



*When Checks and Balances Fail:
Lessons Learned from the Austin Police Department
Crime Laboratory's DNA Section*

NIST Error Management Symposium

July 25, 2017

TEXAS FORENSIC SCIENCE COMMISSION: THE ESSENTIALS

- 9 Commissioners appointed by Governor – 7 scientists & 2 lawyers & 3 full-time staff.
- We have three main responsibilities:
 1. conduct investigations;
 2. manage crime lab accreditation program;
 3. develop analyst licensing program (2019)

TEXAS FORENSIC SCIENCE COMMISSION: THE ESSENTIALS

- Why have a Forensic Science Commission?
- History – HPD lab breakdown & Coverdell
- Isn't accreditation enough?
- The benefits of state-based (local) oversight are many.

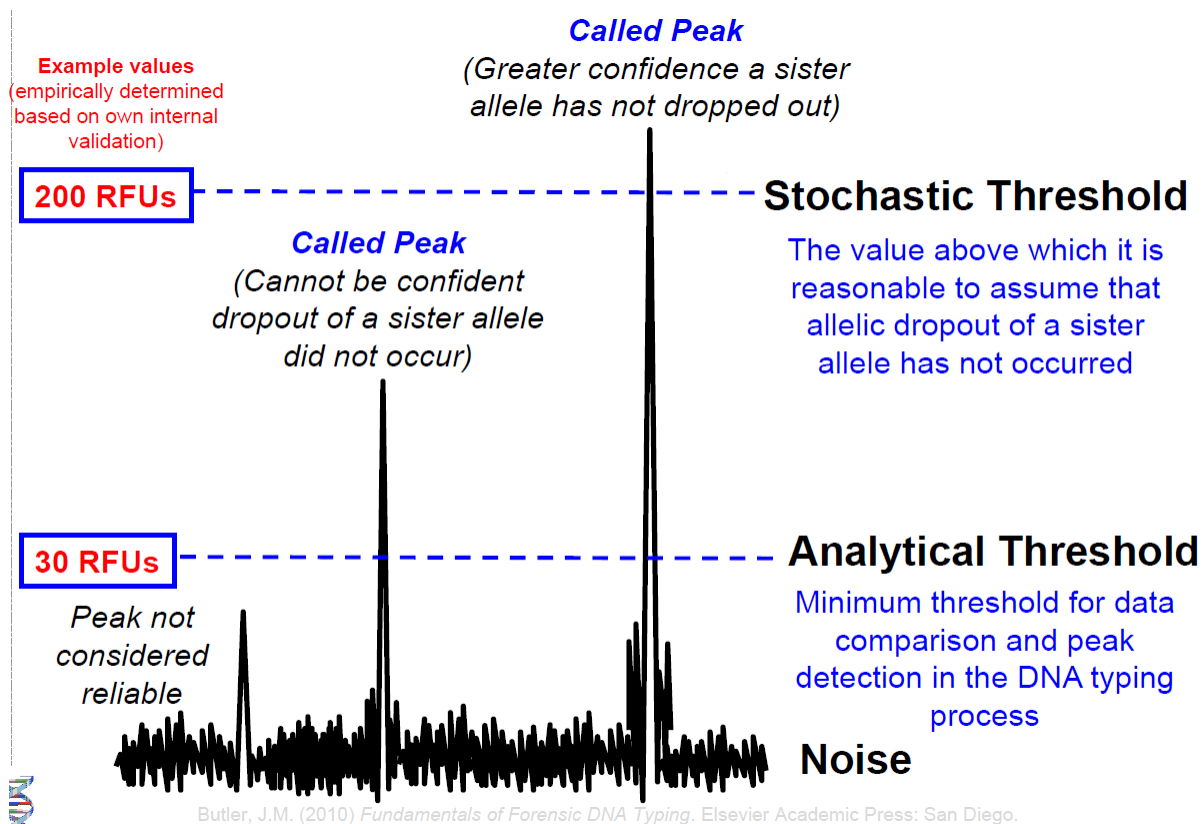
STORY BEGINS AT DNA MIXTURE ANALYSIS



THE INTERPRETIVE CHALLENGE

- Mixtures have become more complex as technology advances and more touch DNA is submitted for analysis. For single source samples & those for which a major component can be teased out, RMP can be used.
- BUT, when you have a mixture and no clear major contributor, the statistic used in the United States was almost always the Combined Probability of Inclusion (CPI).
- There was tremendous misunderstanding (and still is) about how to properly interpret complex mixtures. *See e.g.*, two studies by NIST – MIX05 and MIX13 (71 out of 101 labs used CPI or CPE for complex mixture interpretation).
- CPI has been particularly problematic – a main principle of is that it should not be used for loci where allele dropout is possible. The labs did not always understand how to identify and address the possibility of allele dropout.
- In 2010, SWGDAM issued guidelines to help labs flag dropout. But not all labs adopted guidelines in a timely manner, and not all labs understood the guidelines.

THRESHOLDS USED BY LABS TO EVALUATE DATA



D.N.A. BOX 13.1

URBAN LEGENDS OF CPI

Urban legends are funny (or sometimes horrifying) stories that spread quickly, often via email. While they are seldom based in reality, urban legends often reflect the paranoia of the population that perpetuates them. In recent years a number of misconceptions have arisen within the forensic DNA community surrounding the purpose and practice of the combined probability of inclusion (CPI) statistic in DNA mixture analysis.

In trying to describe problems with the application of CPI to complex mixtures, I have come up with several urban legends that can be associated with this approach to DNA mixture analysis.

1. The number of contributors to a mixture does not matter.
2. It is okay to report “conservative” numbers like 1 in 10.
3. CPI provides a true and relevant statement to aid investigators and the court.
4. CPI is easy to understand for non-DNA users of information.

5. It is okay to apply CPI stats without worrying about relative peak heights for alleles.
6. If all peaks at a locus are above the established stochastic threshold, then the locus is safe to use.
7. It is okay to apply CPI without thinking about the mixture because you assume nothing.
8. Suspect-driven CPI (where the comparison of each suspect results in a different statistical result) is fine.
9. CPI works fine even if potential relatives are in the mixture.
10. It is okay to just consider the presence of potential donor alleles.

Brief explanations of each are provided in the chapter.

Source: Author's presentation at the DNA Technical Leaders Summit held in Norman, Oklahoma on November 20, 2013. For more on the concept of urban legends, see http://en.wikipedia.org/wiki/Urban_Legend and <http://www.snopes.com/>.

HOW DID TEXAS LEARN WE HAD AN ISSUE WITH CPI? UNINTENDED CONSEQUENCES OF MAY 2015 FBI NOTICE

- FBI population data was generated in the 1990s.
- Used as the basis for statistical calculations by most labs.
- Minor errors occurred during typing in 51 of ~30K alleles typed. Errors were human and technology limitations.
- FBI and state partners addressed potential impact with population studies. State partners (like DPS) offered to recalculate in an abundance of caution and upon request.
- The statistical impact of FBI errors should have been insignificant no matter how you look at it.

TEXAS DEPARTMENT OF PUBLIC SAFETY

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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.

A handwritten signature in blue ink that reads 'Brady W Mills'.

Brady W Mills
Deputy Assistant Director
Law Enforcement Support

THE ASK....

- Some prosecutors asked for recalculations in their pending cases, in an abundance of caution.
- AND Results were not what they expected.
- Examples include significant changes in some CPI statistics, like from 1 in 1.4 billion to 1 in 36 or 1 in 4,000 to inconclusive.
- Prosecutors wanted to know what happened??
- **The labs answered by explaining their mixture protocols had changed.**
- The response went something like....

REACTION OF MOST PROSECUTORS



One response could have been....



SOME ARGUMENTS



- SWGDAM issues guidelines, not rules.
- Science changes; SWGDAM not retroactive.
- There is a lot of confusion in the community; the literature is not clear.
- We can't look at old cases because it is just not possible to validate an ST on an old kit/instrument.
- We followed our protocol.
- We were audited/assessed "x" times.
- Probabilistic Genotyping will fix it.

THE TEXAS APPROACH: ADDRESSING POTENTIALLY AFFECTED CASES COLLABORATIVELY



- Commission (with Dr. Budowle and other expert help) has worked with labs to ensure observation of key principle of CPI and revise protocols to be as robust as possible.
- Dr. Budowle reviewed protocols and case examples. Further work only necessary in one lab (APD DNA Lab).
- Also created statewide triage system to identify cases that may be impacted. Steps: (1) Labs generate mixture lists. (2) Prosecutors determine which cases had convictions and send notice. (3) Defense team receives inmate requests. Cases screened for materiality. (4) Team asks lab for recalculations where necessary. (5) Lawyers appointed to file writs or Chapter 64 motions but only where the statistical analysis changed significantly and the DNA may have been material.

**Labs generate DNA mixture lists.
Commission combines into master list.**

COLOR KEY:
LAB ACTION
PROSECUTOR ACTION
DEFENDANT/DEFENSE COUNSEL ACTION
FORENSIC SCIENCE COMMISSION ACTION
COURT

PRIORITIES:
1. CAPITAL
2. CURRENTLY INCARCERATED
3. PAROLE/SUPERVISION
4. OUT OF SYSTEM



*In some cases, counsel may be appointed to fulfill function of defense triage team

2014 CPI RESULTS IN SMITH COUNTY ROBBERY CASE

Client **cannot be excluded** as a contributor to the DNA from the shirt pockets:

The probability of the DNA profile, which matches both the DNA from the pockets and our client's DNA, appearing at random is:

POCKET ONE: 1 in 87,950

POCKET TWO: 1 in 22,480

POCKET THREE: 1 in 2.67 MILLION

RECALCULATION IN 2016

STRMix results of same evidence,
conducted 18 months later:

POCKET 1: client **excluded** as a
contributor

POCKET 2: client is **excluded** as a
contributor

POCKET 3: 1 in 5.27 trillion

What We Hoped For When We Reviewed the Lab Protocols....



What We Saw....



APD Chief Brian Manley



Observations/Findings

Issues of Mixture Interpretation

- The interpretation of DNA forensic evidence is an important part of the analytical process, which often is not sufficiently defined
- Mixtures, at times, can be complex and thus present some challenges for interpreting the profile(s)
- There is variation regarding interpretation across the community
- Variation in interpretation is somewhat acceptable
- But the mere fact that variation exists does not obviate responsibility of applying an approach correctly within in the bounds of the approach established by the lab
- Misunderstandings persist and in some cases good information is being ignored

Issues of Mixture Interpretation

- ANY method implemented - the process must adhere to good scientific practices
- Important that analysts appreciate the different approaches even if they select one for operation
- CPI (Combined Probability of Inclusion) has been the method of choice
- Interpretation requires similar logic (initially) as does probabilistic genotyping
- Requires an ability to deconvolve mixtures
- Requires education and training

APD Situation

- Initial alert that something was amiss:
 - Stochastic threshold not based on signal output
 - Stochastic threshold based on input DNA solely
 - Misunderstood stochastic effects and allele drop out
 - Did not allow for proper interpretation of mixtures
 - A mixture of 0.3 ng total DNA will have contributors each with less than 0.3 ng

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.

APD Mixture Interpretation

- Actually translates to no stochastic threshold
- Inadequately addressing allele drop out
- Estimation of number of contributors
- Additive effects – allele peaks and stutter positions
- Two (or more) CPI calculations
 - Bias in interpretation
- Not understanding bias
 - Determined allele drop out by looking at known reference profiles
 - See – inc for stats; multiple CPIs for same mixture profile

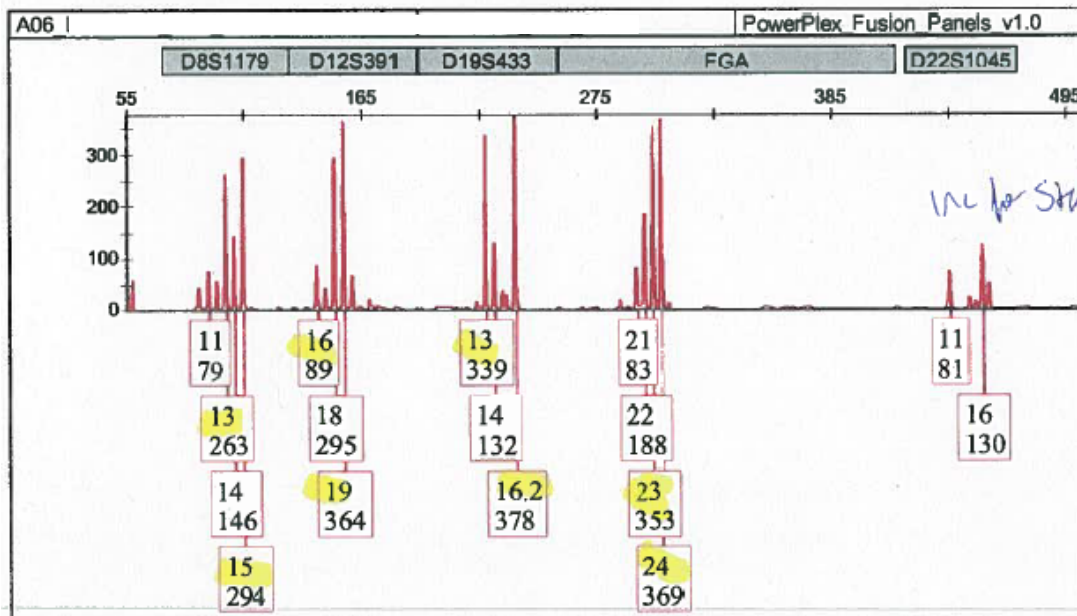
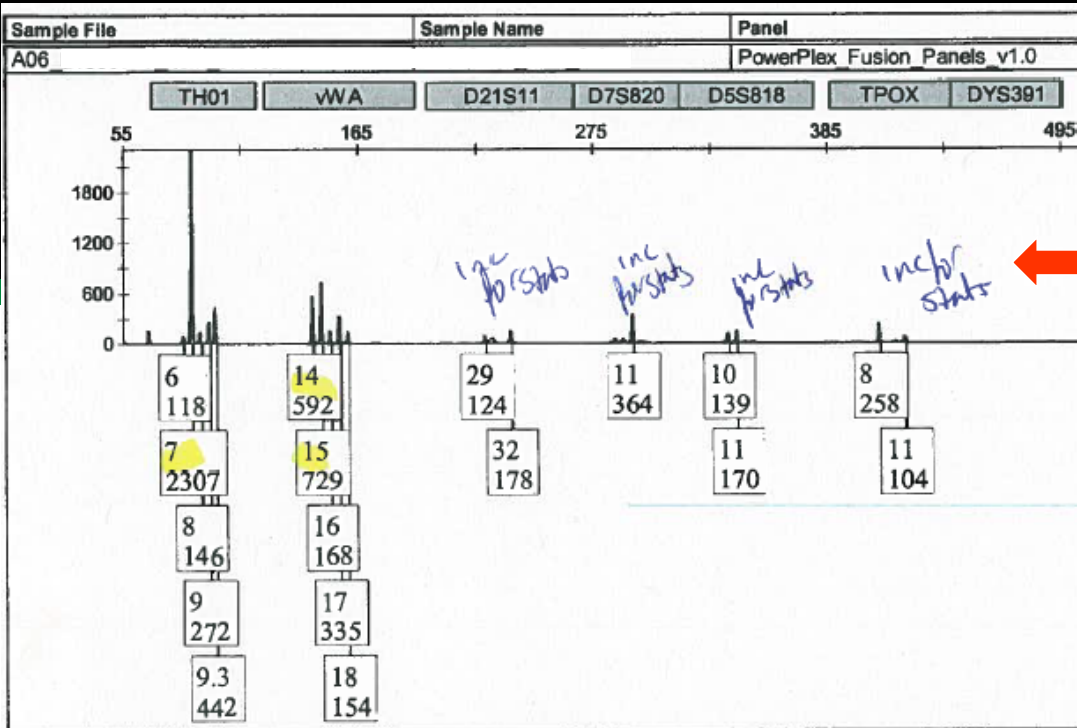
DNA Quant-Based ST

- APD is the only known lab to use this approach
- Not clear how this approach was selected and developed
 - May be in part based on misinterpretation of language in Butler text book
- Limited validation of this approach
- Appears to have been applied in an *ad hoc* manner
 - Inconsistent
 - Also led to bias in interpretation

The DNA profile from the swabbing from the inside of the beanie () is consistent with a mixture of at least three individuals. () cannot be excluded as a contributor to this profile. Statistics were calculated at the following loci: D3S1358, D1S1656, D2S441, D10S1248, D13S317, D16S539, D18S51, D2S1338, CSF1PO, TH01, vWA, D8S1179, D123391, D19S433, and FGA. At these loci, the probability of selecting an unrelated person at random who could be a contributor to this DNA profile is approximately 1 in 4.005 million for Caucasians, 1 in 342.7 thousand for African Americans, and 1 in 3.534 million for Hispanics. Comparisons to () are inconclusive due to insufficient data being present to draw a conclusion for this comparison.

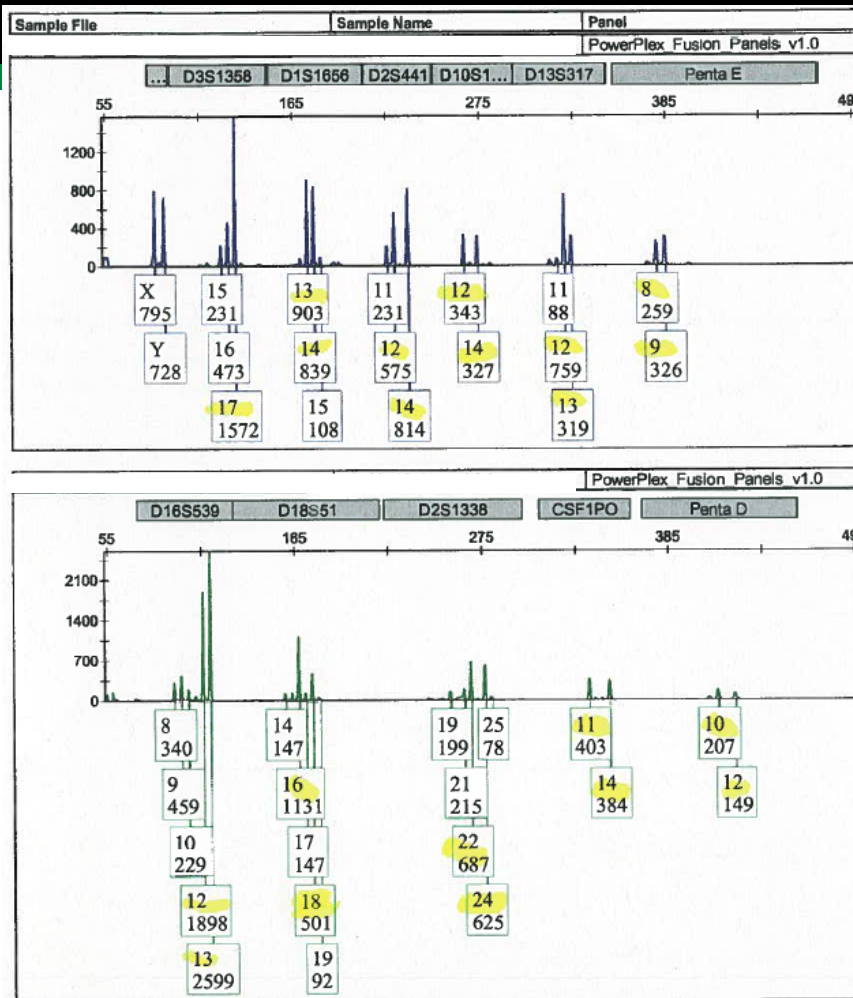
The DNA profile from the swabbing from the inside of the face mask () is consistent with a mixture of at least three individuals. () cannot be excluded as a contributor to this profile. The probability of selecting an unrelated person at random who could be a contributor to this DNA profile is approximately 1 in 3.313 quadrillion for Caucasians, 1 in 37.68 trillion for African Americans, and 1 in 889.7 trillion for Hispanics. () can be excluded as a contributor to this profile.

- At first glance – seems okay
 - Note – at least three individuals
 - Note – no mention of a major contributor



- Note - inc for stats
- Selected only loci matching POI
- No indication of a priori selection of loci with potential ADO
- Note – at least three contributors and peak heights
 - See next example

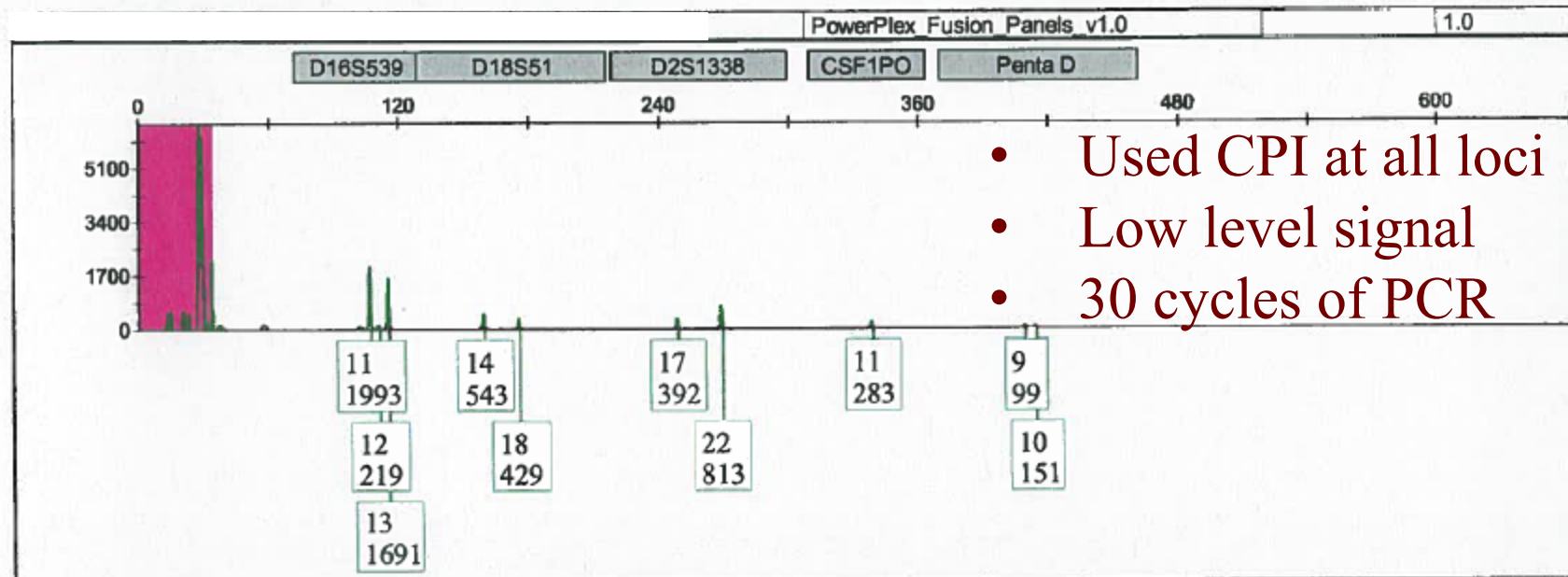
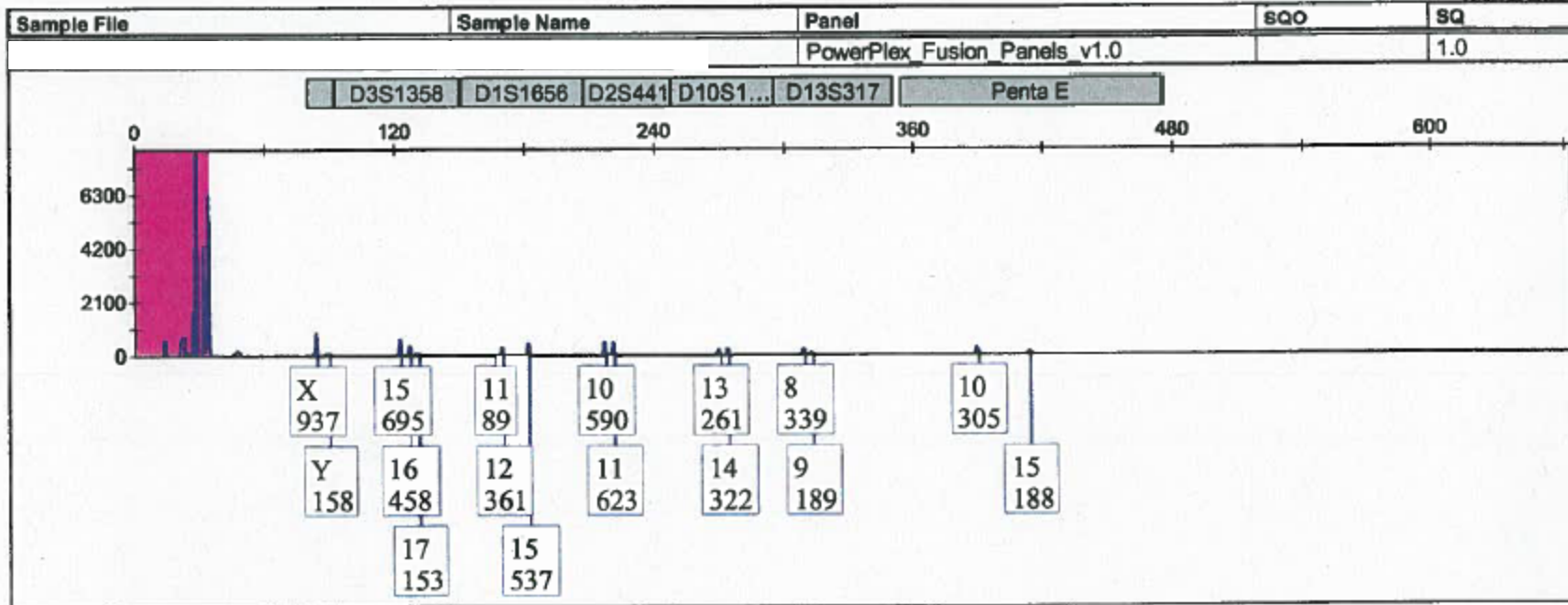
The results of the testing on the swabbing from the inside waist band area of the swim trunks) are inconclusive due to the number of contributors and the potential for allelic dropout in the profile.



- At least three contributors
- Peak heights comparable or higher
- Highlighted peaks match victim
- No documentation on reasoning/assumptions

mixture - at least 3

The DNA profile from the swab of exterior and interior top areas of black purse [REDACTED] is consistent with a mixture of at least two individuals. [REDACTED] cannot be excluded as a contributor to this profile. Statistics were calculated at the following loci: D3S1358, D1S1656, D2S441, D10S1248, D13S317, Penta E, D16S539, D18S51, D2S1338, Penta D, TH01, vWA, D21S11, D7S820, D5S818, TPOX, D8S1179, D123391, D19S433, FGA, and D22S1045. At these loci, the probability of selecting an unrelated person at random who could be a contributor to this DNA profile is approximately 1 in 31.92 quadrillion for Caucasians, 1 in 243.0 quintillion for African Americans, and 1 in 68.45 quadrillion for Hispanics. [REDACTED] can be excluded as a contributor to this profile.



- Used CPI at all loci
- Low level signal
- 30 cycles of PCR

Combined Probability of Inclusion (CPI)

Conveys:

The proportion of the population expected to **be included** as possible contributor to the mixture.

or

The proportion of the population expected to **not be excluded** as possible contributor to the mixture.

Threshold Values

- Two thresholds
 - Analytical (Detection)
 - Stochastic (Interpretation)
- Critical for proper mixture interpretation with STR data with CPI approach
- Only interpret loci where all peaks >200 RFU (for example)
- Concept is that a peak(s) below 200 RFU could have had a partner **allele drop out**
 - Assumes that the loci used exhibit no allele drop out
 - Or at least highly unlikely
 - Must be determined before looking at reference profiles

General Method Philosophy

- Assumes that the loci used exhibit no allele drop out
 - or at least highly unlikely
- Actually means “there are no missing data”
 - What you see is what you get!
- If there are missing data (or highly likely), cannot use the CPI

Validation Issues

- DNA interpretation guidelines and SOPs not consistent with validation data
 - Quantification-based stochastic study
- Insufficient testing of quant-based ST
 - 4 samples – nominal tests
- Dilution series of Fusion 30 cycle validation
 - Peak heights were inconsistent relatively with amounts
- Lack of understanding of significant digits
 - Often recording DNA concentrations up to 7 significant digits
- Lack of understanding of limitations of instrumentation
 - Pipetting with a 0.5-2 uL pipette down to 0.005 uL

Other Critical Findings

- Contamination
 - Penile swab and vaginal epithelial fraction side-by-side
 - Analyst claimed negative controls and RB were negative
 - Later on said – can address it in court
 - Lack of understanding on how contamination can occur
- Acid Phosphatase test
 - Lack of use of freshly made reagent
 - No validation
- Lack of documentation

Other Practices

- Case examples of situations where subtraction should have been considered and not used
 - Reasonable expectation of a known contributor to mixture
 - Especially cases with additional information – such as sexual assault
 - Ex: **differential extraction**, victim's profile complete in e-fraction and CPI used in s-fraction, resulted in unnecessary loss of information and in some cases erroneous statistical conclusions

Inexcusable Excuses

- Until SWGDAM tells us otherwise...
 - Even if it leads to incorrect use of data
- Auditors approved us
 - What does accreditation and auditing mean?
- “Our SOP does not allow us to perform further analyses”
 - Even if not best use of data
 - Can perform deviation!
 - Part of acceptable QA practices

Quality Assurance Standards

- Illusion
 - Something that deceives by producing false or misleading impression of reality
- Accreditation
 - The act of accrediting or the state of being accredited, especially the granting of approval to an institution of learning by an official review board after the school has met specific requirements

Quality Assurance Standards

- Accreditation and Audits do not convey that valid mixture interpretations protocols are in place
- Mixture interpretation protocols often are scant
- Thus even with review details of process are not obvious without thorough review of actual practices
- Variation may and will occur
- A review process is necessary and invaluable
- Many labs were not performing CPI correctly

Example of Misunderstanding



Croat Med J. 2009 Jun; 50(3): 250–267.

PMCID: PMC2702740

doi: [10.3325/cmj.2009.50.250](https://doi.org/10.3325/cmj.2009.50.250)

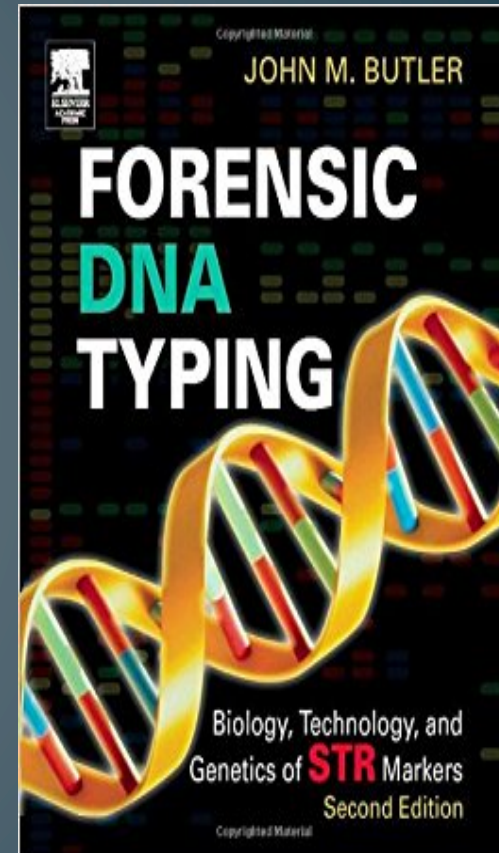
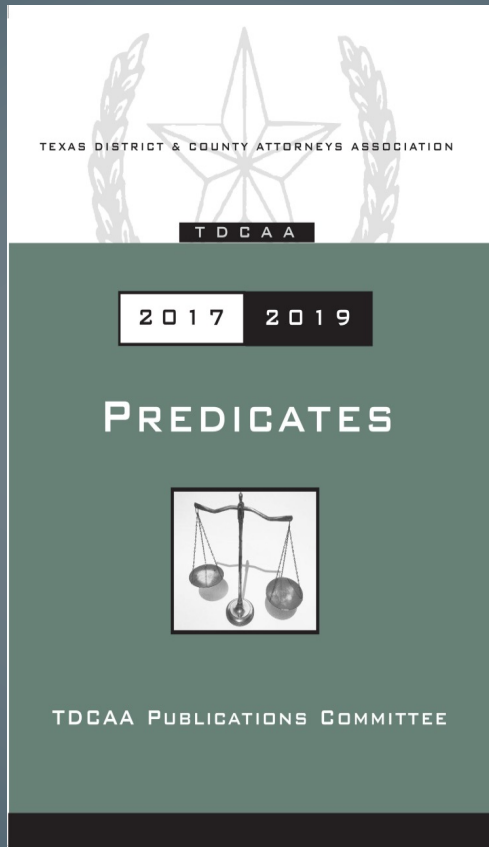
Validation of Testing and Interpretation Protocols for Low Template DNA Samples Using AmpF ℓ STR[®] Identifier[®]

[Theresa Caragine](#),¹ [Rebecca Mikulasovich](#),¹ [Jeannie Tamariz](#),¹ [Ewelina Bajda](#),¹ [James Sebestyen](#),¹ [Howard Baum](#),² and [Mechthild Prinz](#)¹

Following review of our validation studies for LT-DNA testing, our quality control, testing, and interpretation protocols were approved by the DNA Subcommittee of the New York State Forensic Science Commission, and subsequently, by the entire commission, in December 2005. In addition, these studies have since been reviewed and approved by Federal Bureau of Investigation-trained DNA auditors during routine external audits. The validation studies demonstrate that by employing these protocols, LT-DNA testing is reliable and robust.

- NY DNA Subcommittee!
- Certainly the members read the publication!

Range of Understanding



5 Q. Okay. And does the laboratory of which you are
6 the assistant director have any accreditation?

7 A. Yes, we are accredited by ASCLD Lab.

8 Q. Now, when we hear something like accredited,
9 that sounds good, but what does that actually mean as far
10 as the protocols that y'all have to follow in order to
11 maintain that certification?

12 A. Well, to be accredited, you're actually
13 inspected by the accrediting agency, and they review your
14 procedures to make sure that the procedures that you're
15 following are scientifically valid, as well as accepted in
16 the forensic community. They will come in and check out
17 all of your operations, and then they routinely check --
18 the accreditation cycle is actually a five-year cycle, but
19 they do routinely check every year, or two years to make
20 sure that you're following their guidelines and practices.

Understanding of Limitations

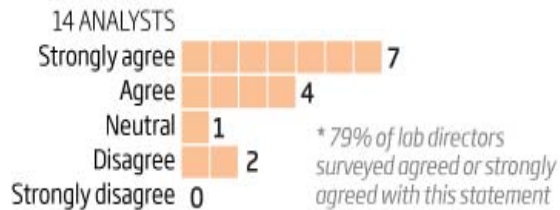
Too much faith, not enough understanding

©NewScientist

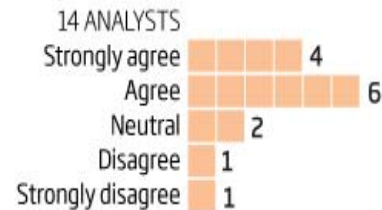
We surveyed the opinions of DNA forensic analysts from the US, UK, Australia, New Zealand, Canada and India on the state of the field. Here are some highlights. Go to [newscientist.com/article/dn19317](https://www.newscientist.com/article/dn19317) for more results

QUESTIONS

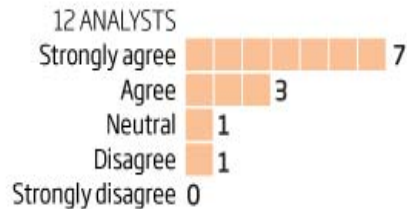
Q: *Statistical analysis is essential to understand the significance of DNA evidence, so every qualitative DNA conclusion should include a quantitative component (statistic)**



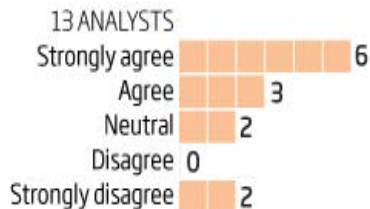
Q: *Bias in the interpretation of DNA profiles may sometimes lead to incorrect matches between a suspect and evidence sample*



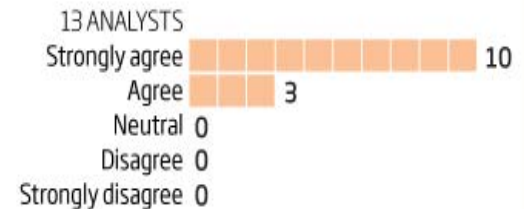
Q: *The police have too much faith in DNA profiling and don't understand its limitations*



Q: *The courts have too much faith in DNA profiling and don't understand its limitations*



Q: *Lab staff need more training on how to deal with complex profiles such as mixtures and very small samples of DNA*

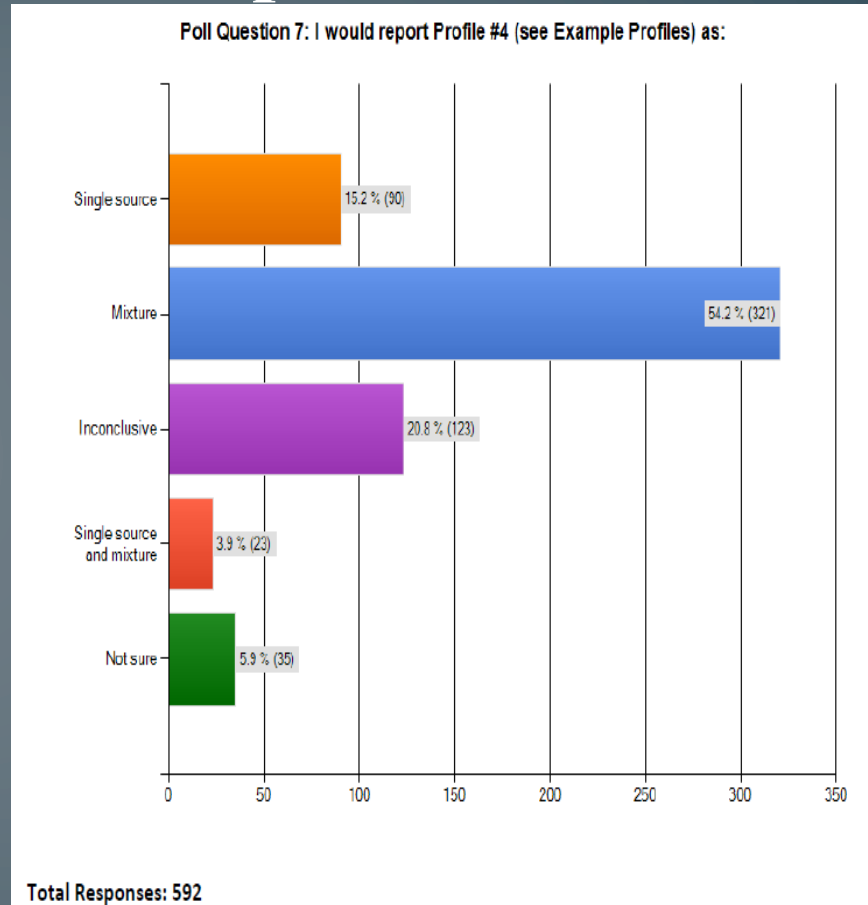


Subjectivity and Variability

- “If you show 10 colleagues a mixture, you will probably end up with 10 different answers.” – Dr. Peter Gill
- 2010 Scientific Working Group on DNA Analysis Methods (SWGDM) Interpretation Guidelines for Autosomal STR Typing by Forensic DNA Testing Laboratories
 - Rules vs Guideline (Policy vs Practice)

NIST DNA Mixture Workshop

April 12, 2013



During the NIST DNA Mixture Interpretation Workshop and Webcast on April 12, 2013, a total of 20 poll questions were made available for workshop attendees and webcast viewers to answer. The responses were used as discussion points by the presenters during the event. Responses were received from individual viewers using computers or web browsers on smart phones. These anonymous responses were only presented in aggregate form as seen in this document. We are unable to determine the identity, occupation, or associated agency of the individuals that answered these poll questions.

The MMA Case

- Active Aggravated Assault Jury Trial
- DNA Analyst is Next Witness

The MMA Case

- Was SOP followed?
- Unsatisfactory explanation given
- Analyst not called to testify
- Conflicting explanation given next day
- *Brady* letter on analyst written

New Language

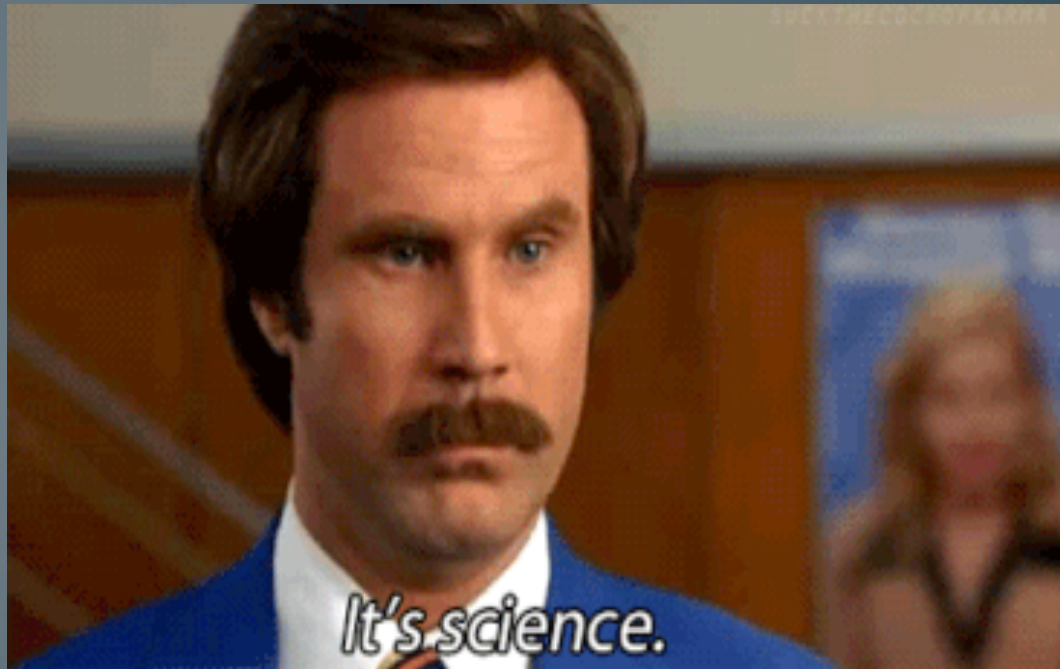
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NOTE: X indicates that this combination of criteria does not meet the minimum criteria for stochastic amplification and the special guidelines for stochastic amplification are not applicable. Interpret according to the standard interpretation guidelines.

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.



Shared Responsibility

- CSI effect
- Dumbing down (caution)
- DNA is ubiquitous in criminal investigations
- *Understand the importance of why things are done the way they are done, the scientific method, the viewpoint of the critiques, the issues of bias and the importance of ethics. – Greg Matheson*

PROJECT PLAN

Project Task #1: CAPDS/TCDA Legal Materiality Review

Conduct legal materiality reviews of past convictions to identify cases potentially impacted by issues with the APD DNA lab. Provide quality representation to those entitled to post-conviction legal relief of the criminal case.

Project Task #2: UNT Retroactive Case Review

The City of Austin will contract with University of North Texas Health Science Center (UNT) to conduct retroactive case reviews inside the DNA lab on cases forwarded by the TCDA and CAPDS. These reviews will assess possible carryover or other contamination, review potential stochastic issues, re-interpret all mixtures, identify possible missed stains within case due to AP-negative results that may have been attributable to laboratory practice regarding mixing of AP reagent, and coordinate with the DPS Capital Area Lab to assess proper storage of samples.

PROJECT PLAN

Project Task #3: Quattrone Look Forward/Look Back

The City of Austin will contract with the University of Pennsylvania Law School's Quattrone Center for the Fair Administration of Justice (Quattrone) to conduct a root cause analysis of the issues documented in the Texas Forensic Science Commission's audit report and the freezer outage, and make recommendations to the City and the County regarding the options for organization, staffing, training and leadership structure for a DNA laboratory in Austin.

Project Task #4: DPS/APD Interim Solution

DPS Capital Area Lab currently operates and has assumed management responsibility for the former APD DNA lab facility under an ILA with the City of Austin.

The City of Austin has contracted with various private DNA laboratories to conduct DNA testing of backlogged cases.