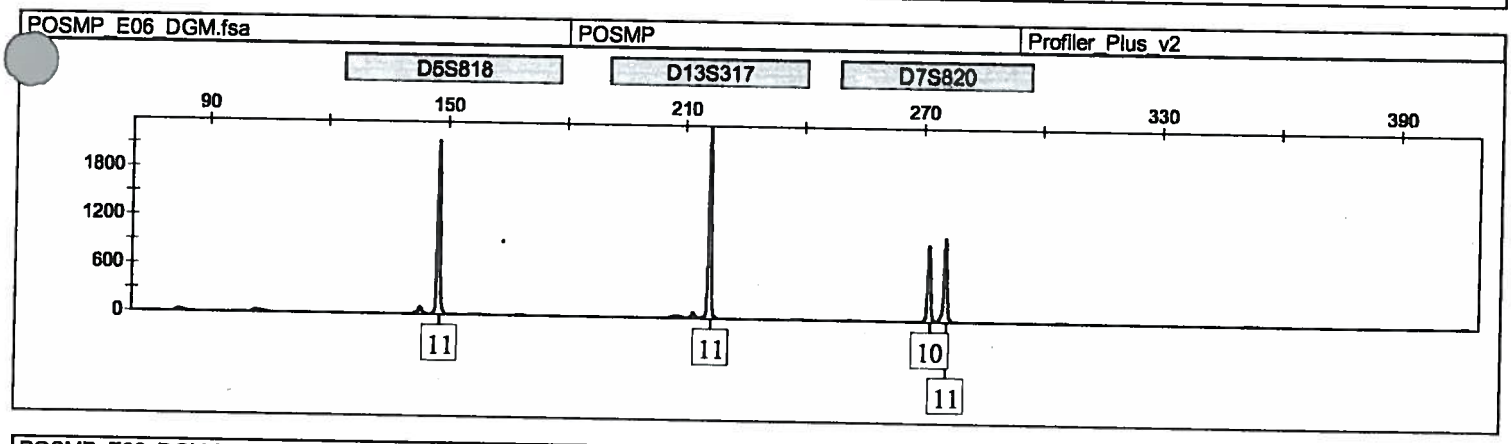
Sample File	Sample Name	Panel
SMP_E06_DGM.fsa	POSMP	Profiler_Plus_v2



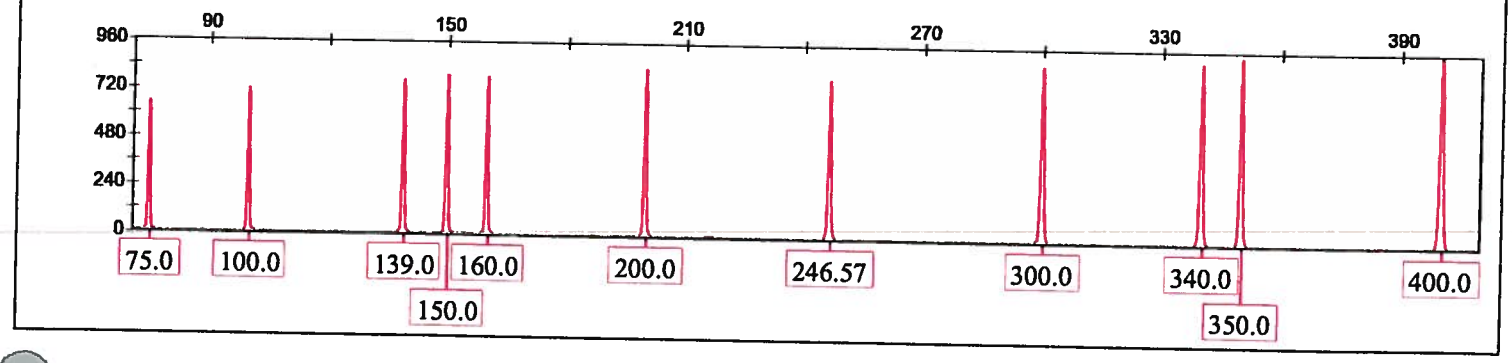
POSMP_E06_DGM.fsa	POSMP	Profiler_Plus_v2



POSMP_E06_DGM.fsa	POSMP	Profiler_Plus_v2



POSMP_E06_DGM.fsa	POSMP	Profiler_Plus_v2



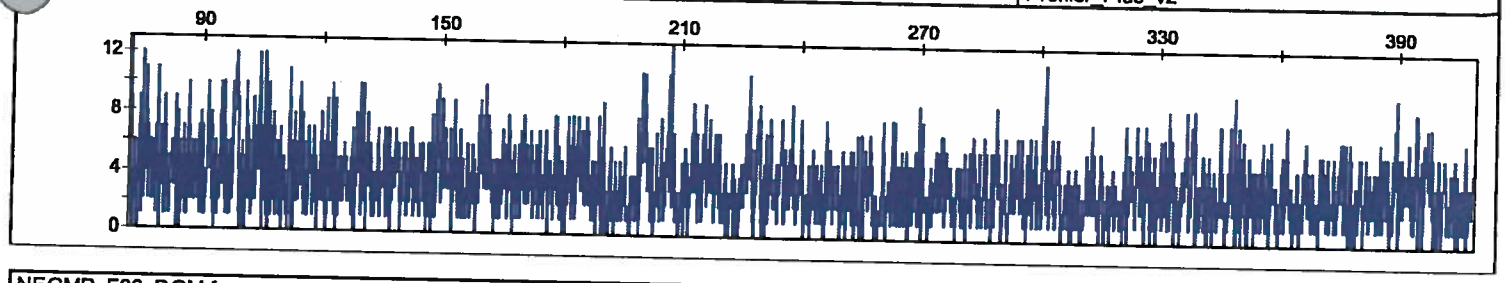
ST CCC

Applied Biosystems

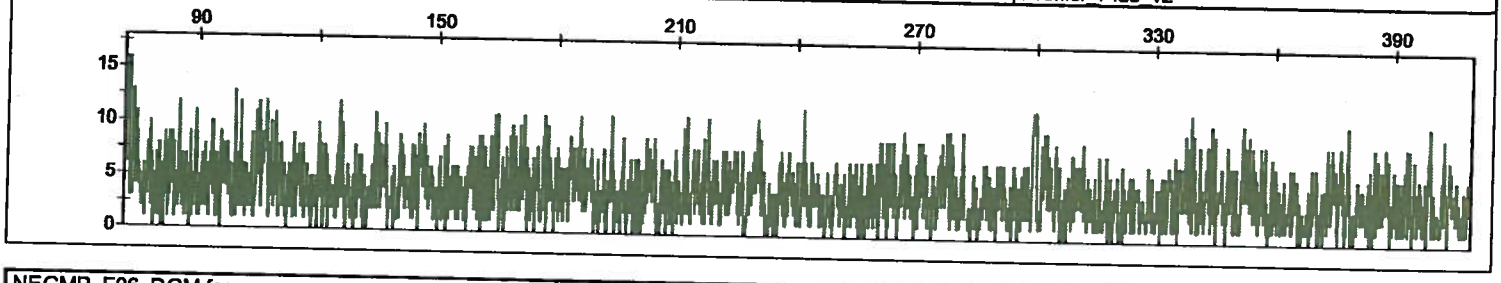
GeneMapper ID v3.2.1

ST010410DM

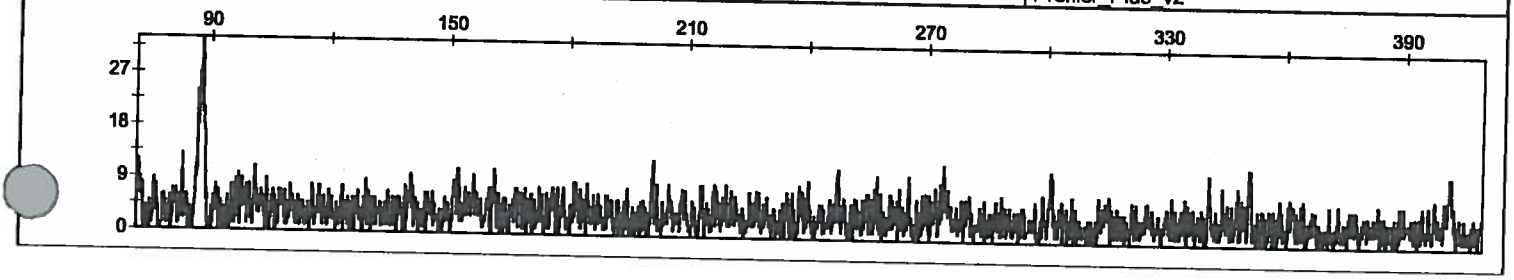
Sample File	Sample Name	Panel
GMP_F06_DGM.fsa	NEGMP	Profiler_Plus_v2



NEGMP_F06_DGM.fsa	NEGMP	Profiler_Plus_v2



NEGMP_F06_DGM.fsa	NEGMP	Profiler_Plus_v2

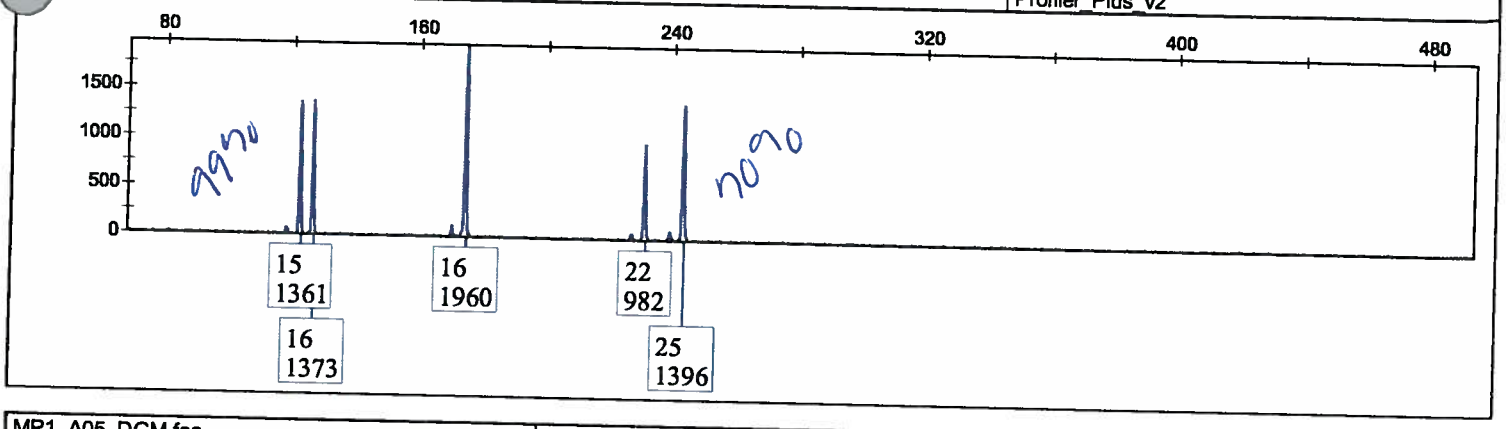


Applied Biosystems

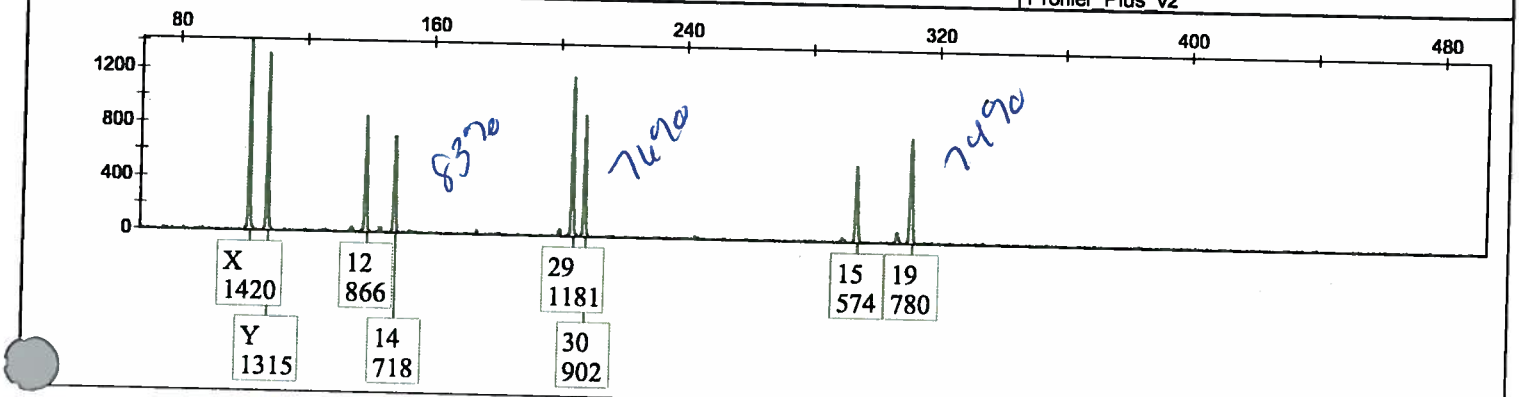
GeneMapper ID v3.2.1

ST010410DM

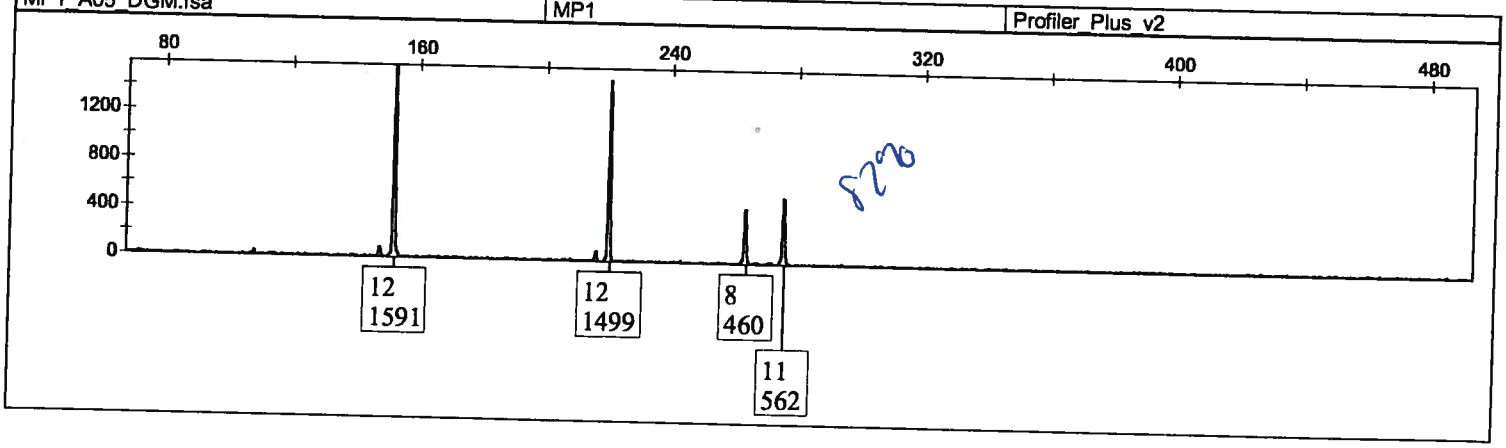
Sample File	Sample Name	Panel
1_A05_DGM.fsa	MP1	Profiler_Plus_v2



MP1_A05_DGM.fsa	MP1	Profiler_Plus_v2



MP1_A05_DGM.fsa	MP1	Profiler_Plus_v2



0.75

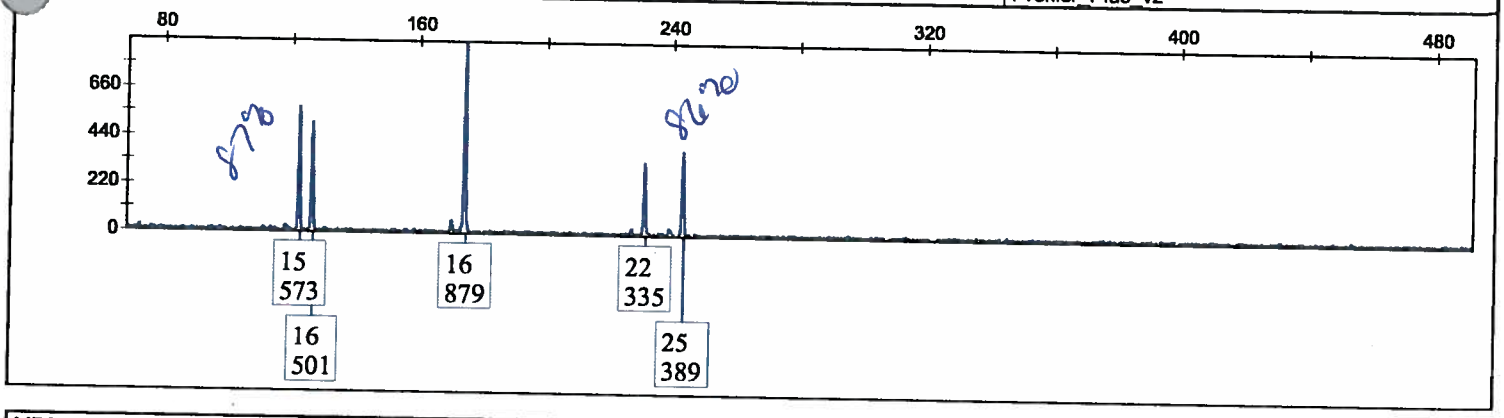
5sec
STCCC

Applied Biosystems

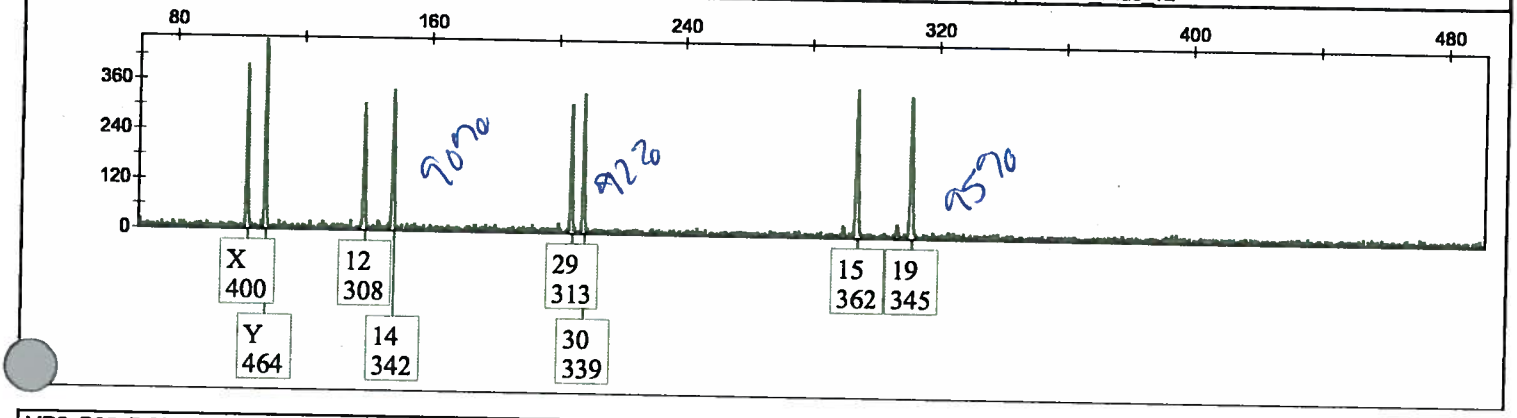
GeneMapper ID v3.2.1

ST010410DM

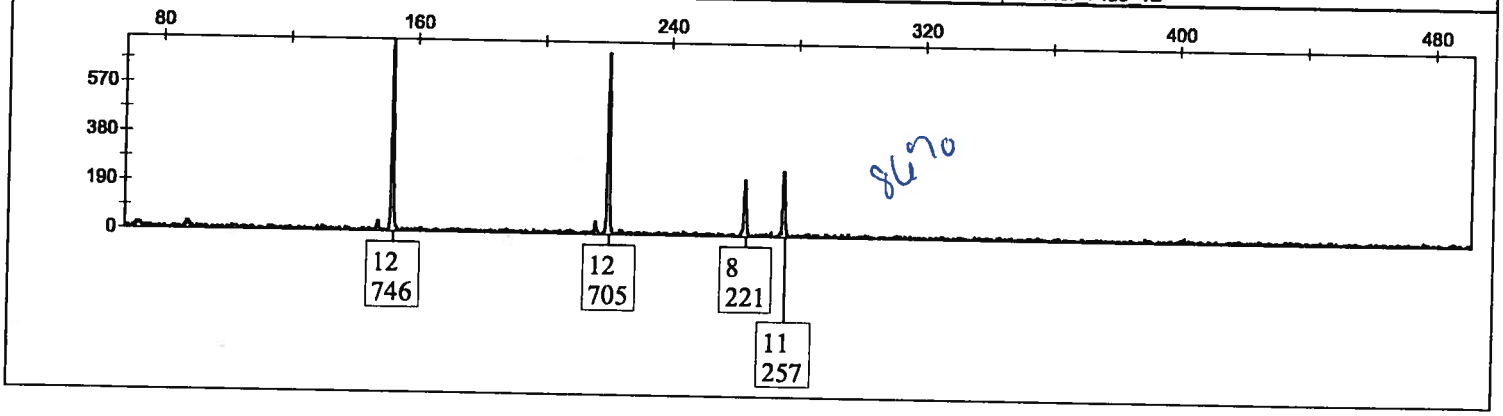
Sample File	Sample Name	Panel
P2_B05_DGM.fsa	MP2	Profiler_Plus_v2



Sample File	Sample Name	Panel
MP2_B05_DGM.fsa	MP2	Profiler_Plus_v2



Sample File	Sample Name	Panel
MP2_B05_DGM.fsa	MP2	Profiler_Plus_v2



0.375

5sec
STCCC

EXHIBIT J

7. Immediately chill the sample tubes for approximately 3 minutes in a PCR cooler tray
    a. Do not reheat samples, this may cause an increase in artifacts
8. Set up plate records on the Genetic Analyzer. Inject the samples for 2-15 seconds.
    a. Any reagent blanks will be run at the injection time of the highest corresponding sample and on the same instrument model. Each run will include a positive control, a negative control, and a ladder.
9. Analyze collected data using Genemapper ID and refer to Interpretation Section for additional information.
10. Each case folder will contain a printout of the complete sample list in Genemapper ID for each run, and the electropherograms for the positive control, negative control, and reagent blanks for that case.
    a. Only electropherograms used in the final interpretation (and the final electropherogram for a sample that will be re-extracted) are required to be in the case folder. However, documentation should be made as to why an injection was not used.
    b. If the interpreted data is in the next run then that should also be stated. If the interpreted data is in a later run, then that date should be stated. Lot numbers of CE reagents and instrument maintenance documents will be stored in the instrument log books. Since a complete ILS is required for the typing of a sample, it is optional for the ILS to be printed on the electropherogram.

Interpretation
None

Literature/Supporting Documentation
PowerPlex Fusion Technical Manual

CHAPTER 6 INTERPRETATION

General Guidelines

Several results are possible when conducting forensic casework analysis. These guidelines are in place to ensure that conclusions are scientifically supported by the analytical data with appropriate standards and controls. Interpretations are made as objectively as possible and as consistently from analyst to analyst as possible. Interpretation guidelines are based upon validation studies, literature, instrumentation and casework experience.

Three conclusions are generally possible:
1. inclusion (individual could have contributed to or been a source of the questioned profile)
2. exclusion (individual could not have contributed to or been a source of the questioned profile),

3. uninterpretable/inconclusive (the profile is not suitable for comparison for technical reasons)

Conclusions are determined by objective qualitative and quantitative evaluation of the entire DNA profile produced by the various loci tested.

DYS391 will be considered an information only locus in our laboratory and will not be used for interpretation and or statistical calculations. Lack of signal at DYS391 will not result in the profile being called "partial" and will not negate identity if all other loci meet the criteria. A result for DYS391 in the positive control is still required to ensure appropriate amplification.

At initial analysis of the data, all data above the analytical threshold should be assessed to determine if the sample is a mixture, if degradation/inhibition patterns exist, etc. At this time, inconclusive results for loci or the determination that the profile is uninterpretable should be documented to indicate exclusion of that locus or profile for interpretation. Inconclusive loci may result from, but are not limited to, the following causes:

- Insufficient amounts of template DNA which can result in observation of stochastic effects
- Degradation due to environmental or chemical influences
- Preferential amplification due to the presence of inhibitors or other factors that limit the amplification of larger fragments
- Differences in the amounts of DNA present in a sample from multiple donors
- Mixtures of an excessive number of donors

It should be noted, however, that it is acceptable for an inclusion or exclusion to be determined when one or more of the loci yield inconclusive results. A consistency statement will be based only on loci that yield interpretable results. In most single source samples, exclusion will be determined if only one locus produces exclusionary results, however there may be rare exceptions to this rule.

Considerations for comparison to known profiles include the possible loss of an allele due to preferential amplification, stochastic effects, mutation, presence of a very minor component, or other factors are believed to have likely occurred. In these cases, a locus or sample may be determined to be inconclusive for statistics for a particular individual.

Preliminary Evaluation of Data

The first step in data evaluation is to determine whether the results are of sufficient intensity/quality for interpretation purposes. The profile should be interpreted before comparison to reference samples.

Analytical thresholds

An analytical threshold defines the minimum height requirement at and above which detected peaks can be reliably distinguished from background noise. Data below the 75 RFU threshold is considered uninterpretable or inconclusive. For any given locus, the minimum analytical threshold for evaluating profiles is 75 RFU. Reagent blanks will be analyzed at the lowest (or lower than) threshold of its interpretable corresponding samples. For any given locus, except Amelogenin, off scale data cannot be used. Off scale data in other dye colors in the Amelogenin basepair region should be interpreted with caution and consideration given to the presence of pull up. All inclusions and exclusions must be supported by above threshold data.

If the minimum analytical threshold provides unsuitable data, the analyst, at his/her discretion may choose the following:

- re-amplify the sample with more template
- re-inject that sample for a longer approved injection time
- call the sample uninterpretable/inconclusive

If the maximum threshold (sample is deemed off-scale) is exceeded at any locus (excluding Amelogenin), the analyst may choose the following:

- inject the sample for not less than 2 seconds
- dilute the amplified product in TE buffer and add the diluted amplified product to the formamide/ CC5 ILS 500 mixture (example: 2ul amplicon in 6ul TE)
- call that locus inconclusive/uninterpretable. Data in other dye colors may be interpreted with caution and evaluated for possible pull up.
- re-amplify the sample with less template.

If the maximum threshold is exceeded for the size standard, the analyst may still use the data as long as pull-up, if present, does not interfere with data interpretation. When samples are re-amplified or re-injected, typically the amplification or injection providing the most interpretable information will be used for comparisons and interpretations. If both provide the same information, either may be used. If the number of alleles obtained is the same but the alleles are not concordant, the analyst will determine which profile to use prior to comparisons of reference samples.

Internal size standard and ladder evaluation

Internal size standards are critical in STR analysis and are run with every sample. The internal lane size standard is used to normalize injection-to-injection migration differences, thereby providing sizing precision within a set of capillary injections. The minimum threshold for analysis of the ILS is 50 RFU. To ensure alleles are assigned appropriately, confirm all 21 peaks are present (additional peaks may be present as long

as they do not interfere in the sample calls):
60,65,80,100,120,140,160,180,200,225,250,275,300,325,350,375,400,425,450,475,500.

If a SQ value of <1.0 is observed for a sample, the size standard should be examined to ensure that all size standard peaks are being properly called.

When interpreting results, genotypes are assigned to sample alleles by comparing their sizes to those obtained for the known alleles in the allelic ladders. Thus, a ladder must be present within each run. Each ladder used for analysis must have the appropriate alleles present for each locus (additional peaks may be present as long as they do not interfere in the sample calls) when analyzed (minimum of 75 RFU may be used). See PowerPlex Fusion Technical Manual for appropriate alleles in ladder. The ladders do not need to be printed for the paper file but the electronic data will be stored on the group drive in the run file.

Spectral

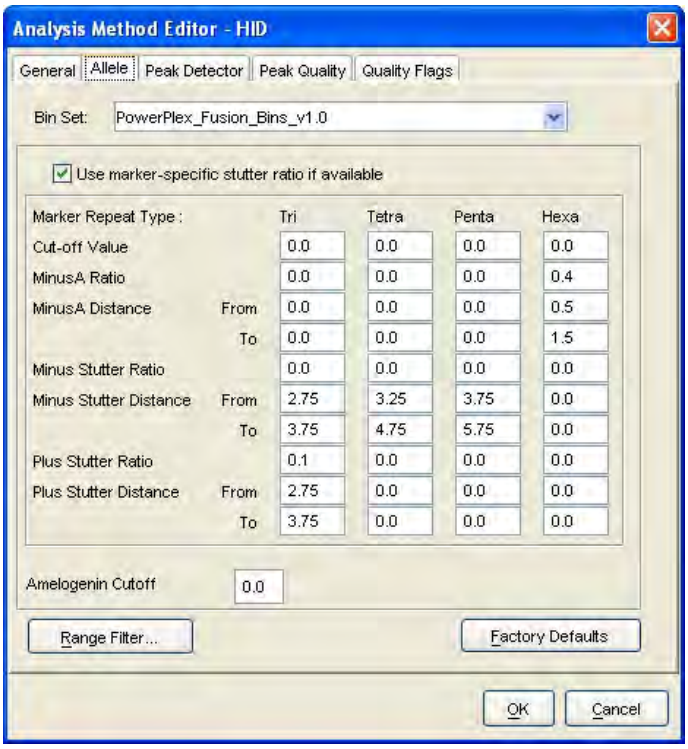
Multicomponent analysis is the process that separates the different fluorescent dye colors into distinctive spectral components. The five dyes used in the PowerPlex Fusion amplification kits are fluorescein, JOE, TMR-ET, CXR-ET, and CC5 ILS 500. Although each dye emits its maximum fluorescence at a different wavelength, there is some overlap in the emission spectra. The precise spectral overlap is measured by analyzing DNA fragments labeled with each of the dyes. These dye-labeled fragments are spectral standards. A new spectral should be run on an instrument following a planned maintenance when necessary, after parts (i.e., laser, CCD camera, etc.) are replaced or realigned, or as needed.

It is critical to select the correct G5 spectral for the PowerPlex 5-dye chemistry.

Analysis

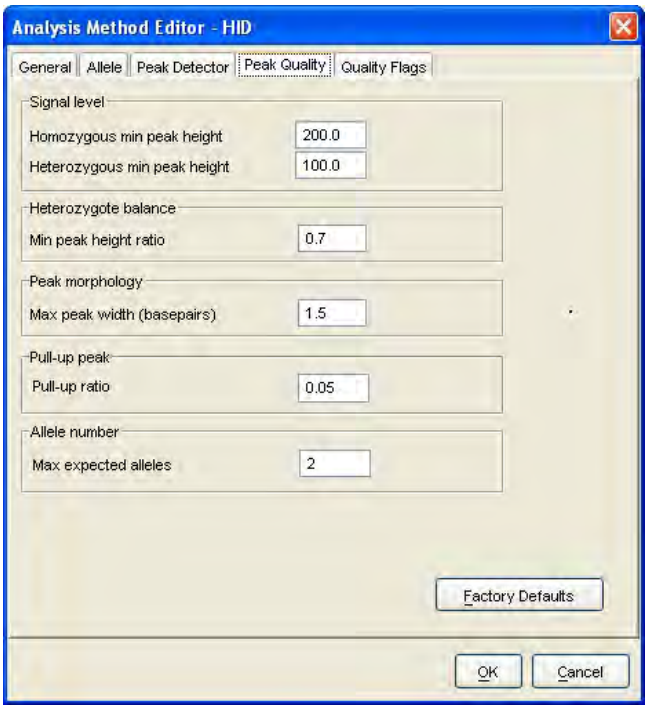
Samples from each run will be analyzed using GeneMapper ID v3.2.1. The following settings will be required for analysis. The parameters in the screenshots below should be used for all analysis. Exceptions to this rule are noted above the screenshot:

Parameters on the Allele Tab that can be adjusted at analyst discretion: None



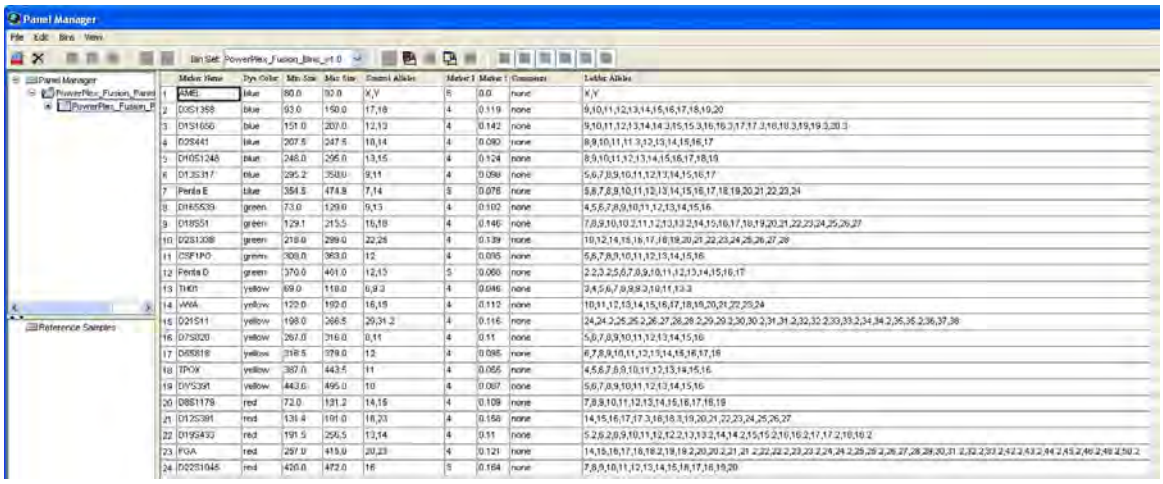
Parameters on the Peak Detector Tab that can be adjusted at analyst discretion: Ranges, Analysis or Sizing

Parameters on the Peak Quality Tab that can be adjusted at analyst discretion: All. These are flag settings and generally flags are not used in our analysis in a formalized way. If individual analysts would like to use flags for their purpose, they are free to do so.



Parameters on the Quality Flags Tab that can be adjusted at analyst discretion: All. These are flag settings and generally flags are not used in our analysis in a formalized way. If individual analysts would like to use flags for their purpose, they are free to do so.

Parameters in the Panel Manager that can be adjusted at analyst discretion: None



Controls

Controls are required to assess the effectiveness, accuracy and precision of the analytical procedures. Appropriate controls must be analyzed with each sample batch.

Appropriate controls may include, but are not limited to, reagent blanks, negative controls, and positive controls.

Reagent Blank

The reagent blank is a test for contamination of the extraction reagents. See section on investigating contaminations for more information.

Negative Amplification Control

The negative amplification control is a test for contamination during amplification set-up. See section on investigating contaminations for more information.

Positive Amplification Control

The positive amplification control tests for proper amplification of samples, as well as ensuring that GeneMapper ID™ v3.2.1 is working properly. A positive amplification control is included in the amplification kit. This control must exhibit the following typing results:

NOTE: Amelogenin may have OL allele due to minus A or pull up

Amel	D3S1358	D1S1656	D2S441	D10S1248	D13S317	Penta E	D16S539
X,Y	17,18	12,13	10,14	13,15	9,11	7,14	9,13

D18S51	D2S1338	CSF1PO	Penta D	TH01	vWA	D21S11	D7S820	D5S818
16,18	22,25	12,12	12,13	6,9.3	16,19	29,31.2	8,11	12,12

TPOX	DYS391	D8S1179	D12S391	D19S433	FGA	D22S1045
11,11	10	14,15	18,23	13,14	20,23	16,16

If the correct alleles are not achievable, re-amplification of the positive control and all samples in the batch will be necessary. If the positive control is off-scale, it will be re-injected for 2 seconds. If it is still off-scale, it may be used with Technical Leader approval.

Allele Identification

True alleles are defined as peaks that are clearly visible above baseline noise, cannot be determined to be caused by an artifact or extra peak, and are of a size and shape indicative of an allele. The following describes other types of information that may be detected.

Artifacts and Extra Peaks

Peaks other than target alleles may be detected on an electropherogram. These artifacts or anomalies occur routinely during STR analysis. It is important to attempt to identify

the cause of extra peaks. The analyst should use their training, knowledge, and skills to determine the likely cause of the artifact, and the analyst interpretation should be documented in the case record. Additional print outs of base pair sizing or raw data may be useful to help demonstrate the probable cause of an artifact.

An attempt should be made to rule out common causes of extra peaks such as spikes, pull up, excessive stutter, or other commonly encountered anomalies. All interpretations of artifacts must be made prior to comparison of the unknown profile to any reference profiles. Some signal that can be observed in the Powerplex Fusion chemistry are indicated in the manufacturer's user manual.

Spikes

Spikes are generally present in at least two colors and have the same data points. Confirmation of spikes may be confirmed in the raw data view of the software and removed from the interpretable profile.

Stutter

A stutter peak is a reproducible minor product peak shorter or longer than the corresponding main allele peak that is produced during amplification of STR loci. Stutter products may be caused by slippage of the DNA polymerase during amplification, probably due to out-of-alignment re-annealing of complementary target sequences during extension or out-of-alignment re-annealing of incomplete PCR products prior to extension. If a stutter peak exceeds the percent stutter associated with a locus, the analyst may choose to interpret that peak as excessive stutter and remove it as an allele designation. Like other data interpretations, this must occur prior to comparison to reference DNA samples. The stutter filter percentages for PowerPlex Fusion represent the manufacturer published values and are as follows:

Amel	D3S1358	D1S1656	D2S441	D10S1248	D13S317	Penta E	D16S539
0%	11.9%	14.2% / (N-2) 3.6%	9.2%	12.4%	9.8%	7.6%	10.2%

D18S51	D2S1338	CSF1PO	Penta D	TH01	vWA	D21S11	D7S820	D5S818
14.6%	13.9%	9.5%	6.8%	4.6%	11.2%	11.6%	11.0%	9.5%

TPOX	DYS391	D8S1179	D12S391	D19S433	FGA	D22S1045
5.5%	8.7%	10.9%	15.8%	11.0%	12.1%	(-)16.4%/(+) 8.6%

Minus A

The DNA polymerase used in STR/PCR amplification catalyzes the addition of a single nucleotide to the 3' ends of double stranded PCR products. This non-template addition results in a PCR product that is one base pair longer than the actual target DNA sequence. STR/PCR amplifications have been optimized to favor the "A" nucleotide addition. Incomplete "A" nucleotide addition may occur when too much amplification product is generated due to over addition of template DNA. Clear documentation will

exist in the case record regarding the analyst's interpretation of –A and agreement should exist between the analyst and the technical reviewer.

Pull-up

Pull-up is the result of the instrument's inability to separate colors (spectral overlap) used to fluorescently label STR products. Pull-up is observed as a peak beneath a peak or as an elevation of the baselines for any color. Pull-up is identified as a minor peak of similar base pair size as the true allele but in a different color, and interpretation is often assisted by utilizing the raw data view in the data analysis software. Pull-up can occur when too much template DNA has been added to the amplification mix or when a new spectral is needed. Samples with pull-up caused by over-amplification may be re-injected for a shorter period of time, diluted and re-run, or re-amplified with less DNA. Alternatively, in single source samples or mixtures when the pull-up is called "OL", the analyst may document the peak as pull-up and use the injection. Clear documentation will exist in the case record regarding the analyst's interpretation of pull up and agreement should exist between the analyst and the technical reviewer.

Microvariants

Microvariants are defined as alleles that contain an incomplete repeat unit. The designation of alleles containing an incomplete repeat unit should include the number of complete repeats, and separated by a decimal point, the number of base pairs in the incomplete repeat. If an analyst is unable to determine the size of a microvariant, it will be documented as undetermined. If an allele falls outside (shorter or longer) the ladder alleles at a locus, it will be designated as greater than or less than the appropriate ladder allele (i.e., >16 for CSF1PO) for CODIS entry.

Off ladder alleles must be verified by re-injection of the sample unless the off ladder allele is called consistently in two or more samples (reference or questioned samples). Off ladder alleles often require the use of the minimum allele frequency for statistical calculations.

Full profiles (Single Source)

An evidentiary sample may be considered to be from a single person if the number of observed alleles at each locus is no more than two (except in the rare event of a tri-allele) and the peak heights are balanced (≥ 60%) for heterozygous alleles. All loci must be evaluated in total when making determination of single source or mixed profile. If a sample has unbalanced peak heights (< 60%) with no other indication of a mixture, the sample can still be considered a single source for statistical purposes with the approval of the technical reviewer.

Partial profiles

Partial profiles are profiles (insufficient data at one or more loci) can result from degraded/inhibited template DNA or low concentrations of template DNA. The entirety

of the profile should be considered when determining whether a profile is a partial profile or not, and all individual locus interpretations must occur prior to comparing to the known reference samples in the case. Some profiles may contain too many contributors, or be of poor quality, to allow the profile to be used for interpretation. The profile should be designated as inconclusive and the analyst's reason for doing so shall be documented in the case record. This determination shall be agreed to by the technical reviewer and, if necessary in the case of dispute, agreed to by the technical leader. See below for more guidance on interpreting and reporting partial profiles.

Stochastic effects

Decreasing levels of template DNA may lead to stochastic effects which may under-represent one of the alleles in a locus. Using a minimum analytical threshold of 75 RFU, the following guidelines will be followed for interpreting data from low concentration samples:

Concentration	Single Source	Mixture with Major Component	Mixture with no Major Component
>0.3 ng	X	X	X
Between 0.0625 ng and 0.3 ng	X	Interpret loci from the major profile that contain heterozygous loci. The minor profile will be deemed uninterpretable.	The entire profile is uninterpretable
<0.0625 ng	May interpret heterozygous loci (>75 RFU) or designate entire profile as uninterpretable	The entire profile is uninterpretable	The entire profile is uninterpretable

NOTE: X indicates that this combination of criteria does not meet the minimum criteria for stochastic amplification and the special guidelines for stochastic amplification are not applicable. Interpret according to the standard interpretation guidelines.

The table above represents commonly encountered general guidelines. If a departure from the above guidelines is determined to be necessary after discussion between the analyst and technical reviewer, approval from the technical leader is necessary prior to issuance of a test report.

Mixtures

Samples from crime scene evidence may contain DNA from more than one individual. The entire profile should be used to determine if there is sufficient information to conclude that the sample contains DNA from more than one person. The analyst should be aware that mixtures can consist of full and/or partial profiles from multiple individuals, and a full profile from each component is not assumed due to potential dropout,

especially in low template samples. Since quantitation does not allow for the individual quantitation of each person in a mixture, the exact concentration of each component cannot be known.

Some common indicators of potential signs of mixed profiles are:

- The presence of greater than two alleles at a locus

- The presence of a peak at a stutter position that is significantly greater in percentage than what is typically observed in validation or single source samples

- Significantly unbalanced alleles for a heterozygous genotype (i.e. <60% PHR). The peak height ratio, or PHR, is defined as the height of the lower peak (in RFU) divided by the height of the higher peak in (RFU), expressed as a percentage.

  Factors causing peak height imbalances include, but are not limited to, the following:
  - degraded DNA
  - inhibitors
  - very low amounts of input DNA
  - SNPs, mutation, and other genetic anomalies (i.e., Down's syndrome, Klinefelters, XYY)
  - multiple components or mixed profile

Minimum number of contributors: If a mixture is present, prior to including an individual, the data should be examined carefully to determine the minimum number of contributors (2, 3, 4, etc.) to a mixture. The minimum number of contributors is determined by the analyst considering the allele pattern of the entire profile, requires agreement from the technical reviewer and, if necessary, disputes can be arbitrated by the technical leader.

Some considerations when the analyst is estimating the minimum number of contributors are:

1. Only alleles that are present above the analytical threshold (75 RFU) may be used for interpretation. Signal below the analytical threshold should not be used for interpretation either for inclusion, exclusion, or an estimation of the number of contributors.
2. Examples of some signal patterns that may be removed at the analyst discretion are pull up, elevated stutter, spikes, free dye signal, and PCR artifacts, among others. This signal, if removed as uninterpretable signal, should be documented as such in the case file. The analyst may use data sources such as the raw data to assist in arriving at an opinion that a signal is likely not a true allele that should be attributed to the profile.

3. After removing artifacts and signal that is not interpreted by the analyst as alleles, divide the number of alleles per locus and divide by two and round up, if necessary.
4. The analyst will document their interpretation of the minimum number of contributors in the case record and determine this prior to examining any reference samples in the case.

NOTE: if, after the above analysis is performed, the profile contains too many contributors (minimum number of contributors determined to be 4 or greater and a major component cannot be deconvoluted), this profile will be deemed inconclusive and not used for comparison to known reference samples or statistical analysis.

Mixture with Major/Minor Components

Specific genotype combinations and input DNA ratios of the samples contained in a mixture contribute to the complexity of resolving the genotypes of contributors to a mixed profile.

Whether a major component is discernable from a mixture is determined by the analyst considering the allele pattern of the entire profile, requires agreement from the technical reviewer and, if necessary, disputes can be arbitrated by the technical leader. Some factors that assist the analyst in determining a major component are:

- The analyst can determine which alleles are sourced from the major component at each interpretable locus
- Definition of Major Component: Where no minor allele is greater than 40% of the height of the shortest major peak in the same locus.
    - For example, if the allele that is determined to be from the major component is 1,000 RFU, no minor alleles should be greater than 400 RFU. This is not to be confused with a CODIS Eligible Profile (CEP) which is discussed in the context of CODIS uploads. (See section on CODIS for more information).

Prior to comparisons to reference samples, analysts will determine if a major component exists. If so, the major component profile will be documented in the case record.

Mixtures with indistinguishable contributors: An evidentiary sample should be considered a mixture with indistinguishable contributors when the major or minor contributors cannot be distinguished because of signal intensities or shared or masked alleles. Individuals may still be included or excluded as possible contributors assuming more than the minimum number of contributors does not exceed 3. If, after the analyst has corrected for artifacts and extra signal, the analyst interpretation is that the minimum number of contributors is 4 or more and a major component cannot be deduced, the profile will be identified as uninterpretable and no comparisons to knowns will be performed. This determination should be made by the analyst, agreed to by the

technical reviewer and, if necessary, disputes can be arbitrated by the technical leader. All loci in the profile should be considered when making this determination.

Comparisons to Known Reference Samples: Mixtures should be carefully evaluated to determine whether or not the reference profile of the individual being compared is consistent with being a component of the mixture. The totality of the profile including, but not limited to peak height ratios, homozygosity or heterozygosity of the individual, evidence of preferential amplification/ degradation/inhibition, should be considered.

Interpretation Guidelines for Very Low Level Profiles

An interpretation that includes an individual as a possible contributor to a questioned sample may be made even if evidence of locus or allele dropout exists. Some indicators, among others, that may lead the analyst to render a profile too weak for comparison are low RFU values, preponderance of homozygous alleles, and entire loci with no alleles above threshold. However, the following rules should be followed:

1. The analyst must determine which loci are interpretable and uninterpretable, or inconclusive, prior to comparing the profile to known reference samples.
2. Loci marked as inconclusive are not to be used in the comparison, for inclusionary or exclusionary purposes, to known reference samples. Once the locus is marked as inconclusive, it is to be ignored for comparison purposes.
3. A minimum number of 4 autosomal loci (in the Powerplex Fusion chemistry) should be determined to be interpretable in order to be suitable for comparison purposes. If only 3 loci or less are determined to be interpretable, the profile will be marked inconclusive. The relative weight or significance of the inclusion will be reflected in the statistical weight assigned to the profile.
4. Depending on the totality of the circumstances surrounding a profile, the analyst has the discretion of marking any profile inconclusive even if more than 3 loci are deemed interpretable. This determination shall be agreed to by the technical reviewer and, in the case of dispute, by the technical leader.
5. Any deviation from these guidelines regarding low level samples should be clearly stated by the analyst in the case record, agreed to by the technical reviewer or, in the case of dispute, agreed to by the technical leader.

CHAPTER 7 STATISTICS

Once an individual cannot be excluded as a possible component of a sample, the weight of the significance of the inclusion is determined statistically. Entire profiles or individual loci in profiles that are determined to be inconclusive are not included in the statistical analysis. Because it is the significance of the match that is important, only the interpretable loci where the person is included can be taken into account in estimating significance. DYS391 and Amelogenin will not be used for statistical purposes since they are not autosomal loci.

Related Documents
None

EXHIBIT K

Carter Pereira, Claudine

From: Pamela Bordner <PBordner@ascld-lab.org>
Sent: Tuesday, April 12, 2016 12:34 PM
To: Carter Pereira, Claudine
Cc: Laurel Farrell; 'Coffman, David'; Tara Dolin
Subject: ASCLD/LAB Board decision on allegations
Attachments: 160412-BrowardSO-notification of Board decision.pdf; 160412-BrowardSO-Investigative Report-Board Reveiwed.pdf

Director Carter-Pereira,

Attached to this email you will find a formal notice of the ASCLD/LAB Board of Directors decisions related to our investigation of allegations submitted by Tiffany Roy. A copy of the Investigative Report is also attached. As the letter states, you have until May 12, 2016 to provide me with the laboratory's corrective action plans related to the sustained allegations or alternatively, you can provide me with a request for a formal Board review. The current ASCLD/LAB policy on "Allegations Related to Accredited Laboratories and Their Employees" can be found here https://ascld-lab.qualtrax.com/Default.aspx?ID=1446

Please acknowledge receipt of this notification.

Best Regards,
Pam
Pam Bordner, Executive Director
ASCLD/LAB
919-773-2600
919-773-2602 FAX
pbordner@ascld-lab.org
www.ascld-lab.org
Quality Matters ®

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AMERICAN SOCIETY OF CRIME LABORATORY DIRECTORS
LABORATORY ACCREDITATION BOARD

April 12, 2016

Claudine Carter-Pereira
Crime Laboratory Director
Broward Sheriff's Office Crime Laboratory
201 S.E. 6th Street, North Wing - Room 1799
Ft. Lauderdale, FL 33301

Director Carter-Pereira:

On March 25, 2016, The ASCLD/LAB Board of Directors (Board) considered the investigative report prepared by ASCLD/LAB Staff Assessor Melissa Smrz. The report documented the results of our investigation of the allegations submitted to ASCLD/LAB by Tiffany Roy on or about October 2, 2015.

For the purposes of our investigation, we identified the allegations as Allegation 1, Allegation 2 and Allegation 3. Specifically, we summarized the allegations in the following way:

Allegation 1: Inappropriate consideration of submitted known reference samples to determine loci that will be selected for statistical calculation purposes.

Allegation 2: Inappropriate use of the statistic known as the Combined Probability of Inclusion (hereafter referred to as the 'CPI') to calculate statistical significance of occurrence of genetic profiles when allelic dropout is known and/or is suspected to have occurred.

Allegation 3: Use of the FBI population database to calculate statistics.

After reviewing and considering the allegations, the results of our investigation and input from our Technical Advisory Committee, the Board reached the following conclusions and took the following actions:

<u>Allegation 1 Conclusion</u>
The Board accepted the investigative report and input from the Technical Advisory Committee and determined that there was sufficient objective evidence to sustain the allegation. The Board has directed that laboratory management is to take appropriate corrective action to resolve the nonconformity to ISO/IEC 17025:2005 requirement 5.4.1.

<u>Allegation 2 Conclusion</u>
The Board accepted the investigative report and input from the Technical Advisory Committee and determined that there was sufficient objective evidence to sustain the allegation. The Board has directed that laboratory management is to take appropriate corrective action to resolve the nonconformity to ISO/IEC 17025:2005 requirement 5.4.1.

Allegation 3 Conclusion

The Board accepted the investigative report and determined that the laboratory has taken appropriate action to address the discrepancies in the FBI STR Population Data as it relates to the cited case.

In accordance with the ASCLD/LAB policy "Allegations Concerning Accredited Laboratories and Their Employees," you may accept the conclusions and directions of the Board or you may disagree with the conclusions and directions of the Board and request a follow-up formal review by the Board. You are being provided with a copy of the Board reviewed investigative report. You have until close of business on May 12, 2016, to a) provide me with the laboratory's corrective action plans related to Allegations 1 & 2 or b) notify me of your request for a formal Board review. Please review Sections 8 & 9 of the enclosed allegations policy for further information.

During the investigation into the allegations listed above, Ms. Smrz identified an issue with the laboratory's technical procedures for statistical calculations as stated below.

> It appears that the laboratory's definition for the CPI calculation it performs is not consistent with the calculation that is stated in the test report. The calculation stated in the procedure is defined **as the odds of the known reference sample profile being a contributor to the evidentiary profile.** The calculation stated in the test report is defined **as the odds of randomly selecting an unrelated individual consistent with the evidentiary sample, based upon the interpretation of the profiles from the named individuals.** These statements appear to reflect two different types of calculations. Further, from the calculations observed in the cited case, it is unclear as to how the probability was converted to odds using a ratio, as described by Weir and the laboratory's unknown source definitions/calculations.

The Board has directed that laboratory management appropriately address this issue in conjunction with the corrective actions taken to resolve the nonconformities cited in the allegation conclusions.

I look forward to working with you to bring this matter to an appropriate resolution. Please contact me if you have any questions.

Sincerely,

Pamela L. Bordner

Pamela L. Bordner
Executive Director

cc: David Coffman, ASCLD/LAB Board Chair
Laurel Farrell, ASCLD/LAB Senior Accreditation Program Manager

American Society for Crime Laboratory Directors/ Laboratory Accreditation Board

Board Reviewed

INVESTIGATIVE REPORT - ALLEGATIONS

Report Date: February 21, 2016
Laboratory: Broward Sheriff's Office Crime Laboratory
International Certificate Number: ALI -013-T
Investigator: Melissa Anne Smrz – Lead Assessor ASCLD-LAB

INTRODUCTION

On October 2, 2015, the ASCLD/LAB Executive Director received a complaint regarding an allegation of using inappropriate procedures and a misapplication of statistical procedure (Attachments 1 and 1a) by the Broward Sheriff's Office Crime Laboratory (hereafter referred to as 'the laboratory'), from private DNA consultant Tiffany Roy (hereafter referred to as 'complainant'). The ASCLD/LAB Board Chair reviewed the allegation and determined on October 29, 2015 the complaint was within ASCLD/LAB's purview. The laboratory was given the opportunity to respond to the allegation and a response (Attachment 2) was received by ASCLD/LAB on November 17, 2015 (letter dated November 6, 2015). On November 19, 2015, ASCLD/LAB's Staff/Lead Assessor Melissa Anne Smrz was assigned to proceed with an investigation.

The complainant was requested to provide additional details to support the complaint allegation and did so on several occasions via email (Attachments 3, 3a, and 3b). The laboratory was provided the additional complaint allegation details on
November 20, 2015 (Attachment 4), and was given an additional opportunity to respond. The laboratory responded to the additional complaint allegation details on December 28, 2015 (Attachment 5 with referenced supporting documentation).

After a review of the records, Ms. Smrz requested technical assistance from a DNA technical expert on approximately January 6, 2016. On January 25, 2016, Dr. Robin Cotton was approved to participate in the technical aspects of the investigation. Additional information was provided by the laboratory via telephonic interview and email, as requested, between February 5 and 19, 2016. Per the investigator's request, the laboratory provided a summary and explanatory memo dated February 19, 2016, which provided answers to questions asked during the cited February time frame and a summary of a February 17, 2016 telephone conference call with the investigator (Attachment 7-see Comment 1).

OVERVIEW OF LABORATORY

The Broward Sheriff's Office Crime Laboratory is a local government laboratory which provides services primarily to the County of Broward (Florida) and, in some instances, surrounding jurisdictions. The laboratory is located at 201 S.E. 6th Street, Ft. Lauderdale, Florida, and is headed by Director Claudine Carter-Pereira. The laboratory was first granted accreditation under the ASCLD/LAB-*International* program in 2005 and was re-accredited in 2010 and 2015.

OVERVIEW OF ALLEGATIONS

In correspondences received by ASCLD/LAB on various dates between October 2, 2015 and January 24, 2015, the complainant alleged inappropriate procedures by the laboratory's DNA unit to be based on the following:

1. Inappropriate consideration of submitted known reference samples to determine loci that will be selected for statistical calculation purposes.
2. Inappropriate use of the statistic known as the Combined Probability of Inclusion (hereafter referred to as the 'CPI') to calculate statistical significance of occurrence of genetic profiles when allelic dropout is known and/or is suspected to have occurred.
3. Use of the FBI population database to calculate statistics.

SCOPE OF REVIEW

The investigation was conducted to obtain data, facts, and opinions that could fairly and objectively assess the validity of the allegation lodged against the laboratory. The investigator reviewed the complaint, the laboratory responses, associated documents, records and statements from interviews conducted with the complainant, laboratory personnel, and Dr. Cotton, the DNA technical expert appointed by ASCLD/LAB, to gather objective information and evaluate the allegation.

It should be noted that due to the number of emails and submissions of information, the responses from the complainant and the laboratory will be summarized with references to attachments which contain the supporting/objective information to verify the responses, or to reference documents cited in the responses.

The allegation was not directly associated with a specific accreditation requirement; however, the complaint that the laboratory failed to follow appropriate and acceptable procedures, and/or used inappropriate procedures which could be biased and overstate the significance of occurrence of a DNA profile could be associated with the following accreditation requirement:

ISO/IEC 17025:2005 - General Requirements for the Competence of Testing and Calibration Laboratories, clause 5.4.1:

"The laboratory shall use appropriate methods and procedures for all tests and/or calibrations within its scope. These include sampling, handling, transport, storage and preparation of items to be tested and/or calibrated, and, where appropriate, an estimation of the measurement uncertainty as well as statistical techniques for analysis of test and/or calibration data."

In addition, the complainant made reference to, and the laboratory included in its response to ASCLD/LAB, several guidelines contained in the 2010 SWGDAM Interpretation Guidelines for Autosomal STR Typing by Forensic DNA Testing Laboratories (2010, www.fbi.gov, hereafter referred to the SWGDAM Guidelines) (Attachment 9), which include guidance pertaining to the interpretation of DNA mixture results. It is understood that this guidelines document is not under the purview of the accreditation body; however, the laboratory has included a number of these guidelines in its technical procedures. Given these inclusions in the laboratory's management system, the complaint that the laboratory failed to follow certain SWGDAM Guidelines could be associated with the following accreditation requirement:

ISO/IEC 17025:2005 - General Requirements for the Competence of Testing and Calibration Laboratories, clause 4.2.1:

The laboratory shall establish, implement and maintain a management system appropriate to the scope of its activities. The laboratory shall document its policies, systems, programmes, procedures and instructions to the extent necessary to assure the quality of the test and/or calibration results. The system's documentation shall be communicated to, understood by, available to, and implemented by the appropriate personnel.

INVESTIGATIVE RESULTS
BACKGROUND:

DNA Terms

The term "rfu" is an abbreviation for 'relative fluorescence units' and a measure of the signal strength [i.e. florescence], detected from DNA fragments which is proportional to the amount of DNA present. The peak height or rfu depends on the amount of DNA being analyzed. When the amount of DNA is very low, then it can be difficult to separate a true low-level rfu peak from signal noise or other technical artifacts. As a result, many forensic DNA laboratories set minimum rfu peak-height levels i.e. *analytical threshold* to distinguish a true low level peak from noise. Only peaks above the analytical threshold are considered 'true' peaks and not noise or an artifact. Due to the inherent nature of PCR amplification of low levels of DNA, results may contain dramatic peak height imbalance and allele drop-out, i.e., where only one allele (e.g., 18) is seen from a heterozygous pair (e.g., 17, 18).

The term "*stochastic threshold"* is the rfu value that, when exceeded by a single allelic peak in a single source sample, the DNA analyst can be confident that the sister peak of a heterozygous pair would be detected (i.e. would be above the analytical threshold). The SWGDAM Guidelines address stochastic threshold in a number of sections which are relevant to and have been cited in the complaint allegation and the laboratory's response. These are specified in the applicable sections of the investigative report.

The term 'partial profile' is a DNA profile for which DNA typing results may not be obtained at all loci (*or may not display all alleles at some loci – added by investigator)* for a given evidentiary sample (e.g., due to DNA degradation, inhibition of amplification and/or low-template quantity)" (from SWGDAM Guidelines, 2010, Section 3.6.2).

The term 'Combined Probability of Inclusion (CPI) is a statistical calculation which "is typically applied to all alleles detected in a mixture, subject to the limitations described in section 4.6.3 of the SWGDAM Guidelines. The Probability of Inclusion (PI) is calculated as the (sum of allele frequencies)^2 for each locus. The CPI is the product of the individual locus PIs: CPI = PI_1 * PI_2 * ... * PI_N." (from SWGDAM Guidelines, Sections 5.3.1, 5.3.2 and 5.3.5).

The case of State of Florida vs. John Paul Spencer, laboratory number 14-03320, is a 2014 case involving several DNA profiles, one being a partial mixed DNA profile recovered from the handle of a knife (Item 14). The DNA mixture results were compared to known reference sample profiles from two individuals, one being the defendant, and calculations to assess the statistical significance of occurrence of the evidentiary genetic profile were performed using the CPI. Sample information worksheets and electropherograms (Attachment 10) and the test report, allele 'call' sheets and the statistical calculation form (Attachment 11) were provided and reviewed as part of the investigation. The transcript of testimony given by the reporting analyst during a deposition (Attachment 17) was also provided and reviewed as part of the investigation.

Allegations, Responses and Objective Evidence:

The allegation consists of three primary issues that rose from the complainant's review of the specific case, although she believes that the issues cited exist in other cases she has reviewed/is reviewing from this laboratory. Two of the issues are related, but have been separated in this report as Allegation 1 and Allegation 2.

ALLEGATION 1:

The complainant alleges that the laboratory is inappropriately considering the known DNA profile results of submitted reference standards prior to determining which genetic loci/allele results will be used to calculated the statistical significance of occurrence of the genetic profile obtained in evidentiary samples. The complainant alleges that the laboratory's approach does not ensure that all possible allelic information in the sample is fully represented, which is necessary for the CPI to be appropriately applied. The laboratory only requires that the full contribution/representation of any submitted known standard(s) used for comparison is present when using the CPI. The complainant states that this constitutes contextual bias and the calculation can overstate the significance of the occurrence of the profile when the CPI method of calculating statistical significance is used with complex mixtures exhibiting allelic dropout (See Allegation 2).

The complainant states that this practice is in direct conflict with the following SWGDAM Guidelines, section 3.6, Comparison of DNA Typing Results:

"3.6.1: The laboratory must establish guidelines to ensure that, to the extent possible, DNA typing results from evidentiary samples **are interpreted before** comparison with any known samples, **other than those of assumed contributors**." (emphasis added)

"3.6.2.1: For partial profiles, **the determination** of which alleles/loci are suitable for comparison **and statistical analysis** should be made **prior to comparison to the known profiles.**" (emphasis added)

The complainant opines that:

The SWGDAM standard states that the determination of which loci will be used for statistical analysis is made before the analyst even looks at the known standards in the case. "This is done by examining the profile and determining if deconvolution is possible. If the profile cannot be deconvoluted into major and minor profiles, the profile must be examined to determine if all the information is full(y) represented at each location. This is done by assessing the peak heights of the alleles present in the DNA mixture to see if they are all above the stochastic threshold determined by the laboratory. If a determination as to the number of contributors can be made, allele information that is below the stochastic threshold may still be used. If not, locations with alleles below the laboratories (sic) stochastic threshold must be excluded from the statistical calculation. All this determination takes place BEFORE the examination of known profiles is made." (Attachments 3 and 3a)

Laboratory's Summary Response:

In response to the allegation that it does not follow SWGDAM Guideline 3.6.1, the laboratory responded that it analyzes and interprets DNA results from evidentiary samples prior to comparing the results to those from any reference samples, and has procedures requiring this step (Attachment 12). Alleles evaluated as 'conclusive' and warranting further consideration are those which meet the laboratory's analytical threshold of 400 rfu, and the laboratory's 'homozygote' threshold for single allelic loci of 750 rfu. The laboratory does not use peak height ratios or a stochastic threshold (See Allegation 2). The laboratory then moves on to analyze and interpret the DNA profiles results from the known reference samples. The next step is to perform the comparison(s) of the evidentiary profile results to those of the known reference samples. The laboratory stated that it includes SWGDAM Guidelines 3.6.2.2, 3.6.3 and 3.6.4 in its technical procedures as they pertain to interpreting and comparing evidentiary and known samples for the purposes of inclusion, exclusion and inconclusive results (Attachments 13, 14 and 15). Once an association is made, the analyst then further evaluates the evidentiary profiles to determine which loci have the full contribution of the known reference sample(s) and performs statistical calculations based upon that evaluation and determination. (Attachment 16).

In response to the allegation that it does not follow SWGDAM Guideline 3.6.2.1, the laboratory confirmed that it does not follow the latter part of the guideline which pertains to using the known reference sample profile to assist in determining which loci/alleles will be used for **statistical** calculation purposes. It is the laboratory's opinion that calculating the statistics solely on evidentiary profile information without considering the known reference sample profiles may incorrectly convey the statistical significance of any resulting association that may occur. The laboratory believes that it would be in potential conflict with ISO/IEC17025 (2005), Clause 5.10.1 and Clause 5.10.3.5 of the 2011 ASCLD/LAB-*International* Supplemental Requirements for the Accreditation of Forensic Science Testing Laboratories (REFERENCE 18) if it were to follow SWGDAM Guideline 3.6.2.1, in that the statistical significance calculated for an evidentiary sample may not correctly reflect the statistical significance of any association made to submitted known reference samples. This concern continues into the laboratory's response to Allegation 2.
(See Attachment 7, pages 4-5)

Objective Evidence Reviewed:

ATTACHMENT 12 - DNA Unit – Analytical Methods Manual, Section 24, STR Results Table, page 1, second paragraph (highlighted) – requires analysts to evaluate evidentiary samples for interpretation purposes prior to comparison to known reference samples.

ATTACHMENT 6 – DNA Unit – Analytical Methods Manual, Section 21, Analysis of STR Data-Control Samples, page 4 (highlighted) - defines the analytical and homozygote threshold rfus.

ATTACHMENT 14 - DNA Unit – Analytical Methods Manual, Section 26, Interpretations of STR Results: Non-Intimate Mixture Samples, pages 3 – 4 – defines procedures for comparing partial DNA mixture results or DNA results in which there is no major/minor contributor distinction

ATTACHMENT 15 - DNA Unit – Analytical Methods Manual, Section 28, Interpretations of STR Results – Inconclusive Results, first two paragraphs (highlighted) – defines threshold for single allele loci and instructions for determining and recording inconclusive alleles and loci

ATTACHMENT 16 - DNA Unit – Analytical Methods Manual, Section 29, Statistical Evaluations of STR Results and CODIS, pages 1, paragraph 4; page 3, Section B and Pages 3-4, Section B 2 (highlighted) – states that "…for CPI calculations, loci where full contribution is established from all compared donors will be included in the statistical calculations."

ATTACHMENTs 10, 11 and 7: sample worksheets and GeneMapperID-X electropherograms/printouts, including that for sample 14, and the test report, allele call sheets, and statistical calculation results (11) from the examination record– the technical expert and the investigator independently reviewed the electropherograms and the allele call sheets and found them to be consistent with each other and compliant with the laboratory's technical procedures for evaluating alleles using the established thresholds and for analyzing the evidentiary samples prior to analyzing the known reference samples. The technical expert and the investigator also independently reviewed the statistical calculation results and found them to be consistent with having followed the laboratory's procedure to include only loci for which the profiles of the known reference samples are fully observed/established, with one exception. The analyst did not include the locus D19S433 in the statistical calculations. When questioned about this, the laboratory advised that it did not have the allele frequencies of this locus for one of the Hispanic populations it typically includes in its statistical evaluations. Therefore, this locus was not included in the statistical calculations for any population group.

ATTACHMENT 17 - Deposition of laboratory analyst Chris Comar, 8/27/2014, page 13, line 18 through page 14, line 7, and pages 24- 25 –analyst explains he first analyzes the evidentiary sample and selects loci based on the analytical and 'homozygote' thresholds and records these on the chart. In this case, because of the type of item (a knife handle), he made no assumptions of the number of contributors. He compared the known reference samples and then determined which loci had the full contribution of both known reference samples and used those loci for the statistical calculations.

Technical Expert's Response:

The laboratory is analyzing the profiles with Gene Mapper and presumably evaluating the evidentiary profiles prior to the analysis of the known reference samples, based upon the dates on the Gene MapperID-X electropherograms/printouts. The dates on the electropherograms for the evidentiary samples (5.16.2014) and known reference samples (5/20/2014) are different. The Table of Alleles (laboratory Document Control Number CLSTR-14A) is a single document with the same date (5/20/14). It is unclear whether the evidentiary samples are evaluated before the known reference samples, based on the examination records provided. The documented procedure requires this step, and the analyst explained this practice in detail during his deposition testimony. In the review of the statistical calculations, it is clear that the laboratory used the known reference sample profile to further reduce the alleles/loci that were used for the statistical calculations, with one exception, which was later clarified by the laboratory. The laboratory did not add alleles/loci for statistical consideration based upon the comparison to the known reference samples.

The appropriateness of the laboratory's practice will be discussed as part of Allegation 2.

SUMMARY/DETERMINATION for ALLEGATION 1:

It was determined that there is insufficient evidence to prove the laboratory's procedure allows analysts to evaluate the genetic profiles of submitted known reference samples prior to making decisions about which DNA results from evidentiary samples are suitable for comparison to the known reference samples. The laboratory provided evidence that ***indicates*** *but does not* ***verify*** *that the evaluation of the evidentiary DNA profile results occurs prior to the evaluation of the known reference samples.*

It was determined that there is sufficient evidence that the laboratory's procedure requires/allows analysts to compare the genetic profiles of submitted known reference samples to those of the evidentiary samples prior to making decisions on which loci will be used to perform statistical significance calculations. The laboratory's management system does not include 2010 SWGDAM Guideline 3.6.2.1, which recommends that loci/alleles in the evidentiary sample be selected for statistical calculation purposes prior to comparison to the known reference samples.

The appropriateness of this part of the laboratory's procedure will be discussed in the next section pertaining to Allegation 2.

ALLEGATION 2:

The complainant alleges that the laboratory is improperly using the CPI calculation on genetic loci in which allelic dropout is occurring, when no assumptions are being made about the contributors (as in cases of defined intimate samples or with other expectations of known DNA being present). In the case cited as part of this complaint, the evidentiary profile is a mixed DNA profile with evidence of allelic dropout.

The complainant explains that:

"Allelic peaks below the laboratory's analytical and 'homozygote' thresholds and low level peaks indicate drop out. The CPI calculation is not just interested in the suspected known profiles peaks being fully represented, it relies on all possible contributors being represented and calculations for all possible allele combinations. The CPI is not suitable in situations where all the known profile alleles are fully represented but other non-attributable alleles which may be dropping out. The complainant alleges that this approach shows deep bias and results in a gross overestimation of statistical weight in low level, partial profiles." (3, 3a, 3b).

The complainant also opines that the laboratory's 'homozygote' threshold is being used as a stochastic threshold, contrary to the laboratory's response. It should be noted that the complainant was made aware of the laboratory's 12/28/15 response to ASCLD/LAB via the discovery process for the cited case (email record, 1/25/2016).

In the cited case, the complainant states that:

> "the profile should have been examined for alleles below the stochastic threshold, and loci where alleles were below the stochastic threshold should have been immediately unsuitable for statistical analysis. This was not done. Instead, even though the profile had alleles below the stochastic threshold all loci except **one**, calculations were performed at **five** loci ..." (3 and 3a).

Upon request of the investigator, the complainant later re-evaluated the electropherograms for sample 14, the results of which are as follows:

> "The D3 and vWA loci are the only ones, in my opinion, that do not exhibit visible signs of allelic dropout. But, I would decline to use them because there is drop out at the D19 locus, which is smaller than the vWA locus, and because there is visible drop out as early in the 120 mark, which would fall in the middle of the reported alleles at the D3 locus (16 allele @126) I would decline to use them all. Given the early evidence of allele drop out at ~120 and the rest of the obvious drop out in the profile, the likelihood of drop out at every locus is either obvious or highly likely, therefore the literature says a CPI calculation is not suitable for them, and the profile as a whole."

The complainant alleges the lab is non-compliant with SWGDAM Guideline 4.6.3 in this case because the laboratory used the CPI to calculate the statistical significance of occurrence at loci with some alleles which were below the laboratory's homozygote/single allele threshold of 750 rfu and/or with evidence of allelic dropout. SWGDAM Guideline 4.6.3 states:

> *"4.6.3: When using CPE/CPI (with no assumptions of number of contributors) to calculate the probability that a randomly selected person would be excluded/included as a contributor to the mixture,* ***loci with alleles below the stochastic threshold may not be used for statistical purposes to support an inclusion. In these instances, the potential for allelic dropout raises the possibility of contributors having genotypes not encompassed by the interpreted alleles.****"*

Laboratory's Summary Response:

In response to the allegation that the laboratory is using DNA data that is below a stochastic threshold, the laboratory stated that it does not use a stochastic threshold and does not use peak height ratio values to establish the presence or inclusion of a possible donor in a single source or mixture sample. Because the laboratory does not use a stochastic threshold based upon peak height, in order to meet SWGDAM Guidelines 3.2 and 3.2.1, the laboratory defers to SWGDAM Guideline 3.2.2 (which follows 3.2 and 3.2.1):

"3.2. Application of Peak Height Thresholds to Allelic Peaks

Amplification of low-level DNA samples may be subject to stochastic effects, where two alleles at a heterozygous locus exhibit considerably different peak heights (i.e., peak height ratio generally <60%) or an allele fails to amplify to a detectable level (i.e., allelic dropout). Stochastic effects within an amplification may affect one or more loci irrespective of allele size. Such low-level samples exhibit peak heights within a given range which is dependent on quantitation system, amplification kit and detection instrumentation. A threshold value can be applied to alert the DNA analyst that all of the DNA typing information may not have been detected for a given sample. This threshold, referred to as a stochastic threshold, is defined as the value above which it is reasonable to assume that allelic dropout has not occurred within a single-source sample. The application of a stochastic threshold to the interpretation of mixtures should take into account the additive effects of potential allele sharing.

3.2.1. The laboratory establishes a stochastic threshold based on empirical data derived within the laboratory and specific to the quantitation and amplification systems (e.g., kits) and the detection instrumentation used. It is noted that a stochastic threshold may be established by assessing peak height ratios across multiple loci in dilution series of DNA amplified in replicate. The RFU value above which it is reasonable to assume that, at a given locus, allelic dropout of a sister allele has not occurred constitutes a stochastic threshold.

3.2.2. If a stochastic threshold based on peak height is not used in the evaluation of DNA typing results, the laboratory must establish alternative criteria (e.g., quantitation values or use of a probabilistic genotype approach) for addressing potential stochastic amplification. The criteria must be supported by empirical data and internal validation and must be documented in the standard operating procedures."

The laboratory conducted a quantitation cut-off study and validation of a homozygote threshold. From these studies, the laboratory established two thresholds for interpreting DNA results. One is defined as an 'allele interpretation threshold' (analytical threshold) and is set at 400 rfus. Any peak below this level is not marked by the genetic analyzer software. The second is defined as a 'homozygote interpretation threshold' which is 750 rfus for any locus at which a single allele is present. Neither threshold is used as a stochastic threshold. Summaries of the two studies were provided and reviewed by the technical expert (Attachments 19 and 20).

The stated purpose of the cut-off study was to determine the minimal concentration of DNA from extracts of real casework samples that would yield a DNA profile suitable for CODIS upload. The laboratory confirmed this during the 2/17/16 telephone discussion that this concentration was determined to be 0.015 ng/ul; anything less than that was demonstrated to be 'junk.' (7)

The stated goal of the 'homozygote' threshold study was:

> "to examine that data from the validation study of the 3500 system performed January 2012 at the BSO and make recommendations on appropriate levels for data interpretation. Specifically signal intensities for three values….
>
> 1. Analytical threshold….
> 2. Stochastic threshold….
> 3. Limit of linearity…."

The summary concluded with the following:

> "The recommended analytical threshold is 400 RFU based on noise and pull-up percentage. The recommended stochastic threshold is 750 RFU based on PHR (peak height ratio-defined in previous section of summary) and recommended minimum input levels of DNA. The recommended limit of linearity is 20,000 RFU base on pull-up and linearity of dynamic range."

When questioned about this (Attachment 7), the laboratory stated that the researcher used the wrong terminology, because the resulting 'homozygote' threshold was based on a single source sample study for a single allele result generated from a 0.1 ng DNA input. The laboratory discussed this with the researcher at the time, but he did not feel he could change it, so the laboratory prepared a clarification memo to explain that this study did not establish a stochastic threshold because the data pertained to single source sample and not mixed DNA samples. The laboratory provided the memo (Attachment 21), which states the same, and adds that "allelic patterns and characteristics in single source samples are not maintained or consistent when the same sample is part of a mixture due to increased competitive amplification of target DNA across all the loci."

In response to the allegation that the laboratory is calculating the CPI using loci that exhibit allelic dropout, the laboratory stated in its 12/28/2015 response that it calculates CPI's for all inclusions in mixture samples using full donor contribution as the main rule (pursuant to SWGDAM 3.6.2.2, 3.6.3 and 3.6.4). The laboratory stated that this approach was formulated in consultation with and supported by experts in the field. (Attachment 22).

In response to the allegation that the laboratory is not-compliant with SWGDAM Guideline 4.6.3, the laboratory suggested that there may be differing or conflicting recommendations with the SWGDAM Guidelines:

> "When CPIs are calculated to support an inclusion call, guideline 4.6.3 states/suggests that loci with loci with data below the ST may not be used in the stats, but that guideline 4.6.3.1 states/suggests that data or alleles below threshold may be used for comparisons which include inclusions." (Guidelines follow for ease of reference):
>
> "4.6.3: When using CPE/CPI (with no assumptions of number of contributors) to calculate the probability that a randomly selected person would be excluded/included as a contributor to the mixture, loci with alleles below the stochastic threshold may not be used for statistical purposes to support an inclusion. In these instances, the potential for allelic dropout raises the possibility of contributors having genotypes not encompassed by the interpreted alleles.
>
> 4.6.3.1. Alleles below the stochastic threshold may be used for comparisons and/or to establish the presence of a mixture or male DNA (e.g., Y allele at amelogenin)."

The laboratory reiterated its concern these SWGDAM Guidelines, if followed, may be in conflict with ISO/IEC 17025 (2005) and Supplemental (2011 5.10.3.5, in that there may be discrepant and/or unclear reporting of meaningful statistics.

The laboratory responded that the procedures have been audited against the FBI QAS standards on a number of occasions, including those conducted during previous ASCLD/LAB assessments.

Technical Expert's Response:

It is unclear how the laboratory's 'homozygote threshold' is being used differently than a stochastic threshold, even though the laboratory claims it does not use a stochastic threshold or peak height ratio to make determinations for which loci to use for comparison or statistical purposes. Regardless, upon review of the Gene Mapper electropherograms/printouts and allele tables (10 and 11), the allele calls based on analytical and 'homozygote' thresholds appear to be correct. However, there is allelic dropout at a number of loci which indicates the potential for dropout at the loci which the analyst included in the statistical calculations (abbreviated as D8, D3, D13, vWA and D5). There are concerns about these and other loci (abbreviated as D7, D21, CFS1PO, TH01) which have unlabeled peaks and other apparent information above baseline that cannot be confirmed with the electropherograms provided. It is apparent that these peaks fall below the lab's analytical threshold, but are not marked, because of the instrument settings. Some are peaks that appear to be in stutter positions, but without documentation that these have been considered and ruled out as stutter, it is not clear that the lab considered the entire profile for potential allelic dropout.

It is unclear as to how the laboratory's claim of not using a stochastic threshold is supported by the validation summary that was provided (20). The summary provided appears to support the use of and provide for the laboratory a recommended stochastic threshold. It does not mention nor provide information supporting the use of a 'homozygote threshold.' There does not appear to be enough detail in the summary to verify what was done and to verify it with the clarification memo provided by the lab (21).

It is unclear as to how the lab's cut-off study meets the SWGDAM 3.2.2 guideline (and lab's response) to assess/evaluate the potential stochastic amplification effects. The stated purpose of the study was to determine if the DNA Unit 'could reasonably determine a cut-off value in which an examiner would be reasonably assured that a results would be obtained which would not be valid for CODIS….upload.' The summary of the study does not mention anything about stochastic amplification, nor is it clear how potential stochastic amplifications were considered, from the summary provided. Further, the laboratory's determination of the minimum concentration is not necessarily valid when dealing with mixtures of DNA, since the concentration of DNA from individual contributors cannot be known. In those instances, it may be necessary to have that minimum concentration from each contributor in the sample in order to be able to detect it at a suitable level.

The laboratory's justification to use the known reference standard profile(s) to make decisions on the statistical significance of the evidentiary sample is flawed, as it uses the CPI method of calculation. As referenced in SWGDAM 4.6.3 and by Butler (Attachment 25). In order to properly state the significance of occurrence of an evidentiary profile using the CPI with no assumption of the number contributors (as was with this case), the laboratory must not use loci in which there are alleles below the laboratory's established stochastic threshold, or in the laboratory's case, either the analytical threshold or 'homozygote' threshold, if one relies upon the two validation studies provided. In the cited case, there is evidence of allelic dropout and potential alleles that are below the laboratory's analytical threshold (at loci displaying multiple peaks). As stated in the first paragraph of this response, it is not clear if the analyst/laboratory appropriately analyzed these peaks for artifacts. Without the documentation of that consideration in the examination records provided, it is not clear if potential stochastic amplification effects were considered and ruled out. Given the extent of visible allelic dropout seen at the larger loci, it would be valid to consider the possibility of dropout at the smaller loci as well. With those considerations, some of the loci the laboratory used for statistical purposes based on its comparison to the known reference samples, might not be appropriate for that purpose.

The laboratory's definition of a conclusive locus (Attachment 16, and explained in Attachment 7, page 6-9) based upon, in part, the comparison to the known reference sample(s) is not /may not be appropriate, as it does not appear to include appropriate consideration of all potential genetic profiles and the possibility of allelic dropout.

Objective Evidence Provided and Reviewed:

ATTACHMENTs 13 and 13a – Sections 21 and 22, Analysis of STR Data – Control Samples and Analysis of STR Data – Samples, respectively: Sets forth the requirements of data analysis and evaluation, including the analytical and 'homozygote' thresholds.

ATTACHMENT 16 – Section 29, Statistical Evaluations of STR Results and CODIS: Sets forth the requirements for the statistical interpretations and calculations using the CPI.

ATTACHMENT 20 – 'Homozygote' Threshold Validation Study Summary: The validation summary submitted was a document prepared by an external researcher, Dr. Bruce McCord, using data provided by the laboratory as part of its validation of the ABI3500 genetic analysis instrumentation.

ATTACHMENT 19- CUT OFF (QUANTITATION) STUDY: A cut-off study was conducted to determine the concentration of DNA in a sample that could be used and obtain an uploadable CODIS profile. The summary provided showed that the cut-off study established a minimum concentration of 0.015 ng/ul.

ATTACHMENTs 10 and 11: case electropherograms, allele call sheets, test report and statistical calculations, previously described.

ATTACHMENT 17: transcript of analyst's deposition testimony, previously described.

ALLEGATION 3:

The complainant alleges that the statistical frequencies relied upon in the calculation of this case were outdated and inaccurate. The frequencies have since been updated. All calculations performed by the analyst at the laboratory were affected by these mistakes in the FBI database, which was acknowledged by analyst on page 27 of his deposition, lines 12-16. The statistical analysis of the samples in this case were done incorrectly according to the best practice in the field and as such should not be relied upon by the court.

Laboratory's Summary Response:

The laboratory acknowledged using the FBI database frequencies, has stopped using them, is using a revised version of the database, and has notified the appropriate legal authorities in the cited (and other) cases advising that it will re-calculate the statistics upon request. (ATTACHMENT 23). The laboratory's response did not include any additional information pertaining to the corrective action of this specific matter.

Objective Evidence Provided and Reviewed:

ATTACHMENT 17: deposition of analyst, pages 26 – 28. The analyst explained the laboratory's policy about recalculating the statistics upon request. It is noted that the attorneys acknowledge having received this information.

ATTACHMENT 23: laboratory letter to various state and county attorneys advises of the database frequency errors, the causes of the errors, the expected impact, the actions taken by the FBI, this laboratory, and other laboratories, and the notification that re-calculations on old cases will be done upon request with specified notification.

Technical Expert's Response:

The technical expert was not requested to provide input on this point of the allegation.

<u>SUMMARY/DETERMINATION(S)</u>

Allegation 1

It was determined that there is sufficient evidence that the laboratory's procedure to evaluate the genetic profiles of submitted known reference samples prior to making decisions about which loci upon which statistical significance calculations will be based is not appropriate, in that it has the potential to not fully recognize the genotypes of all potential contributors and the potential to overstate the statistical significance of occurrence of the evidentiary profile.

REQUIREMENT: "5.4.1: The laboratory shall use appropriate methods and procedures for all tests and/or calibrations within its scope. These include sampling, handling, transport, storage and preparation of items to be tested and/or calibrated, and, where appropriate, an estimation of the measurement uncertainty as well as statistical techniques for analysis of test and/or calibration data."

NONCONFORMANCE: The laboratory's procedure for calculating statistics on indistinguishable/unresolvable mixed DNA evidentiary profiles allows an analyst to select alleles/loci and to perform statistical calculations after considering the DNA profile results of the known reference samples submitted for comparison. While alleles/loci are first selected based on two interpretation thresholds, the laboratory's procedure then allows for the subsequent consideration of known reference sample profiles to make additional decisions about which alleles/loci in the evidence sample will be used for statistical assessment and calculation purposes. The laboratory's procedure does not require consideration of the full range of possible contributing genotypes at each locus, which could increase the number of potential contributors to an evidentiary profile.

Allegation 2

It was determined that there is sufficient evidence that the laboratory's procedure of:

1) *using a combination of an analytical and 'homozygote' threshold;*
2) *using the known reference sample profiles to select loci for statistical consideration and;*
3) *calculating a CPI statistical probability of occurrence based upon the full contribution of the known reference sample profiles;*

does not appear to sufficiently address the potential for allelic dropout, and does not appear to be supported by the validation summaries provided by the laboratory. The validation summary for the quantitative 'cut-off' concentration offered by the laboratory to support its conformance with the laboratory's stated compliance with SWGDAM 3.2.2 does not include information indicating that potential stochastic amplification effects were adequately considered. The validation summary for the laboratory's 'homozygote' threshold procedure includes a stochastic threshold which the laboratory is not using. While the laboratory provided an explanation for the terminology discrepancies in the validation summary, it remains unclear how the laboratory's studies address potential stochastic amplifications. The laboratory's procedure for using the known reference sample profiles does not appear to fully consider loci with apparent visible allelic dropout, which is required for the CPI calculation.

REQUIREMENT: "5.4.1: The laboratory shall use appropriate methods and procedures for all tests and/or calibrations within its scope. These include sampling, handling, transport, storage and preparation of items to be tested and/or calibrated, and, where appropriate, an estimation of the measurement uncertainty as well as statistical techniques for analysis of test and/or calibration data."

NONCONFORMANCE: The laboratory's procedure using the CPI calculation for evaluating the statistical significance of DNA mixture results does not appropriately require that loci exhibiting or having the potential for allelic dropout be excluded from use in the statistical calculations.

Allegation 3

It was determined that the laboratory used the FBI database involved in the large-scale non-conformance. It was determined from the laboratory's response that the appropriate legal representatives in this case (and others) have been notified regarding the FBI DNA population database errors, and have offered to re-calculate the statistics upon request.

COMMENTS

1. It is recommended that Attachment 7 be viewed as a more complete response to the complaint allegations and should be used in conjunction with the laboratory's 12/28/15 response.

2. It should be noted that both the complainant and the laboratory acknowledge that the topic of statistical calculations used to evaluate the significance of occurrence of DNA mixed profiles is under debate in the scientific community.

3. It is acknowledged that the validations and methods used by the laboratory have previously undergone review and assessment pursuant to FBI QAS requirements and this accrediting body's requirements and have been accepted as meeting relevant requirements during those reviews. The investigator did not request QAS audit records to verify this acknowledgement.

4. During a 2/5/16 telephone interview with the DNA Unit manager and technical leader, it was learned that the analyst, misspoke during the cited case deposition regarding the laboratory's allele threshold. In the deposition, the analyst stated that the analytical threshold the lab uses was 375 rfus, when it is 400 rfus. The error was a human error because the lab had previously used a 375 rfu threshold. This statement was reviewed and was determined to not be relevant to the complaint, nor have an impact on the case.

ATTACHMENTS AND REFERENCES

1 and 1a. Complainant's first and subsequent complaint allegation
2. Laboratory's first response to complaint allegations, 11/06/2015
3, 3a & 3b. Complainant's additional details to clarify allegation
4. Communication to lab regarding complainant's additional complaint allegation details
5. Laboratory's second response, 12/28/2015
6. DNA Unit – Analytical Methods Manual, Section 21, Analysis of STR Data-Control Samples.
7. Laboratory's memo to investigator, 2/19/2016
8 (R) *ISO/IEC 17025:2005 - General Requirements for the Competence of Testing and Calibration Laboratories*
9. 2010 SWGDAM Interpretation Guidelines for Autosomal STR Typing by Forensic DNA Testing Laboratories (2010, www.fbi.gov)
10. Examination records from cited case, State of Florida vs. John Paul Spencer, laboratory number 14-03320, including sample information worksheets and GeneMapperID-X electropherograms/printouts.
11. Test report, allele call sheets (laboratory Document Control Number CLSTR-14A) and statistical calculation from cited case, State of Florida vs. John Paul Spencer, laboratory number 14-03320.
12. DNA Unit – Analytical Methods Manual, Section 24, STR Results Table
13 DNA Unit – Analytical Methods Manual, Section 21, Analysis of STR Data – Control Samples
13a. DNA Unit – Analytical Methods Manual, Section 22, Analysis of STR Data - Samples
14. DNA Unit – Analytical Methods Manual, Section 26, Interpretations of STR Results: Non-Intimate Mixture Samples.
15. DNA Unit – Analytical Methods Manual, Section 28, Interpretations of STR Results – Inconclusive Results.
16. DNA Unit – Analytical Methods Manual, Section 29, Statistical Evaluations of STR Results and CODIS
17. Deposition of Chris Comar, laboratory DNA analyst, from cited case, State of Florida vs. John Spencer, laboratory number 14-03320,
18 (R). *ASCLD/LAB-International Supplemental Requirements for the Accreditation of Forensic Science Testing Laboratories* (2011 Edition)
19. Quantitation cut-off validation study summary, provided by laboratory: Plexor HY System Internal Cut-Off Validation, no date
20. 'Homozygote' threshold validation study summary, provided by laboratory: Evaluation of BSO DNA Data from ABI 3500 Validation Study
21. Clarification memo for 'homozygote' threshold validation study summary, 9/4/2014, provided by laboratory
22. Letter from laboratory consultants McElfresh, Tracey and McCord, Florida International University, 12/7/2015
23. Letter from laboratory to relevant legal community re: FBI population database allele frequency errors, 7/6/2015
24. Definitions of 'odds', unknown sources, provided by laboratory, 2/19/2016
25. Scanned pages from:
    a. Butler, John M. **Advanced Topics in Forensic DNA Typing: Interpretation**. Elsevier/Academic Press, 2015: 320-322.
    b. Evett, Ian W. and Weir, Bruce S. **Interpreting DNA Evidence: Statistical Genetics for Forensic Scientists.** Sinauer Associates, 2001: 16-17.

Carter Pereira, Claudine

From: Pamela Bordner <PBordner@ascld-lab.org>
Sent: Tuesday, April 12, 2016 2:35 PM
To: Carter Pereira, Claudine
Subject: allegation policy
Attachments: ASCLDLAB Allegation Policy.pdf

The ASCLD/LAB allegation policy is attached.

Pam

Pam Bordner, Executive Director
ASCLD/LAB
919-773-2600
919-773-2602 FAX
pbordner@ascld-lab.org
www.ascld-lab.org
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Carter Pereira, Claudine

From: Carter Pereira, Claudine
Sent: Tuesday, April 12, 2016 1:45 PM
To: Duncan, George; Tsingelis, Petros
Subject: Fwd: ASCLD/LAB Board decision on allegations
Attachments: 160412-BrowardSO-notification of Board decision.pdf; 160412-BrowardSO-Investigative Report-Board Reveiwed.pdf

Claudine Carter Pereira
Director-Crime Laboratory

954-831-3578 (w)
954-856-3627 (c)

Sent from my Sprint Phone.

----- Forwarded message -----
From: "Pamela Bordner" <PBordner@ascld-lab.org>
To: "Carter Pereira, Claudine" <Claudine_CarterPereira@sheriff.org>
Cc: "Laurel Farrell" <LFarrell@ascld-lab.org>, "'Coffman, David'" <DavidCoffman@fdle.state.fl.us>, "Tara Dolin" <tdolin@ascld-lab.org>
Subject: ASCLD/LAB Board decision on allegations
Date: Tue, Apr 12, 2016 12:33 PM

Director Carter-Pereira,

Attached to this email you will find a formal notice of the ASCLD/LAB Board of Directors decisions related to our investigation of allegations submitted by Tiffany Roy. A copy of the Investigative Report is also attached. As the letter states, you have until May 12, 2016 to provide me with the laboratory's corrective action plans related to the sustained allegations or alternatively, you can provide me with a request for a formal Board review. The current ASCLD/LAB policy on "Allegations Related to Accredited Laboratories and Their Employees" can be found here https://ascld-lab.qualtrax.com/Default.aspx?ID=1446

Please acknowledge receipt of this notification.

Best Regards,
Pam
Pam Bordner, Executive Director
ASCLD/LAB
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919-773-2602 FAX
pbordner@ascld-lab.org
www.ascld-lab.org
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Carter Pereira, Claudine

From: Carter Pereira, Claudine
Sent: Tuesday, April 12, 2016 6:35 PM
To: Duncan, George; Tsingelis, Petros
Subject: Re: ASCLD/LAB Board decision on allegations

Let's plan to discuss after the completion of our DNA proficiency cycle (May 8), so that we can send our initial response regarding the decision by the May 12th deadline.

Thanks,

Claudine Carter Pereira
Director-Crime Laboratory

954-831-3578 (w)
954-856-3627 (c)

Sent from my Sprint Phone.

----- Reply message -----
From: "Carter Pereira, Claudine" <Claudine_CarterPereira@sheriff.org>
To: "Duncan, George" <George_Duncan@sheriff.org>, "Tsingelis, Petros" <Petros_Tsingelis@sheriff.org>
Subject: ASCLD/LAB Board decision on allegations
Date: Tue, Apr 12, 2016 1:45 PM

Claudine Carter Pereira
Director-Crime Laboratory

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To: "Carter Pereira, Claudine" <Claudine_CarterPereira@sheriff.org>
Cc: "Laurel Farrell" <LFarrell@ascld-lab.org>, "'Coffman, David'" <DavidCoffman@fdle.state.fl.us>, "Tara Dolin" <tdolin@ascld-lab.org>
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Carter Pereira, Claudine

From: Carter Pereira, Claudine
Sent: Thursday, May 12, 2016 4:02 PM
To: 'Pamela Bordner'
Cc: Laurel Farrell; 'Coffman, David'; Tara Dolin
Subject: RE: ASCLD/LAB Board decision on allegations

Good afternoon,

Upon receipt and review of the preliminary investigative report, we would like to request a formal Board review (or the current equivalent review process that now in place).

Please let me know how to proceed.

Thank you for your time and consideration.

Regards,
Claudine

Claudine Carter Pereira, MS, CLPE
Director - Crime Laboratory
Broward Sheriff's Office | www.Sheriff.org
201 SE 6th Street, N. Wing Rm 1799
Fort Lauderdale, FL 33301
tel: 954-831-3578 | tel2: 954-831-7320
cell: 954-856-3627| fax: 954-831-6138

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From: Pamela Bordner [mailto:PBordner@ascld-lab.org]
Sent: Tuesday, April 12, 2016 12:34 PM
To: Carter Pereira, Claudine <Claudine_CarterPereira@sheriff.org>
Cc: Laurel Farrell <LFarrell@ascld-lab.org>; 'Coffman, David' <DavidCoffman@fdle.state.fl.us>; Tara Dolin <tdolin@ascld-lab.org>
Subject: ASCLD/LAB Board decision on allegations

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Carter Pereira, Claudine

From: Pamela Bordner <PBordner@ascld-lab.org>
Sent: Thursday, May 12, 2016 5:56 PM
To: Carter Pereira, Claudine
Cc: Laurel Farrell; 'Coffman, David'; Tara Dolin
Subject: RE: ASCLD/LAB Board decision on allegations

Follow Up Flag: Follow up
Flag Status: Flagged

Claudine,

I am in receipt of your request for a formal Board review of the appeals. Due to the merger of ASCLD/LAB and ANAB, we have a new appeals procedure that can be found here https://ascld-lab.qualtrax.com/ShowDocument.aspx?ID=1768. Since the initial appeal was already heard, we will enter the new process at a level 2 appeal. The Accreditation Council is currently comprised of the former ASCLD/LAB Board of Directors so this will be very similar to the formal Board review that would have occurred under the previous appeals procedure.

I will appoint a panel to hear the appeal, provide you those names and contact you to set a date for the appeal hearing.

Best Regards.
Pam
Pam Bordner, Vice President
ASCLD/LAB
919-773-2600
919-773-2602 FAX
pbordner@ascld-lab.org
www.ascld-lab.org
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Sent: Thursday, May 12, 2016 4:02 PM
To: Pamela Bordner <PBordner@ascld-lab.org>
Cc: Laurel Farrell <LFarrell@ascld-lab.org>; 'Coffman, David' <DavidCoffman@fdle.state.fl.us>; Tara Dolin <tdolin@ascld-lab.org>
Subject: RE: ASCLD/LAB Board decision on allegations

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Please let me know how to proceed.

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Claudine

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Cc: Laurel Farrell <LFarrell@ascld-lab.org>; 'Coffman, David' <DavidCoffman@fdle.state.fl.us>; Tara Dolin <tdolin@ascld-lab.org>
Subject: ASCLD/LAB Board decision on allegations

Director Carter-Pereira,

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Carter Pereira, Claudine

From: Pamela Bordner <PBordner@ascld-lab.org>
Sent: Wednesday, June 15, 2016 10:45 AM
To: Carter Pereira, Claudine
Subject: ASCLD/LAB hearing scheduled

Importance: High

Director Pereira,

I have selected three members of the Forensics Accreditation Council to hear the Broward Sheriff's Office of the ASCLD/LAB Board of Director's decision related to the DNA complaint submitted by Ms. Tiffany Roy. All three panel members have a DNA background. Those members are:

- Catherine Knutson, Minnesota Bureau of Criminal Apprehension
- Erin Henry, Oklahoma State Bureau of Investigation
- Kathleen Corrado, Onondaga County Center for Forensic Sciences

Please let me know if you are aware of any conflict of interest.

The hearing is set for Tuesday, June 21, 2016. Is 9:30 AM an acceptable start time? We previously discussed 10:00AM, but the earlier time is necessary in order to ensure that all participants can be available for the necessary amount of time.

Your prompt response will be appreciated.

Best Regards,
Pam
Pam Bordner, Vice President, Forensics
ANAB dba ASCLD/LAB
919-773-2600
919-773-2602 FAX
pbordner@ascld-lab.org
www.ascld-lab.org

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Carter Pereira, Claudine

From: Carter Pereira, Claudine
Sent: Wednesday, June 15, 2016 9:36 PM
To: Pamela Bordner
Subject: RE: ASCLD/LAB hearing scheduled

Good Evening Pam,

Sorry for the delay in getting back to you, I was out of the office most of the day today.

I am unaware of any conflict of interest and the 9:30 am start time is acceptable.
One quick question regarding the logistics of the hearing, will there be a conference line or WebEx set up to call into?

Please advise.

Thank you.

Best Regards,
C

Claudine Carter Pereira, MS, CLPE
Director - Crime Laboratory
tel: 954-831-3578 | tel2: 954-831-7320
cell: 954-856-3627| fax: 954-831-6138
Please note that Florida has a broad public records law, and that all correspondence sent to me via email may be subject to disclosure.

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From: Pamela Bordner [PBordner@ascld-lab.org]
Sent: Wednesday, June 15, 2016 10:44 AM
To: Carter Pereira, Claudine
Subject: ASCLD/LAB hearing scheduled

Director Pereira,

I have selected three members of the Forensics Accreditation Council to hear the Broward Sheriff's Office of the ASCLD/LAB Board of Director's decision related to the DNA complaint submitted by Ms. Tiffany Roy. All three panel members have a DNA background. Those members are:

- Catherine Knutson, Minnesota Bureau of Criminal Apprehension
- Erin Henry, Oklahoma State Bureau of Investigation
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Carter Pereira, Claudine

Subject:	Broward Sheriff's Office Appeal of Complaint Decision
Start:	Tue 6/21/2016 9:30 AM
End:	Tue 6/21/2016 11:30 AM
Recurrence:	(none)
Meeting Status:	Accepted
Organizer:	Pamela Bordner

This meeting will be held using FUZE Meeting:
FUZE Connection Information:
http://fuze.me/33014154

Once connected you will be provided audio connection information. You can connect through speakers on your computer or by phone line.
The phone connection number will be:
201-479-4595 OR Toll-Free 855-346-3893
Meeting Room ID: 33014154

I have attached a brief document to help orient new users to the FUZE Meeting interface. Please make sure to use the Meeting Room ID listed above and not the one listed in the example on this document.

The hearing will proceed as follows:
•Introductions
• Presentation by the appellant, limited to 30 minutes
• Presentation by ASCLD/LAB, limited to 30 minutes
• Rebuttals, limited to 10 minutes for each party
• Questions by the panel
• Closing of the hearing, at which the chair shall:
o Make a formal projection regarding the expected time frame for communicating the documented final decision (normally not to exceed two weeks).
o Inform all parties that the appeal may be escalated to the next level of appeal within 30 days of receipt of the panel decision.
o Dismiss the parties.

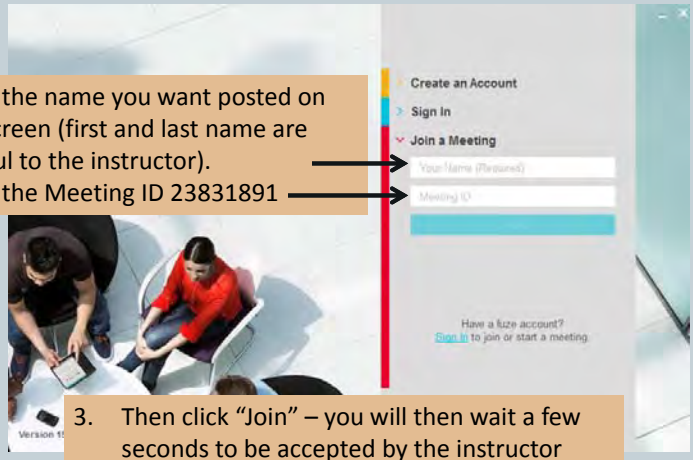
Fuze Meeting Interface

ATTENDEE

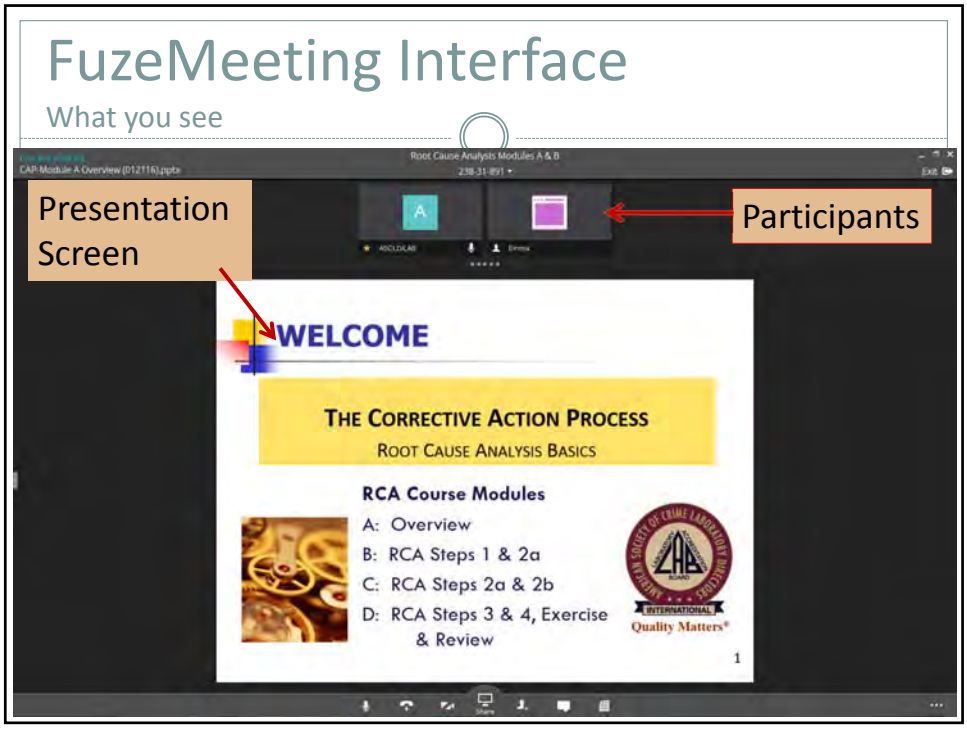
QUICK SCREEN ORIENTATION

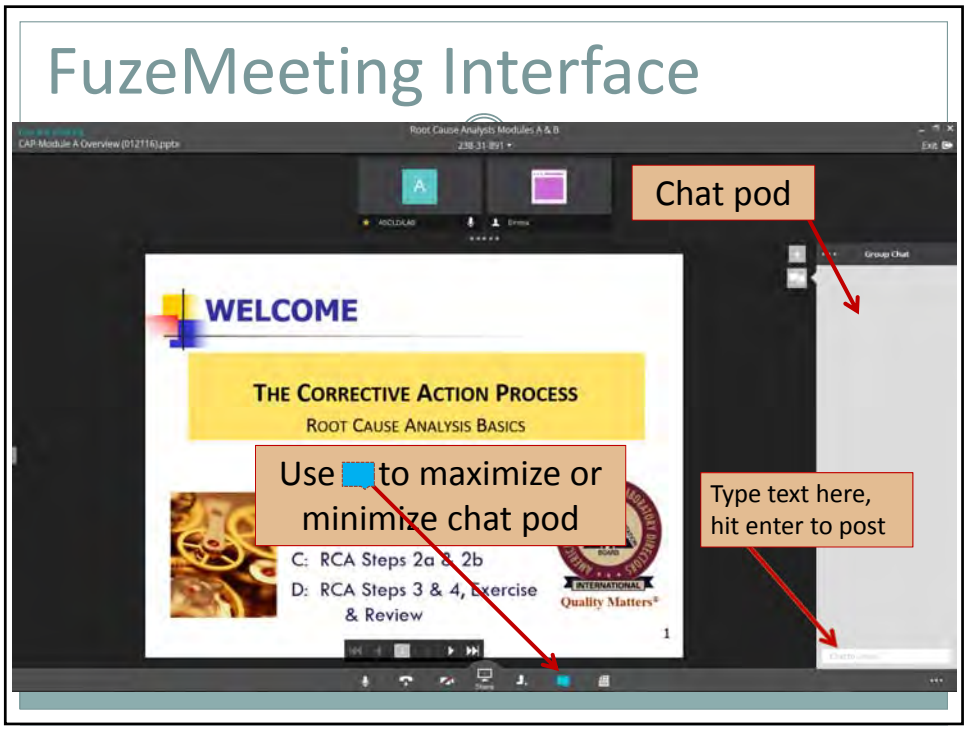
When you click on the invite link

Click Invite Link: http://fuze.me/23831891 You will be directed to this page.



1. Enter the name you want posted on the screen (first and last name are helpful to the instructor).
2. Enter the Meeting ID 23831891
3. Then click "Join" – you will then wait a few seconds to be accepted by the instructor


FuzeMeeting Interface
What you see
Presentation Screen
Participants
WELCOME
THE CORRECTIVE ACTION PROCESS
ROOT CAUSE ANALYSIS BASICS
RCA Course Modules
A: Overview
B: RCA Steps 1 & 2a
C: RCA Steps 2a & 2b
D: RCA Steps 3 & 4, Exercise & Review
Quality Matters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D: RCA Steps 3 & 4, Exercise & Review
Quality Matters

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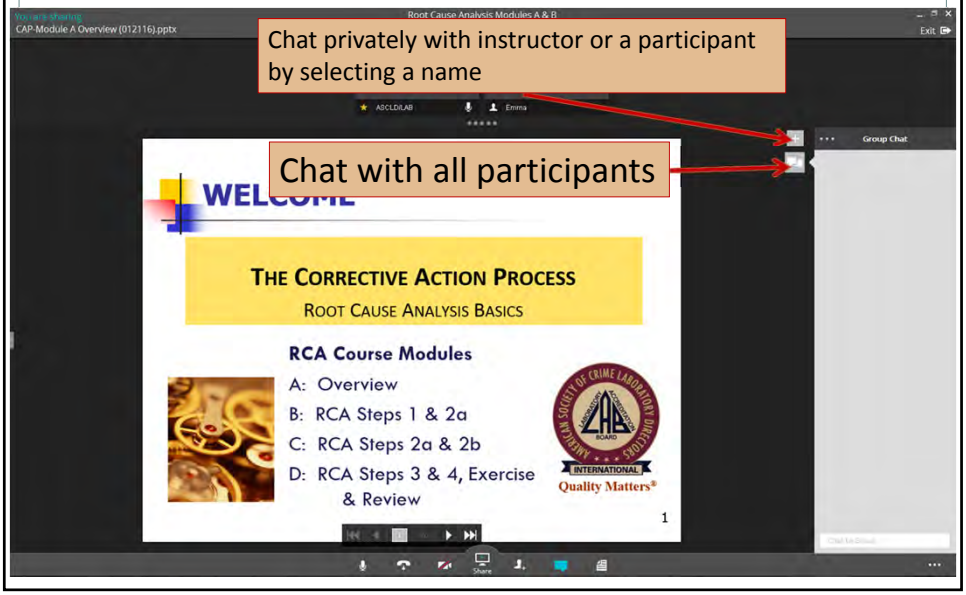

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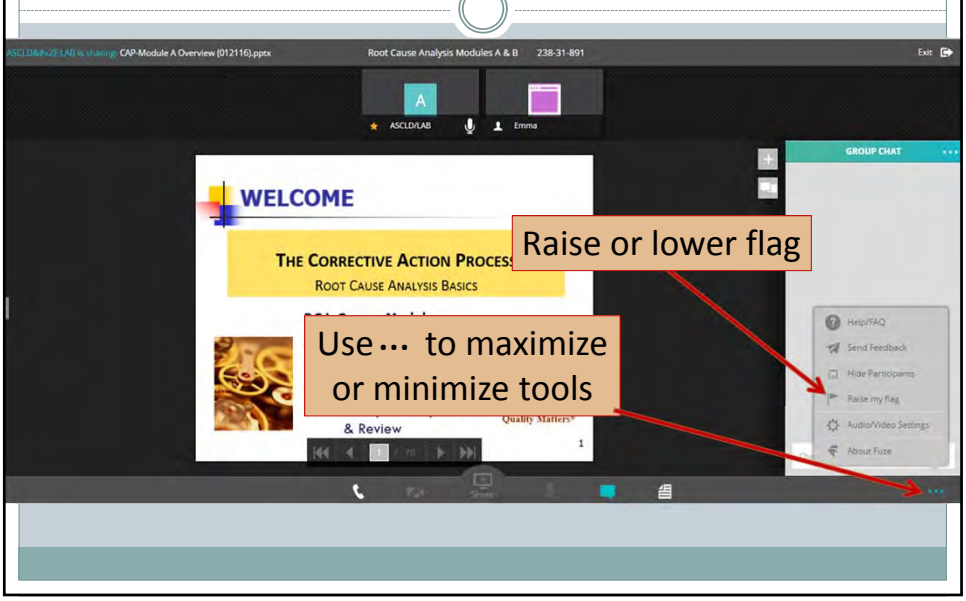

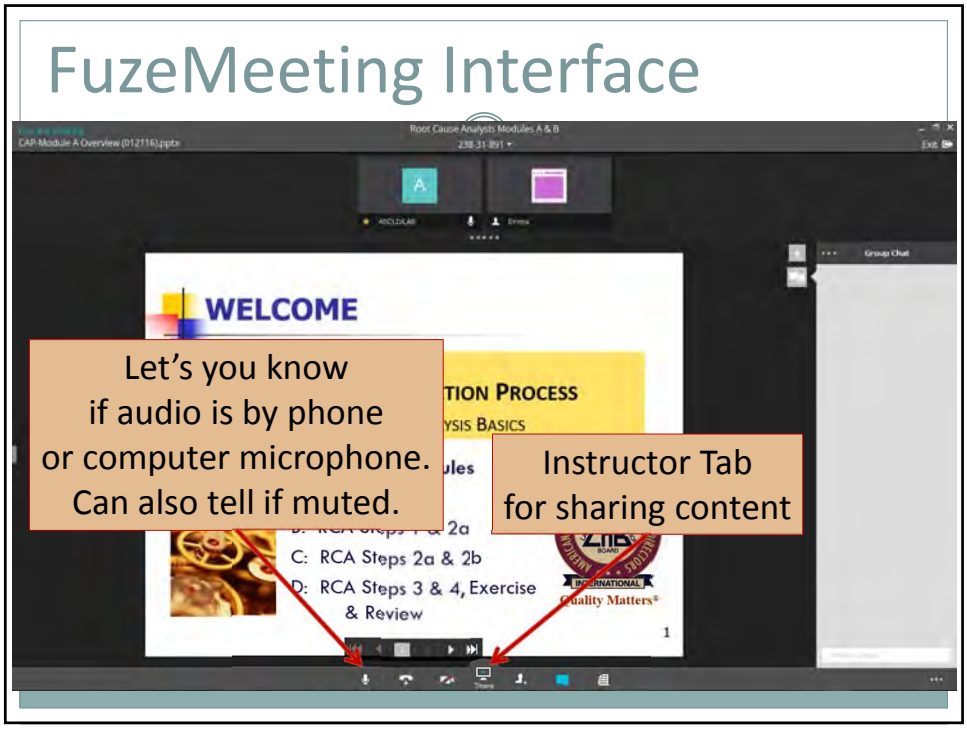
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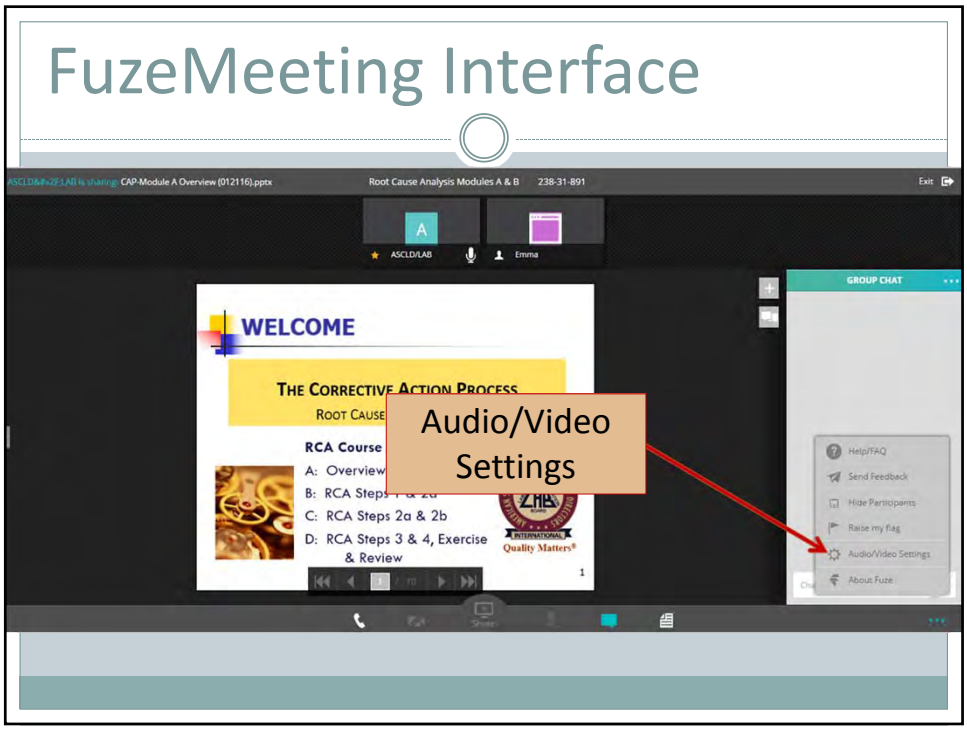
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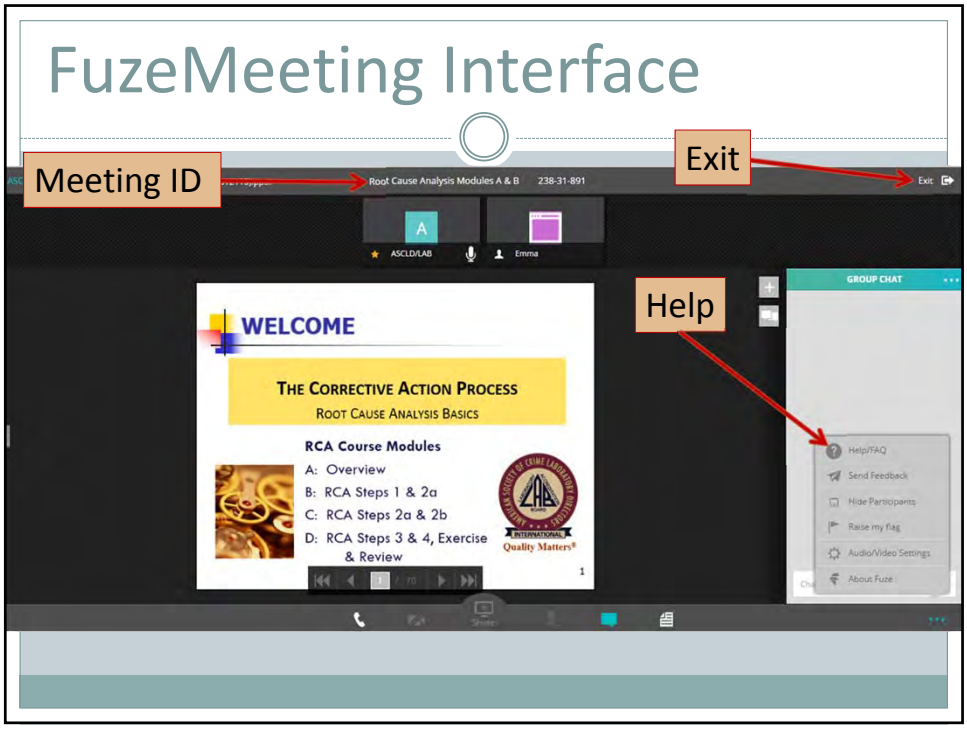
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ROOT CAUSE ANALYSIS BASICS
RCA Course Modules
A: Overview
B: RCA Steps 1 & 2a
C: RCA Steps 2a & 2b
D: RCA Steps 3 & 4, Exercise & Review
Quality Matters
Help/FAQ
Send Feedback
Hide Participants
Raise my flag
Audio/Video Settings
About Fuze

Carter Pereira, Claudine

From: Carter Pereira, Claudine
Sent: Tuesday, June 21, 2016 4:10 PM
To: pbordner@ascld-lab.org
Subject: FIU Letter
Attachments: 0664_001.pdf - Adobe Acrobat Pro.pdf

Hi Pam,

Thank you for your time today. I wanted to forward this letter to you and the board.

If you require anything further, please let me know.

Best regards,

Claudine Carter Pereira
Director-Crime Laboratory

954-831-3578 (w)
954-856-3627 (c)

Sent from my Sprint Phone.

FIU FLORIDA INTERNATIONAL UNIVERSITY

20 June 2016

To Whom It May Concern:

Last year we wrote to Director Claudine Carter Pereira expressing our support for the way the DNA section has designed and validated their protocols, and to express our concern that the allegations were filed by an individual who was an expert in an on-going case.

We have reviewed the responses crafted by the Technical Leader Petros Tsingelis, and we agree with his clarifications. Rather than add to his explanations, we simply offer to answer questions or amplify on any issues where the panel feels that our input would be helpful.

Respectfully yours,

Bruce McCord PhD

Kevin McElfresh PhD

Martin Tracey PhD

DEPARTMENT OF BIOLOGICAL SCIENCES
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EXHIBIT L

Final Report on

Review of Mixture Interpretation in Selected Casework of the

DNA Section

of the

Forensic Science Laboratory Division (FSL),

Department of Forensic Sciences (DFS),

District of Columbia

Prepared by: Bruce Budowle, Frederick R. Bieber

Prepared for: Vincent H. Cohen, Jr., Acting U.S. Attorney,

District of Columbia

22 April 2015

Preface

The Department of Forensic Sciences (DFS) was created through the "Department of Forensic Sciences Act of 2011" by the Council of the District of Columbia. Operations as an agency commenced on October 1, 2012. The DFS is comprised of several divisions, including the Forensic Science Laboratory Division (FSL), the Public Health Laboratory Division, and the Crime Scene Sciences Division, all located along with the Office of the Chief Medical Examiner (OCME) in a Consolidated Forensic Laboratory at 401 E Street S.W. Washington, DC.

The DFS provides various services to a number of agencies, including the Metropolitan Police Department, the Office of the Chief Medical Examiner, the Office of the Attorney General, the Department of Health, the Fire and Emergency Medical Services Department, the United States Attorney's Office for the District of Columbia, and other law enforcement or investigative agencies.

Over the past few years the United States Attorney's Office for the District of Columbia (USAO) has requested the assistance of various experts, outside of the DFS and its predecessor laboratory, in preparation for admissibility hearings and trial testimony, some relating to forensic DNA evidence. Such experts have included the authors of this Report.

In May 2014, the USAO requested the assistance of Dr. Bruce Budowle in providing additional statistical calculations, not performed by DFS, relating to a DNA mixture profile from a particular evidence item in preparation for an upcoming trial (U.S. v. Tavon Barber, 2013-CF1-011157). During review of the DNA results in that case analyzed by the FSL of the DFS, in preparation of his trial testimony, Dr. Budowle identified several concerns regarding interpretation of the DNA evidence by DFS, specifically regarding selection of interpretable genetic markers for statistical calculations and DNA mixture deconvolution. Dr. Budowle prepared his own independent analysis and testified in the trial. After this review, a USAO representative attended a DFS Scientific Advisory Board meeting on October 7, 2014 to present the concerns raised by Dr. Budowle about mixture interpretation at the DFS.[1]

At the advice of Dr. Budowle (based on his concerns in the review of the Barber case), the USAO began reviewing pending cases in which DFS had issued reports with Combined Probability of Inclusion (CPI) statistics. During this time frame, the USAO, with the assistance of Dr. Budowle, communicated telephonically with DFS management including the DNA technical leader and two members of the DFS Scientific Advisory Board and later delivered a telephonic PowerPoint presentation illustrating the issues he and the USAO had identified regarding DFS mixture interpretation practices. Dr. Budowle reviewed a number of additional pending cases, at the request of the USAO, and identified additional issues regarding mixture interpretation, including CPI statistics and mixture deconvolution, and recommended a more comprehensive review.

[1] Prior to the formation of the "Panel" issuing this Report, DFS performed a "non-exhaustive" review of 27 cases involving DNA evidence. Seven involved DNA mixtures, 3 of which included DNA mixture statistics. Of these 3 cases, 2 had CPI calculations one of which was modified by DFS after its review. This limited review was deemed insufficient by Dr. Budowle which then led to a more comprehensive review by USAO.

In response, the USAO retained a panel of experts consisting of Dr. Bruce Budowle, Dr. Frederick R Bieber, and Ms. Lisa Brewer (hereinafter referred to as "the Panel") to perform review of all pending cases and others which involved prior convictions, in which DFS had issued a DNA report with statistical calculations.[2]

In review of such cases and DFS reports, the USAO instructed the Panel to identify any instances in which, in their opinion, the interpretation of DNA evidence or the accompanying statistical analysis was questionable, but not those which could be attributed to acceptable variation of DNA interpretation within the relevant scientific community. However, the Panel did take note of inconsistent practices by DFS analysts that might impact the interpretation of DNA results and statistical analyses.

Lastly, the Panel was asked to assess what measures need to be implemented before the USAO can resume using DFS for DNA testing. To accomplish that assessment, the Panel, with the permission of the D.C. Mayor's Office, conducted a two-day site-visit of DFS. The USAO also asked the Panel to make recommendations about the need for additional training on DNA mixture analysis, interpretation, and reporting, as well as the formal technical review process and to offer to develop and deliver a practical competency assessment to help assure that the above protocols, procedures, review processes, and continuing education meet the needs of the DFS in order to attain high quality DNA typing results. This Report summarizes the results of the Panel's work and findings, to date, and addresses some of the needs and recommendations for the future.

[2] Past convictions and pending cases involving DFS mixture interpretation were selected for review by the USAO and then screened initially by Dr. Bruce Budowle. If issues of concern were identified, the case materials were forwarded to the entire Panel for review. After the USAO decided to send case work to the Verdugo Regional Crime Laboratory in California, Lisa Brewer no longer served on the Panel in order to avoid a potential conflict of interest.

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Abstract

Scope and Goals of the Project (December, 2014 to the present)

The work of the Panel involved review of selected DNA casework performed in the DNA section of the Forensic Science Laboratory Division (FSL), Department of Forensic Sciences (DFS), District of Columbia (DC). This project was performed at the request of the U.S. Attorney's Office for the District of Columbia (USAO) in the aftermath of concerns raised by Dr. Budowle regarding interpretation and statistical analysis of forensic DNA evidence in specific cases.

The goals of the Panel included general review of interpretation and statistical evaluation of forensic DNA mixtures in cases identified and selected by the USAO. The purpose was to identify specific issues of concern in the selected cases analyzed by the DFS and, through the on-site visit and interviews with members of the FSL staff, to identify ways to improve laboratory performance through additional education and training of DNA analysts, standardization of technical reviews, and to plan for the future needs.

The Panel was asked to review prior convictions and pending cases (N=68 as of the date of this Report) involving forensic DNA mixtures with specific focus on selection of alleles or loci used for generating statistical estimates to determine whether they comport with acceptable practices. The Panel evaluated these cases following the DFS standard operating protocols (SOPs) and relied on the analytical and stochastic thresholds established by DFS.

The Panel identified several thematic concerns from an initial review of selected DFS cases as well as a number of problems in interpretation of DNA mixtures in many of the cases selected by the USAO for review. The Panel's thematic concerns and specific findings were forwarded directly to the USAO. Several examples are included herein (see Appendix).

Based on an on-site visit, review of DFS documents and interviews with members of the DNA section and laboratory management, the Panel has identified necessary changes to the evaluation of forensic DNA mixtures at the DFS and recommended initiatives for improvement. The Panel also identified needs for additional training, education, and proficiency testing of members of the DNA unit and recommends improvements in the technical review procedures prior to issuing final reports along with better formalized communication with laboratory management. These changes and initiatives will be needed considering the anticipated increased demand for forensic DNA services and need for reduction of existing case backlogs at the DFS.

The Executive Summary that follows addresses the findings and opinions of the Panel, and its recommendations for the USAO and the DFS to consider.

Executive Summary

1) A the request of the USAO, the Panel reviewed selected forensic casework involving DNA mixtures that were analyzed by the FSL of the DFS. In addition, the Panel performed an on-site review of DNA operations at the FSL of DFS for the purpose of assessment of general operations and laboratory protocols as they relate to interpretation of forensic DNA profiles. The USAO also asked the Panel to address whether any additional DNA interpretation training is warranted for DNA analysts at the DFS. As requested, the Panel communicated its opinions and concerns about selected cases directly to the USAO. Work completed, in progress, and recommendations for the near future are summarized below.

2) With regard to the thematic issues raised and concerns about the initial set of cases reviewed, the Panel respectfully disagrees with DFS management's response that its practices related to DNA mixture interpretation are appropriate and within the range of generally acceptable practices. Several examples from cases reviewed by the Panel are provided (see Appendix) to illustrate what the Panel considers fundamental problems with the interpretation of forensic DNA mixtures in certain evidence in specified casework.

3) Problems identified by the Panel in specific DFS cases included:

a) inappropriate use of the combined probability of inclusion statistical approach (CPI) in mixtures by inclusion of loci where allele drop out was highly probable;
b) inappropriate use of the CPI in mixtures by including individuals whose known alleles were not present, at those loci, in the evidence samples;
c) inappropriate calculation of two separate CPIs for the same forensic DNA mixture profile;
d) not using established stochastic thresholds to assess potential allele drop out, and
e) inconsistencies and deficiencies in the technical review process of the DNA analysis pipeline.

4) A comprehensive review of mixture cases should be performed by DFS. The Panel had concerns, in some cases, regarding the DFS methods of mixture deconvolution and also identified instances in which cognitive (interpretation) bias in mixture interpretation may have been present. The Panel noted inconsistent practices regarding subtraction of profiles of known victims from DNA mixtures taken from intimate evidentiary samples. Such variation, along with the findings noted above, led the Panel to conclude that both the analysis and the technical review steps in the DFS DNA analysis pipeline require improvement.

5) The Panel performed a 2-day site visit of the FSL at the DFS and interviewed DNA analysts in the DNA section. The Panel found the FSL facilities to be outstanding providing every opportunity for success. The Panel was favorably impressed with the dedication of the staff of the DNA unit, yet was concerned when informed by most of those interviewed that they had not been apprised of case-specific concerns previously raised by the Panel regarding interpretation and statistical evaluation of specific evidentiary items or cases. The Panel found that the DFS responses did not address the Panel's thematic concerns about DNA mixture interpretation and

reporting. Without improved communication and open discussion of case-specific concerns the staff members' ability to address deficiencies and enhance performance is hampered.

6) The technical review component of the DNA pipeline needs substantial improvement with concomitant better documentation. The processes in place at the time of the visit were not sufficiently stringent to identify interpretation errors and challenges and to reduce substantial variation in DNA mixture interpretation and statistical analysis among the analysts.

7) The Panel recommends additional training on DNA mixture interpretation for the DNA analysts and technical leader at DFS prior to performing additional casework involving forensic mixtures. This training should include evaluation of DNA mixtures, technical review processes, validation strategies, and other topics focused on minimizing potential for cognitive (interpretation) bias. After additional training and demonstration of competency, assessment of performance should be monitored if the USAO returns DNA casework to DFS. Only with such an effort can the DFS analysts achieve the goal of being highest quality service providers which each and every one of them professed as his/her desire and commitment.

Review of Selected DNA Casework

At the request of the USAO, the Panel reviewed selected convictions and pending cases (N=68 as of the date of this Report) primarily regarding the alleles or loci used for generating the reported statistic(s) to determine whether they comport with correct practices within the general scope of the current DFS SOP in effect at the time the DFS final reports were generated.

The Panel members are well aware of the methods used by forensic DNA analysts to evaluate and interpret forensic DNA mixture evidence. Such protocols involve applying similar methods to those used to evaluate single source DNA profiles with the additional special focus on factors such as PCR artifacts, PCR stutter, allele and locus drop-out and drop-in, allele stacking or sharing, DNA degradation, etc. Mixture evidence is far more common and complex today than it was at the inception of forensic DNA typing. Statistical evaluation of such DNA mixture evidence requires careful interpretation of results and application of appropriate statistical tools to perform calculations which estimate random match probabilities, the so-called Combined Probability of Exclusion/Inclusion (herein after referred to as the CPI), and likelihood ratios comparing the probabilities of DNA evidence under competing hypotheses.

On January 29, 2015 the DFS issued a memo in response to the Panel's concerns based on its initial review of selected DNA mixture casework. In this memo the DFS response was

"All of the reported issues fall under the general category concerning the DNA mixture interpretation guidelines within the Unit. On January 27, 2015, the reported issues and related cases were reviewed in depth by DFS personnel. The general finding of the review were ultimately seen as a difference of opinion between experts in regards to all five of the noted issues. The arguments and criticisms raised in the USAO report were not found to be persuasive. In all cases, it was seen that the Unit personnel issuing the reports adhered to the Unit's DNA mixture interpretation guidelines that were in place at the time the work was performed on the cases. "

The Panel respectfully disagrees with the response by the DFS. Indeed, many of the concerns raised by the Panel relate to basic and fundamental aspects of a mixture interpretation process and the use of the CPI. The Panel appreciates that the CPI is considered to be an acceptable statistical approach to evaluation of forensic DNA mixtures. It also is well-established that the CPI cannot be applied to individual loci in situations where allelic drop out is either evident or probable. The Panel is aware that some of the DFS analysts have received some training on interpretation of DNA mixtures during the summer of 2014, prior to the time that concerns were raised by USAO. The Panel reviewed this particular training material which noted explicitly that the CPI cannot be applied in situations where there is a high probability of allele drop-out. The Panel agrees with this position on allele drop-out and application of the CPI. This same accepted principle was used by the Panel in its interpretation of CPI calculations performed by the DFS in selected cases that were reviewed.

Moreover, the approaches for using CPI are well-documented and described in the literature and in a textbook written by Dr. John M. Butler ("Advanced Topics in Forensic DNA Typing: Interpretation", Academic Press/Elsevier, 2014). Textbooks assemble common knowledge and practices and thus can convey the community wide understanding of what is considered to be generally acceptable, within the wide range of acceptable practices.

The passages below, taken directly from Dr. Butler's book, provide some examples of what are considered acceptable standards for calculation of the CPI when analyzing forensic DNA mixture evidence.

Page 321 - "CPI is based on the evidence only. Selecting different loci for comparison purposes, something often referred to as "suspect-driven CPI" is inappropriate since decisions on which loci are suitable for comparison should be made prior to doing a comparison to reference sample(s)."

Page 335 – "CPI can be a valid statistical representation of DNA mixture data provided that there are no missing alleles."

Page 335 – "Keep in mind also that a higher number of contributors dilutes out the amount of DNA for each contributor, which leads to more stochastic effects and the possibility of allele dropout and therefore less certainty in the overall interpretation."

Page 336 – "Urban Legend #6: If all peaks at a locus are above the established stochastic threshold, then the locus is safe to use. Allele stacking is a possibility (see Figure 6.6), especially with less polymorphic STR loci, such as TPOX and D5S818. Therefore, having for example TPOX alleles 8 and 11 above an established stochastic threshold (a situation that could occur due to allele stacking) does not mean that allele drop-out did not occur with one of the contributors to this mixture. This urban legend relates to Urban Legend #1 regarding the number of potential contributors."

Page 336 – "Urban Legend #8: Suspect-driven CPI (where the comparison of each suspect results in a different statistical result) is fine. The CPI statistic is calculated from the evidence profile and should not vary based on the reference profile."

Page 549 – "There are several requirements to consider before the RMNE approach can be appropriately used: (1) the individuals in the mixture are unrelated, (2) the individuals are from the same population group, and (3) all of the alleles in the profile are present (no drop-out), which is presumed by having all alleles at a locus possess peak heights above the stochastic threshold. Only loci where all of the alleles are present above the stochastic threshold should be used in the CPI statistical calculation. If there is any indication that data may be missing at the examined locus, perhaps due to the presence of allele peaks below the stochastic threshold that may raise the possibility of a missing sister allele, then anyone could technically be included in the mixture and the statistical weight of the locus would have a probability of 1."

These textbook passages on some of the fundamental principles of the application of the CPI, and its limitations, are consistent with the opinions of the Panel and not with the manner that DFS selected alleles and loci utilized in CPI calculations in selected casework reviewed at the request of the USAO. The Panel is well aware of the variety of practices in the forensic DNA community and reiterates that it was asked by the USAO to identify instances in which the interpretation of DNA evidence or the accompanying statistical analysis was questionable, but

not those which could be attributed to acceptable variation of DNA interpretation within the relevant scientific community.

Accordingly, the Panel identified a number of instances of flawed interpretation of DNA mixtures which were summarized previously and communicated to the USAO. Several examples are described herein (see Appendix) to help illustrate the Panel's findings that some of the interpretive practices at DFS were not supported by generally accepted scientific principles and are beyond what the Panel considers acceptable variation in the relevant scientific community.

Referring again to the DFS Memo of January 29, 2015, DFS is implementing a new SOP,

"Specifically, the proposed changes to the mixture interpretation protocols will address all of the issues raised by the scientific panel appointed by the USAO and by the DFS' Scientific Advisory Board. These protocols will include the documented justification of mixture identification, mixture deconvolution, and the determination of the number of potential contributors to a DNA mixture. The statistic calculation protocols will address the statistical inclusion or exclusion of individuals within a DNA mixture based on Combined Probability of Inclusion (CPI) methodologies, and when CPI should be applied as a calculation."

Action Item: The Panel recommends review of all previously reported DFS cases involving DNA mixtures to determine whether the new SOP addresses the thematic concerns identified by the Panel. In addition, the Panel recommends assessment of performance for a defined time period if the USAO returns DNA casework to DFS.

On-site DFS Visit (February 19-20, 2015)

The USAO requested the Panel to review DNA operations at the FSL of DFS for the purpose of assessment of general DNA Unit operations and laboratory protocols as they relate to interpretation of forensic DNA profiles. The USAO also asked the Panel to recommend whether any additional DNA interpretation training is warranted for DNA analysts at the DFS.

A 2-day on-site visit of DFS was conducted on February 19 and 20, 2015. The Panel conducted interviews with members of the Forensic Biology Unit and some members of the managerial staff at DFS. The DFS Director, General Counsel, and Quality Assurance Manager were not present during the 2-day visit and were interviewed subsequently in a follow-up telephone conference on February 25, 2015.

The Panel, accordingly, reviewed the DNA pipeline in place at DFS which included:

1. an on-site visit for 2 days to review the updated protocols, equipment and validation studies, recent audits, and workflow pipelines, to include the internal case technical and administrative review procedures;
2. review of all DNA training materials and recent updates to laboratory protocols, bench worksheets and statistical worksheets; and
3. meetings with some of the key DFS personnel who perform and oversee DNA analyses to review background and level of experience with forensic DNA analyses.

The Panel requested specific materials for review, including:

1. DNA SOPs and work instructions, to include the newly implemented SOPs and the criteria for inclusion and exclusion,
2. summary of review process in general, including case review, technical review, and administrative review,
3. DNA and General Lab Documents,
4. past two internal and external audits,
5. corrective action policies,
6. records of problems and remediations,
7. DNA mixture validation studies,
8. Internal validations to determine detection thresholds and stochastic thresholds at all conditions utilized (e.g. 28 cycles, 29 cycles),
9. machine noise testing,
10. personnel files relating to:
    a. university transcripts;
    b. transcripts of testimony;
    c. proficiency test scores;
    d. training programs including in-house and continuing education;
    e. corrective action pertaining to specific analysts;
    f. qualifying exams; and
11. documentation relating to meetings of management with personnel regarding policy and guidance regarding DNA typing for the last 12 months.

Findings from the On-Site Visit of DFS

Interpretation and Statistical Analysis of DNA Mixtures

During the on-site visit the Panel met with most members of the DNA unit to discuss general issues surrounding the new SOP and DNA mixture interpretation. The goal was to try to understand the background and level of training of the DNA staff and to try to determine the rationale to explain why for example, in some of the cases reviewed by the Panel, a CPI statistic was applied using loci at which the allele(s) of individual(s) who were not excluded as possible contributors were not observed in the evidence profile itself *(The CPI estimates the portion of the relevant population(s) that could be potential contributors to a DNA mixture profile. If the same alleles present in a suspect's known sample are not seen in the mixture evidence, then the CPI cannot be used. Thus, if a CPI estimate were generated, it would have no relevance regarding the potential inclusion of that particular suspect).* For a locus to be included in a CPI there should not be convincing evidence or a high probability of allele or locus drop out. During the on-site interview the technical leader stated she did not recall such a practice at DFS, although the Panel identified such examples in the casework reviewed.

During the on-site visit, the Panel was informed that four upper management personnel (two of whom had forensic DNA analysis technical experience) were those who reviewed the thematic issues and specific cases of concern that had been initially identified by the Panel. The Panel was informed that these specific cases were not discussed with the Technical Leader or the DNA analysts assigned to the cases. There were no other materials available to the Panel for review other than the unsigned memo sent to USAO that conveyed upper management's position.

Despite a number of attempts by the Panel during the on-site visit to learn about the scientific bases of upper management's position and if there were any differences in opinion regarding the specific cases, those who were interviewed declined to engage in any discussion other than to state that the DFS position taken was an "agency position". Therefore, those at DFS who were familiar with specific concerns raised by the Panel provided little insight regarding the underlying scientific principles used to support the DFS position on the issues raised by the Panel. One point that was reiterated repeatedly was that a statistical estimate could vary, for example, between 1/2000 and 1/1000 and such differences are minor or meaningless; thus there should be little concern if a locus or two used in calculations were questioned by the Panel or the USAO. Such a position is not sustainable as some of the statistical estimates, when calculated in accord with the Panel's recommendation, changed by several orders of magnitude (even from 1 in millions to 1 in tens) or could not be calculated at all.

It is the opinion of the Panel that it is not appropriate to justify use of a particular protocol or interpretive practice based on differences in numerical statistical estimates. The Panel agrees that statistical estimates can vary somewhat, that some of the differences might not be significantly different from one another and that the confidence intervals of different estimates might overlap. That fact notwithstanding, it is concerning if interpretive errors are not identified prior to issuing a final report and, if such errors do occur, that they might not be addressed simply because the degree of variation in a statistical calculation may be deemed nominal.

At the time of the Panel's site visit, DFS was in the process of implementing new SOP for DNA mixture interpretation. The DFS memo of January 29, stated,

"Specifically, the proposed changes to the mixture interpretation protocols will address all of the issues raised by the scientific panel appointed by the USAO and by the DFS' Scientific Advisory Board. These protocols will include the documented justification of mixture identification, mixture deconvolution, and the determination of the number of potential contributors to a DNA mixture. The statistic calculation protocols will address the statistical inclusion or exclusion of individuals within a DNA mixture based on Combined Probability of Inclusion (CPI) methodologies, and when CPI should be applied as a calculation."

Action Item: Additional training on DNA mixture interpretation should be offered along with competence assessment of DNA analysts (and relevant supervisors) prior to performing any additional casework. A more formalized root cause analysis process should be implemented at DFS to address the issues noted above. Once additional training and competency assessment has been successful there should be an interim period of review of cases going forward under the recently modified SOP.

Technical Review Process

The technical review process in the FSL DNA analysis pipeline appears to the Panel to be woefully inadequate. The Panel noted inconsistencies among analysts in how they selected loci for inclusion in statistical calculations as well as errors in reports and case files which escaped notice in technical or administrative review. For example, the Panel was informed that use of two different CPIs for the same mixture profile was due to practices of two former DNA analysts and, that, because they are no longer employed, this practice of calculating two CPIs for a single mixture is no longer an issue. Yet, the current staff (including the technical leader) served as technical reviewers in such cases (for example, see **US v. Roble 2013-CF1-6095, DFS Lab # M130206-1**). The Panel believes that both the SOP and technical review should have identified this issue prior to issuing a report.

Action Item: While the new SOP contains a helpful documentation worksheet, the technical review process did not appear to the Panel to be addressed adequately. As some of the DNA technical reviews were performed by current DNA staff members, additional training and education on performance and documentation of a thorough technical review are needed. Based on review of casework and discussions during the on-site visit, it is the opinion of the Panel that the current DNA technical leader deserves more guidance and support from DFS management in order to address issues of concern to the Panel and to successfully lead the DNA Unit.

Potential for Interpretation Bias

Interpretation bias, a form of cognitive bias, is often an inherent, sometimes subconscious, human tendency to interpret unclear or vague results into a positive or negative outcome.

Processes should be implemented in forensic laboratories to recognize and reduce interpretation bias. One such process involves the technical review component of the analysis pipeline. The Panel found several examples involving DNA mixture interpretation problems that are consistent with the possibility of such bias. In **US v Jeffrey Neal (2014-CF1-010507, DFS Lab # M140492)**, the original DFS report for item 26c1.1_26c1.2 stated "The major male contributor is consistent with the DNA profile obtained from the known sample from Jeffrey Neal (Item 76)." The Panel disagreed with the interpretation by DFS. To assess whether interpretation bias might have factored into the original interpretation by DFS an electropherogram from this case (item 26c1.1_26c1.2) was redacted and shown to a DNA analyst and the technical leader during the on-site interviews. The DNA analyst and the technical leader were each asked to interpret the profile under both the new SOP and the interpretation guidelines employed when this case was analyzed. The Panel notes that the analyst was the original technical reviewer of the electropherogram. To reduce the chance for interpretation bias the analyst and the technical leader were not shown the reference profile of the suspect when asked to interpret the mixture profile in determination of the types of the major contributor (as this is the proper practice). Both opined that the profile of the suspect would be excluded as the major contributor of the profile (an interpretation consistent with that of the Panel but discrepant with the actual final laboratory report). Both stated that they would reach this same interpretation whether they used the previous or currently enacted SOPs for mixture interpretation. It is noteworthy that DFS upper management reviewed this same case and supported the original interpretation.

This observation (and others) supports the view that DNA analysts need to be aware of the possibility of and try to avoid interpretation bias in the analysis and interpretation of DNA mixture evidence as the technical review process alone might not be sufficient at identifying and correcting all analytical and interpretation errors.

Action item: The technical review process should be formalized to address the potential of interpretation bias in forensic laboratories. Casework should be reviewed to identify and address any instances in which such bias affected interpretation of results. Training and continuing education of staff should include lectures on cognitive bias, how it affects interpretation, and tell-tale signs to identify when it may arise.

Analytical Threshold Validation

The Panel was informed that DFS has implemented a new DNA SOP (for interpretation) that contains a requirement of more documentation during the analytical phase of the DNA analysis pipeline. The new SOP makes reference to different analytical thresholds (ATs) for DNA results above and below 1000 RFUs. The Panel expressed concern that the new derived AT thresholds may be inappropriate as different types of samples were used to determine the AT values. The AT thresholds above 1000 RFU were derived from samples amplified with ideal target quantities of DNA while the AT thresholds below 1000 RFU were based on data derived from 29 negative samples. The Panel notes that using different data sets for AT thresholds – a lower set for less than 1000 rfu data and a higher set for above 1000 rfu - is not appropriately derived from the DFS validation study, as baseline "noise" from samples that contain DNA is higher than from

samples that do not contain DNA. Samples with low and ideal target quantities of DNA inherently will have more "noise" than "negative" samples. Accordingly, higher AT thresholds are appropriate for such samples.

Action Item: The Panel recommends additional input for performing, interpreting, and applying validation data is required for upper management and staff.

New DFS Policy on Minimum # of Loci

The Panel was informed that DFS has instituted a new policy which requires a minimum of six loci to be interpretable to report a statistic for a DNA mixture deemed to be composed of three or more individuals. The rationale for this decision was not clear to the Panel, as there could be instances for which DNA results on less than six loci may be probative for either the defense or the government. Therefore, the Panel, through the USAO, requested additional information on the rationale for selecting a six loci threshold. The Panel was informed through this communication from DFS that this decision was based, in part, on CODIS upload requirements.

The Panel notes that the principal reasons that CODIS (either NDIS, SDIS or LDIS) selects a minimum locus threshold for uploads and searches is for managing the number of adventitious hits and subsequent downstream workloads. The DFS justification for reporting DNA mixture statistics, based on a potential upload to CODIS, does not consider the statistical power that can be present even with less than six loci and it does not consider how DNA evidence can support alternate hypotheses during litigation. The potential value of even limited evidence could be meaningful for both defense and prosecution.

Action Item: The Panel recommends that DFS fully engage its customers before implementing such a new policy.

29-Cycle PCR

In the cases reviewed by the Panel, DFS employed two different PCR protocols for DNA analysis, i.e., a 28 cycle protocol and a 29 cycle one. The former is used routinely and if a sample yields low DNA profile signal results and at the discretion of the analyst, the 29 cycle PCR protocol is employed. The different PCR cycles have different stochastic thresholds (STs) – the 28 cycle has a 200 rfu ST, and the 29 cycle has a 300 rfu ST.

The Panel noticed that with the 29 cycle protocol stochastic effects may be increasing compared with the 28 cycle protocol, and some interpretations of results appeared to be associated with cognitive (interpretation) bias (as described above in US v Jeffrey Neal). Initially, the Panel considered further review of DFS's validation studies regarding the 29 cycle protocol and whether the findings of these studies comport with the current SOP. As the new DNA SOP does not describe interpretation of DNA results generated from the 29 cycle protocol, the Panel

questioned whether the 29 cycle protocol was still being employed at DFS[3]. On April 14, 2015 the DFS informed the Panel, through the USAO, that this 29 cycle protocol was discontinued in June 2013. Given the potential of increased stochastic effects to affect reliability of results, cessation of the 29 cycle protocol should be investigated further. Elimination of the 29 cycle protocol is noteworthy as some of the cases identified by the Panel employed the 29 cycle methodology with interpretation problems. One of the cases described herein (**United States v. Breal Hicks, et al, 2013-CR-203 (RJL), DFS Lab # M130107-1**) is an example of the panel's findings of problematic interpretation which involves a 29 cycle-generated STR profile.

Action Item: Additional review will be needed of cases in which the 29 cycle protocol was used along with notification or clarification in adjudicated and pending cases in which it was utilized. Furthermore, quality assurance practices need to be formalized to include better communication to DFS customers about any material changes in protocols.

Post-audit telephonic conversation with DFS officials

During the telephonic discussion with top DFS officials on February 25, 2015, among the explanations offered for the Laboratory's responses on thematic issues raised by the Panel and with regard to the specific casework in question included the following three;

1) The DFS lab followed its own protocol, there is no absolute standard, and "other labs do it that way".

The Panel again refers DFS to the Butler text and to their own past and recent training material and reiterates its opinion that the thematic issues raised require corrective action.

2) Nowhere is it explicitly stated that DFS practices of concern identified by the Panel cannot be performed in the manner DFS has done it.

Again, while the Panel is aware of a variety of practices in the community, some of the approaches used by DFS are simply not sustainable, and,

3) A materiality criterion (i.e., whether someone was wrongly convicted as a result of the interpretations used by DFS).

The Panel finds that materiality does not address the specific thematic issues raised by the Panel. Materiality is the responsibility of those in the legal system and can change throughout the course of an investigation, legal arbitration, or trial. Laboratories often do not necessarily have access to materiality, and in a case in which materiality is of issue resolution may take years.

[3] Deduction of termination of the 29 cycle protocol was based on the lack of description for interpretation in the newly instituted SOP and from a telephone communication between Dr. Budowle and a former employee.

As mentioned earlier, the Panel had been informed by those most knowledgeable and intimately involved with the DNA casework (i.e., the technical leader and DNA analysts) that they were not involved in any attempt at a root cause analysis of the issues raised initially by the Panel. Such analysis and corrective actions, when needed, require enhanced communication with those involved in DNA typing process.

Action Item: A full root cause analysis is recommended so that the issues raised by the Panel can be carefully and systematically addressed.

Summary of Recommendations to Consider

1) Additional Training and qualifying exams for DNA analysts. The Panel recommends additional training on DNA mixture interpretation along with competence assessment of DNA analysts (and relevant supervisors) prior to performing any additional casework involving forensic mixtures. Then there should be an interim period of review of cases going forward under the recently modified SOP.

The Panel is aware that DNA analysts in the FSL of DFS had received some training on DNA mixture interpretation and statistical calculations by selected members of the DFS Advisory Board in the summer of 2014 and again in early 2015. A review of these training materials confirmed that basic DNA mixture interpretation was included but there were few if any examples of the case specific concerns identified by the Panel in its review of selected casework.

Use of a new worksheet to carefully document steps in the DNA interpretation pipeline is a welcomed and very good step forward and should reduce chances for cognitive (interpretation) bias. The Panel notes that additional recent internal training at DFS was designed to familiarize the analysts with the new mixture interpretation protocols and documentation worksheets. However, going forward it will be important that additional training be focused on the issues raised by the USAO and the Panel so that the areas of specific concern can be fully addressed.

The Panel reviewed the DNA profile used by DFS for final evaluation of the DNA analysts. The Panel finds that the "test" utilized does not constitute an adequate assessment of each analyst's ability to properly evaluate and interpret DNA mixture evidence. Serious consideration should be taken to develop and deliver tests that effectively assess each analyst to help assure that the above protocols, procedures, review processes, and continuing education enable DFS staff to attain high quality DNA typing results.

2) Internal Quality Improvement Program Needed. Open discussion of the concerns of the USAO and the Panel is needed with the DNA analysts, technical leader, supervisors, and management. DNA analysts may have had cogent reasons for how they proceeded in individual cases or their practices may not be acceptable. It is most productive to have communication with all practitioners when assessing the root cause of potential problems.

3) Improvements in Technical Review: The Panel was informed that DFS plans to perform group technical reviews of upcoming DNA mixture cases, for an unspecified time period, using its new interpretation protocol. Review of this process will be needed to assess whether such changes will address the concerns and issues raised to date by the Panel and the USAO. In addition, review of the process should address any documentation requirements for such group technical reviews.

4) Validation of Analytical Thresholds. As noted above the process described appears, to the Panel, to be inappropriate and needs more attention before final implementation.

5) Revisit policy on minimum # of loci for DNA mixture statistics. This matter warrants discussion with customers and should be addressed on a case-by-case basis.

6) Audit of past cases. Review of additional previous casework regarding DNA mixture analysis is warranted to identify items in which departures from recommended interpretive practices exist and which were not identified during the non-exhaustive review by DFS or by the Panel. Specific attention will be needed in the review of cases in which the 29 cycle protocol was used.

7) Training and continuing education for upper management. Continuing education and management training should be encouraged for upper management and supervisors to include topics on DNA analysis, quality assurance practices and root cause analysis. Upper management should communicate more effectively with its customers and DNA analysts regarding any concerns raised by its clients and advisors. Such communications and deliberations should be carefully documented and all material changes in its SOP (e.g., 29 cycle PCR) should be communicated in a timely fashion to its key clients.

Suggested Readings

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APPENDIX

Case Examples

United States v David Shepherd, 2012CF1009602, DFS Lab# M140247

In US v Shepherd DFS concluded that Henry Miller (item 5.0) could not be excluded as a potential contributor to DNA extracted from a weapon swab (items 3.1W_3.1D). DFS applied a CPI calculation and obtained estimates of the portion of the population that could be included that ranged from 1 in 368,000 to approximately 1 in 1 million. Intuitively, for Henry Miller to be included as a potential contributor then the alleles he carries also should be observed at the loci included in the CPI. Otherwise the calculation has no relevance regarding an association of Henry Miller and the evidence item. Based on the tables in the laboratory report and the electropherogram, DFS applied the CPI to the following loci D7S820, D3S1358, D2S1338, D19S433, vWA, and TPOX. At D7S820 the evidence item displayed 8,12 and Henry Miller displayed 10,12; at D2S1338 the evidence item displayed 17,20 and Henry Miller displayed 17,23; and at TPOX the evidence item displayed 9,11 and Henry Miller displayed 6,8. For Henry Miller to be included and a CPI rendered DFS must assume that allele drop out has not occurred at these three loci. However, the CPI calculation at these three loci did not include the 10 allele at D7S820, the 23 allele at D2S1338, and the 6 and 8 alleles at TPOX that are present in DNA from Henry Miller's known sample. If the laboratory used the CPI at these loci under the assumption that no allele drop out has occurred then Henry Miller would have been excluded as a potential contributor. Yet, DFS and the Panel concur that Henry Miller cannot be excluded as a potential contributor to the DNA mixture. The laboratory report attributes the major contributor of the mixture of items 3.1W_3.1D to an unknown male. Therefore, DFS can only reasonably include Henry Miller as a potential minor contributor. The laboratory results indicate that alleles at the three loci (D7, D2, TPOX) are derived from the "major" contributor and that the "minor" contributor alleles potentially have "dropped out". Therefore, DFS rendered a CPI mixture calculation on portions of the evidence where only a single source is the most plausible explanation of the profile and by the laboratory's own statement the alleles are not attributed by Henry Miller. In essence, DFS has provided a statistical calculation of the portion of the population that could potentially contribute to the mixture of item 3.1W_3.1D in which Henry Miller is not part of the portion of that population.

It is a reasonable expectation that alleles form the "minor" contributor of items 3.1W_3.1D could be dropping out at a number of loci. Yet, DFS failed to take into consideration the potential of allele dropout of the minor contributor at the loci D3S1358 and vWA. The outcome is that of the six loci that were used to calculate the CPI only one marker should have been considered. The strength of the evidence will be substantially reduced.

The Panel concludes that DFS has not interpreted the evidence correctly for this item in this case to account for allele drop out and should more carefully evaluate allele profiles of evidence and reference samples when an inclusion is made.

United States v. George Cocroft, 2012-CF1-20633, DFS Lab # M130061-1

In this particular case DFS concluded that the Brown (victim) and Cocroft (defendant) cannot be excluded as possible contributors to the mixture from item 1A.1SF, a vaginal swab. However, DFS calculated a CPI only for Cocroft and despite Brown's inclusion in the mixture no CPI was calculated regarding Brown as a possible contributor. The computation of a CPI statistic for one possible contributor to a mixture and not another shows a fundamental lack of understanding regarding CPI calculations. Presumably no statistical calculation was associated with Brown is because some of Brown's alleles are not observed at loci used for the CPI related to Cocroft. Thus, allele dropout must have occurred to explain Brown as a contributor. The CPI statistical results in reference to the mixture obtained from Item 1A.1SF failed to address the potential for drop out. Specifically, DFS calculated a CPI at the D3S1358 and D13S17 loci. At D3S1358, DFS called "17,17" alleles, but Brown is a "15, 17" at that locus. Thus, Brown's 15 allele would have had to drop out to be included as a contributor. At D13S317, DFS called "11,11" alleles, but Brown is a "12, 13" at this locus; again indicating allele drop out to explain Brown as a contributor. To conduct a CPI calculation at these two loci, DFS would have had to exclude Brown from her own intimate sample. The panel notes that the DNA profile of Brown was present in the epithelial fraction of the same vaginal swab.

A prominent inconsistent practice identified by the Panel in review of selected cases was not subtracting out the victim contribution from a vaginal swab and yet in other cases intimate samples subtraction was performed. The use of CPI in this case stems from DFS failing to subtract out the alleles of Brown from her own intimate sample (despite that fact that DFS's protocols permit subtraction of a known contributor from a mixture). In fact, the sample given in the DFS protocol is subtracting out a victim's profile from the DNA profile of the sperm fraction from a vaginal swab. While not subtracting the victim profile from an intimate sample is within acceptable variation and one might agree with this position, there were a number of examples in the cases reviewed by the Panel where subtraction was performed and this case was the only one identified where it was not performed. The Panel notes if subtraction was performed the statistical calculation would have resulted in a value of 1 in 900 million using the RMP. While the Panel would have chosen to perform a subtraction, it does not take a position on whether this is within acceptable interpretation variation. Instead the Panel points out that the practices of subtraction varied within the same lab, which is an indication of a lack of consistent technical review in the DNA analysis pipeline.

United States v. Breal Hicks, et al., 2013-CR-203 (RJL), DFS Lab # M130107-1

DFS concluded that a male profile was obtained from the dry swab from the magazine within a .380 cal pistol (Item 40) and that "Dwayne Brown (Item 58.1) cannot be excluded as a possible contributor of this profile. All other submitted known samples were excluded as possible contributors of this profile. DFS then calculated the RMP for an unrelated, random individual having a STR profile which cannot be excluded as a contributor of the DNA profile obtained from the dry swab from Item 40. It is noted that DFS did not declare explicitly the profile developed from Item 40 was a mixture, although the profile clearly is a mixture. In addition, the

29-cycle amplification protocol (discussed above) was used for analysis of Item 40. Based on the results Brown should have been excluded from the profile from Item 40. The Panel was concerned that interpretation bias might be part of the explanation for the inclusion - based on the known profile of Brown - even though there are inconsistent results between the evidence and the known profile of Brown at the D8S1179 and TH01 loci. Because DFS employed a 29 cycle amplification the ST is set at 300 rfu. The ST of 300 rfu is supposedly based on validation studies by DFS. Therefore, a single peak at a locus would be deemed a homozygote as there should be a very low probability that allele drop out would have occurred (which is the purpose of establishing ST). DFS reported a genotype assignment at the D8S1179 locus as a "15,15" for item 40, indicating that DFS interpreted the genotype as a homozygote, as it should have based on its established ST. In contrast, the known reference sample of Brown has two alleles – a 12 and a 15 at the D81179 locus. That is, Brown is a heterozygote, not a homozygote at the D81179 locus. Also, DFS labeled the TH01 locus for item 40 as "6,(8)" and the known profile of Brown has only a 6 allele (no 8 allele) at the TH01 locus. As the DNA profile for Item 40 was not described by DFS in the report as a mixture, the TH01 profile from item 40 cannot be interpreted as consistent with that of Dwayne Brown. One might argue that the peak height ratio (PHR) for the 6 and 8 allele at the TH01 locus in item 40 is below 50% (i.e., 48.1%) and that could explain the reason for not including the locus in the RMP calculation. However, DFS routinely has used lower PHRs for the RMP. More importantly, if the 8 allele was rejected as part of the major profile then the sample should have been declared a mixture. Based on DFS's interpretation guidelines, and the other considerations described above, it was the opinion of the Panel that Dwayne Brown should have been excluded as a potential contributor of this particular item.

The Panel opined that bias could have factored into the interpretation by DFS because the RMP did not include inconsistent loci (and particularly not including the D8S1179 data in the statistical calculation performed by DFS). Simply removing inconsistent loci from a statistical calculation, in and of itself, is not necessarily a conservative practice (especially if the data are exculpatory). The data are discordant based on DFS interpretation guidelines, and DFS should have reported the discrepancies in its report (and at a minimum in its case file). The practices described in this case are not, in the opinion of the Panel, in accordance with accepted practices within the scientific community when conducting statistical analyses of forensic DNA mixtures.

United States v. Mohammed Roble, 2013-CF1-6095, DFS Lab # M130206-1

DFS concluded that Tiblets and Roble cannot be excluded as potential contributors to the DNA mixture obtained from Roble's hand swab item 1_2 (a swab from back of left hand and fingers/palm side left hand and fingers). For reasons unclear to the panel, the DFS calculated two separate CPIs for this exemplar - one calculation using allele calls at locus D16S539 and one NOT using allele calls from locus D16S539. The allele calls for the mixture profile were 10,11. The known profile of Roble shows a 9,11 at the D16S539 locus. Since DFS included Roble in the mixture, the fact that there is no indication of a 9 allele DFS must assume that complete allele drop out occurred. Applying a CPI that fits only Tiblet's profile does not take into account allele drop out and misstates the portion of the population that could contribute to the mixture (at the loci used for the CPI). Calculating two CPIs is inconsistent with the concept of applying the CPI.

ANSI-ASQ National Accreditation Board

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April 24, 2015

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INTRODUCTION:

The ANAB assessment team was tasked with the review of the mixture interpretation procedures previously used by the District of Columbia Department of Forensic Laboratory and the new procedures implemented in February 2015 after recent validations conducted by the laboratory. The ANAB assessment team also reviewed DNA mixture cases analyzed with the previous DNA mixture interpretation procedures as well as a thorough review of available DNA mixture cases analyzed with the new DNA mixture procedures implemented in February 2015. Analysts of the District of Columbia Department of Forensic laboratory were interviewed on the previous procedures, recent validations and new mixture interpretation procedures. Management of the laboratory was also interviewed during the onsite visit of the ANAB assessment team. This assessment was conducted under the authority of the District of Columbia Department of Forensic Laboratory's accreditation, at their request, to both ISO/IEC 17025 and the FBI QAS.

Activities performed by assessment team:

A. ***Departmental Operations Manual***
   Current departmental operations manuals were reviewed and found to be in compliance with the FBI Quality Assurance Standards and ISO/IEC 17025 guidelines.
B. ***FSL Quality Corrective Action Practices***
C. Corrective actions for 2013 and 2014 were reviewed (ISO/IEC 17025). ***FSL Laboratory Operations manuals, Quality Documents and training manuals***
   The current FSL laboratory operations manual, quality documents and training manuals were reviewed by the assessment team.
D. ***FBU Quality control, reagent and standard operating procedures***
   The current FBU quality control, reagent and standard operating procedures were reviewed by the assessment team. .
E. ***Previous two FBI QAS external audits***
F. The external FBI QAS audits for 2013 and 2014 were reviewed. The FBI Quality Assurance Standards for DNA Testing Laboratories effective September 1, 2011 were used to evaluate the laboratory. c***DNA Mixture validation studies, training and interpretation***
   The mixture validations, training and interpretation guidelines approved by the laboratory in February 2015 were reviewed by the assessment team.
G. ***Qualifying examinations and standard for analyst positions***
   The competency testing of the analysts has not been completed. The completion date of the competency testing is set for July 2015.
H. **Machine noise testing**
   The analytical thresholds were evaluated by the assessment team.
I. ***Records of problems and remediation***
   The corrective actions of the DNA sections and all remediation were reviewed by the assessment team.
J. ***Personnel files***
   The personnel files of all analysts were reviewed.
K. ***Previous twelve months of documentation from meetings of management with personnel regarding policy and guidance regarding DNA typing***
   This information was not provided to the assessment team.
L. ***Recent validations regarding new protocols and training***

The audit team has determined that the recent validations were conducted in accordance with the FBI Quality Assurance standards and the guidelines set forth by the Scientific Working Group of DNA Analysis and Methods regarding validations:

I. *Quantifiler®Duo DNA Quantification Kit Using the ABI 7500 Real Time PCR Instrument validation was reviewed. The following studies were conducted by the laboratory: precision, reproducibility, accuracy, sensitivity and reproducibility, contamination, standard curve evaluation, mixture and NIST assessment.*

II. *Applied Biosystems® AmpFlSTR® Identifiler® Plus PCR Amplification Kit Validation Using the Applied Biosystems® 3130xL Genetic Analyzer validation was reviewed. The following studies were conducted by the laboratory: precision, sensitivity (with 1ng and 0.5ng using 28 and 29 cycles), accuracy (with 1ng and 0.5ng using 28 and 29 cycles), reproducibility (with 1ng and 0.5ng using 28 and 29 cycles) and mixture (with 1ng and 0.5ng using 28 and 29 cycles). See recommendation #6.*

III. *Validation of the QIAGEN EZ1 Advanced XL workstation with the DNA Investigator DNA Extraction Kit was reviewed. The following studies were conducted by the laboratory: sensitivity study, reproducibility and precision study, accuracy and concordance study, contamination assessment, mixture study and mock evidence sample evaluation.*

IV. *Statistical Cut-off study using AmpFISTR ®Identifiler® Plus Amplification kit approved by the technical leader on February 1, 2015 was reviewed. The following studies were conducted by the laboratory: Random match probability study, combined probability of inclusion (CPI) study, mixture study, random match probability worksheet validation and combined probability inclusion worksheet validation. See Recommendation #5.*

V. *Reevaluation of AmpFISTR ®Identifiler® Plus internal validation, approved by the technical leader on February 3, 2015 was reviewed. The following studies were conducted by the laboratory: Threshold reassessment- analytical threshold and stochastic threshold were evaluated, peak height ratio, stutter study and different approaches for data analysis and profile interpretation were evaluated.*

M. Sampling Review of casework and case files of all employees within the past 12

Case files were reviewed that were conducted with the old mixture interpretation procedures and the new mixture interpretation procedures.

N. Review of cases work involving DNA mixtures and CPI calculations

The case files reviewed involved DNA mixtures and CPI calculations.

O. Interview 8 – 10 FSL FBU scientists on casework, case flow, case processing, DNA mixture interpretation and training and case jacket reviews.

The following 10 analysts were interviewed: MacBean, Ciacco, Feiter, Zeffer, Larry, Mills, Curtis, Skillman, Johnson and Ferragut.

P. Interview five (5) DFS manager

The District of Columbia Department of Forensic laboratory was interviewed on FBU casework, case flow, case management and management oversight.

Eight completed cases and two in progress cases were provide to the assessment team to review that were analyzed and interpreted under the new mixture interpretation procedures. The team determined that these cases did not give a full range of statistical calculations. The cases did not include any CPI calculations. It is noted that the lab has done a tremendous amount of work reevaluating their mixture interpretation procedures, analytical and stochastic thresholds including starting a process of determining major and minor contributors from mixtures, something that was not done in the past. The new procedures also lay out the

approach the analyst is required to take while interpreting. However, only a limited amount of cases from a limited amount of staff have used these procedures. A fair assessment of whether everyone understands the new concept and whether all factors of a mixture are being evaluated in the mixture interpretation has not been determined

The report contains identified Nonconformities listed as Major/Minor

1. **Major Nonconformity:** A major nonconformity is the absence of or the failure to implement and maintain one or more of the accreditation checklist requirements or a situation which would, on the basis of available objective evidence, raise significant doubt as to operations or appropriateness of the results reported by the accreditation customer. The assessment team may judge numerous minor nonconformities against a single requirement to be a significant breakdown of the management system and thus a major nonconformity. Any minor nonconformity that is a repeat from the previous assessment will be considered a major nonconformity.
2. **Minor Nonconformity:** A minor nonconformity is any other nonconformity which seems to be an isolated occurrence and is normally easily corrected and verified.

Cited clause numbers refer to the International Standard ISO/IEC 17025 and/or the FBI QAS.

Nonconformity #1 (Major)
ISO/IEC 17025, 4.8 Complaints

Does the laboratory have a policy and procedure for resolution of complaints received from customers or other parties?

Are records maintained of all complaints and of investigations and corrective actions taken by the laboratory?

The complaint filed by the U.S Attorney's Office (USAO) with Department of Forensic Sciences was not addressed in accordance to the procedures defined in the Department of Forensic Sciences DOM07- Practices for Quality Corrective Action procedures. A corrective action in response to the complaint by the USAO office was not provided to the assessment team for review. The assessment team does not know if an official corrective action has been opened in response to the complaint filed by the USAO office.

Nonconformity #2 (Major)
ISO/IEC 170254.11 Corrective Actions

Section 4.11.1 General

Has the laboratory established a policy and a procedure and designated appropriate authorities for implementing corrective action when nonconforming work or departures from the policies and procedures in the management system or technical operations have been identified?

The problem identified by the USAO office concerning the statistical interpretations of mixture cases was not formal addressed by the Department of Forensic Lab in accordance with the laboratory's procedures, DOM07-Practices for Quality Corrective Action procedures.

Section 4.11.2 Cause analysis

Does the procedure for corrective action start with an investigation to determine the root cause(s) of the problem?

The assessment team was not provided with a corrective action investigating the root cause of the problem regarding the statistical mixture interpretation complaint filed by the USAO office.

Nonconformity #3 (Major)

ISO/IEC 4.11 Corrective Action

Section 4.11.3 Selection and implementation of corrective actions

Where corrective action is needed, does the laboratory identify potential corrective actions? Does it select and implement the action(s) most likely to eliminate the problem and to prevent recurrence? Are corrective actions to a degree appropriate to the magnitude and risk of the problem? Does the laboratory document and implement any required changes resulting from corrective action investigations?

Section 4.11.4 Monitoring of corrective actions

Does the laboratory monitor the results to ensure that the corrective actions taken have been effective? In 2013 and 2014, the District of Columbia Department of Forensic Science laboratory had the some of the same findings identified in both years. It is apparent the corrective action plans that were put in place in 2013 were not effective since they occurred in 2014. The monitoring of the issues was not effective for the finding to occur again in 2014. In 2014 the laboratory also received a finding for not having a uniform procedure for reporting mixtures. The corrective action of this finding and the monitoring was not effective.

Nonconformity #4 (Major)

ISO/IEC 17025 5.2 Personnel

Section 5.2.1 The laboratory shall ensure the competence of all who operate specific equipment, perform tests, evaluate results and sign test reports.

Section 5.2.2 The laboratory shall have a policy and procedures for identifying training needs and providing training of personnel.

Section 5.2.5 The management shall authorize specific personnel to perform particular types of sampling, test, to issue test reports, to give opinions and interpretations and to operate particular types of equipment.

The laboratory did not ensure that all staff performing DNA mixture cases were competent to evaluate results. The laboratory did not provide training for all personnel involved with DNA mixture case interpretations. The laboratory allowed staff that was not competent or trained to perform DNA mixture cases.

The analysts were interviewed regarding their individual case files regarding case flow, case processing, DNA mixture interpretation, training, recent validations and training. The analysts were also asked questions concerning the lab's old procedures and mixture interpretation guidelines that were used on the case files reviewed by the assessors. It was apparent that the previous procedures lack guidance on mixture interpretation, how to determine the number of contributors, how to determine major and minor contributors, the proper application of Combined Probability of Inclusion (CPI) to mixture profiles and the guidelines on the review of DNA mixture cases. The analysts were not able to show when the loci where determined to be used for statistical purposes, if this occurs after the mixture was evaluated or after comparison to the known standards. The analysts were not able to demonstrate how inhibition, dropout was considered when evaluating mixtures. The recent internal validation of the Reevaluation of AmpFISTR ®Identifiler® Plus and the Statistical Cut-off study using AmpFISTR ®Identifiler® Plus Amplification kit was discussed with the analyst. Some of the analysts were able to explain the new procedures. However,

most of the analysts stated that they have not used these new procedures on casework and are still getting familiar with the procedures. It is apparent that the analysts need further training on the deconvolution of mixtures, determining major and minor profiles and the proper use of the Combined Probability of Inclusion (CPI) methods.

Nonconformity #5 (Major)

ISO/IEC 5.4.5 Validation of Methods

Section 5.4.5.2 The laboratory shall validate non-standard methods, laboratory-designed/developed methods to confirm that the methods are fit for the intended use. The validation shall be as extensive as is necessary to meet the needs of the given application.

The laboratory used procedures for the interpretation of DNA data that were insufficient or inadequate. The new procedures effective Feb 2015 addressed some of these issues. However not all potential issues were addressed and validated in these new procedures. For example, in the case files provided to the assessment team that contained mixtures interpreted under the new test methods did not contain a CPI calculation as part of the interpretation.

Nonconformity #6 (Major)

FBI QAS standard, 8.4 Validations

Has the analyst or examination team successfully completed a competency test using the DNA analysis procedure prior to its incorporation into casework applications?

The analysts have not completed a competency test on the new validated mixture interpretation procedures but are currently using the procedures on casework.

Nonconformity #7 (Major)

FBI QAS standard, 5.2.3, 5.2.3.1 and 5.2.3.1.1 Personnel

5.2.3 Does the technical leader of the laboratory have responsibility for the following:

5.2.3.1 Does the technical leader have the following general duties and authority:

5.2.3.1.1 Oversee the technical operations of the laboratory?

There was no documentation that the technical was involved when the laboratory received the initial complaint regarding mixture interpretation. Therefore, the technical leader has not had oversight of the technical operations of the DNA laboratory.

Nonconformity #8 (Minor)

FBI QAS standard 9.1.1

Does the laboratory have a documented standard operating procedure for each analytical method used?

The FSB 18 procedure 7.3.1.1.1 states that the use of 2P rule can be used but must be approved by the technical leader. During interviews and reviewed of the case files, it was determined that the technical leader did not approve the use of the 2P in statistical calculations. This procedure was not followed in the cases reviewed using the previous procedures where this was a requirement. However, the new procedures, FSB22, removed the requirement of the technical leader approval on 2P calculations. The new procedures do not provide a formula using the 2P calculations. See recommendation # 11.

Nonconformity #9 (Major)

FBI QAS standard 9.6, 9.6.4 and 9.6.4.c

9.6 Does the laboratory have and follow written guidelines for the interpretation of data?

9.6.4 Does the laboratory have and follow documented procedures for mixture interpretation to include the following:
c. Policies for reporting results and statistics?

The laboratory's procedures FBS15ID+ and FBS18 which were used in the interpreting results on the cases the assessment team reviewed, lack guidelines on mixture interpretation and the proper use of statistical calculations. It was determined through the review of the cases files and interviews that the laboratory was not using the combined probability of inclusion calculation correctly. Cases reviewed by the assessment team demonstrated the inclusion of loci in the combined probability of inclusion calculation that had potential allelic drop out. SWGDAM Interpretation of DNA typing results for mixed samples published January 4, 2010 states the following: Section 4.6.3- When using CPE/CPI (with no assumptions of number of contributors) to calculate the probability that a randomly selected person would be excluded/included as a contributor to the mixture, loci with alleles below the stochastic threshold may not be used for statistical purposes to support an inclusion. In these instances, the potential for allelic dropout raises the possibility of contributors having genotypes not encompassed by the interpreted alleles. The laboratory was not following this recommendation in the cases reviewed by the assessment team.

Laboratory Actions Required by ANAB:

The laboratory's DNA section is not in compliance with the FBI QAS or the ISO/IEC 17025 standard. The non-compliance is in two general areas: technical and quality management system. For the technical area, staff were not competent (lack of completed training) and were using inadequate procedures (not fully validated and/or inadequately written). For the quality management system, there was a failure to address these issues before any casework was performed and a failure of not stopping casework when a compliant was received and/or when management including the DNA technical leader became aware of these issues.

DNA case work shall be suspended until all the nonconformities are successfully resolved. A completed assessment of whether everyone understands the new concept and whether all factors of mixture interpretation must be determined. This will include at a minimum the revalidation of test procedures, new interpretation guidelines based on these method validations for DNA mixture cases, creation of new procedures based on the interpretation guidelines, training of staff on the new procedures, competency testing of these new procedures, and authorizations of trained and competent DNA staff.

The laboratory will use its corrective action process to document these activities and to monitor the effectiveness of the new processes placed into service.

The new process shall include at least the following:

1. Update training manuals to include more detailed guidance on mixture interpretations.
2. A more detailed procedures on the review process involved in reviewing mixture cases.
3. Validations shall be conducted on three or more person's mixtures, since the laboratory has procedures to interpret three or more person mixtures without proper validation to support the procedures and lack of training for the analysts for three or more person mixtures.
4. The laboratory shall account stutter and dropout when conducting analytical threshold validations.
5. The analysts shall receive competency testing on the new procedures, and report writing training on the new procedures.

6. Reevaluation of the statistical cutoff study and the use of various analytical thresholds when applying to samples with apparent drop out.
7. A more detailed sensitivity study shall be conducted by the laboratory.
8. Statistical reevaluation of all the DNA cases that the assessment team reviewed, to include issuing amendment reports of any cases where the stats were applied incorrectly.
9. More detailed training with the analyst on the new validated procedures, mixture deconvolution, major and minor profiles, threshold evaluations and the proper use of the statistical method, Combined Probability of Inclusion, CPI.
10. Include a procedure for the correct use of significant figures in reporting statistics in case reports. This should include the proper method of rounding the statistical information.
11. The laboratory shall verify the ability to interpret the types of mixtures created from known standards that the interpretation protocols address.
12. Include a formula for 2P calculations. It should not be calculated just 2P, as that would not adjust for possible subpopulations and if it was actually a homozygous peak.

CONCLUSION:

DNA case work shall be suspended immediately until all the nonconformities are successfully resolved and all the required ANAB action items listed above are completed The laboratory is required to respond to nonconformities in writing within 30 days of receipt of the assessment report. The response shall identify the corrective action taken, including root cause analysis, selection and implementation of corrective action, and any follow-up confirmation of effectiveness. It is recognized that some non-conformities may require more than 30 days for completion of the process of root cause analysis, selection and implementation of corrective action, and confirmation of effectiveness, and in such instances the 30 day response must include a description of action taken to date and a plan with milestones for completion of the corrective action. Once the nonconformities have been resolved and accepted by ANAB, an additional onsite visit is required. This additional visit will determine ANAB's subsequent course of action, which may include suspension, reduction of scope of accreditation, or withdrawal of accreditation. During this period no testing of DNA cases will be performed.

COMMENDATIONS:

The assessors and ANAB would like to thank the DC Department of Forensic Sciences for their openness and willingness to provide any requested materials necessary to conduct an efficient assessment during the audit.

Respectfully,

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EXHIBIT M

of the profile should be considered when determining whether a profile is a partial profile or not, and all individual locus interpretations must occur prior to comparing to the known reference samples in the case. Some profiles may contain too many contributors, or be of poor quality, to allow the profile to be used for interpretation. The profile should be designated as inconclusive and the analyst's reason for doing so shall be documented in the case record. This determination shall be agreed to by the technical reviewer and, if necessary in the case of dispute, agreed to by the technical leader. See below for more guidance on interpreting and reporting partial profiles.

Stochastic effects

Decreasing levels of template DNA may lead to stochastic effects which may under-represent one of the alleles in a locus. Using a minimum analytical threshold of 75 RFU, the following guidelines will be followed for interpreting data from low concentration samples:

Concentration	Single Source	Mixture with Major Component	Mixture with no Major Component
>0.3 ng	X	X	X
Between 0.0625 ng and 0.3 ng	X	Interpret loci from the major profile that contain heterozygous loci. The minor profile will be deemed uninterpretable.	The entire profile is uninterpretable
<0.0625 ng	May interpret heterozygous loci (>75 RFU) or designate entire profile as uninterpretable	The entire profile is uninterpretable	The entire profile is uninterpretable

NOTE: X indicates that this combination of criteria does not meet the minimum criteria for stochastic amplification and the special guidelines for stochastic amplification are not applicable. Interpret according to the standard interpretation guidelines.

The table above represents commonly encountered general guidelines. If a departure from the above guidelines is determined to be necessary after discussion between the analyst and technical reviewer, approval from the technical leader is necessary prior to issuance of a test report.

Mixtures

Samples from crime scene evidence may contain DNA from more than one individual. The entire profile should be used to determine if there is sufficient information to conclude that the sample contains DNA from more than one person. The analyst should be aware that mixtures can consist of full and/or partial profiles from multiple individuals, and a full profile from each component is not assumed due to potential dropout,

Forensic Detection of Semen I. The Acid Phosphatase Test

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Introduction

Acid phosphatase is an enzyme secreted by the prostate gland that is present in large amounts in seminal fluid. It, like psa (prostatic specific antigen), is not unique to the prostate and can be found in other biological fluids including vaginal secretions. It is therefore considered a presumptive chemical test for the presence of semen and semen must be confirmed by other means (sperm detection or psa detection using membrane test systems).

Testing for the presence of acid phosphatase can be extremely helpful however, in locating semen stains on clothing and for testing swabs from sexual assault cases. A strong positive reaction generally indicates that semen is present and that further testing is warranted.

For an excellent review on the history of acid phosphatase detection, see Gaensslen [1]. A number of testing methods exist for the sampling of items for the presence of acid phosphatase. The enzymatic breakdown of sodium-α-naphthyl phosphate by acid phosphatase and the subsequent conversion of o-dianisidine to a colored compound by the free naphthyl is a recognized test procedure for the detection of semen [2]. The Serological Research Institute (SERI) produces a powder they call ap spot test. When the powder is reconstituted in water, it can be used to screen stains and swabs for the presence of semen.

The sensitivity and stability of the product are discussed.

Materials and Methods

<u>Sensitivity</u>

Acid phosphatase was obtained from Sigma Chemical Company. The product number was P-1146, Lot 051K7038 and was isolated from potato. 50 units were purchased, consisting of 7.5 mg of solid having an activity of 6.7 units/mg (50.25 units). The solid was dissolved in 200 µL of deionized water. 100 µL of this solution (25 units) was added to a cotton-tipped swab that was allowed to air dry. 50 µL deionized water was added to the remaining 100 µL, mixed and 100 µL of this solution (17 units) was added to a

cotton-tipped swab that was allowed to air dry. Subsequent dilutions were made in this manner resulting in dry cotton-tipped swabs having the following units of acid phosphatase: 25, 17, 5.6, 1.8, 0.6, 0.2, 0.05 and 0.02.

Testing of these dry swabs was conducted in the following manner. Deionized water was added to a small piece of Whatman filter paper #3. Each swab was pressed against the filter paper strongly between thumb and forefinger for ten seconds. A single drop of freshly prepared SERI ap spot test (Lot 1562) was added to each piece of filter paper and color changes were recorded after 10 minutes.

Stability

SERI ap spot test (Lot 1562) was prepared fresh daily and used for case analysis. The reagent was maintained in a small glass dropper bottle protected from light with tape at room temperature. At the end of the business day (approximately 8 hours), the reagent was placed in a plastic15 mL Falcon tube and refrigerated. The following morning, fresh ap spot test was prepared and kept on the lab bench along with the previous preparation. This procedure was followed for the three remaining days of the week.

Whatman #3 filter paper was moistened and a cotton-tipped swab containing 25 units of acid phosphatase was pressed to 5 areas of the paper (following the procedure described previously). The same procedure was followed with 17 units of acid phosphatase.

SERI ap spot test reagent (fresh, 1 day, 2 days, 3 days, 4 days and 5 days old) was added to the filter paper and color changes were recorded after 10 minutes.

The same methods were followed using SERI ap spot test reagents that were stored frozen for 1 to 5 days; however, the reagents were not removed from the freezer daily.

Results and Discussion

Sexual assault kits and clothing are routinely submitted to crime laboratories for examination for the presence of semen. Typically, forensic scientists conduct visual examinations for stains followed by examination with an alternate light source on clothing and bedding items. This is generally followed by testing of stains for the presence of acid phosphatase, an enzyme secreted by the prostate and found in high levels in semen. Swabs collected from sexual assault survivors are generally tested for the presence of acid phosphatase followed by tests for the presence of spermatozoa, and P30 if necessary.

It is customary to test stained areas and swabs collected from the survivor indirectly. In other words, a transfer method involving wet or dry cotton-tipped swabs or moistened filter paper applied as an overlay to a stained area or swabbing is employed. As recommended by Barnett, et.al. [3], presumptive test reagents should NEVER be applied directly to items of evidence.

Following this methodology, experiments were designed to determine the sensitivity of one acid phosphatase test. The Serological Research Institute (SERI) sells a product they call ap spot test. It contains sodium-α-naphthyl phosphate and o-dianisidine (Fast Blue B). If acid phosphatase is present in a sample and a drop of the ap spot test is added, the enzyme catalyzes the breakdown of sodium-α-naphthyl phosphate producing free naphthyl that reacts with o-dianisidine producing a purple colored compound.

Results of Sensitivity Tests

Freshly prepared ap spot test gave positive results with acid phosphatase diluted to 0.18 to 0.6 units.* A photograph of the results appears in Figure 1.

Figure 1. One drop of ap spot test added to moistened filter paper containing diluted acid phosphatase (25 to 0.02 units). Positive reaction (purple color change) obtained at 0.6 units.

Sensabaugh [4] published results of experiments designed to quantitate the levels of endogenous and postcoital vaginal acid phosphatase. He standardized data from several investigators including his data and obtained a range of endogenous vaginal acid phosphatase of 0.023 to 4.902 units. SERI's ap spot test would certainly react with these endogenous levels of acid phosphatase. Hence the presumptive nature of the ap test and the requirement that the presence of semen be confirmed in another manner.

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*One unit will hydrolyze 1.0 μmole of p-nitrophenyl phosphate per minute at pH 4.8 at 37 °C

Results of Stability Tests

The directions supplied with SERI's ap spot test state to prepare the reagent daily. The Fast Blue B dye is light sensitive. At room temperature, on the lab bench and in the light, the ap spot test will begin to turn yellow and brown material will precipitate out.

The results of the stability experiments are shown in Figure 2.

Figure 2. Photograph of filter paper with 25 and 12 units of acid phosphatase and drops of fresh, 1 day, 2 day, 3 day and 4 day old ap spot test reagent.

A decrease in activity was observed in 1 day old ap spot test reagent, however it still reacted fairly well. By two days, the activity of the reagent dropped significantly and by four days, the reagent has lost the ability to detect 25 units of acid phosphatase.

Tests were conducted to determine whether freezing the reagent could enhance stability. The results of this experiment are shown in Figure 3. As can be seen in Figure 3, freezing did enhance the stability of the reagent. However, the frozen reagents were not

removed from the freezer and thawed on the laboratory bench daily, but remained in the freezer.

Figure 3. Varying units of acid phosphatase (25 u, 17 u, 6u and 2 u) deposited on filter paper to which ap spot test, frozen for various days (fresh, 1 day, 2 days, 5 days), was added.

The ap spot test stored frozen for 5 days worked as well as the freshly prepared reagent. However, once thawed and left on the lab bench, this reagent would degrade just as fresh or refrigerated reagent.

Interpretation of the color change indicating a positive result can be subjective. As seen in Figure 1, a deep, dark purple color change, especially if it occurs rapidly, strongly indicates the presence of semen and would demand further testing. Light results such as 0.6 to 0.18 units (Figure 1) may be the result of very weak semen stains or endogenous acid phosphatase levels.

Occasionally, color changes having a tannish hue are found on swabs taken from survivors, especially rectal swabs. Figure 4 shows one such result. This is a typical result from rectal and anal swabs and should not be confused with a positive AP reaction.

Subsequent testing for P30 and spermatozoa was negative. Certainly, these results can't be ignored, but the experienced analyst will recognize these as negative results, and not a true purple color change indicating the presence of semen.

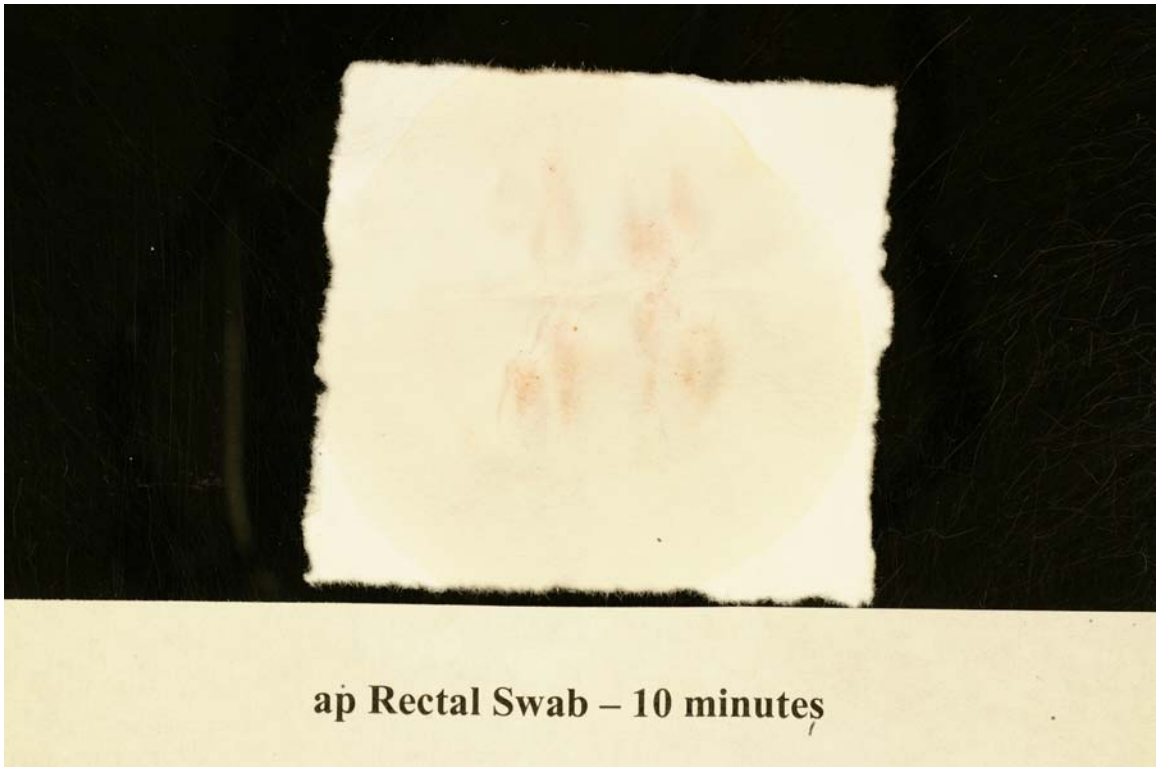

Figure 4. Typical results obtained from ap spot test added to filter paper transfer from a rectal swab. No semen was present on the swab.

Experience cannot be overemphasized and senior forensic biologists should make a habit of passing on their knowledge to new analysts. Schiff [5] stated "after 14 year's (sp) experience with the AP test, the author has found it to have great merit as a test for the identification of seminal fluid in the absence of spermatozoa". He continues, "because he has used the test qualitatively rather than quantitatively, he has established no arbitrary, numerical cutoff as to when the test is to be declared positive and when negative". He states that the test "is only as dependable as the physician, chemist, or pathologist who performs it".

Schiff lists three guidelines that he maintains should be followed in conducting the AP test:

1. The reagents must be freshly prepared.

   He found that the diazo-coupling agent that originally was clear and lightly tinted began to precipitate after 12 hours. This author has experienced the same result and recommends that it be prepared fresh daily.

2. The examiner must follow the same protocol in every case.

   In other words, press a swab to a piece of filter paper for the same time period applying the same pressure each and every time. Add the same number of drops of AP spot test and wait the same length of time each and every time. Developing consistency in the conducting the test will make the analyst more comfortable in interpreting the results.

3. The examiner must not deviate from his/her method of reading the test.

   After the analyst gains confidence in conducting the test, and performs a sufficient number of confirmatory tests on various test results, the analyst will come to realize what is a true positive reaction.

Conclusion

Testing for acid phosphatase remains a valuable presumptive test for the screening of swabs collected from sexual assault survivors and for the testing of stains found on clothing and bedding. The experienced forensic biologist knows that all stains that fluoresce are not necessarily semen and all semen stains do not fluoresce. In addition, semen is a heterogeneous fluid and portions of a deposited stain will contain various levels of acid phosphatase, P30 and spermatozoa. Examination of a pair of panties with an alternate light source and extraction of all the stains that fluoresce followed by psa analysis may yield semen, however, it may not, and it does not appear to this author to be the best use of time and expenses. Acid phosphatase mapping is an inexpensive and quick method for screening such stains.

Years ago, forensic biologists (serologists) were taught what was termed “a systematic approach to the analysis of semen evidence” developed by Blake, Sensabaugh and Bashinski [6]. The three major steps consisted of locating the stain, estimating the amount of semen found and genetic analysis of the stain. With the advent of DNA, it seems possible that one could just cut a stain from a pair of underwear, extract it and generate a DNA profile. Obtaining the subject’s DNA profile on the underwear, where it shouldn’t be, should be conclusive proof of guilt. And perhaps it is. However, this analyst, trained in the “old school” feels that a more thorough analysis is warranted. Acid phosphatase mapping in locating stains and sperm quantitation of positive stains are important steps that can only aid the DNA analyst in interpreting the results.

It behooves the forensic biologist to utilize all of the methods available for optimum semen detection.

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EXHIBIT N

Forensic Detection of Semen I. The Acid Phosphatase Test

Dale L. Laux, M.S.

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Introduction

Acid phosphatase is an enzyme secreted by the prostate gland that is present in large amounts in seminal fluid. It, like psa (prostatic specific antigen), is not unique to the prostate and can be found in other biological fluids including vaginal secretions. It is therefore considered a presumptive chemical test for the presence of semen and semen must be confirmed by other means (sperm detection or psa detection using membrane test systems).

Testing for the presence of acid phosphatase can be extremely helpful however, in locating semen stains on clothing and for testing swabs from sexual assault cases. A strong positive reaction generally indicates that semen is present and that further testing is warranted.

For an excellent review on the history of acid phosphatase detection, see Gaensslen [1]. A number of testing methods exist for the sampling of items for the presence of acid phosphatase. The enzymatic breakdown of sodium-α-naphthyl phosphate by acid phosphatase and the subsequent conversion of o-dianisidine to a colored compound by the free naphthyl is a recognized test procedure for the detection of semen [2]. The Serological Research Institute (SERI) produces a powder they call ap spot test. When the powder is reconstituted in water, it can be used to screen stains and swabs for the presence of semen.

The sensitivity and stability of the product are discussed.

Materials and Methods

<u>Sensitivity</u>

Acid phosphatase was obtained from Sigma Chemical Company. The product number was P-1146, Lot 051K7038 and was isolated from potato. 50 units were purchased, consisting of 7.5 mg of solid having an activity of 6.7 units/mg (50.25 units). The solid was dissolved in 200 µL of deionized water. 100 µL of this solution (25 units) was added to a cotton-tipped swab that was allowed to air dry. 50 µL deionized water was added to the remaining 100 µL, mixed and 100 µL of this solution (17 units) was added to a

cotton-tipped swab that was allowed to air dry. Subsequent dilutions were made in this manner resulting in dry cotton-tipped swabs having the following units of acid phosphatase: 25, 17, 5.6, 1.8, 0.6, 0.2, 0.05 and 0.02.

Testing of these dry swabs was conducted in the following manner. Deionized water was added to a small piece of Whatman filter paper #3. Each swab was pressed against the filter paper strongly between thumb and forefinger for ten seconds. A single drop of freshly prepared SERI ap spot test (Lot 1562) was added to each piece of filter paper and color changes were recorded after 10 minutes.

Stability

SERI ap spot test (Lot 1562) was prepared fresh daily and used for case analysis. The reagent was maintained in a small glass dropper bottle protected from light with tape at room temperature. At the end of the business day (approximately 8 hours), the reagent was placed in a plastic15 mL Falcon tube and refrigerated. The following morning, fresh ap spot test was prepared and kept on the lab bench along with the previous preparation. This procedure was followed for the three remaining days of the week.

Whatman #3 filter paper was moistened and a cotton-tipped swab containing 25 units of acid phosphatase was pressed to 5 areas of the paper (following the procedure described previously). The same procedure was followed with 17 units of acid phosphatase.

SERI ap spot test reagent (fresh, 1 day, 2 days, 3 days, 4 days and 5 days old) was added to the filter paper and color changes were recorded after 10 minutes.

The same methods were followed using SERI ap spot test reagents that were stored frozen for 1 to 5 days; however, the reagents were not removed from the freezer daily.

Results and Discussion

Sexual assault kits and clothing are routinely submitted to crime laboratories for examination for the presence of semen. Typically, forensic scientists conduct visual examinations for stains followed by examination with an alternate light source on clothing and bedding items. This is generally followed by testing of stains for the presence of acid phosphatase, an enzyme secreted by the prostate and found in high levels in semen. Swabs collected from sexual assault survivors are generally tested for the presence of acid phosphatase followed by tests for the presence of spermatozoa, and P30 if necessary.

It is customary to test stained areas and swabs collected from the survivor indirectly. In other words, a transfer method involving wet or dry cotton-tipped swabs or moistened filter paper applied as an overlay to a stained area or swabbing is employed. As recommended by Barnett, et.al. [3], presumptive test reagents should NEVER be applied directly to items of evidence.

Following this methodology, experiments were designed to determine the sensitivity of one acid phosphatase test. The Serological Research Institute (SERI) sells a product they call ap spot test. It contains sodium-α-naphthyl phosphate and o-dianisidine (Fast Blue B). If acid phosphatase is present in a sample and a drop of the ap spot test is added, the enzyme catalyzes the breakdown of sodium-α-naphthyl phosphate producing free naphthyl that reacts with o-dianisidine producing a purple colored compound.

Results of Sensitivity Tests

Freshly prepared ap spot test gave positive results with acid phosphatase diluted to 0.18 to 0.6 units.* A photograph of the results appears in Figure 1.

Figure 1. One drop of ap spot test added to moistened filter paper containing diluted acid phosphatase (25 to 0.02 units). Positive reaction (purple color change) obtained at 0.6 units.

Sensabaugh [4] published results of experiments designed to quantitate the levels of endogenous and postcoital vaginal acid phosphatase. He standardized data from several investigators including his data and obtained a range of endogenous vaginal acid phosphatase of 0.023 to 4.902 units. SERI's ap spot test would certainly react with these endogenous levels of acid phosphatase. Hence the presumptive nature of the ap test and the requirement that the presence of semen be confirmed in another manner.

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*One unit will hydrolyze 1.0 µmole of p-nitrophenyl phosphate per minute at pH 4.8 at 37 °C

Results of Stability Tests

The directions supplied with SERI's ap spot test state to prepare the reagent daily. The Fast Blue B dye is light sensitive. At room temperature, on the lab bench and in the light, the ap spot test will begin to turn yellow and brown material will precipitate out.

The results of the stability experiments are shown in Figure 2.

Figure 2. Photograph of filter paper with 25 and 12 units of acid phosphatase and drops of fresh, 1 day, 2 day, 3 day and 4 day old ap spot test reagent.

A decrease in activity was observed in 1 day old ap spot test reagent, however it still reacted fairly well. By two days, the activity of the reagent dropped significantly and by four days, the reagent has lost the ability to detect 25 units of acid phosphatase.

Tests were conducted to determine whether freezing the reagent could enhance stability. The results of this experiment are shown in Figure 3. As can be seen in Figure 3, freezing did enhance the stability of the reagent. However, the frozen reagents were not

removed from the freezer and thawed on the laboratory bench daily, but remained in the freezer.

Figure 3. Varying units of acid phosphatase (25 u, 17 u, 6u and 2 u) deposited on filter paper to which ap spot test, frozen for various days (fresh, 1 day, 2 days, 5 days), was added.

The ap spot test stored frozen for 5 days worked as well as the freshly prepared reagent. However, once thawed and left on the lab bench, this reagent would degrade just as fresh or refrigerated reagent.

Interpretation of the color change indicating a positive result can be subjective. As seen in Figure 1, a deep, dark purple color change, especially if it occurs rapidly, strongly indicates the presence of semen and would demand further testing. Light results such as 0.6 to 0.18 units (Figure 1) may be the result of very weak semen stains or endogenous acid phosphatase levels.

Occasionally, color changes having a tannish hue are found on swabs taken from survivors, especially rectal swabs. Figure 4 shows one such result. This is a typical result from rectal and anal swabs and should not be confused with a positive AP reaction.

EXHIBIT O

AMERICAN SOCIETY OF CRIME LABORATORY DIRECTORS
LABORATORY ACCREDITATION BOARD

June 22, 2016

Bill Gibbens, Manager
Forensic Science Division
Austin Police Department
P.O. Box 689001
Austin, TX 78768

Dear Mr. Gibbens,

On May 27-28, 2016 and June 6, 2016, ASCLD/LAB technical assessor Jody Koehler participated in an audit of the Austin Police Department Forensic Science Laboratory DNA Section in cooperation with the Texas Forensic Science Commission. As a result of the audit, Ms. Koehler identified eight (8) nonconformities to accreditation requirements. Those nonconformities are listed below to assist you as you make improvements to your biology program. Please refer to ISO/IEC 17025:2005 and the ASCLD/LAB-*International* Supplemental Requirements for the Accreditation of Forensic Science Testing Laboratories (2011) for the language of the cited requirements.

Nonconformity #1 to ISO/IEC 17025:2005 5.2.1

Previous Technical Leaders have not been properly qualified. Records did not demonstrate that one had kept abreast of technical developments and technologies utilized in the laboratory. Duties did not include signing reports or performance of technical reviews but did include making technical decisions about casework and determining if deviations from the standard operating procedure are technically justified and authorized. Another previous technical leader, while a proficiency tested DNA analyst, did not have the depth of technical knowledge to institute appropriate changes to the training manual or DNA standard operating procedures.

Nonconformity #2 to ISO/IEC 17025:2005 4.9.1 and 4.9.2

Procedures for nonconforming testing work and corrective action are not implemented when the lab became aware of discrepant testing results after retesting of a DNA swab occurred. In one example, the laboratory results identify that there is a carry-over contamination event between the epithelial cell fraction from the victim's vaginal swab and the epithelial cell fraction from a penile swab from an unrelated individual. The analyst reported that the victim could not be excluded from the epithelial cell fraction from the penile swab of the unrelated individual. The swab was retested by a private laboratory and generated a 2 person mixture where the victim was excluded. In another example, 10 different cases reviewed there was a reagent blank that was contaminated. In the reagent blank, there were 8 peaks above the lab's analytical threshold of 75 RFU. Peaks ranged in height from 103-744 RFU. The contamination was traced back to the analyst's extraction reagents. All results from these cases were reported without an evaluation of the significance and the acceptability of the data.

Nonconformity #3 to ISO/IEC 17025:2005 5.4.1

In a case there was a deviation in order to utilize the data from this case as a major/minor mixture. This data was also used for interpretation. The item had a quantity of DNA that was at 0.05025 ng amplified. This quantity of DNA is lower than the stochastic threshold stated in the APD DNA SOP. The SOP states that for mixtures (including major/minor) that for DNA quantities amplified below 0.0625, "The entire profile is uninterpretable." There is no documentation that technically justifies utilizing this profile for interpretation purposes and or that ensures that data at this quantity is sufficient for comparison purposes. There is no record that this aspect of the deviation was authorized.

Nonconformity #4 to ISO/IEC 17025:2005 5.4.5.2 and 5.4.5.3

While the quantification-based stochastic study was validated, the validation is lacking in robustness and evaluation of fitness for purpose. In the validation study, only 3 samples were utilized with 9 different dilutions (0.75-0.0029296 ng). Also, for the mixture portion of this validation, only 3 samples were utilized. Not all dilutions were made correctly as the equipment used was not appropriate for the volume measured. The validation data was not properly evaluated as it showed stochastic effects, even when >0.625 ng of DNA was amplified.

Nonconformity #5 to ISO/IEC 17025:2005 5.5.3

There were no instructions for maintenance of the alternate light source to ensure proper functioning. If the alternate light source is not functioning correctly, there is the possibility that biological stains may be missed.

Nonconformity #6 to ASCLD/LAB-*International* Supplemental Requirement for Forensic Science Testing Laboratories (2011) 5.1.3

The procedure for assessing continued reliability of the AP Spot Reagent does not ensure performance reliability. The manufacturer's instruction for the AP Spot Reagent is to "Make fresh daily". After preparation and initial reliability checking the reagent has been utilized for up to 1 month. Although analysts utilize the reagent in a quality control procedure each day of use, there is no supporting validation documentation for what a subjective "4+" result equates to as it relates to possible loss of reagent performance.

Nonconformity #7 to ASCLD/LAB-*International* Supplemental Requirement for Forensic Science Testing Laboratories (2011) 5.2.1.1

While the DNA section has a training program, it does not adequately cover forensic biology screening and DNA analysis. Through interviews it was determined that the analysts lack general knowledge on quality assurance procedures such as critical evaluation of data. A lack of knowledge was also identified regarding general DNA topics such as Hardy-Weinberg and how to calculate random match probability.

Nonconformity #8 to ISO/IEC 17025:2005 4.2.1 and 5.4.1

The laboratory's use of a quantification-based determination of stochastic threshold is not scientifically valid, nor supported by the forensic community. There are no peer-reviewed journal articles citing the acceptance of this type of methodology, especially for forensic DNA mixtures.

The laboratory's review and use of information from suspect or victim reference DNA profiles in the determination of which loci will be used in statistical calculations is not appropriate.

The current instructions for mixture interpretation are not sufficient to ensure the quality of the test results. The current procedure allows for inconsistency among the analysts performing mixture interpretation and is not sufficient to prevent incorrect interpretation.

I was advised prior to the audit that your laboratory had ceased providing DNA test results that involved mixture interpretation. Subsequent to the audit you contacted me for direction on how to voluntarily suspend the laboratory's accreditation in the biology discipline for a temporary period of time. As a result, the Austin Police Department Forensic Science Laboratory's scope of accreditation document was updated to reflect that the biology discipline is no longer accredited. The ASCLD/LAB website has also been updated to reflect this change. When the above nonconformities have been resolved and you are ready to extend the laboratory's scope of accreditation to include the biology discipline, please contact ASCLD/LAB so that we can determine the appropriate course of action.

If ASCLD/LAB can be of any assistance, please contact myself of Senior Accreditation Program Manager Laurel Farrell.

Best Regards,

Pamela L. Bordner

Pamela L. Bordner
Vice President

Subsequent testing for P30 and spermatozoa was negative. Certainly, these results can't be ignored, but the experienced analyst will recognize these as negative results, and not a true purple color change indicating the presence of semen.

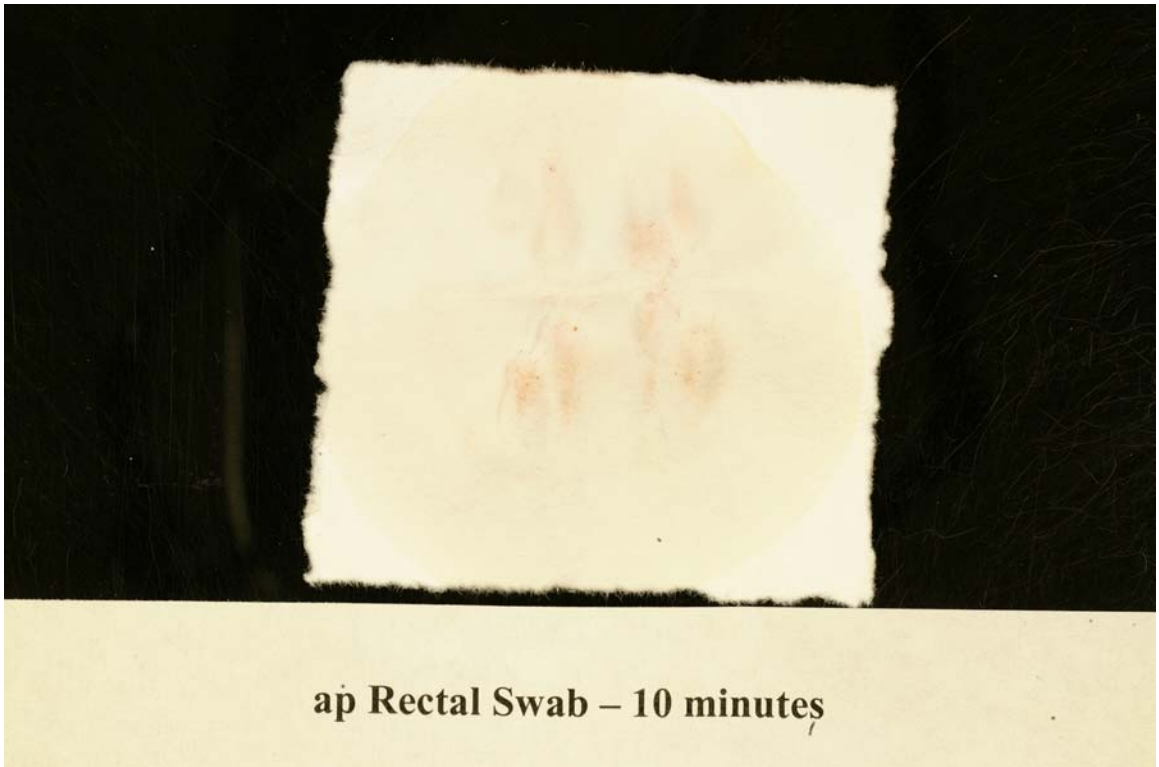

Figure 4. Typical results obtained from ap spot test added to filter paper transfer from a rectal swab. No semen was present on the swab.

Experience cannot be overemphasized and senior forensic biologists should make a habit of passing on their knowledge to new analysts. Schiff [5] stated "after 14 year's (sp) experience with the AP test, the author has found it to have great merit as a test for the identification of seminal fluid in the absence of spermatozoa". He continues, "because he has used the test qualitatively rather than quantitatively, he has established no arbitrary, numerical cutoff as to when the test is to be declared positive and when negative". He states that the test "is only as dependable as the physician, chemist, or pathologist who performs it".

Schiff lists three guidelines that he maintains should be followed in conducting the AP test:

1. The reagents must be freshly prepared.

   He found that the diazo-coupling agent that originally was clear and lightly tinted began to precipitate after 12 hours. This author has experienced the same result and recommends that it be prepared fresh daily.

2. The examiner must follow the same protocol in every case.

   In other words, press a swab to a piece of filter paper for the same time period applying the same pressure each and every time. Add the same number of drops of AP spot test and wait the same length of time each and every time. Developing consistency in the conducting the test will make the analyst more comfortable in interpreting the results.

3. The examiner must not deviate from his/her method of reading the test.

   After the analyst gains confidence in conducting the test, and performs a sufficient number of confirmatory tests on various test results, the analyst will come to realize what is a true positive reaction.

Conclusion

Testing for acid phosphatase remains a valuable presumptive test for the screening of swabs collected from sexual assault survivors and for the testing of stains found on clothing and bedding. The experienced forensic biologist knows that all stains that fluoresce are not necessarily semen and all semen stains do not fluoresce. In addition, semen is a heterogeneous fluid and portions of a deposited stain will contain various levels of acid phosphatase, P30 and spermatozoa. Examination of a pair of panties with an alternate light source and extraction of all the stains that fluoresce followed by psa analysis may yield semen, however, it may not, and it does not appear to this author to be the best use of time and expenses. Acid phosphatase mapping is an inexpensive and quick method for screening such stains.

Years ago, forensic biologists (serologists) were taught what was termed “a systematic approach to the analysis of semen evidence” developed by Blake, Sensabaugh and Bashinski [6]. The three major steps consisted of locating the stain, estimating the amount of semen found and genetic analysis of the stain. With the advent of DNA, it seems possible that one could just cut a stain from a pair of underwear, extract it and generate a DNA profile. Obtaining the subject’s DNA profile on the underwear, where it shouldn’t be, should be conclusive proof of guilt. And perhaps it is. However, this analyst, trained in the “old school” feels that a more thorough analysis is warranted. Acid phosphatase mapping in locating stains and sperm quantitation of positive stains are important steps that can only aid the DNA analyst in interpreting the results.

It behooves the forensic biologist to utilize all of the methods available for optimum semen detection.

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EXHIBIT P

AMERICAN SOCIETY OF CRIME LABORATORY DIRECTORS
LABORATORY ACCREDITATION BOARD

June 22, 2016

Bill Gibbens, Manager
Forensic Science Division
Austin Police Department
P.O. Box 689001
Austin, TX 78768

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If ASCLD/LAB can be of any assistance, please contact myself of Senior Accreditation Program Manager Laurel Farrell.

Best Regards,

Pamela L. Bordner

Pamela L. Bordner
Vice President

EXHIBIT Q

Austin Police Department

City of Austin: Founded by Congress, Republic of Texas, 1839
P.O. Box 689001, Austin, Texas 78768-9001 Telephone (512) 974-5000
www.cityofaustin.org/police

June 13, 2016

Pam Bordner, Vice President
ASCLD/LAB
139 J Technology Drive
Garner, NC 27529

Dear Mrs. Bordner,

The Austin Police Department Forensic Science Division – DNA Laboratory recently participated in an audit conducted by the Texas Forensic Science Commission. As a result several concerns surfaced that must be evaluated and/or remediated.

This letter is to notify ASCLD/LAB that the Austin Police Department Forensic Science Division is voluntarily discontinuing the Biology (DNA-Nuclear and Body Fluid Identification) services until these issues have been fully resolved.

Once corrective action has been completed we will request reinstatement of this category of testing to our accreditation scope.

If you require additional information please do not hesitate to contact me.

Thank you for your assistance in this matter.

Respectfully,

ART ACEVEDO
Chief of Police

EXHIBIT R

STATEMENT OF ROBERT SMITH CONCERNING DIANA MORALES

On May 4, 2016, it was brought to my attention that APD analyst Diana Morales had issued a report on a case that may have used information that was under APD's internal threshold guidelines. The case was currently being tried in the 167th District Court. (State v McGee, APD 2014-2411989; L-1409568). I returned to the office, had a brief conversation with Brandon Grunewald about the facts, and headed up to court to check status. The APD serology witness was testifying in the trial when I arrived, and Diana Morales was in the hallway waiting. Brandon and I met with Diana in the small conference room to discuss her findings before she testified.

APD has an internal SOP stating that mixtures below a quant value 0.0625 should be called as unable to determine. From the bench notes it appears that the stain in question (Item 1.3) had a quant of 0.00335. We asked Diana how she was able to report findings on a stain since it was below the 0.0625 threshold. She looked through her file for several minutes, did some work on her calculator, and ultimately called someone else in the lab. (Identified as Clair McKenna to Brandon and I). After she hung up she demonstrated how if you multiply 0.00335 by 30, the amount would be 1.005 – clearly above threshold. I asked her why she would multiply the number by 30 as it is clearly a number preprinted on the form and she answered something I could not understand or follow. Not knowing any better, we excused ourselves to check in with Jody Koehler from DPS and were told Diana's answer was not a scientifically valid approach to the issue. We decided to get all the people on a conference call to hash out the problem.

The next morning, I participated in a conference call with Diana, Jody Koehler, and others where this issue was discussed. Diana's approach seemed to center around the fact that Jeff Sailus had signed a deviation memo allowing the mixture to be interpreted even though one of the loci was inconclusive and would not be used in the interpretation. The deviation memo did not address using a profile from a quant that was below threshold. When Brandon asked her why she told us something different the day before, she said she had not understood the question we were asking.

Diana had shown me the deviation memo the day before while Brandon was on the phone with Jody Koehler, but since it did not address quant based ST I did not think it applied.

Robert Smith
Assistant District Attorney, Travis County
Date: May 5, 2016

STATEMENT OF BRANDON GRUNEWALD CONCERNING DIANA MORALES

On May 4th, 2016, I received a phone call from Lynn Garcia with the Texas Forensic Science Commission (TFSC) raising concerns that the Austin Police Department DNA lab (APD) may not have followed their Standard Operating Procedure (SOP) in particular case. After discussing this issue with her, I realized that the case she was referencing was currently being tried to a jury in the 167th District Court. This information came to Lynn Garcia from Jody Koehler with the Department of Public Safety (DPS), who had been asked to review the case. This issue raised a concern that the APD DNA lab reached a conclusion on a sample below their quant-based stochastic threshold. Jody informed me that according to APD's own SOPs, this sample should have been labeled uninterpretable. I contacted Robert Smith, Trial Director, and informed him of the situation. Robert Smith and I then proceed to the 167th where Dianna Morales, the APD lab analyst on the case, was waiting to testify. Robert and I met with Dianna and asked her to explain how she was able to reach a conclusion on this sample. She initially calculated the quantity by taking .00335 and multiplying it by 15 (the amount of DNA amplified), which gave a result of .05025. She did this numerous times, still reaching the result of .05025, which fell below APD's established quant-based stochastic threshold of .0625. She then called Claire McKenna, who conducted the technical review of this case, to consult with her. After Dianna spoke with Claire, we were informed that APD validated their quant based stochastic threshold by using the amount of available DNA, not the amount of amplified DNA. This changed the multiplier from 15 (the amount of DNA amplified) to 30 (the amount of DNA available), resulting in a quant of .1005. I called Jody Koehler and informed her of what I was told. It was her opinion that if this is the method that APD used to validate their quant based stochastic threshold then the current SOP itself was not supportable.

On May 5th, 2016, at my request, a conference call that included Dianna Morales, Bill Gibbons, Cmdr. Nick Wright, Jody Koehler and Lynn Garcia was held. On that phone call Dianna Morales stated that she had sought and received a deviation from her SOP to allow her to treat the sample as a major contributor. The deviation request does not address analyzing a sample below quant based stochastic threshold. In response to this being pointed out, Dianna Morales stated that the former technical leader, Jeff Salius, must have been aware that the sample was below .0625 because he would have reviewed the case file, and still approved that deviation. She also stated that the .0625 quant based stochastic threshold was a "guideline."

When I asked Dianna Morales why she was now saying the deviation she requested allowed her to reach this conclusion, she stated that she did not understand my question from the previous day.

Brandon Grunewald
Assistant District Attorney, Travis County
Date: May 5, 2016

EXHIBITS

Notice of Amendment of the FBI's STR Population Data Published in 1999 and 2001

Recently, new amplification kits that expand the number of loci in a multiplex reaction have become commercially available. To establish allele distributions for the additional loci, the FBI Laboratory retyped population samples that were originally genotyped using AmpFlSTR Profiler Plus, COfiler, Identifiler (Thermo Fisher Scientific, South San Francisco, CA) and/or GenePrint PowerPlex (Promega Corp., Madison, WI) (1,2) using GlobalFiler (Thermo Fisher Scientific) and PowerPlex Fusion (Promega Corp.) (3). During a comparison of over 1100 DNA profiles from African Americans, Caucasians, Southwest Hispanics, Bahamians, Jamaicans, Trinidadians, Filipinos and Chamorros in the original (4,5) and new studies (3), genotyping discrepancies were discovered. Electronic genotype data corresponding to the published allele frequencies are not available for the Southeast Hispanic, Apache, Navaho and Minnesota Native American populations (6), as well as Filipino and Chamorro populations (except for D2S1338 and D19S433) (7). Genotypes from these populations thus could not be assessed for concordance.

The discrepancies discovered were attributable to (a) human error, typically due to manual data editing and recording, and (b) technological limitations (e.g., insufficient resolution for distinguishing microvariants using polyacrylamide gel electrophoresis). The published genotype data (3,4) from which allele frequencies were calculated also include sample or data processing errors (e.g., genotype duplications).

Allele frequencies cited across these publications (1,2) have been used by the FBI and many forensic laboratories for calculating match statistics in criminal investigations and other types of human identification applications since 1999. Given that statistical estimates based on these data have been included in thousands of laboratory reports and testimonies, the FBI Laboratory believes the discrepancies require acknowledgement. The FBI Laboratory has submitted an erratum notice, which is scheduled to appear in the July issue of the Journal of Forensic Science (please see http://onlinelibrary.wiley.com/doi/10.1111/1556-4029.12806/abstract for an online version). This article describes these errors and their effect on profile probability calculations. Empirical testing described in this publication supports that any discrepancy between profile probabilities calculated using the original and corrected data is expected to be less than a factor of two in a full profile. The FBI Laboratory is additionally providing herein the amended allele frequency tables for use by anyone interested in performing comparisons between the multi-locus profile probabilities calculated using the previously published data and the amended allele frequencies.

If you have any questions, please contact the FBI's DNA Support Unit at 703-632-7572.

African American Amended Allele Frequency Table

Caucasian Amended Allele Frequency Table

Southwestern Hispanic Amended Allele Frequency Table

Bahamian Amended Allele Frequency Table

Jamaican Amended Allele Frequency Table

Trinidadian Amended Allele Frequency Table

Chamorro Amended Allele Frequency Table

Filipino Amended Allele Frequency Table

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African American Amended Allele Frequencies

Allele	D3S1358	vWA	FGA	D8S1179	D21S11	D18S51	D5S818	D13S317	D7S820	CSF1PO	TPOX	TH01	D16S539	D2S1338	D19S433	Allele
6											0.0861	0.1095				6
7							0.0028		0.0071	0.0429	0.0215	0.4405				7
8				0.0028			0.0500	0.0335	0.1738	0.0857	0.3684	0.1857	0.0359			8
9				0.0056			0.0139	0.0279	0.1571	0.0333	0.1818	0.1452	0.1986			9
9.3												0.1048				9.3
10				0.0250			0.0639	0.0503	0.3238	0.2714	0.0933	0.0143	0.1100		0.0150	10
<11						0.0056										<11
11		0.0028		0.0361		0.0056	0.2611	0.2374	0.2238	0.2048	0.2249		0.2943		0.0689	11
<12	0.0048															<12
12	0.0024			0.1083		0.0587	0.3556	0.4832	0.0905	0.3000	0.0239		0.1866		0.1138	12
12.2															0.0808	12.2
13	0.0119	0.0056		0.2222		0.0559	0.2444	0.1257	0.0190	0.0548			0.1651		0.2964	13
13.2						0.0056									0.0509	13.2
14	0.1214	0.0667		0.3333		0.0642	0.0056	0.0391	0.0048	0.0071			0.0096		0.1946	14
14.2															0.0539	14.2
15	0.2905	0.2361		0.2139		0.1676		0.0028							0.0419	15
15.2															0.0389	15.2
16	0.3071	0.2694		0.0444		0.1872								0.0449	0.0210	16
>16							0.0028									>16
16.2															0.0180	16.2
17	0.2000	0.1833		0.0083		0.1620								0.1018		17
17.2			0.0028												0.0030	17.2
18	0.0548	0.1361	0.0083			0.1313								0.0659		18
18.2			0.0083												0.0030	18.2
19	0.0048	0.0722	0.0528			0.0782								0.1377		19
>19	0.0024															>19
19.2			0.0028													19.2
20		0.0278	0.0722			0.0559								0.0629		20
21			0.1250			0.0112								0.1527		21
22			0.2250			0.0056								0.1377		22
22.2			0.0056													22.2
23			0.1250			0.0056								0.0988		23
24			0.1861											0.0928		24
24.2					0.0028											24.2
25			0.1000											0.0838		25
26			0.0361		0.0028									0.0210		26
27			0.0222		0.0615											27
28			0.0167		0.2151											28
29			0.0056		0.1899											29
29.3					0.0028											29.3
30			0.0028		0.1788											30
30.2			0.0028		0.0084											30.2
31					0.0922											31
31.2					0.0754											31.2
32					0.0084											32
32.2					0.0698											32.2
33					0.0084											33
33.2					0.0335											33.2
34					0.0084											34
34.2					0.0028											34.2
35					0.0279											35
36					0.0056											36
37					0.0056											37
Allele	D3S1358	vWA	FGA	D8S1179	D21S11	D18S51	D5S818	D13S317	D7S820	CSF1PO	TPOX	TH01	D16S539	D2S1338	D19S433	Allele
N	210	180	180	180	179	179	180	179	210	210	209	210	209	167	167	

Caucasian Amended Allele Frequencies

Allele	D3S1358	vWA	FGA	D8S1179	D21S11	D18S51	D5S818	D13S317	D7S820	CSF1PO	TPOX	TH01	D16S539	D2S1338	D19S433	Allele
6									0.0025			0.2252				6
7									0.0173	0.0025		0.1733				7
8				0.0179				0.0995	0.1634	0.0050	0.5470	0.1262	0.0199			8
8.3												0.0025				8.3
9				0.0102			0.0308	0.0765	0.1460	0.0198	0.1238	0.1658	0.1045			9
9.3												0.3045				9.3
10				0.1020			0.0487	0.0510	0.2896	0.2525	0.0371	0.0025	0.0647			10
10.3										0.0025						10.3
<11						0.0128										<11
11				0.0587		0.0128	0.4103	0.3214	0.2030	0.2995	0.2550		0.2736			11
12				0.1454		0.1276	0.3538	0.3061	0.1411	0.3267	0.0371		0.3383		0.1086	12
12.2															0.0066	12.2
13	0.0025	0.0051		0.3393		0.1224	0.1462	0.1097	0.0297	0.0718			0.1642		0.2829	13
13.2															0.0263	13.2
14	0.1386	0.1020		0.2015		0.1735	0.0077	0.0357	0.0074	0.0149			0.0323		0.3355	14
14.2															0.0033	14.2
15	0.2475	0.1122		0.1097		0.1276	0.0026			0.0050			0.0025		0.1349	15
15.2															0.0263	15.2
16	0.2327	0.2015		0.0128		0.1071								0.0296	0.0428	16
16.2															0.0263	16.2
17	0.2104	0.2628		0.0026		0.1556								0.1941		17
17.2															0.0033	17.2
18	0.1634	0.2219	0.0306			0.0918								0.0526		18
18.2															0.0033	18.2
19	0.0050	0.0842	0.0561			0.0357								0.1447		19
20		0.0102	0.1454			0.0255								0.1546		20
20.2			0.0026													20.2
21			0.1735			0.0051								0.0197		21
22			0.1888			0.0026								0.0296		22
22.2			0.0102													22.2
23			0.1582											0.1349		23
24			0.1378											0.1217		24
24.2					0.0051											24.2
25			0.0689											0.0954		25
26			0.0179											0.0230		26
27			0.0102		0.0459											27
28					0.1658											28
29					0.1811											29
30					0.2321											30
30.2					0.0383											30.2
31					0.0714											31
31.2					0.1020											31.2
32					0.0153											32
32.2					0.1097											32.2
33.2					0.0306											33.2
35.2					0.0026											35.2
Allele	D3S1358	vWA	FGA	D8S1179	D21S11	D18S51	D5S818	D13S317	D7S820	CSF1PO	TPOX	TH01	D16S539	D2S1338	D19S433	Allele
N	202	196	196	196	196	196	195	196	202	202	202	202	201	152	152	

Southwest Hispanic Amended Allele Frequencies

Allele	D3S1358	vWA	FGA	D8S1179	D21S11	D18S51	D5S818	D13S317	D7S820	CSF1PO	TPOX	TH01	D16S539	D2S1338	D19S433	Allele
5												0.0024				5
6									0.0024		0.0048	0.2321				6
7							0.0616		0.0215	0.0024	0.0048	0.3373				7
8				0.0025			0.0025	0.0665	0.0981		0.5550	0.0813	0.0168			8
9				0.0025			0.0542	0.2192	0.0478	0.0072	0.0335	0.1029	0.0793		0.0035	9
9.3												0.2416				9.3
10				0.0936			0.0640	0.1010	0.3062	0.2536	0.0335	0.0024	0.1755			10
<11						0.0049										<11
11		0.0025		0.0616		0.0123	0.4261	0.2020	0.2895	0.2656	0.2727		0.3149		0.0035	11
12				0.1207		0.1059	0.2882	0.2167	0.1914	0.3923	0.0933		0.2885		0.0563	12
12.2															0.0211	12.2
13	0.0024			0.3251		0.1700	0.0961	0.1379	0.0383	0.0646	0.0024		0.1010		0.1620	13
13.2															0.1092	13.2
14	0.0789	0.0616		0.2463		0.1700	0.0049	0.0567	0.0048	0.0096			0.0240		0.3204	14
14.2															0.0458	14.2
15	0.4258	0.0714		0.1158		0.1379	0.0025			0.0048					0.1197	15
15.2															0.0810	15.2
16	0.2656	0.3645		0.0246		0.1158								0.0176	0.0423	16
16.2															0.0352	16.2
17	0.1268	0.2217		0.0074		0.1379								0.2218		17
18	0.0837	0.1946	0.0025			0.0517								0.0423		18
19	0.0144	0.0714	0.0813			0.0369								0.2606		19
>19	0.0024															>19
20		0.0123	0.0690			0.0172								0.1408		20
20.2			0.0025													20.2
21			0.1305			0.0197								0.0106		21
21.2			0.0025													21.2
22			0.1773			0.0074								0.0704		22
>22						0.0123										>22
22.2			0.0049													22.2
23			0.1404											0.1232		23
23.2			0.0074													23.2
24			0.1256											0.0669		24
24.2					0.0025											24.2
25			0.1379											0.0387		25
26			0.0837											0.0070		26
27			0.0320		0.0099											27
28			0.0025		0.0690											28
29					0.2044											29
29.2					0.0025											29.2
30					0.3300											30
30.2					0.0320											30.2
31					0.0690											31
31.2					0.0862											31.2
32					0.0123											32
32.2					0.1355											32.2
33.2					0.0419											33.2
34.2					0.0049											34.2
Allele	D3S1358	vWA	FGA	D8S1179	D21S11	D18S51	D5S818	D13S317	D7S820	CSF1PO	TPOX	TH01	D16S539	D2S1338	D19S433	Allele
N	209	203	203	203	203	203	203	203	209	209	209	209	208	142	142	

Bahamian Amended Allele Frequencies

Allele	D3S1358	vWA	FGA	D8S1179	D21S11	D18S51	D5S818	D13S317	D7S820	CSF1PO	TPOX	TH01	D16S539	Allele
5												0.0032		5
6										0.0032	0.0673	0.1538		6
7								0.0031	0.0126	0.0641	0.0256	0.3846		7
8							0.0723	0.0220	0.1541	0.0577	0.3237	0.2276	0.0385	8
9				0.0032			0.0094	0.0314	0.1258	0.0481	0.2212	0.1282	0.2147	9
9.3												0.0897		9.3
10				0.0194			0.0597	0.0252	0.3396	0.2340	0.0897	0.0128	0.0994	10
<11						0.0097								<11
11		0.0094		0.0516		0.0097	0.2390	0.3050	0.2201	0.2244	0.2372		0.3013	11
11.3									0.0031					11.3
12				0.1290		0.0484	0.3711	0.3994	0.1195	0.2853	0.0353		0.1731	12
13		0.0283		0.1903		0.0516	0.2264	0.1604	0.0252	0.0705			0.1442	13
13.2						0.0032								13.2
14	0.0742	0.0629		0.3387		0.0452	0.0157	0.0535		0.0096			0.0256	14
15	0.3194	0.1541		0.1839		0.1548	0.0063			0.0032			0.0032	15
15.2	0.0032					0.0032								15.2
16	0.3387	0.2642		0.0613		0.1645								16
17	0.1968	0.2013		0.0226		0.1871								17
<18			0.0129											<18
18	0.0645	0.1824				0.1258								18
18.2			0.0129											18.2
19	0.0032	0.0723	0.0581			0.0968								19
20		0.0252	0.0742			0.0484								20
21			0.1129			0.0226								21
21.2			0.0032			0.0032								21.2
22			0.1452			0.0258								22
22.3			0.0032											22.3
23			0.1774											23
24			0.1968											24
24.3					0.0065									24.3
25			0.0968											25
26			0.0323											26
27			0.0516		0.0710									27
28			0.0097		0.2226									28
29			0.0065		0.1742									29
30					0.1774									30
>30			0.0065											>30
30.2					0.0097									30.2
30.3					0.0032									30.3
31					0.0935									31
31.2					0.0484									31.2
32					0.0194									32
32.2					0.0968									32.2
33					0.0032									33
33.2					0.0387									33.2
34					0.0097									34
34.2					0.0032									34.2
35					0.0226									35
Allele	D3S1358	vWA	FGA	D8S1179	D21S11	D18S51	D5S818	D13S317	D7S820	CSF1PO	TPOX	TH01	D16S539	Allele
N	155	159	155	155	155	155	159	159	159	156	156	156	156	

Jamaican Amended Allele Frequencies

Allele	D3S1358	vWA	FGA	D8S1179	D21S11	D18S51	D5S818	D13S317	D7S820	CSF1PO	TPOX	TH01	D16S539	Allele
5												0.0024		5
6									0.0041		0.0673	0.1394		6
7								0.0020	0.0061	0.0481	0.0313	0.3558		7
8							0.0533	0.0205	0.1988	0.0625	0.3822	0.2548	0.0340	8
9				0.0077			0.0102	0.0246	0.1393	0.0313	0.2644	0.1587	0.2087	9
9.3												0.0841		9.3
10				0.0129			0.0553	0.0246	0.3443	0.2716	0.0745	0.0048	0.1092	10
10.1									0.0020					10.1
<11						0.0026								<11
11		0.0041		0.0309		0.0052	0.2049	0.2766	0.1844	0.2332	0.1538		0.3131	11
12	0.0052			0.1160		0.0438	0.3996	0.4549	0.1025	0.2933	0.0264		0.1869	12
13	0.0155	0.0082		0.2139		0.0258	0.2561	0.1434	0.0123	0.0529			0.1383	13
13.2						0.0052								13.2
14	0.0670	0.0738		0.3273		0.0412	0.0143	0.0533	0.0061	0.0072			0.0097	14
14.2						0.0026								14.2
15	0.3376	0.2275		0.2165		0.1572	0.0061							15
15.2	0.0026													15.2
16	0.3067	0.2910		0.0670		0.1907								16
17	0.2113	0.1824		0.0052		0.1830								17
<18			0.0077											<18
18	0.0464	0.1311		0.0026		0.1237								18
18.2			0.0206											18.2
19	0.0077	0.0533	0.0670			0.0954								19
19.2			0.0077											19.2
20		0.0225	0.0464			0.0696								20
21		0.0061	0.0747			0.0284								21
21.2						0.0026								21.2
22			0.1881			0.0155								22
23			0.1959			0.0052								23
24			0.1469			0.0026								24
24.3			0.0026		0.0026									24.3
25			0.1160											25
26			0.0412											26
27			0.0515		0.0644									27
28			0.0155		0.2732									28
29			0.0077		0.1830									29
30					0.1649									30
>30			0.0103											>30
30.2					0.0180									30.2
31					0.0644									31
31.2					0.0490									31.2
32					0.0155									32
32.1					0.0026									32.1
32.2					0.0619									32.2
33					0.0052									33
33.2					0.0309									33.2
34					0.0077									34
34.2					0.0026									34.2
35					0.0412									35
36					0.0103									36
37					0.0026									37
Allele	D3S1358	vWA	FGA	D8S1179	D21S11	D18S51	D5S818	D13S317	D7S820	CSF1PO	TPOX	TH01	D16S539	Allele
N	194	244	194	194	194	194	244	244	244	208	208	208	206	

Trinidadian Amended Allele Frequencies

Allele	D3S1358	vWA	FGA	D8S1179	D21S11	D18S51	D5S818	D13S317	D7S820	CSF1PO	TPOX	TH01	D16S539	Allele
5												0.0061		5
6											0.0976	0.1829		6
7							0.0118		0.0060	0.0671	0.0122	0.3110		7
8				0.0063			0.0235	0.0536	0.2083	0.0549	0.3232	0.2073	0.0610	8
9							0.0294	0.0476	0.1131	0.0244	0.1646	0.2073	0.1646	9
9.3												0.0732		9.3
10				0.0500			0.1529	0.0536	0.3333	0.2744	0.0671	0.0122	0.1280	10
<11						0.0064								<11
11		0.0059		0.0750		0.0256	0.2941	0.2798	0.2202	0.2134	0.2866		0.2866	11
12				0.1563		0.0833	0.3235	0.3214	0.1012	0.2744	0.0488		0.1829	12
13		0.0059		0.2250		0.0962	0.1353	0.1607	0.0179	0.0793			0.1402	13
13.2						0.0064								13.2
13.3													0.0061	13.3
14	0.0563	0.0882		0.2500		0.1090	0.0235	0.0833		0.0122			0.0305	14
15	0.3125	0.1412		0.1813		0.1538								15
16	0.3188	0.2941		0.0563		0.2051	0.0059							16
17	0.2000	0.2647				0.0513								17
18	0.1125	0.1353	0.0125			0.0577								18
19		0.0471	0.0563			0.0962								19
20		0.0176	0.0938			0.0705								20
21			0.1000			0.0385								21
22			0.1688											22
23			0.1625											23
24			0.2063											24
25			0.1063											25
26			0.0438											26
27			0.0188		0.0625									27
28			0.0125		0.2250									28
29			0.0063		0.2000									29
29.2					0.0063									29.2
30					0.1750									30
>30			0.0125											>30
30.2					0.0125									30.2
31					0.0500									31
31.2					0.0813									31.2
32					0.0313									32
32.2					0.0688									32.2
33					0.0063									33
33.2					0.0500									33.2
34					0.0188									34
35					0.0125									35
Allele	D3S1358	vWA	FGA	D8S1179	D21S11	D18S51	D5S818	D13S317	D7S820	CSF1PO	TPOX	TH01	D16S539	Allele
N	80	85	80	80	80	78	85	84	84	82	82	82	82	

Chamorro Amended Allele Frequencies

Allele	D2S1338	D19S433	Allele
12		0.0347	12
12.2		0.0139	12.2
13		0.3542	13
13.2		0.0417	13.2
14		0.2292	14
14.2		0.0972	14.2
15		0.0903	15
15.2		0.0972	15.2
16	0.0278		16
16.2		0.0139	16.2
17	0.1042		17
17.2		0.0278	17.2
18	0.0833		18
19	0.1875		19
20	0.1111		20
21	0.0139		21
22	0.0972		22
23	0.1736		23
24	0.1319		24
25	0.0556		25
26	0.0069		26
27	0.0069		27
Allele	D2S1338	D19S433	Allele
N	72	72	

Filipino Amended Allele Frequencies

Allele	D2S1338	D19S433	Allele
12		0.0286	12
13		0.2857	13
13.2		0.0357	13.2
14		0.1571	14
14.2		0.0500	14.2
15		0.1071	15
15.2		0.2500	15.2
16	0.0286	0.0143	16
16.2		0.0643	16.2
17	0.0786		17
17.2		0.0071	17.2
18	0.0571		18
19	0.2214		19
20	0.0786		20
21	0.0286		21
22	0.0643		22
23	0.1357		23
24	0.2643		24
25	0.0357		25
26	0.0071		26
Allele	D2S1338	D19S433	Allele
N	70	70	

Validation Summary: ID Plus EXCEL PopStats Template v1.2

1. Purpose

In May of 2015, the FBI CODIS unit notified all CODIS laboratories of errors found in the population database data published in the **Journal of Forensic Sciences** ***Population data on the thirteen CODIS core short tandem repeat loci in African Americans, U.S. Caucasians, Hispanics, Bahamians, Jamaicans and Trinidadians*****, Journal of Forensic Sciences 1999 44(6):1277-86** [1,2]. This population data was used to build the allele frequency tables in the FBI's Popstats database which HCIFS subsequently used to build the in-house ID Plus EXCEL Popstats template.

Corrected allele frequencies were provided as well as a pre-publication copy of the erratum notice to be published in Journal of Forensic Science in July 2015[3].

The purpose of this performance check was to update the EXCEL Popstats Template with the corrected allele frequencies as well as to evaluate the impact of the errors with a comparison of corrected and original allele frequencies[3].

2. Materials

ID Plus EXCEL PopStats Template v1.1
ID Plus EXCEL PopStats Template v1.2

3. Procedure

Allele frequencies for the 1999 Caucasian (CAU), African American (AA), and South West Hispanics (SWH) population databases were compared to the amended 2015 data released by the FBI. Changes to Fraction (allele frequency), Minimum Allele Frequency, Allele counts, or Total Samples were documented and a list of every affected allele was compiled and verified by a second analyst. The updated allele frequencies were incorporated into the ID PLUS Excel PopStats Template v1.2. The allele frequency changes were verified by a second analyst.

One hundred alleles were affected by the 2015 FBI corrections (data not shown, see Forensic Genetics\DNA\Compliance Group\Amended FBI STR 2015\DNA\Profiles Tested.xlsm). The difference in the frequencies between the original and corrected values was calculated as well as the difference in p^2 values, homozygous single source values ($p^2 + p(1-p)\theta$, and the 2p portion of a 2pq heterozygote single source.

Five profiles were initially created to test genotype frequencies for profiles containing alleles with the most significant differences. The five profiles included three with a single allele, one with seven loci and one full simulated single source profile including all 10 of the alleles with the largest observable frequency changes. Random match probabilities (RMP) were calculated using both original and corrected allele frequencies.

An expanded group of 15 single source and 15 mixture samples were then tested using both original and corrected allele frequencies. The first sample of these sets was

generated with a genotype at one locus and subsequent samples were created through addition of genotypes to each locus until all 15 loci contained a genotype. RMP was calculated for the single source profiles and Combined Probability of Inclusion (CPI) calculated for the mixtures.

The ID Plus EXCEL PopStats Template v1.1 and v1.2 were further compared using RMP and CPI values for five single source profiles, five two-person and five three-person mixtures previously amplified in Identifiler Plus for another study.

4. Results

Of the 100 alleles with corrected allele frequencies, 48 showed no change in p^2 value, 43 varied between 0.0001 and 0.0008, and 9 varied between 0.0010 and 0.0042. Similar results were obtained using $p^2 + p(1-p)\theta$. The largest variation was seen when calculating 2p (Table 1).

	No difference	0.0001 - 0.0008	0.0010 - 0.0100
p^2	48	43	9
$p^2 + p(1-p)\theta$	47	44	9
2p	1	49	50

Table 1. Differences between 1999 and 2015 genotype frequency data.

The 10 alleles with the largest variation between original and corrected frequencies were selected (Table 2) and used to generate five profiles (Table 3). These profiles were used to test the effect of the amended allele frequencies in addition to testing the template update to version 1.2 using the newly amended frequencies.

Allele	Ethnicity	Δp^2
D16S539-10	SWH	0.0008
CSF1PO-12	CAU	0.0010
D19S433-14	AA	0.0012
D16S539-12	SWH	0.0014
D5S818-12	SWH	0.0014
D13S317-12	CAU	0.0016
D13S317-11	CAU	0.0016
TPOX-8	CAU	0.0029
VWA-16	SWH	0.0035
D5S818-11	SWH	0.0042

Table 2. Alleles with the largest observable frequency change

Profile 1 is a single source profile containing the 10 alleles with the largest frequency differences. Profile 2 simulates a partial single source profile containing a mix of affected and non-affected allele frequencies, with all loci containing heterozygous alleles.

Profile 3 is a homozygous version of profile 2. Profile 4 contains the single allele with the largest difference in allele frequency. Profile 5 is similar to profile 4, with an allele still in the top 10 most affected, but with a less severe difference in frequency value. The largest difference in RMP of 1.2 fold was observed in Profile 4 when only D5, allele 11 was used in the calculation. For Profiles 1, 2, and 3 observed differences ranged from no change to a maximum 1.07 fold difference. No change was seen in Profile 5. Across all profiles, all differences in calculated statistical values were less than 2-fold.

Profile	D8S1179	D21S11	D7S820	CSF1PO	D3S1358	TH01	D13S317	D16S539	D2S1338	D19S433	vWA	TPOX	D18S51	AMEL	D5S818	FGA
1	13	29,30	9,10	10,12	16,18	6,7	11,12	12	19,22	13.2,14	16	8,12	14,16	X	11,12	19,20
2				10,12			11,12	10,12		14,15	16,17	8,9			11,12	
3				12			11	12		14	16	8			12	
4															11	
5				12												

Table 3. Five profiles generated using the 10 most affected allele frequencies.

		Single Source	
Profile	Ethnicity	New	Old
1	Caucasian	1.17E+18	1.09E+18
	African American	1.91E+19	1.88E+19
	South Western Hispanics	5.82E+16	6.06E+16
2	Caucasian	1.87E+06	1.82E+06
	African American	1.63E+07	1.72E+07
	South Western Hispanics	5.01E+06	5.20E+06
3	Caucasian	4.00E+06	4.12E+06
	African American	9.83E+07	9.54E+07
	South Western Hispanics	4.72E+06	4.85E+06
4	Caucasian	6	6
	African American	14	14
	South Western Hispanics	5	6
5	Caucasian	9	9
	African American	11	11
	South Western Hispanics	6	6

Table 4. Calculations of RMP for 5 profiles containing the 10 most affected alleles.

Profile	Ethnicity	Fold Change SS	Change % Difference SS
1	Caucasian	1.07	7%
	African American	1.02	2%
	South Western Hispanics	1.04	-4%
2	Caucasian	1.03	3%
	African American	1.06	-6%
	South Western Hispanics	1.04	-4%
3	Caucasian	1.03	-3%
	African American	1.03	3%
	South Western Hispanics	1.03	-3%
4	Caucasian	1.00	0%
	African American	1.00	0%
	South Western Hispanics	1.20	-17%
5	Caucasian	1.00	0%
	African American	1.00	0%
	South Western Hispanics	1.00	0%

Table 5. Difference in RMP between the v1.1 and 1.2.

For the expanded set of single source samples seen in Table 6, calculations in Table 7 show that the differences between the corrected and old frequencies are minimal. There was less than a 2-fold difference overall in the statistical values calculated using the old and corrected frequencies (Table 8).

Profile	D8S1179	D21S11	D7S820	CSF1PO	D3S1358	TH01	D13S317	D16S539	D2S1338	D19S433	vWA	TPOX	D18S51	AMEL	D5S818	FGA
1				12												
2				12			11,12									
3				12			11,12	12								
4				12			11,12	12		14						
5				12			11,12	12		14	16					
6				12			11,12	12		14	16	8				
7				12			11,12	12		14	16	8			11,12	
8	13			10,12			11,12	12		13.2,14	16	8,12			11,12	
9	13	29,30		10,12			11,12	12		13.2,14	16	8,12			11,12	
10	13	29,30	9,10	10,12			11,12	12		13.2,14	16	8,12			11,12	
11	13	29,30	9,10	10,12	16,18		11,12	12		13.2,14	16	8,12			11,12	
12	13	29,30	9,10	10,12	16,18	6,7	11,12	12		13.2,14	16	8,12			11,12	
13	13	29,30	9,10	10,12	16,18	6,7	11,12	12	19,22	13.2,14	16	8,12			11,12	
14	13	29,30	9,10	10,12	16,18	6,7	11,12	12	19,22	13.2,14	16	8,12	14,16		11,12	
15	13	29,30	9,10	10,12	16,18	6,7	11,12	12	19,22	13.2,14	16	8,12	14,16		11,12	19,20

Table 6: Fifteen single source profiles generated from a single locus to all 15 loci containing genotypes.

	Template v1.1			Template v1.2		
Profile	Caucasian	African American	South Western Hispanics	Caucasian	African American	South Western Hispanics
1	9	11	6	9	11	6
2	47	47	73	47	47	73
3	402	1,302	871	400	1,302	857
4	3,498	32,050	8,305	3,483	33,030	8,174
5	82,870	430,000	63,100	82,500	443,000	60,470
6	277,400	3,115,000	203,200	273,500	3,209,000	194,700
7	955,100	16,770,000	830,200	942,000	17,280,000	792,900
8	241,000,000	2,940,000,000	27,160,000	256,000,000	2,985,000,000	25,940,000
9	2,867,000,000	43,290,000,000	201,300,000	3,046,000,000	43,960,000,000	192,300,000
10	33,370,000,000	425,500,000,000	6,862,000,000	36,020,000,000	432,100,000,000	6,569,000,000
11	443,300,000,000	12,640,000,000,000	154,300,000,000	473,600,000,000	12,840,000,000,000	147,800,000,000
12	5,673,000,000,000	131,000,000,000,000	985,700,000,000	6,068,000,000,000	133,100,000,000,000	943,700,000,000
13	661,900,000,000,000	3,455,000,000,000,000	26,860,000,000,000	707,900,000,000,000	3,508,000,000,000,000	25,720,000,000,000
14	17,810,000,000,000,000	143,100,000,000,000,000	682,200,000,000,000	19,050,000,000,000,000	145,900,000,000,000,000	653,100,000,000,000
15	1,092,000,000,000,000,000	18,770,000,000,000,000,000	60,630,000,000,000,000	1,168,000,000,000,000,000	19,140,000,000,000,000,000	58,210,000,000,000,000

Table 7: RMP Calculations for the fifteen single source profiles using ID Plus PopStats template v1.1 (old frequencies) and v1.2 (corrected frequencies).

	Fold Change			Change % Diffference		
Profile	Caucasian	African American	South Western Hispanics	Caucasian	African American	South Western Hispanics
1	1.00	1.00	1.00	0%	0%	0%
2	1.00	1.00	1.00	0%	0%	0%
3	1.01	1.00	1.02	0%	0%	2%
4	1.00	1.03	1.02	0%	3%	2%
5	1.00	1.03	1.04	0%	3%	4%
6	1.01	1.03	1.04	1%	3%	4%
7	1.01	1.03	1.05	1%	3%	4%
8	1.06	1.02	1.05	6%	2%	4%
9	1.06	1.02	1.05	6%	2%	4%
10	1.08	1.02	1.04	7%	2%	4%
11	1.07	1.02	1.04	6%	2%	4%
12	1.07	1.02	1.04	7%	2%	4%
13	1.07	1.02	1.04	6%	2%	4%
14	1.07	1.02	1.04	7%	2%	4%
15	1.07	1.02	1.04	7%	2%	4%

Table 8: Differences calculated for the single source samples when comparing ID Plus Popstats template v1.1(old frequencies) and v1.2 (corrected frequencies).

For the expanded set of mixture samples seen in Table 9, the differences in the calculations shown in Table 10 using the old and corrected frequencies were minimal. The differences in the calculations using template v1.1 versus 2.2 did not exceed 1.02 fold (Table 10).

Profile	D8S1179	D21S11	D7S820	CSF1PO	D3S1358	TH01	D13S317	D16S539	D2S1338	D19S433	vWA	TPOX	D18S51	AMEL	D5S818	FGA
1				10,11,12,13												
2				10,11,12,13			11,12,13,14									
3				10,11,12,13			11,12,13,14	12,13,14,15								
4				10,11,12,13			11,12,13,14	12,13,14,15		11,12,13,14						
5				10,11,12,13			11,12,13,14	12,13,14,15		11,12,13,14	16,17,18,19					
6				10,11,12,13			11,12,13,14	12,13,14,15		11,12,13,14	16,17,18,19	8,9,10,11,12				
7				10,11,12,13			11,12,13,14	12,13,14,15		11,12,13,14	16,17,18,19	8,9,10,11,12			11,12,13,14	
8	13,14,15,16			10,11,12,13			11,12,13,14	12,13,14,15		11,12,13,14	16,17,18,19	8,9,10,11,12			11,12,13,14	
9	13,14,15,16	28,29,30,31		10,11,12,13			11,12,13,14	12,13,14,15		11,12,13,14	16,17,18,19	8,9,10,11,12			11,12,13,14	
10	13,14,15,16	28,29,30,31	8,9,10,11	10,11,12,13			11,12,13,14	12,13,14,15		11,12,13,14	16,17,18,19	8,9,10,11,12			11,12,13,14	
11	13,14,15,16	28,29,30,31	8,9,10,11	10,11,12,13	15,16,17,18		11,12,13,14	12,13,14,15		11,12,13,14	16,17,18,19	8,9,10,11,12			11,12,13,14	
12	13,14,15,16	28,29,30,31	8,9,10,11	10,11,12,13	15,16,17,18	6,7,8,9	11,12,13,14	12,13,14,15		11,12,13,14	16,17,18,19	8,9,10,11,12			11,12,13,14	
13	13,14,15,16	28,29,30,31	8,9,10,11	10,11,12,13	15,16,17,18	6,7,8,9	11,12,13,14	12,13,14,15	19,20,21,22	11,12,13,14	16,17,18,19	8,9,10,11,12			11,12,13,14	
14	13,14,15,16	28,29,30,31	8,9,10,11	10,11,12,13	15,16,17,18	6,7,8,9	11,12,13,14	12,13,14,15	19,20,21,22	11,12,13,14	16,17,18,19	8,9,10,11,12	14,15,16,17		11,12,13,14	
15	13,14,15,16	28,29,30,31	8,9,10,11	10,11,12,13	15,16,17,18	6,7,8,9	11,12,13,14	12,13,14,15	19,20,21,22	11,12,13,14	16,17,18,19	8,9,10,11,12	14,15,16,17		11,12,13,14	19,20,21,22

Table 9: Fifteen mixture profiles generated from a single locus to all 15 loci containing genotypes.

	Template v1.1			Template v1.2		
Profile	Caucasian	African American	South Western Hispanics	Caucasian	African American	South Western Hispanics
1	1	1	1	1	1	1
2	2	2	3	2	2	3
3	6	13	15	6	13	15
4	11	27	47	11	27	47
5	18	62	65	18	62	65
6	18	77	66	18	77	66
7	21	100	98	21	100	96
8	47	149	191	47	150	188
9	110	323	418	109	324	411
10	168	415	753	168	416	741
11	229	566	918	228	568	903
12	477	724	1,601	473	726	1,575
13	3,826	2,951	6,575	3,794	2,958	6,469
14	11,680	8,548	20,530	11,760	8,639	20,200
15	36,760	37,220	96,070	36,440	37,620	94,470

Table 10: CPI calculations for the fifteen mixture profiles using ID Plus PopStats template v1.1 and v1.2.

	Fold Change			Change % Diffference		
Profile	Caucasian	African American	South Western Hispanics	Caucasian	African American	South Western Hispanics
1	1.00	1.00	1.00	0%	0%	0%
2	1.00	1.00	1.00	0%	0%	0%
3	1.00	1.00	1.00	0%	0%	0%
4	1.00	1.00	1.00	0%	0%	0%
5	1.00	1.00	1.00	0%	0%	0%
6	1.00	1.00	1.00	0%	0%	0%
7	1.00	1.00	1.02	0%	0%	2%
8	1.00	1.01	1.02	0%	1%	2%
9	1.01	1.00	1.02	1%	0%	2%
10	1.00	1.00	1.02	0%	0%	2%
11	1.00	1.00	1.02	0%	0%	2%
12	1.01	1.00	1.02	1%	0%	2%
13	1.01	1.00	1.02	1%	0%	2%
14	1.01	1.01	1.02	1%	1%	2%
15	1.01	1.01	1.02	1%	1%	2%

Table 11: Difference calculated for the mixture profiles when comparing ID Plus Popstats template v1.1 and v1.2.

The single source and two and three person mixtures from a prior study (Table 12) showed results similar to those from the manually generated profiles. The differences in RMP and CPI Table 13 did not exceed 1.16 fold (Table 14).

	Profile	D8S1179	D21S11	D7S820	CSF1PO	D3S1358	TH01	D13S317	D16S539	D2S1338	D19S433	vWA	TPOX	D18S51	AMEL	D5S818	FGA
Single	1	13,15	29,31	9		15,18	7		9	16,21	13,14			12			
	2	14	28		10	14	9.3	12									
	3	8	32.2	10,11	11,12	11,14	7,8	13	10,13	20	13,14	15	8,11		X	11	24
	4	12,14	30	8,10	10,11	14,17	9,9.3	8	12,13	17	13,15.2	17,18	10,11	14,19	X,Y	10,12	21
	5	13,14	27,28	11,12	11,12	16,18	6,7	11	9,13	19,20	14,15	15,17	8,10	14,17	X	12	20,22
2 Person Mixture	1	14,15	28,31	10,11	8,10	14	7,9.3	12	9,11	19,21, 22	13,14	13,17,18	11	16,17	X,Y	11,12,13	23,25, 26
	2	14	30			14,18	6,7,9		13	24			11		X		
	3		30		10	15,17	7,8, 9.3	10,12		17,19	14	17	8,11	17	X	12	22
	4	13,14, 15	28,29, 31	9,10, 11,13	7,8,10, 12	14,15,18	7,9.3	11,12	9,11	16,19, 21,22	13,14	13,15,17, 18	9,11	12,16, 17	X,Y	11,12,13	22,23, 25,26
	5	12,13, 14	30,31.2,3 2.2	8,9	7,11	15,17	6,7,9,9.3	9,12,13	12	17,19,20	13,14,15	17,18,20	8	13,16	X	10,12	18,23,25
3 Person Mixture	1	12,13,14	30		11	14,16,18	6,7,9	11,12	12,13	19,24	12,14, 14.2,15.2		8,11	13,15, 16	X	11	20,24
	2	8,11,14	28,30, 32.2	10,11	8,10,11	11,14,15, 16	7,8, 9.3	8,9,12, 13	9,11,12,1 3	17,19,20, 22,24,25	13,14, 14.2	13,15,18, 19	8,11	16,17	X,Y	11,12, 13	22,23, 24,25, 26
	3	12,13,14	28,30, 31.2	8,9,12	7,11,12	15,16	7,9	12,13	8,10,12	17,19	15	14,17	8,10	15	X	8,12	21,22
	4	10,13,14, 15	28,30,32, 35	10,11, 12	8,10,11	14,15,16, 17	7,8,9,9.3	9,10,11,1 2,13	8,11,13	17,19,20, 22,23	11,13,14, 15.2	15,16,17, 18	8,11	13,16, 17	X	11,12	22,23, 25
	5	13,14,15		12		15	7,8	12		17	13,14, 14.2	17				11	

Table 12: Profiles for 5 single source profiles, 5 two person mixtures, and 5 three person mixtures calculated using ID Plus PopStats template v1.1 and v1.2.

		Template v1.1			Template v1.2		
	Profile	Caucasian	African American	South Western Hispanics	Caucasian	African American	South Western Hispanics
Single	1	198,700,000,000,000	7,095,000,000,000	3,768,000,000,000,000	197,600,000,000,000	7,114,000,000,000	3,783,000,000,000,000
	2	63,700,000	54,720,000	2,188,000,000	67,410,000	54,720,000	2,196,000,000
	3	2,460,000,000,000,000,000,000	8,247,000,000,000,000,000,000	5,144,000,000,000,000,000,000	2,592,000,000,000,000,000,000	8,382,000,000,000,000,000,000	5,777,000,000,000,000,000,000
	4	172,300,000,000,000,000,000	52,980,000,000,000,000,000,000	3,380,000,000,000,000,000,000	174,700,000,000,000,000,000	60,490,000,000,000,000,000,000	3,612,000,000,000,000,000,000
	5	574,000,000,000,000,000	29,810,000,000,000,000,000	45,160,000,000,000,000,000	544,500,000,000,000,000	28,310,000,000,000,000,000	52,050,000,000,000,000,000
2 Person Mixture	1	150,200,000,000,000	1,676,000,000,000	313,700,000,000,000	154,800,000,000,000	1,731,000,000,000	312,800,000,000,000
	2	473,300,000	1,170,000,000	2,489,000,000	466,700,000	1,170,000,000	2,608,000,000
	3	351,000,000,000	1,816,000,000,000	192,700,000,000	355,600,000,000	1,914,000,000,000	196,100,000,000
	4	698,800,000	30,450,000	1,173,000,000	687,400,000	31,310,000	1,191,000,000
	5	33,770,000,000	4,121,000,000,000	64,110,000,000	34,480,000,000	4,167,000,000,000	64,950,000,000
3 Person Mixture	1	2,052,000,000	73,480,000,000	4,170,000,000	2,043,000,000	74,750,000,000	4,181,000,000
	2	26,470,000	8,408,000	14,330,000	26,680,000	8,744,000	14,660,000
	3	3,278,000,000,000	35,560,000,000,000	2,995,000,000,000	3,231,000,000,000	30,830,000,000,000	2,987,000,000,000
	4	861,900	1,218,000	302,600	849,000	1,250,000	304,200
	5	1,036,000,000	2,720,000,000	260,900,000	1,023,000,000	2,750,000,000	225,300,000

Table 13: Calculations for the RMP and CPI using ID Plus PopStats template v1.1 and v1.2.

		Fold Change			Change % Diffference		
	Profile	Caucasian	African American	South Western Hispanics	Caucasian	African American	South Western Hispanics
Single	1	1.01	1.00	1.00	1%	0%	0%
	2	1.06	1.00	1.00	6%	0%	0%
	3	1.05	1.02	1.12	5%	2%	11%
	4	1.01	1.14	1.07	1%	12%	6%
	5	1.05	1.05	1.15	5%	5%	13%
2 Person Mixture	1	1.03	1.03	1.00	3%	3%	0%
	2	1.01	1.00	1.05	1%	0%	5%
	3	1.01	1.05	1.02	1%	5%	2%
	4	1.02	1.03	1.02	2%	3%	2%
	5	1.02	1.01	1.01	2%	1%	1%
3 Person Mixture	1	1.00	1.02	1.00	0%	2%	0%
	2	1.01	1.04	1.02	1%	4%	2%
	3	1.01	1.15	1.00	1%	13%	0%
	4	1.02	1.03	1.01	1%	3%	1%
	5	1.01	1.01	1.16	1%	1%	14%

Table 14: Difference calculated for RMP and CPI when comparing the results from ID Plus Popstats template v1.1 and v1.2.

5. Conclusion

The update to the "ID Plus EXCEL PopStats Template" incorporates the corrected allele frequency data for Caucasian, African American, and South West Hispanic populations. This evaluation shows statistical calculations made using the corrected allele frequencies resulted in much less than the two-fold difference cited in the FBI's Notice of Amendments. This difference is also well within the factor of ten suggested by the National Research Council[4] in 1996 for statistical calculations based on population database allele frequencies. A two-fold difference in statistical value calculations translates to a difference significantly less than one order of magnitude. In this study, the most significantly affected RMP statistical calculation evaluated for a single source sample previously calculated as approximately 1 in 6 individuals was estimated at 1 in 5 individuals when determined using the corrected frequencies (a 1.2 fold difference). The most significantly affected CPI statistical calculation evaluated for a mixed sample previously calculated as approximately 1 in 260,900,000 was, when repeated using the corrected frequencies, determined to be approximately 1 in 225,300,000 (a 1.16 fold difference).

The original electronic genotype data for the FBI Southeast Hispanic population could not be verified[1]. Therefore, calculations will no longer be made using this database. This field will be removed from the ID Plus EXCEL PopStats Template v.1.2.

With the removal of this racial group from the final printed calculation summary page to be included in casefiles, the ID Plus EXCEL PopStats Template v1.2 including the amended FBI database information operated correctly and without any errors during this study.

6. References

[1] B. Budowle, T. R. Moretti. J. S. Buckleton, Notice of Amendment of the FBI's STR Population Data Published in 1999 and 2001. 2015.

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[3] B. Budowle, T. Moretti, A. L. Baumstark, D. A. Defenbaugh, K. M. Keys, Population Data on the Thirteen Core Short Tandem Repeat Loci in African

Americans, U.S. Caucasians, Hispanics, Bahamians, Jamaicans, and Trinidadians, Journal of Forensic Sciences, 1999 44(6):1277-86.

[4] National Research Council. The evaluation of forensic DNA evidence. Washington, DC: National Academy Press, 1996.

7. Review

The validation of the modification to the ID Plus EXCEL PopStats Template V1.2 is complete. ID Plus EXCEL PopStats Template V1.2 is considered suitable for its intended use.

Completed by: _____ Date: 6.16.2015

Reviewed by: _____ Date: 6-16-2015
Validation Manager

Reviewed by: _____ Date: 6/16/15
QA/Compliance Manager

Reviewed by: _____ Date: 6/16/15
Forensic Genetics Director/Technical Leader

Reviewed by: _____ Date: 6/16/15
Quality Director

Reviewed by: _____ Date: 6-17-15
Crime Laboratory Director

[1]

TECHNICAL NOTE

Bruce Budowle,[1] Ph.D.; Tamyra R. Moretti,[1] Ph.D.; Anne L. Baumstark,[1] B.S.; Debra A. Defenbaugh,[1] B.S.; and Kathleen M. Keys,[1] B.S.

Population Data on the Thirteen CODIS Core Short Tandem Repeat Loci in African Americans, U.S. Caucasians, Hispanics, Bahamians, Jamaicans, and Trinidadians*

REFERENCE: Budowle B, Moretti TR, Baumstark AL, Defenbaugh DA, Keys KM. Population data on the thirteen CODIS core short tandem repeat loci in African Americans, U.S. Caucasians, Hispanics, Bahamians, Jamaicans, and Trinidadians. J Forensic Sci 1999;44(6):1277–1286.

[illegible]

KEYWORDS: forensic science, Caucasian, African American, Hispanic, Bahamian, Jamaican, Trinidadian, DNA typing, population genetics, CSF1PO, FGA, TH01, TPOX, VWA, D3S1358, D5S818, D7S820, D13S317, D16S539, D18S51, D21S11, CODIS core loci

The full use of DNA typing technology in forensic science has come to fruition by the development of national DNA databanks, such as the Combined DNA Index System (CODIS) (1). The main objective for a national DNA databank is to assist investigators in the identification of perpetrators of violent crimes. For purposes of applying DNA technology to human identity testing and to make effective use of a national DNA databank, defined polymorphic genetic markers are required, and all laboratories that contribute to the database should use the same genetic loci. Short tandem repeat (STR) loci are the most informative PCR-based genetic markers available to date for attempting to individualize biological material (2–5). The 13 STR loci CSF1PO, FGA, TH01, TPOX, vWA, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, and D21S11 have been selected as the core loci for use in CODIS (1).

This paper presents allele distribution data in African Americans, United States Caucasians, Hispanics, Bahamians, Jamaicans, and Trinidadians for the 13 core STR loci. The data demonstrate that these loci can be useful for providing estimates of the frequency of a DNA profile in forensic identity testing and that a multiple locus profile is extremely rare in all the population groups analyzed.

Materials and Methods

Sample Preparation—Whole blood, obtained in EDTA vacutainer tubes by venipuncture from African American, Caucasian, and southwestern Hispanic individuals, was kindly provided by Dr. A. Eisenberg, University of North Texas Health Science Center, Fort Worth, Texas. Bloodstains from Bahamians, Jamaicans, and Trinidadians were kindly provided by Dr. George Duncan, Broward County Sheriff's Department, Ft. Lauderdale, FL. The DNA was extracted by the phenol-chloroform method (6). The quantity of extracted DNA was estimated using the slot-blot procedure described by Waye, et al. (7) and Budowle, et al. (8).

STR Amplification—The African American, Caucasian, and Hispanic samples were amplified at the loci FGA, VWA, D3S1358, D5S818, D7S820, D8S1179, D13S317, D18S51, and D21S11 using the AmpF/STR™ Profiler Plus kit (PE Biosystems, Foster City, CA) (i.e., Profiler Plus kit) and at the loci CSF1PO, TPOX, TH01, D3S1358, D7S820, and D16S539 using the AmpF/STR COfiler™ kit (PE Biosystems, Foster City, CA) (i.e., COfiler kit). The loci D3S1358 and D7S820 were typed with both kits. The Bahamian, Jamaican, and Trinidadian samples were amplified at the loci CSF1PO, TPOX, TH01, VWA, D5S818, D7S820, D13S317, and D16S539 using the GenePrint™ PowerPlex™ kit (Promega Corporation, Madison, WI) (i.e., PowerPlex

[1] Forensic Science Research and Training Center, FBI Academy, Quantico, Virginia.

* This is publication number 99-02 of the Laboratory Division of the Federal Bureau of Investigation. Names of commercial manufacturers are provided for identification only, and inclusion does not imply endorsement by the Federal Bureau of Investigation.

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[2]

CODIS

CODIS Bulletin

Bulletin #: BT050815 Date Distributed: 05/08/15

AMENDMENT OF THE 1999 AND 2001 FBI STR POPULATION DATA

In the evaluation of original population database samples with the new expanded CODIS core loci, the FBI Laboratory has identified some errors in the data published in the Journal of Forensic Sciences *Population data on the thirteen CODIS core short tandem repeat loci in African Americans, U.S. Caucasians, Hispanics, Bahamians, Jamaicans and Trinidadians*, Journal of Forensic Sciences 1999 44(6):1277-86. An erratum notice will be published as a Letter to the Editor in the July 2015 issue of the Journal of Forensic Sciences containing the empirical data demonstrating their nominal effect on profile probabilities. The new amended population data will be available at FBI.gov (http://www.fbi.gov/about-us/lab/biometric-analysis/dna-casework-unit-dcu-1), on the CJIS WAN, and in a future update to Popstats.

Since the development in the late 1990s of the original short tandem repeat (STR) typing systems that included the 13 CODIS core loci, new amplification kits that expand the number of loci to 24 in a multiplex reaction are now commercially available. To establish allele distributions for the additional loci, population samples that were originally genotyped using AmpFLSTR Profiler Plus, COfiler, Identifiler (Thermo Fisher Scientific, South San Francisco, CA) and/or GenePrint PowerPlex (Promega Corp., Madison, WI) were retyped using GlobalFiler (Thermo Fisher Scientific) and PowerPlex Fusion (Promega Corp.). For any sample where a given locus is typed with different amplification kits, concordant genotypes should be obtained irrespective of the kit(s) used, with the exception of genotype differences due to rare primer binding site variants and improvements in allelic ladders that expand allele identification capabilities (e.g., an allele may be designated as <11 in one system and as 9 in another).

The FBI Laboratory has identified errors in the data published in the Journal of Forensic Sciences and has submitted erratum notice regarding "Population data on the thirteen CODIS core short tandem repeat loci in African Americans, U.S. Caucasians, Hispanics, Bahamians,

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[3]

Notice of Amendment of the FBI's STR Population Data Published in 1999 and 2001

Recently, new amplification kits that expand the number of loci in a multiplex reaction have become commercially available. To establish allele distributions for the additional loci, the FBI Laboratory retyped population samples that were originally genotyped using AmpFISTR Profiler Plus, COfiler, Identifiler (Thermo Fisher Scientific, South San Francisco, CA) and/or GenePrint PowerPlex (Promega Corp., Madison, WI) (1,2) using GlobalFiler (Thermo Fisher Scientific) and PowerPlex Fusion (Promega Corp.) (3). During a comparison of over 1100 DNA profiles from African Americans, Caucasians, Southwest Hispanics, Bahamians, Jamaicans, Trinidadians, Filipinos and Chamorros in the original (4,5) and new studies (3), genotyping discrepancies were discovered. Electronic genotype data corresponding to the published allele frequencies are not available for the Southeast Hispanic, Apache, Navaho and Minnesota Native American populations (6), as well as Filipino and Chamorro populations (except for D2S1338 and D19S433) (7). Genotypes from these populations thus could not be assessed for concordance.

The discrepancies discovered were attributable to (a) human error, typically due to manual data editing and recording, and (b) technological limitations (e.g., insufficient resolution for distinguishing microvariants using polyacrylamide gel electrophoresis). The published genotype data (3,4) from which allele frequencies were calculated also include sample or data processing errors (e.g., genotype duplications).

Allele frequencies cited across these publications (1,2) have been used by the FBI and many forensic laboratories for calculating match statistics in criminal investigations and other types of human identification applications since 1999. Given that statistical estimates based on these data have been included in thousands of laboratory reports and testimonies, the FBI Laboratory believes the discrepancies require acknowledgement. The FBI Laboratory has submitted the attached erratum notice, which is scheduled to appear in the July issue of the Journal of Forensic Science. This article describes these errors and their effect on profile probability calculations. Empirical testing described in this publication supports that any discrepancy between profile probabilities calculated using the original and corrected data is expected to be less than a factor of two in a full profile. The FBI Laboratory is additionally providing herein the amended allele frequency tables for use by anyone interested in performing comparisons between the multi-locus profile probabilities calculated using the previously published data and the amended allele frequencies.

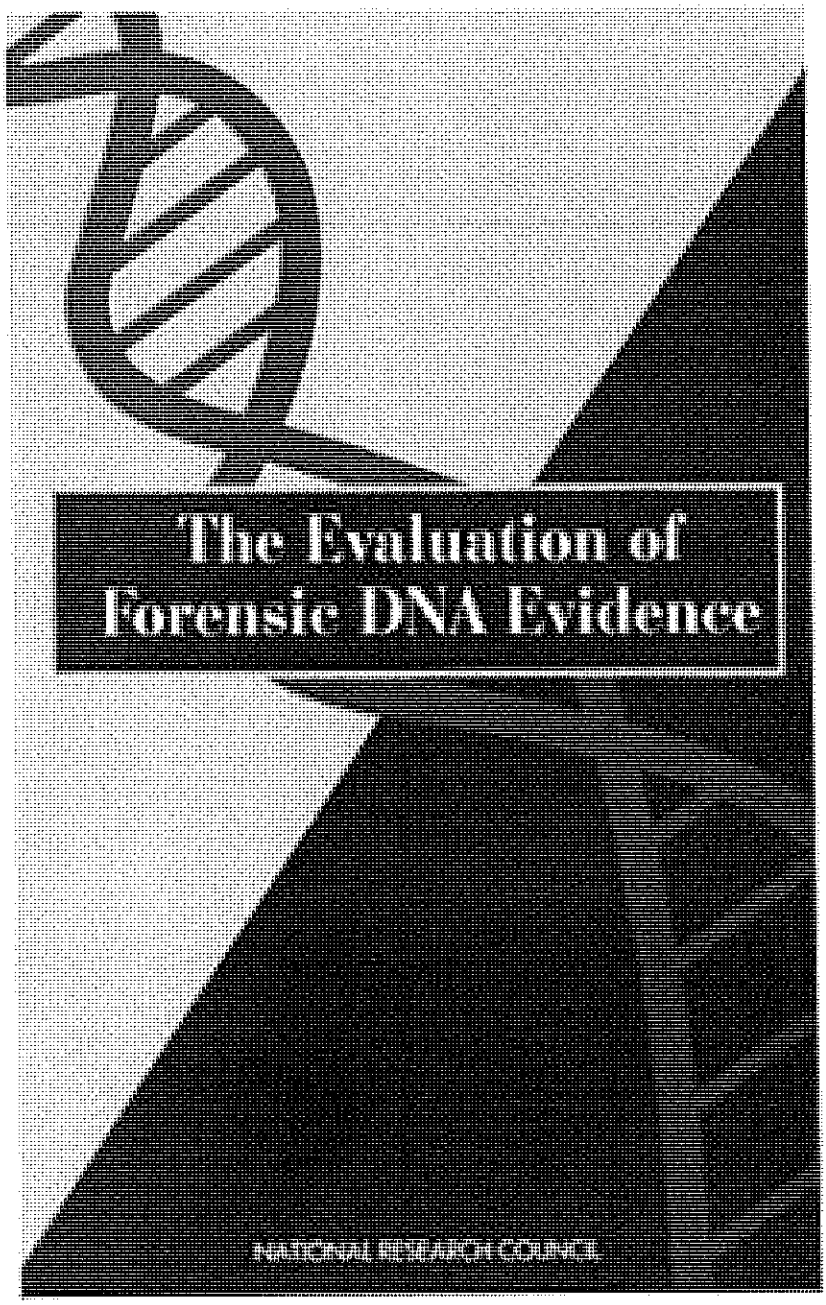
If you have any questions, please contact Anthony J. Onorato of the FBI's DNA Support Unit at Anthony.Onorato@ic.fbi.gov or 703-632-7572.

African American Amended Allele Frequency Table

Caucasian Amended Allele Frequency Table

Southwestern Hispanic Amended Allele Frequency Table

[4]


The Evaluation of
Forensic DNA Evidence
NATIONAL RESEARCH COUNCIL

EXHIBITS

Notice of Amendment of the FBI's STR Population Data Published in 1999 and 2001

Recently, new amplification kits that expand the number of loci in a multiplex reaction have become commercially available. To establish allele distributions for the additional loci, the FBI Laboratory retyped population samples that were originally genotyped using AmpFlSTR Profiler Plus, COfiler, Identifiler (Thermo Fisher Scientific, South San Francisco, CA) and/or GenePrint PowerPlex (Promega Corp., Madison, WI) (1,2) using GlobalFiler (Thermo Fisher Scientific) and PowerPlex Fusion (Promega Corp.) (3). During a comparison of over 1100 DNA profiles from African Americans, Caucasians, Southwest Hispanics, Bahamians, Jamaicans, Trinidadians, Filipinos and Chamorros in the original (4,5) and new studies (3), genotyping discrepancies were discovered. Electronic genotype data corresponding to the published allele frequencies are not available for the Southeast Hispanic, Apache, Navaho and Minnesota Native American populations (6), as well as Filipino and Chamorro populations (except for D2S1338 and D19S433) (7). Genotypes from these populations thus could not be assessed for concordance.

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If you have any questions, please contact the FBI's DNA Support Unit at 703-632-7572.

African American Amended Allele Frequency Table

Caucasian Amended Allele Frequency Table

Southwestern Hispanic Amended Allele Frequency Table

Bahamian Amended Allele Frequency Table

Jamaican Amended Allele Frequency Table

Trinidadian Amended Allele Frequency Table

Chamorro Amended Allele Frequency Table

Filipino Amended Allele Frequency Table

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1. Budowle B, Moretti TR, Baumstark AL, Defenbaugh DA and Keys KM. Population data on the thirteen CODIS core short tandem repeat loci in African Americans, US Caucasians, Hispanics, Bahamians, Jamaicans and Trinidadians. J Forensic Sci 1999;44:1277-1286.

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African American Amended Allele Frequencies

Allele	D3S1358	vWA	FGA	D8S1179	D21S11	D18S51	D5S818	D13S317	D7S820	CSF1PO	TPOX	TH01	D16S539	D2S1338	D19S433	Allele
6											0.0861	0.1095				6
7							0.0028		0.0071	0.0429	0.0215	0.4405				7
8				0.0028			0.0500	0.0335	0.1738	0.0857	0.3684	0.1857	0.0359			8
9				0.0056			0.0139	0.0279	0.1571	0.0333	0.1818	0.1452	0.1986			9
9.3												0.1048				9.3
10				0.0250			0.0639	0.0503	0.3238	0.2714	0.0933	0.0143	0.1100		0.0150	10
<11						0.0056										<11
11		0.0028		0.0361		0.0056	0.2611	0.2374	0.2238	0.2048	0.2249		0.2943		0.0689	11
<12	0.0048															<12
12	0.0024			0.1083		0.0587	0.3556	0.4832	0.0905	0.3000	0.0239		0.1866		0.1138	12
12.2															0.0808	12.2
13	0.0119	0.0056		0.2222		0.0559	0.2444	0.1257	0.0190	0.0548			0.1651		0.2964	13
13.2						0.0056									0.0509	13.2
14	0.1214	0.0667		0.3333		0.0642	0.0056	0.0391	0.0048	0.0071			0.0096		0.1946	14
14.2															0.0539	14.2
15	0.2905	0.2361		0.2139		0.1676		0.0028							0.0419	15
15.2															0.0389	15.2
16	0.3071	0.2694		0.0444		0.1872								0.0449	0.0210	16
>16							0.0028									>16
16.2															0.0180	16.2
17	0.2000	0.1833		0.0083		0.1620								0.1018		17
17.2			0.0028												0.0030	17.2
18	0.0548	0.1361	0.0083			0.1313								0.0659		18
18.2			0.0083												0.0030	18.2
19	0.0048	0.0722	0.0528			0.0782								0.1377		19
>19	0.0024															>19
19.2			0.0028													19.2
20		0.0278	0.0722			0.0559								0.0629		20
21			0.1250			0.0112								0.1527		21
22			0.2250			0.0056								0.1377		22
22.2			0.0056													22.2
23			0.1250			0.0056								0.0988		23
24			0.1861											0.0928		24
24.2					0.0028											24.2
25			0.1000											0.0838		25
26			0.0361		0.0028									0.0210		26
27			0.0222		0.0615											27
28			0.0167		0.2151											28
29			0.0056		0.1899											29
29.3					0.0028											29.3
30			0.0028		0.1788											30
30.2			0.0028		0.0084											30.2
31					0.0922											31
31.2					0.0754											31.2
32					0.0084											32
32.2					0.0698											32.2
33					0.0084											33
33.2					0.0335											33.2
34					0.0084											34
34.2					0.0028											34.2
35					0.0279											35
36					0.0056											36
37					0.0056											37
Allele	D3S1358	vWA	FGA	D8S1179	D21S11	D18S51	D5S818	D13S317	D7S820	CSF1PO	TPOX	TH01	D16S539	D2S1338	D19S433	Allele
N	210	180	180	180	179	179	180	179	210	210	209	210	209	167	167	

Caucasian Amended Allele Frequencies

Allele	D3S1358	vWA	FGA	D8S1179	D21S11	D18S51	D5S818	D13S317	D7S820	CSF1PO	TPOX	TH01	D16S539	D2S1338	D19S433	Allele
6									0.0025			0.2252				6
7									0.0173	0.0025		0.1733				7
8				0.0179				0.0995	0.1634	0.0050	0.5470	0.1262	0.0199			8
8.3												0.0025				8.3
9				0.0102			0.0308	0.0765	0.1460	0.0198	0.1238	0.1658	0.1045			9
9.3												0.3045				9.3
10				0.1020			0.0487	0.0510	0.2896	0.2525	0.0371	0.0025	0.0647			10
10.3										0.0025						10.3
<11						0.0128										<11
11				0.0587		0.0128	0.4103	0.3214	0.2030	0.2995	0.2550		0.2736			11
12				0.1454		0.1276	0.3538	0.3061	0.1411	0.3267	0.0371		0.3383		0.1086	12
12.2															0.0066	12.2
13	0.0025	0.0051		0.3393		0.1224	0.1462	0.1097	0.0297	0.0718			0.1642		0.2829	13
13.2															0.0263	13.2
14	0.1386	0.1020		0.2015		0.1735	0.0077	0.0357	0.0074	0.0149			0.0323		0.3355	14
14.2															0.0033	14.2
15	0.2475	0.1122		0.1097		0.1276	0.0026			0.0050			0.0025		0.1349	15
15.2															0.0263	15.2
16	0.2327	0.2015		0.0128		0.1071								0.0296	0.0428	16
16.2															0.0263	16.2
17	0.2104	0.2628		0.0026		0.1556								0.1941		17
17.2															0.0033	17.2
18	0.1634	0.2219	0.0306			0.0918								0.0526		18
18.2															0.0033	18.2
19	0.0050	0.0842	0.0561			0.0357								0.1447		19
20		0.0102	0.1454			0.0255								0.1546		20
20.2			0.0026													20.2
21			0.1735			0.0051								0.0197		21
22			0.1888			0.0026								0.0296		22
22.2			0.0102													22.2
23			0.1582											0.1349		23
24			0.1378											0.1217		24
24.2					0.0051											24.2
25			0.0689											0.0954		25
26			0.0179											0.0230		26
27			0.0102		0.0459											27
28					0.1658											28
29					0.1811											29
30					0.2321											30
30.2					0.0383											30.2
31					0.0714											31
31.2					0.1020											31.2
32					0.0153											32
32.2					0.1097											32.2
33.2					0.0306											33.2
35.2					0.0026											35.2
Allele	D3S1358	vWA	FGA	D8S1179	D21S11	D18S51	D5S818	D13S317	D7S820	CSF1PO	TPOX	TH01	D16S539	D2S1338	D19S433	Allele
N	202	196	196	196	196	196	195	196	202	202	202	202	201	152	152	

Southwest Hispanic Amended Allele Frequencies

Allele	D3S1358	vWA	FGA	D8S1179	D21S11	D18S51	D5S818	D13S317	D7S820	CSF1PO	TPOX	TH01	D16S539	D2S1338	D19S433	Allele
5												0.0024				5
6									0.0024		0.0048	0.2321				6
7							0.0616		0.0215	0.0024	0.0048	0.3373				7
8				0.0025			0.0025	0.0665	0.0981		0.5550	0.0813	0.0168			8
9				0.0025			0.0542	0.2192	0.0478	0.0072	0.0335	0.1029	0.0793		0.0035	9
9.3												0.2416				9.3
10				0.0936			0.0640	0.1010	0.3062	0.2536	0.0335	0.0024	0.1755			10
<11						0.0049										<11
11		0.0025		0.0616		0.0123	0.4261	0.2020	0.2895	0.2656	0.2727		0.3149		0.0035	11
12				0.1207		0.1059	0.2882	0.2167	0.1914	0.3923	0.0933		0.2885		0.0563	12
12.2															0.0211	12.2
13	0.0024			0.3251		0.1700	0.0961	0.1379	0.0383	0.0646	0.0024		0.1010		0.1620	13
13.2															0.1092	13.2
14	0.0789	0.0616		0.2463		0.1700	0.0049	0.0567	0.0048	0.0096			0.0240		0.3204	14
14.2															0.0458	14.2
15	0.4258	0.0714		0.1158		0.1379	0.0025			0.0048					0.1197	15
15.2															0.0810	15.2
16	0.2656	0.3645		0.0246		0.1158								0.0176	0.0423	16
16.2															0.0352	16.2
17	0.1268	0.2217		0.0074		0.1379								0.2218		17
18	0.0837	0.1946	0.0025			0.0517								0.0423		18
19	0.0144	0.0714	0.0813			0.0369								0.2606		19
>19	0.0024															>19
20		0.0123	0.0690			0.0172								0.1408		20
20.2			0.0025													20.2
21			0.1305			0.0197								0.0106		21
21.2			0.0025													21.2
22			0.1773			0.0074								0.0704		22
>22						0.0123										>22
22.2			0.0049													22.2
23			0.1404											0.1232		23
23.2			0.0074													23.2
24			0.1256											0.0669		24
24.2					0.0025											24.2
25			0.1379											0.0387		25
26			0.0837											0.0070		26
27			0.0320		0.0099											27
28			0.0025		0.0690											28
29					0.2044											29
29.2					0.0025											29.2
30					0.3300											30
30.2					0.0320											30.2
31					0.0690											31
31.2					0.0862											31.2
32					0.0123											32
32.2					0.1355											32.2
33.2					0.0419											33.2
34.2					0.0049											34.2
Allele	D3S1358	vWA	FGA	D8S1179	D21S11	D18S51	D5S818	D13S317	D7S820	CSF1PO	TPOX	TH01	D16S539	D2S1338	D19S433	Allele
N	209	203	203	203	203	203	203	203	209	209	209	209	208	142	142	

Bahamian Amended Allele Frequencies

Allele	D3S1358	vWA	FGA	D8S1179	D21S11	D18S51	D5S818	D13S317	D7S820	CSF1PO	TPOX	TH01	D16S539	Allele
5												0.0032		5
6										0.0032	0.0673	0.1538		6
7								0.0031	0.0126	0.0641	0.0256	0.3846		7
8							0.0723	0.0220	0.1541	0.0577	0.3237	0.2276	0.0385	8
9				0.0032			0.0094	0.0314	0.1258	0.0481	0.2212	0.1282	0.2147	9
9.3												0.0897		9.3
10				0.0194			0.0597	0.0252	0.3396	0.2340	0.0897	0.0128	0.0994	10
<11						0.0097								<11
11		0.0094		0.0516		0.0097	0.2390	0.3050	0.2201	0.2244	0.2372		0.3013	11
11.3									0.0031					11.3
12				0.1290		0.0484	0.3711	0.3994	0.1195	0.2853	0.0353		0.1731	12
13		0.0283		0.1903		0.0516	0.2264	0.1604	0.0252	0.0705			0.1442	13
13.2						0.0032								13.2
14	0.0742	0.0629		0.3387		0.0452	0.0157	0.0535		0.0096			0.0256	14
15	0.3194	0.1541		0.1839		0.1548	0.0063			0.0032			0.0032	15
15.2	0.0032					0.0032								15.2
16	0.3387	0.2642		0.0613		0.1645								16
17	0.1968	0.2013		0.0226		0.1871								17
<18			0.0129											<18
18	0.0645	0.1824				0.1258								18
18.2			0.0129											18.2
19	0.0032	0.0723	0.0581			0.0968								19
20		0.0252	0.0742			0.0484								20
21			0.1129			0.0226								21
21.2			0.0032			0.0032								21.2
22			0.1452			0.0258								22
22.3			0.0032											22.3
23			0.1774											23
24			0.1968											24
24.3					0.0065									24.3
25			0.0968											25
26			0.0323											26
27			0.0516		0.0710									27
28			0.0097		0.2226									28
29			0.0065		0.1742									29
30					0.1774									30
>30			0.0065											>30
30.2					0.0097									30.2
30.3					0.0032									30.3
31					0.0935									31
31.2					0.0484									31.2
32					0.0194									32
32.2					0.0968									32.2
33					0.0032									33
33.2					0.0387									33.2
34					0.0097									34
34.2					0.0032									34.2
35					0.0226									35
Allele	D3S1358	vWA	FGA	D8S1179	D21S11	D18S51	D5S818	D13S317	D7S820	CSF1PO	TPOX	TH01	D16S539	Allele
N	155	159	155	155	155	155	159	159	159	156	156	156	156	

Jamaican Amended Allele Frequencies

Allele	D3S1358	vWA	FGA	D8S1179	D21S11	D18S51	D5S818	D13S317	D7S820	CSF1PO	TPOX	TH01	D16S539	Allele
5												0.0024		5
6									0.0041		0.0673	0.1394		6
7								0.0020	0.0061	0.0481	0.0313	0.3558		7
8							0.0533	0.0205	0.1988	0.0625	0.3822	0.2548	0.0340	8
9				0.0077			0.0102	0.0246	0.1393	0.0313	0.2644	0.1587	0.2087	9
9.3												0.0841		9.3
10				0.0129			0.0553	0.0246	0.3443	0.2716	0.0745	0.0048	0.1092	10
10.1									0.0020					10.1
<11						0.0026								<11
11		0.0041		0.0309		0.0052	0.2049	0.2766	0.1844	0.2332	0.1538		0.3131	11
12	0.0052			0.1160		0.0438	0.3996	0.4549	0.1025	0.2933	0.0264		0.1869	12
13	0.0155	0.0082		0.2139		0.0258	0.2561	0.1434	0.0123	0.0529			0.1383	13
13.2						0.0052								13.2
14	0.0670	0.0738		0.3273		0.0412	0.0143	0.0533	0.0061	0.0072			0.0097	14
14.2						0.0026								14.2
15	0.3376	0.2275		0.2165		0.1572	0.0061							15
15.2	0.0026													15.2
16	0.3067	0.2910		0.0670		0.1907								16
17	0.2113	0.1824		0.0052		0.1830								17
<18			0.0077											<18
18	0.0464	0.1311		0.0026		0.1237								18
18.2			0.0206											18.2
19	0.0077	0.0533	0.0670			0.0954								19
19.2			0.0077											19.2
20		0.0225	0.0464			0.0696								20
21		0.0061	0.0747			0.0284								21
21.2						0.0026								21.2
22			0.1881			0.0155								22
23			0.1959			0.0052								23
24			0.1469			0.0026								24
24.3			0.0026		0.0026									24.3
25			0.1160											25
26			0.0412											26
27			0.0515		0.0644									27
28			0.0155		0.2732									28
29			0.0077		0.1830									29
30					0.1649									30
>30			0.0103											>30
30.2					0.0180									30.2
31					0.0644									31
31.2					0.0490									31.2
32					0.0155									32
32.1					0.0026									32.1
32.2					0.0619									32.2
33					0.0052									33
33.2					0.0309									33.2
34					0.0077									34
34.2					0.0026									34.2
35					0.0412									35
36					0.0103									36
37					0.0026									37
Allele	D3S1358	vWA	FGA	D8S1179	D21S11	D18S51	D5S818	D13S317	D7S820	CSF1PO	TPOX	TH01	D16S539	Allele
N	194	244	194	194	194	194	244	244	244	208	208	208	206	

Trinidadian Amended Allele Frequencies

Allele	D3S1358	vWA	FGA	D8S1179	D21S11	D18S51	D5S818	D13S317	D7S820	CSF1PO	TPOX	TH01	D16S539	Allele
5												0.0061		5
6											0.0976	0.1829		6
7							0.0118		0.0060	0.0671	0.0122	0.3110		7
8				0.0063			0.0235	0.0536	0.2083	0.0549	0.3232	0.2073	0.0610	8
9							0.0294	0.0476	0.1131	0.0244	0.1646	0.2073	0.1646	9
9.3												0.0732		9.3
10				0.0500			0.1529	0.0536	0.3333	0.2744	0.0671	0.0122	0.1280	10
<11						0.0064								<11
11		0.0059		0.0750		0.0256	0.2941	0.2798	0.2202	0.2134	0.2866		0.2866	11
12				0.1563		0.0833	0.3235	0.3214	0.1012	0.2744	0.0488		0.1829	12
13		0.0059		0.2250		0.0962	0.1353	0.1607	0.0179	0.0793			0.1402	13
13.2						0.0064								13.2
13.3													0.0061	13.3
14	0.0563	0.0882		0.2500		0.1090	0.0235	0.0833		0.0122			0.0305	14
15	0.3125	0.1412		0.1813		0.1538								15
16	0.3188	0.2941		0.0563		0.2051	0.0059							16
17	0.2000	0.2647				0.0513								17
18	0.1125	0.1353	0.0125			0.0577								18
19		0.0471	0.0563			0.0962								19
20		0.0176	0.0938			0.0705								20
21			0.1000			0.0385								21
22			0.1688											22
23			0.1625											23
24			0.2063											24
25			0.1063											25
26			0.0438											26
27			0.0188		0.0625									27
28			0.0125		0.2250									28
29			0.0063		0.2000									29
29.2					0.0063									29.2
30					0.1750									30
>30			0.0125											>30
30.2					0.0125									30.2
31					0.0500									31
31.2					0.0813									31.2
32					0.0313									32
32.2					0.0688									32.2
33					0.0063									33
33.2					0.0500									33.2
34					0.0188									34
35					0.0125									35
Allele	D3S1358	vWA	FGA	D8S1179	D21S11	D18S51	D5S818	D13S317	D7S820	CSF1PO	TPOX	TH01	D16S539	Allele
N	80	85	80	80	80	78	85	84	84	82	82	82	82	

Chamorro Amended Allele Frequencies

Allele	D2S1338	D19S433	Allele
12		0.0347	12
12.2		0.0139	12.2
13		0.3542	13
13.2		0.0417	13.2
14		0.2292	14
14.2		0.0972	14.2
15		0.0903	15
15.2		0.0972	15.2
16	0.0278		16
16.2		0.0139	16.2
17	0.1042		17
17.2		0.0278	17.2
18	0.0833		18
19	0.1875		19
20	0.1111		20
21	0.0139		21
22	0.0972		22
23	0.1736		23
24	0.1319		24
25	0.0556		25
26	0.0069		26
27	0.0069		27
Allele	D2S1338	D19S433	Allele
N	72	72	

Filipino Amended Allele Frequencies

Allele	D2S1338	D19S433	Allele
12		0.0286	12
13		0.2857	13
13.2		0.0357	13.2
14		0.1571	14
14.2		0.0500	14.2
15		0.1071	15
15.2		0.2500	15.2
16	0.0286	0.0143	16
16.2		0.0643	16.2
17	0.0786		17
17.2		0.0071	17.2
18	0.0571		18
19	0.2214		19
20	0.0786		20
21	0.0286		21
22	0.0643		22
23	0.1357		23
24	0.2643		24
25	0.0357		25
26	0.0071		26
Allele	D2S1338	D19S433	Allele
N	70	70	

Validation Summary: ID Plus EXCEL PopStats Template v1.2

1. Purpose

In May of 2015, the FBI CODIS unit notified all CODIS laboratories of errors found in the population database data published in the **Journal of Forensic Sciences** ***Population data on the thirteen CODIS core short tandem repeat loci in African Americans, U.S. Caucasians, Hispanics, Bahamians, Jamaicans and Trinidadians*****, Journal of Forensic Sciences 1999 44(6):1277-86** [1,2]. This population data was used to build the allele frequency tables in the FBI's Popstats database which HCIFS subsequently used to build the in-house ID Plus EXCEL Popstats template.

Corrected allele frequencies were provided as well as a pre-publication copy of the erratum notice to be published in Journal of Forensic Science in July 2015[3].

The purpose of this performance check was to update the EXCEL Popstats Template with the corrected allele frequencies as well as to evaluate the impact of the errors with a comparison of corrected and original allele frequencies[3].

2. Materials

ID Plus EXCEL PopStats Template v1.1
ID Plus EXCEL PopStats Template v1.2

3. Procedure

Allele frequencies for the 1999 Caucasian (CAU), African American (AA), and South West Hispanics (SWH) population databases were compared to the amended 2015 data released by the FBI. Changes to Fraction (allele frequency), Minimum Allele Frequency, Allele counts, or Total Samples were documented and a list of every affected allele was compiled and verified by a second analyst. The updated allele frequencies were incorporated into the ID PLUS Excel PopStats Template v1.2. The allele frequency changes were verified by a second analyst.

One hundred alleles were affected by the 2015 FBI corrections (data not shown, see Forensic Genetics\DNA\Compliance Group\Amended FBI STR 2015\DNA\Profiles Tested.xlsm). The difference in the frequencies between the original and corrected values was calculated as well as the difference in p^2 values, homozygous single source values ($p^2 + p(1-p)\theta$, and the 2p portion of a 2pq heterozygote single source.

Five profiles were initially created to test genotype frequencies for profiles containing alleles with the most significant differences. The five profiles included three with a single allele, one with seven loci and one full simulated single source profile including all 10 of the alleles with the largest observable frequency changes. Random match probabilities (RMP) were calculated using both original and corrected allele frequencies.

An expanded group of 15 single source and 15 mixture samples were then tested using both original and corrected allele frequencies. The first sample of these sets was

generated with a genotype at one locus and subsequent samples were created through addition of genotypes to each locus until all 15 loci contained a genotype. RMP was calculated for the single source profiles and Combined Probability of Inclusion (CPI) calculated for the mixtures.

The ID Plus EXCEL PopStats Template v1.1 and v1.2 were further compared using RMP and CPI values for five single source profiles, five two-person and five three-person mixtures previously amplified in Identifiler Plus for another study.

4. Results

Of the 100 alleles with corrected allele frequencies, 48 showed no change in p^2 value, 43 varied between 0.0001 and 0.0008, and 9 varied between 0.0010 and 0.0042. Similar results were obtained using $p^2 + p(1-p)\theta$. The largest variation was seen when calculating 2p (Table 1).

	No difference	0.0001 - 0.0008	0.0010 - 0.0100
p^2	48	43	9
$p^2 + p(1-p)\theta$	47	44	9
2p	1	49	50

Table 1. Differences between 1999 and 2015 genotype frequency data.

The 10 alleles with the largest variation between original and corrected frequencies were selected (Table 2) and used to generate five profiles (Table 3). These profiles were used to test the effect of the amended allele frequencies in addition to testing the template update to version 1.2 using the newly amended frequencies.

Allele	Ethnicity	Δp^2
D16S539-10	SWH	0.0008
CSF1PO-12	CAU	0.0010
D19S433-14	AA	0.0012
D16S539-12	SWH	0.0014
D5S818-12	SWH	0.0014
D13S317-12	CAU	0.0016
D13S317-11	CAU	0.0016
TPOX-8	CAU	0.0029
VWA-16	SWH	0.0035
D5S818-11	SWH	0.0042

Table 2. Alleles with the largest observable frequency change

Profile 1 is a single source profile containing the 10 alleles with the largest frequency differences. Profile 2 simulates a partial single source profile containing a mix of affected and non-affected allele frequencies, with all loci containing heterozygous alleles.

Profile 3 is a homozygous version of profile 2. Profile 4 contains the single allele with the largest difference in allele frequency. Profile 5 is similar to profile 4, with an allele still in the top 10 most affected, but with a less severe difference in frequency value. The largest difference in RMP of 1.2 fold was observed in Profile 4 when only D5, allele 11 was used in the calculation. For Profiles 1, 2, and 3 observed differences ranged from no change to a maximum 1.07 fold difference. No change was seen in Profile 5. Across all profiles, all differences in calculated statistical values were less than 2-fold.

Profile	D8S1179	D21S11	D7S820	CSF1PO	D3S1358	TH01	D13S317	D16S539	D2S1338	D19S433	vWA	TPOX	D18S51	AMEL	D5S818	FGA
1	13	29,30	9,10	10,12	16,18	6,7	11,12	12	19,22	13.2,14	16	8,12	14,16	X	11,12	19,20
2				10,12			11,12	10,12		14,15	16,17	8,9			11,12	
3				12			11	12		14	16	8			12	
4															11	
5				12												

Table 3. Five profiles generated using the 10 most affected allele frequencies.

		Single Source	
Profile	Ethnicity	New	Old
1	Caucasian	1.17E+18	1.09E+18
	African American	1.91E+19	1.88E+19
	South Western Hispanics	5.82E+16	6.06E+16
2	Caucasian	1.87E+06	1.82E+06
	African American	1.63E+07	1.72E+07
	South Western Hispanics	5.01E+06	5.20E+06
3	Caucasian	4.00E+06	4.12E+06
	African American	9.83E+07	9.54E+07
	South Western Hispanics	4.72E+06	4.85E+06
4	Caucasian	6	6
	African American	14	14
	South Western Hispanics	5	6
5	Caucasian	9	9
	African American	11	11
	South Western Hispanics	6	6

Table 4. Calculations of RMP for 5 profiles containing the 10 most affected alleles.

Profile	Ethnicity	Fold Change SS	Change % Difference SS
1	Caucasian	1.07	7%
	African American	1.02	2%
	South Western Hispanics	1.04	-4%
2	Caucasian	1.03	3%
	African American	1.06	-6%
	South Western Hispanics	1.04	-4%
3	Caucasian	1.03	-3%
	African American	1.03	3%
	South Western Hispanics	1.03	-3%
4	Caucasian	1.00	0%
	African American	1.00	0%
	South Western Hispanics	1.20	-17%
5	Caucasian	1.00	0%
	African American	1.00	0%
	South Western Hispanics	1.00	0%

Table 5. Difference in RMP between the v1.1 and 1.2.

For the expanded set of single source samples seen in Table 6, calculations in Table 7 show that the differences between the corrected and old frequencies are minimal. There was less than a 2-fold difference overall in the statistical values calculated using the old and corrected frequencies (Table 8).

Profile	D8S1179	D21S11	D7S820	CSF1PO	D3S1358	TH01	D13S317	D16S539	D2S1338	D19S433	vWA	TPOX	D18S51	AMEL	D5S818	FGA
1				12												
2				12			11,12									
3				12			11,12	12								
4				12			11,12	12		14						
5				12			11,12	12		14	16					
6				12			11,12	12		14	16	8				
7				12			11,12	12		14	16	8			11,12	
8	13			10,12			11,12	12		13.2,14	16	8,12			11,12	
9	13	29,30		10,12			11,12	12		13.2,14	16	8,12			11,12	
10	13	29,30	9,10	10,12			11,12	12		13.2,14	16	8,12			11,12	
11	13	29,30	9,10	10,12	16,18		11,12	12		13.2,14	16	8,12			11,12	
12	13	29,30	9,10	10,12	16,18	6,7	11,12	12		13.2,14	16	8,12			11,12	
13	13	29,30	9,10	10,12	16,18	6,7	11,12	12	19,22	13.2,14	16	8,12			11,12	
14	13	29,30	9,10	10,12	16,18	6,7	11,12	12	19,22	13.2,14	16	8,12	14,16		11,12	
15	13	29,30	9,10	10,12	16,18	6,7	11,12	12	19,22	13.2,14	16	8,12	14,16		11,12	19,20

Table 6: Fifteen single source profiles generated from a single locus to all 15 loci containing genotypes.

	Template v1.1			Template v1.2		
Profile	Caucasian	African American	South Western Hispanics	Caucasian	African American	South Western Hispanics
1	9	11	6	9	11	6
2	47	47	73	47	47	73
3	402	1,302	871	400	1,302	857
4	3,498	32,050	8,305	3,483	33,030	8,174
5	82,870	430,000	63,100	82,500	443,000	60,470
6	277,400	3,115,000	203,200	273,500	3,209,000	194,700
7	955,100	16,770,000	830,200	942,000	17,280,000	792,900
8	241,000,000	2,940,000,000	27,160,000	256,000,000	2,985,000,000	25,940,000
9	2,867,000,000	43,290,000,000	201,300,000	3,046,000,000	43,960,000,000	192,300,000
10	33,370,000,000	425,500,000,000	6,862,000,000	36,020,000,000	432,100,000,000	6,569,000,000
11	443,300,000,000	12,640,000,000,000	154,300,000,000	473,600,000,000	12,840,000,000,000	147,800,000,000
12	5,673,000,000,000	131,000,000,000,000	985,700,000,000	6,068,000,000,000	133,100,000,000,000	943,700,000,000
13	661,900,000,000,000	3,455,000,000,000,000	26,860,000,000,000	707,900,000,000,000	3,508,000,000,000,000	25,720,000,000,000
14	17,810,000,000,000,000	143,100,000,000,000,000	682,200,000,000,000	19,050,000,000,000,000	145,900,000,000,000,000	653,100,000,000,000
15	1,092,000,000,000,000,000	18,770,000,000,000,000,000	60,630,000,000,000,000	1,168,000,000,000,000,000	19,140,000,000,000,000,000	58,210,000,000,000,000

Table 7: RMP Calculations for the fifteen single source profiles using ID Plus PopStats template v1.1 (old frequencies) and v1.2 (corrected frequencies).

	Fold Change			Change % Diffference		
Profile	Caucasian	African American	South Western Hispanics	Caucasian	African American	South Western Hispanics
1	1.00	1.00	1.00	0%	0%	0%
2	1.00	1.00	1.00	0%	0%	0%
3	1.01	1.00	1.02	0%	0%	2%
4	1.00	1.03	1.02	0%	3%	2%
5	1.00	1.03	1.04	0%	3%	4%
6	1.01	1.03	1.04	1%	3%	4%
7	1.01	1.03	1.05	1%	3%	4%
8	1.06	1.02	1.05	6%	2%	4%
9	1.06	1.02	1.05	6%	2%	4%
10	1.08	1.02	1.04	7%	2%	4%
11	1.07	1.02	1.04	6%	2%	4%
12	1.07	1.02	1.04	7%	2%	4%
13	1.07	1.02	1.04	6%	2%	4%
14	1.07	1.02	1.04	7%	2%	4%
15	1.07	1.02	1.04	7%	2%	4%

Table 8: Differences calculated for the single source samples when comparing ID Plus Popstats template v1.1(old frequencies) and v1.2 (corrected frequencies).

For the expanded set of mixture samples seen in Table 9, the differences in the calculations shown in Table 10 using the old and corrected frequencies were minimal. The differences in the calculations using template v1.1 versus 2.2 did not exceed 1.02 fold (Table 10).

Profile	D8S1179	D21S11	D7S820	CSF1PO	D3S1358	TH01	D13S317	D16S539	D2S1338	D19S433	vWA	TPOX	D18S51	AMEL	D5S818	FGA
1				10,11,12,13												
2				10,11,12,13			11,12,13,14									
3				10,11,12,13			11,12,13,14	12,13,14,15								
4				10,11,12,13			11,12,13,14	12,13,14,15		11,12,13,14						
5				10,11,12,13			11,12,13,14	12,13,14,15		11,12,13,14	16,17,18,19					
6				10,11,12,13			11,12,13,14	12,13,14,15		11,12,13,14	16,17,18,19	8,9,10,11,12				
7				10,11,12,13			11,12,13,14	12,13,14,15		11,12,13,14	16,17,18,19	8,9,10,11,12			11,12,13,14	
8	13,14,15,16			10,11,12,13			11,12,13,14	12,13,14,15		11,12,13,14	16,17,18,19	8,9,10,11,12			11,12,13,14	
9	13,14,15,16	28,29,30,31		10,11,12,13			11,12,13,14	12,13,14,15		11,12,13,14	16,17,18,19	8,9,10,11,12			11,12,13,14	
10	13,14,15,16	28,29,30,31	8,9,10,11	10,11,12,13			11,12,13,14	12,13,14,15		11,12,13,14	16,17,18,19	8,9,10,11,12			11,12,13,14	
11	13,14,15,16	28,29,30,31	8,9,10,11	10,11,12,13	15,16,17,18		11,12,13,14	12,13,14,15		11,12,13,14	16,17,18,19	8,9,10,11,12			11,12,13,14	
12	13,14,15,16	28,29,30,31	8,9,10,11	10,11,12,13	15,16,17,18	6,7,8,9	11,12,13,14	12,13,14,15		11,12,13,14	16,17,18,19	8,9,10,11,12			11,12,13,14	
13	13,14,15,16	28,29,30,31	8,9,10,11	10,11,12,13	15,16,17,18	6,7,8,9	11,12,13,14	12,13,14,15	19,20,21,22	11,12,13,14	16,17,18,19	8,9,10,11,12			11,12,13,14	
14	13,14,15,16	28,29,30,31	8,9,10,11	10,11,12,13	15,16,17,18	6,7,8,9	11,12,13,14	12,13,14,15	19,20,21,22	11,12,13,14	16,17,18,19	8,9,10,11,12	14,15,16,17		11,12,13,14	
15	13,14,15,16	28,29,30,31	8,9,10,11	10,11,12,13	15,16,17,18	6,7,8,9	11,12,13,14	12,13,14,15	19,20,21,22	11,12,13,14	16,17,18,19	8,9,10,11,12	14,15,16,17		11,12,13,14	19,20,21,22

Table 9: Fifteen mixture profiles generated from a single locus to all 15 loci containing genotypes.

	Template v1.1			Template v1.2		
Profile	Caucasian	African American	South Western Hispanics	Caucasian	African American	South Western Hispanics
1	1	1	1	1	1	1
2	2	2	3	2	2	3
3	6	13	15	6	13	15
4	11	27	47	11	27	47
5	18	62	65	18	62	65
6	18	77	66	18	77	66
7	21	100	98	21	100	96
8	47	149	191	47	150	188
9	110	323	418	109	324	411
10	168	415	753	168	416	741
11	229	566	918	228	568	903
12	477	724	1,601	473	726	1,575
13	3,826	2,951	6,575	3,794	2,958	6,469
14	11,680	8,548	20,530	11,760	8,639	20,200
15	36,760	37,220	96,070	36,440	37,620	94,470

Table 10: CPI calculations for the fifteen mixture profiles using ID Plus PopStats template v1.1 and v1.2.

	Fold Change			Change % Diffference		
Profile	Caucasian	African American	South Western Hispanics	Caucasian	African American	South Western Hispanics
1	1.00	1.00	1.00	0%	0%	0%
2	1.00	1.00	1.00	0%	0%	0%
3	1.00	1.00	1.00	0%	0%	0%
4	1.00	1.00	1.00	0%	0%	0%
5	1.00	1.00	1.00	0%	0%	0%
6	1.00	1.00	1.00	0%	0%	0%
7	1.00	1.00	1.02	0%	0%	2%
8	1.00	1.01	1.02	0%	1%	2%
9	1.01	1.00	1.02	1%	0%	2%
10	1.00	1.00	1.02	0%	0%	2%
11	1.00	1.00	1.02	0%	0%	2%
12	1.01	1.00	1.02	1%	0%	2%
13	1.01	1.00	1.02	1%	0%	2%
14	1.01	1.01	1.02	1%	1%	2%
15	1.01	1.01	1.02	1%	1%	2%

Table 11: Difference calculated for the mixture profiles when comparing ID Plus Popstats template v1.1 and v1.2.

The single source and two and three person mixtures from a prior study (Table 12) showed results similar to those from the manually generated profiles. The differences in RMP and CPI Table 13 did not exceed 1.16 fold (Table 14).

	Profile	D8S1179	D21S11	D7S820	CSF1PO	D3S1358	TH01	D13S317	D16S539	D2S1338	D19S433	vWA	TPOX	D18S51	AMEL	D5S818	FGA
Single	1	13,15	29,31	9		15,18	7		9	16,21	13,14			12			
	2	14	28		10	14	9.3	12									
	3	8	32.2	10,11	11,12	11,14	7,8	13	10,13	20	13,14	15	8,11		X	11	24
	4	12,14	30	8,10	10,11	14,17	9,9.3	8	12,13	17	13,15.2	17,18	10,11	14,19	X,Y	10,12	21
	5	13,14	27,28	11,12	11,12	16,18	6,7	11	9,13	19,20	14,15	15,17	8,10	14,17	X	12	20,22
2 Person Mixture	1	14,15	28,31	10,11	8,10	14	7,9.3	12	9,11	19,21, 22	13,14	13,17,18	11	16,17	X,Y	11,12,13	23,25, 26
	2	14	30			14,18	6,7,9		13	24			11		X		
	3		30		10	15,17	7,8, 9.3	10,12		17,19	14	17	8,11	17	X	12	22
	4	13,14, 15	28,29, 31	9,10, 11,13	7,8,10, 12	14,15,18	7,9.3	11,12	9,11	16,19, 21,22	13,14	13,15,17, 18	9,11	12,16, 17	X,Y	11,12,13	22,23, 25,26
	5	12,13, 14	30,31.2,3 2.2	8,9	7,11	15,17	6,7,9,9.3	9,12,13	12	17,19,20	13,14,15	17,18,20	8	13,16	X	10,12	18,23,25
3 Person Mixture	1	12,13,14	30		11	14,16,18	6,7,9	11,12	12,13	19,24	12,14, 14.2,15.2		8,11	13,15, 16	X	11	20,24
	2	8,11,14	28,30, 32.2	10,11	8,10,11	11,14,15, 16	7,8, 9.3	8,9,12, 13	9,11,12,1 3	17,19,20, 22,24,25	13,14, 14.2	13,15,18, 19	8,11	16,17	X,Y	11,12, 13	22,23, 24,25, 26
	3	12,13,14	28,30, 31.2	8,9,12	7,11,12	15,16	7,9	12,13	8,10,12	17,19	15	14,17	8,10	15	X	8,12	21,22
	4	10,13,14, 15	28,30,32, 35	10,11, 12	8,10,11	14,15,16, 17	7,8,9,9.3	9,10,11,1 2,13	8,11,13	17,19,20, 22,23	11,13,14, 15.2	15,16,17, 18	8,11	13,16, 17	X	11,12	22,23, 25
	5	13,14,15		12		15	7,8	12		17	13,14, 14.2	17				11	

Table 12: Profiles for 5 single source profiles, 5 two person mixtures, and 5 three person mixtures calculated using ID Plus PopStats template v1.1 and v1.2.

		Template v1.1			Template v1.2		
	Profile	Caucasian	African American	South Western Hispanics	Caucasian	African American	South Western Hispanics
Single	1	198,700,000,000,000	7,095,000,000,000	3,768,000,000,000,000	197,600,000,000,000	7,114,000,000,000	3,783,000,000,000,000
	2	63,700,000	54,720,000	2,188,000,000	67,410,000	54,720,000	2,196,000,000
	3	2,460,000,000,000,000,000,000	8,247,000,000,000,000,000,000	5,144,000,000,000,000,000,000	2,592,000,000,000,000,000,000	8,382,000,000,000,000,000,000	5,777,000,000,000,000,000,000
	4	172,300,000,000,000,000,000	52,980,000,000,000,000,000,000	3,380,000,000,000,000,000,000	174,700,000,000,000,000,000	60,490,000,000,000,000,000,000	3,612,000,000,000,000,000,000
	5	574,000,000,000,000,000	29,810,000,000,000,000,000	45,160,000,000,000,000,000	544,500,000,000,000,000	28,310,000,000,000,000,000	52,050,000,000,000,000,000
2 Person Mixture	1	150,200,000,000,000	1,676,000,000,000	313,700,000,000,000	154,800,000,000,000	1,731,000,000,000	312,800,000,000,000
	2	473,300,000	1,170,000,000	2,489,000,000	466,700,000	1,170,000,000	2,608,000,000
	3	351,000,000,000	1,816,000,000,000	192,700,000,000	355,600,000,000	1,914,000,000,000	196,100,000,000
	4	698,800,000	30,450,000	1,173,000,000	687,400,000	31,310,000	1,191,000,000
	5	33,770,000,000	4,121,000,000,000	64,110,000,000	34,480,000,000	4,167,000,000,000	64,950,000,000
3 Person Mixture	1	2,052,000,000	73,480,000,000	4,170,000,000	2,043,000,000	74,750,000,000	4,181,000,000
	2	26,470,000	8,408,000	14,330,000	26,680,000	8,744,000	14,660,000
	3	3,278,000,000,000	35,560,000,000,000	2,995,000,000,000	3,231,000,000,000	30,830,000,000,000	2,987,000,000,000
	4	861,900	1,218,000	302,600	849,000	1,250,000	304,200
	5	1,036,000,000	2,720,000,000	260,900,000	1,023,000,000	2,750,000,000	225,300,000

Table 13: Calculations for the RMP and CPI using ID Plus PopStats template v1.1 and v1.2.

		Fold Change			Change % Diffference		
	Profile	Caucasian	African American	South Western Hispanics	Caucasian	African American	South Western Hispanics
Single	1	1.01	1.00	1.00	1%	0%	0%
	2	1.06	1.00	1.00	6%	0%	0%
	3	1.05	1.02	1.12	5%	2%	11%
	4	1.01	1.14	1.07	1%	12%	6%
	5	1.05	1.05	1.15	5%	5%	13%
2 Person Mixture	1	1.03	1.03	1.00	3%	3%	0%
	2	1.01	1.00	1.05	1%	0%	5%
	3	1.01	1.05	1.02	1%	5%	2%
	4	1.02	1.03	1.02	2%	3%	2%
	5	1.02	1.01	1.01	2%	1%	1%
3 Person Mixture	1	1.00	1.02	1.00	0%	2%	0%
	2	1.01	1.04	1.02	1%	4%	2%
	3	1.01	1.15	1.00	1%	13%	0%
	4	1.02	1.03	1.01	1%	3%	1%
	5	1.01	1.01	1.16	1%	1%	14%

Table 14: Difference calculated for RMP and CPI when comparing the results from ID Plus Popstats template v1.1 and v1.2.

5. Conclusion

The update to the "ID Plus EXCEL PopStats Template" incorporates the corrected allele frequency data for Caucasian, African American, and South West Hispanic populations. This evaluation shows statistical calculations made using the corrected allele frequencies resulted in much less than the two-fold difference cited in the FBI's Notice of Amendments. This difference is also well within the factor of ten suggested by the National Research Council[4] in 1996 for statistical calculations based on population database allele frequencies. A two-fold difference in statistical value calculations translates to a difference significantly less than one order of magnitude. In this study, the most significantly affected RMP statistical calculation evaluated for a single source sample previously calculated as approximately 1 in 6 individuals was estimated at 1 in 5 individuals when determined using the corrected frequencies (a 1.2 fold difference). The most significantly affected CPI statistical calculation evaluated for a mixed sample previously calculated as approximately 1 in 260,900,000 was, when repeated using the corrected frequencies, determined to be approximately 1 in 225,300,000 (a 1.16 fold difference).

The original electronic genotype data for the FBI Southeast Hispanic population could not be verified[1]. Therefore, calculations will no longer be made using this database. This field will be removed from the ID Plus EXCEL PopStats Template v.1.2.

With the removal of this racial group from the final printed calculation summary page to be included in casefiles, the ID Plus EXCEL PopStats Template v1.2 including the amended FBI database information operated correctly and without any errors during this study.

6. References

[1] B. Budowle, T. R. Moretti. J. S. Buckleton, Notice of Amendment of the FBI's STR Population Data Published in 1999 and 2001. 2015.

[2] BC. Chick, CODIS Bulleting: Update on the Amendment of the 1999 and 2001 FBI Population Data. May 29, 2015.

[3] B. Budowle, T. Moretti, A. L. Baumstark, D. A. Defenbaugh, K. M. Keys, Population Data on the Thirteen Core Short Tandem Repeat Loci in African

Americans, U.S. Caucasians, Hispanics, Bahamians, Jamaicans, and Trinidadians, Journal of Forensic Sciences, 1999 44(6):1277-86.

[4] National Research Council. The evaluation of forensic DNA evidence. Washington, DC: National Academy Press, 1996.

7. Review

The validation of the modification to the ID Plus EXCEL PopStats Template V1.2 is complete. ID Plus EXCEL PopStats Template V1.2 is considered suitable for its intended use.

Completed by: _____ Date: 6.16.2015

Reviewed by: _____ Date: 6-16-2015
Validation Manager

Reviewed by: _____ Date: 6/16/15
QA/Compliance Manager

Reviewed by: _____ Date: 6/16/15
Forensic Genetics Director/Technical Leader

Reviewed by: _____ Date: 6/16/15
Quality Director

Reviewed by: _____ Date: 6-17-15
Crime Laboratory Director

[1]

TECHNICAL NOTE

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Population Data on the Thirteen CODIS Core Short Tandem Repeat Loci in African Americans, U.S. Caucasians, Hispanics, Bahamians, Jamaicans, and Trinidadians*

REFERENCE: Budowle B, Moretti TR, Baumstark AL, Defenbaugh DA, Keys KM. Population data on the thirteen CODIS core short tandem repeat loci in African Americans, U.S. Caucasians, Hispanics, Bahamians, Jamaicans, and Trinidadians. J Forensic Sci 1999;44(6):1277–1286.

[illegible]

KEYWORDS: forensic science, Caucasian, African American, Hispanic, Bahamian, Jamaican, Trinidadian, DNA typing, population genetics, CSF1PO, FGA, TH01, TPOX, VWA, D3S1358, D5S818, D7S820, D13S317, D16S539, D18S51, D21S11, CODIS core loci

The full use of DNA typing technology in forensic science has come to fruition by the development of national DNA databanks, such as the Combined DNA Index System (CODIS) (1). The main objective for a national DNA databank is to assist investigators in the identification of perpetrators of violent crimes. For purposes of applying DNA technology to human identity testing and to make effective use of a national DNA databank, defined polymorphic genetic markers are required, and all laboratories that contribute to the database should use the same genetic loci. Short tandem repeat (STR) loci are the most informative PCR-based genetic markers available to date for attempting to individualize biological material (2–5). The 13 STR loci CSF1PO, FGA, TH01, TPOX, vWA, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, and D21S11 have been selected as the core loci for use in CODIS (1).

This paper presents allele distribution data in African Americans, United States Caucasians, Hispanics, Bahamians, Jamaicans, and Trinidadians for the 13 core STR loci. The data demonstrate that these loci can be useful for providing estimates of the frequency of a DNA profile in forensic identity testing and that a multiple locus profile is extremely rare in all the population groups analyzed.

Materials and Methods

Sample Preparation—Whole blood, obtained in EDTA vacutainer tubes by venipuncture from African American, Caucasian, and southwestern Hispanic individuals, was kindly provided by Dr. A. Eisenberg, University of North Texas Health Science Center, Fort Worth, Texas. Bloodstains from Bahamians, Jamaicans, and Trinidadians were kindly provided by Dr. George Duncan, Broward County Sheriff's Department, Ft. Lauderdale, FL. The DNA was extracted by the phenol-chloroform method (6). The quantity of extracted DNA was estimated using the slot-blot procedure described by Waye, et al. (7) and Budowle, et al. (8).

STR Amplification—The African American, Caucasian, and Hispanic samples were amplified at the loci FGA, VWA, D3S1358, D5S818, D7S820, D8S1179, D13S317, D18S51, and D21S11 using the AmpF/STR™ Profiler Plus kit (PE Biosystems, Foster City, CA) (i.e., Profiler Plus kit) and at the loci CSF1PO, TPOX, TH01, D3S1358, D7S820, and D16S539 using the AmpF/STR COfiler™ kit (PE Biosystems, Foster City, CA) (i.e., COfiler kit). The loci D3S1358 and D7S820 were typed with both kits. The Bahamian, Jamaican, and Trinidadian samples were amplified at the loci CSF1PO, TPOX, TH01, VWA, D5S818, D7S820, D13S317, and D16S539 using the GenePrint™ PowerPlex™ kit (Promega Corporation, Madison, WI) (i.e., PowerPlex

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* This is publication number 99-02 of the Laboratory Division of the Federal Bureau of Investigation. Names of commercial manufacturers are provided for identification only, and inclusion does not imply endorsement by the Federal Bureau of Investigation.

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[2]

CODIS

CODIS Bulletin

Bulletin #: BT050815 Date Distributed: 05/08/15

AMENDMENT OF THE 1999 AND 2001 FBI STR POPULATION DATA

In the evaluation of original population database samples with the new expanded CODIS core loci, the FBI Laboratory has identified some errors in the data published in the Journal of Forensic Sciences *Population data on the thirteen CODIS core short tandem repeat loci in African Americans, U.S. Caucasians, Hispanics, Bahamians, Jamaicans and Trinidadians*, Journal of Forensic Sciences 1999 44(6):1277-86. An erratum notice will be published as a Letter to the Editor in the July 2015 issue of the Journal of Forensic Sciences containing the empirical data demonstrating their nominal effect on profile probabilities. The new amended population data will be available at FBI.gov (http://www.fbi.gov/about-us/lab/biometric-analysis/dna-casework-unit-dcu-1), on the CJIS WAN, and in a future update to Popstats.

Since the development in the late 1990s of the original short tandem repeat (STR) typing systems that included the 13 CODIS core loci, new amplification kits that expand the number of loci to 24 in a multiplex reaction are now commercially available. To establish allele distributions for the additional loci, population samples that were originally genotyped using AmpFLSTR Profiler Plus, COfiler, Identifiler (Thermo Fisher Scientific, South San Francisco, CA) and/or GenePrint PowerPlex (Promega Corp., Madison, WI) were retyped using GlobalFiler (Thermo Fisher Scientific) and PowerPlex Fusion (Promega Corp.). For any sample where a given locus is typed with different amplification kits, concordant genotypes should be obtained irrespective of the kit(s) used, with the exception of genotype differences due to rare primer binding site variants and improvements in allelic ladders that expand allele identification capabilities (e.g., an allele may be designated as <11 in one system and as 9 in another).

The FBI Laboratory has identified errors in the data published in the Journal of Forensic Sciences and has submitted erratum notice regarding "Population data on the thirteen CODIS core short tandem repeat loci in African Americans, U.S. Caucasians, Hispanics, Bahamians,

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[3]

Notice of Amendment of the FBI's STR Population Data Published in 1999 and 2001

Recently, new amplification kits that expand the number of loci in a multiplex reaction have become commercially available. To establish allele distributions for the additional loci, the FBI Laboratory retyped population samples that were originally genotyped using AmpFISTR Profiler Plus, COfiler, Identifiler (Thermo Fisher Scientific, South San Francisco, CA) and/or GenePrint PowerPlex (Promega Corp., Madison, WI) (1,2) using GlobalFiler (Thermo Fisher Scientific) and PowerPlex Fusion (Promega Corp.) (3). During a comparison of over 1100 DNA profiles from African Americans, Caucasians, Southwest Hispanics, Bahamians, Jamaicans, Trinidadians, Filipinos and Chamorros in the original (4,5) and new studies (3), genotyping discrepancies were discovered. Electronic genotype data corresponding to the published allele frequencies are not available for the Southeast Hispanic, Apache, Navaho and Minnesota Native American populations (6), as well as Filipino and Chamorro populations (except for D2S1338 and D19S433) (7). Genotypes from these populations thus could not be assessed for concordance.

The discrepancies discovered were attributable to (a) human error, typically due to manual data editing and recording, and (b) technological limitations (e.g., insufficient resolution for distinguishing microvariants using polyacrylamide gel electrophoresis). The published genotype data (3,4) from which allele frequencies were calculated also include sample or data processing errors (e.g., genotype duplications).

Allele frequencies cited across these publications (1,2) have been used by the FBI and many forensic laboratories for calculating match statistics in criminal investigations and other types of human identification applications since 1999. Given that statistical estimates based on these data have been included in thousands of laboratory reports and testimonies, the FBI Laboratory believes the discrepancies require acknowledgement. The FBI Laboratory has submitted the attached erratum notice, which is scheduled to appear in the July issue of the Journal of Forensic Science. This article describes these errors and their effect on profile probability calculations. Empirical testing described in this publication supports that any discrepancy between profile probabilities calculated using the original and corrected data is expected to be less than a factor of two in a full profile. The FBI Laboratory is additionally providing herein the amended allele frequency tables for use by anyone interested in performing comparisons between the multi-locus profile probabilities calculated using the previously published data and the amended allele frequencies.

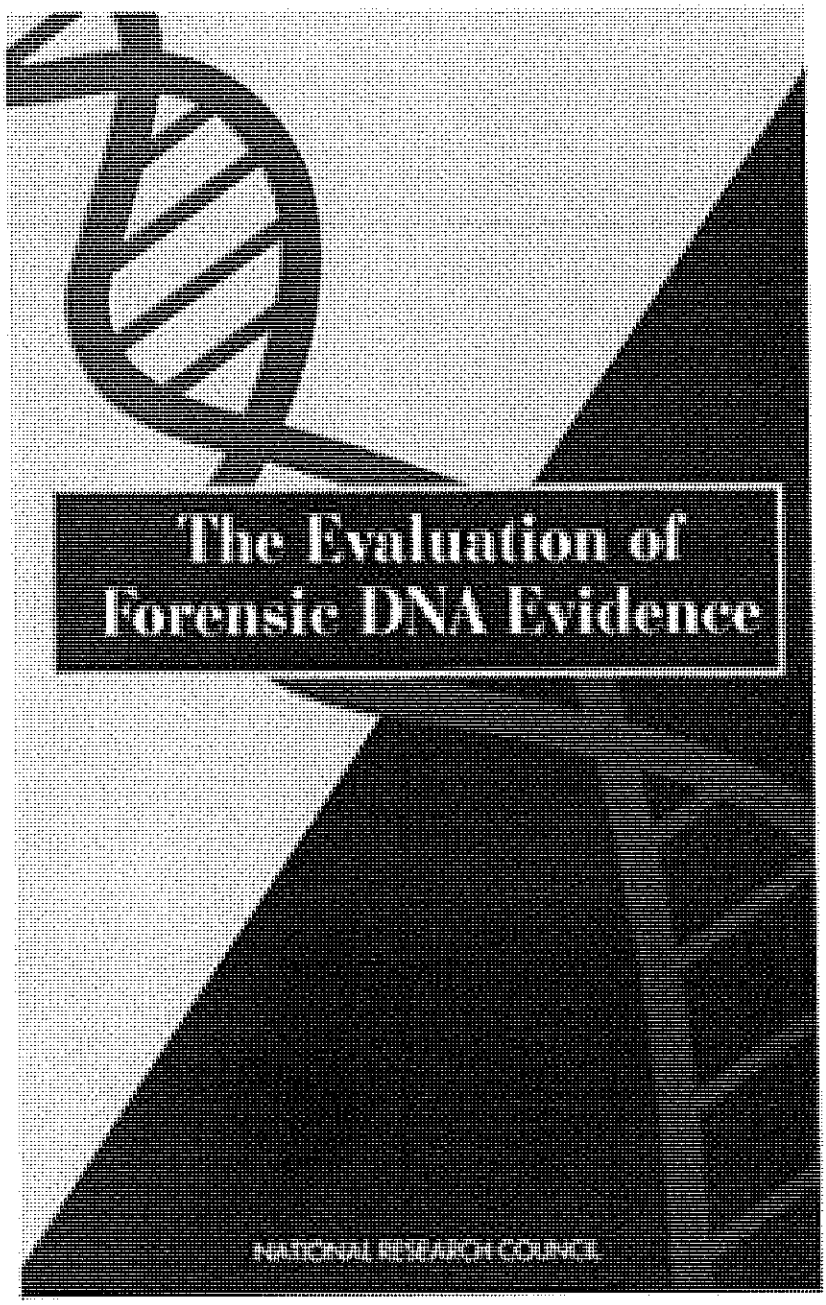
If you have any questions, please contact Anthony J. Onorato of the FBI's DNA Support Unit at Anthony.Onorato@ic.fbi.gov or 703-632-7572.

African American Amended Allele Frequency Table

Caucasian Amended Allele Frequency Table

Southwestern Hispanic Amended Allele Frequency Table

[4]


The Evaluation of
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