From Training to Trial: A Reflection on Nearly Three Years of Probabilistic Genotyping

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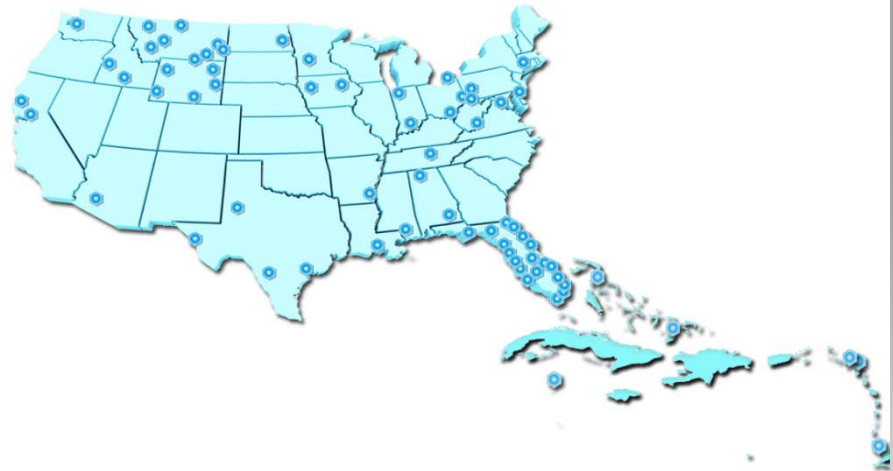
DNA Labs International
January 31, 2018

DNA LABS INTERNATIONAL

ABOUT US

*ISO/IEC 17025 Accredited

*Founded in 2004

*We are one of the leading private forensic DNA laboratories across the US & Caribbean, working with hundreds of law enforcement agencies.

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FBI Quality Assurance Standards

Standard 17 – Outsourcing
- Technology – Autosomal STR
- Platform – Capillary Electrophoresis
- Test Kit

- Identifiler
- Identifiler Plus
- PowerPlex 16 HS
- MiniFiler

- GlobalFiler
- PowerPlex Fusion 5C
- PowerPlex Fusion 6C

Why the need for change?

- The nature of gun crime results in frequent exchange of weapons between individuals.

  ❖Original Owner
  ❖Seller
  ❖Buyer
  ❖Friends
  ❖Significant Others
  ❖Police Officers
  ❖Crime Scene Technicians

- Multiple individuals handling weapons causes mixed DNA profiles
- The majority of DNA profiles obtained from weapons are inconclusive mixtures of 2 or more people
- Gun swabs are primarily 'touch' DNA samples and as such typically result in lower quantities of DNA.

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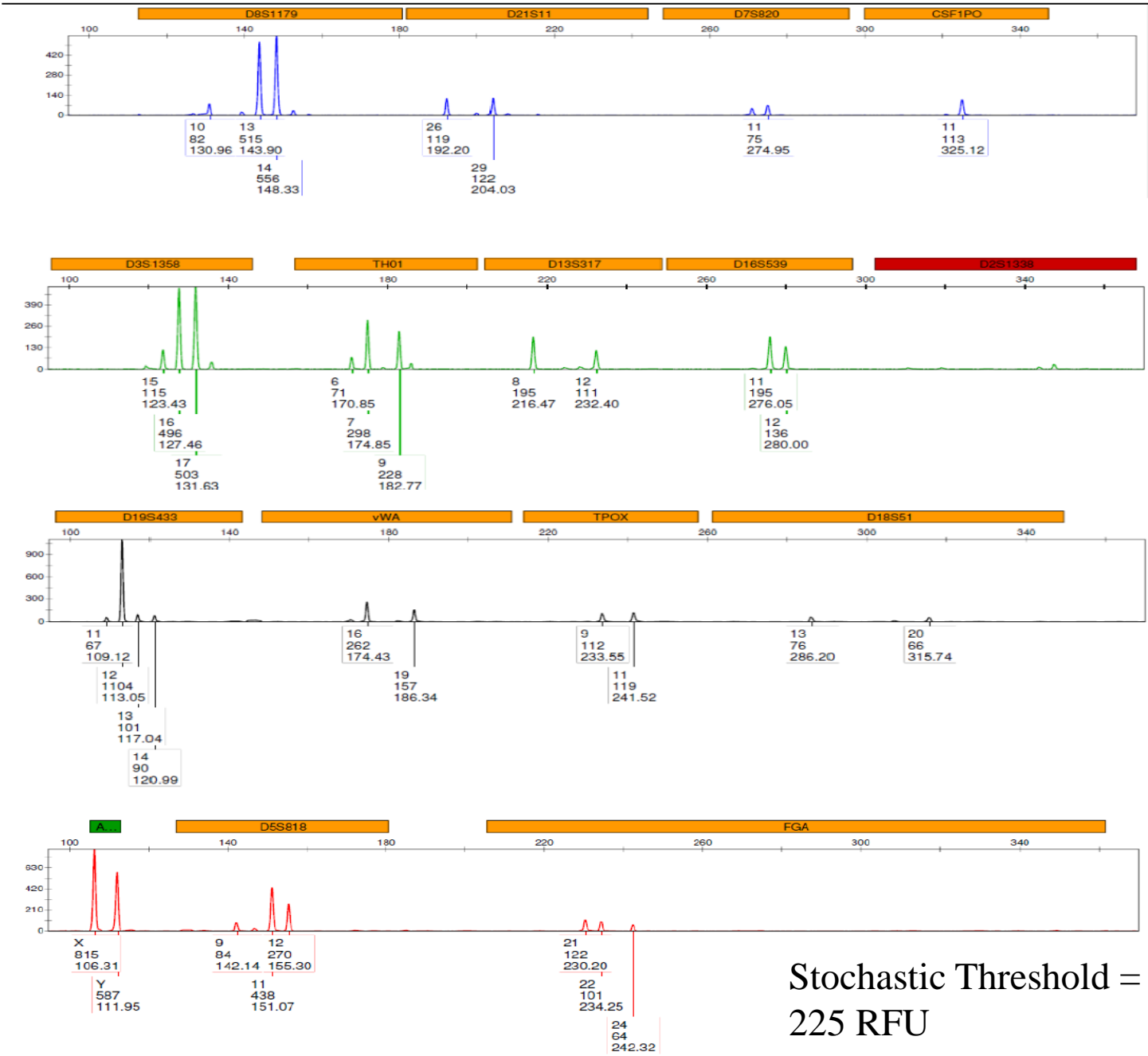
Stochastic Threshold = 225 RFU

Why the need for change?

The DNA profile obtained from the swabs indicates a mixture of at least two individuals with at least one male contributor and is inconclusive for comparison purposes.

Or…

A very low statistic.

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So what can we do?

Semi-Continuous
(does not consider peak height)
- LR Mix
- Lab Retriever
- ArmedXpert™

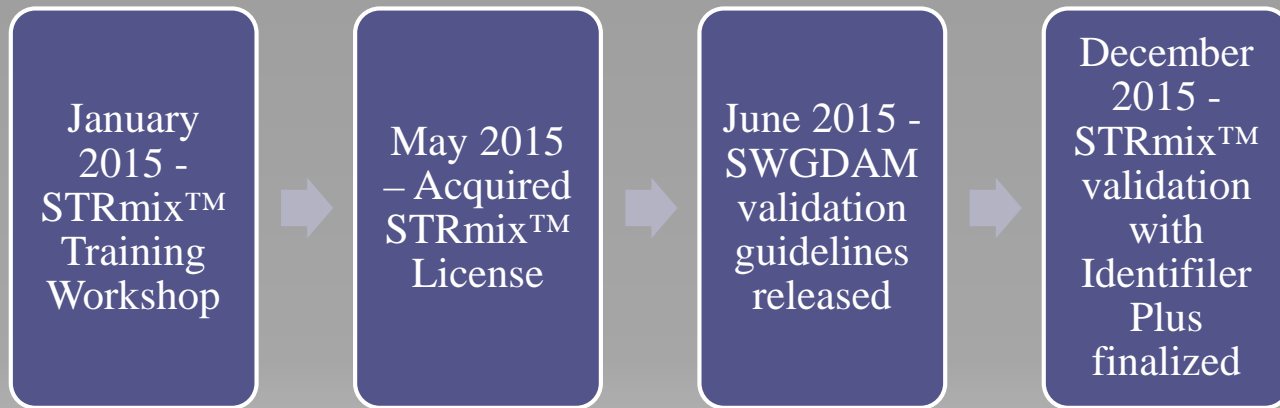**Fully Continuous**
(incorporates most parameters)
STRmix™
TrueAllele®
DNA-View®

Validate a probabilistic genotyping software!

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Timeline: From Validation to Implementation

| January 2015 - STRmix™ Training Workshop | May 2015 – Acquired STRmix™ License | June 2015 - SWGDAM validation guidelines released | December 2015 - STRmix™ validation with Identifiler Plus finalized |

January 2016 – First STRmix™ report issued at DLI

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Timeline: From Validation to Implementation

January 2017 – Expanded CODIS core loci required for upload	→	April 2017 – STRmix™ validation with GlobalFiler finalized	→	November 2017 – STRmix™ validation with PowerPlex Fusion 6C finalized

November 2016 – Daubert motion for STRmix™ denied in FL
April 2017 – 1st Daubert/Frye hearing for STRmix™ in FL

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2 Years Later… **Statistics through 2017**

- Over 250 cases using STRmix ™

- 40 Total Jurisdictions Using STRmix™

- 16 Florida Counties, 4 US States/Territories and 5 Countries

- Approximately 65% of samples analyzed were obtained from gun swabs

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How does STRmix™ work?

- Fully continuous approach that takes into consideration most of the data present in the profile.
- Uses a sampling strategy to compare what is expected of all the possible genotypes to what was observed assigning weights to possible genotype combinations
- Considers two propositions to apply a likelihood ratio (LR) incorporating the assigned weights
- The LR is the probability of the prosecution hypothesis over the probability of the defense hypothesis.

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Factors STRmix™ Accounts For

- Drop In
- Drop Out
- Stutter- Including Longest Uninterrupted Stretch (LUS) Values
- Allele Sharing
- Degradation
- Locus Specific Amplification Efficiency by Kit

- Locus Specific Amplification Efficiency by Sample
- Template
- Replicates
- Peaks Below Stochastic Threshold
- Relatedness
- Stratified Likelihood Ratio (LR) for all 3 populations

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# Validation Studies

- Establishing the Parameters
  - Drop-in
  - Dropout
  - Saturation
  - Stutter/LUS
  - Model Maker
  - Population Data
  - Kit Specific Settings
  - Iterations
  - Number of MCMC chains

- Sensitivity & Specificity
- Checking the Likelihood Ratio
- Checking the Weights
- Addition of a Contributor
- Subtraction of a Contributor
- Reproducibility
- Iterations
- Alternate Hypotheses
- Probative Samples
- Artifacts
- Performance Check

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Additional Studies with GlobalFiler and PowerPlex Fusion 6C

- President's Council of Advisors on Science and Technology (PCAST) - September 2016
- New York v Hillary Frye decision – August 2016
  - Extreme Mixture Proportions
  - Multi-Laboratory Study – 5-person mixture analyzed at different laboratories based on their own models, conclusion for POI remained the same
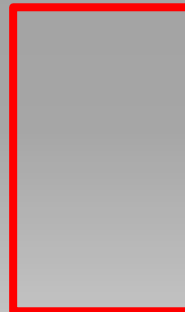
Additional Studies with GlobalFiler and PowerPlex Fusion 6C

- Expanded kits and degradation
  - Saturation study – evaluate the effects of saturated loci in STRmix™ analysis
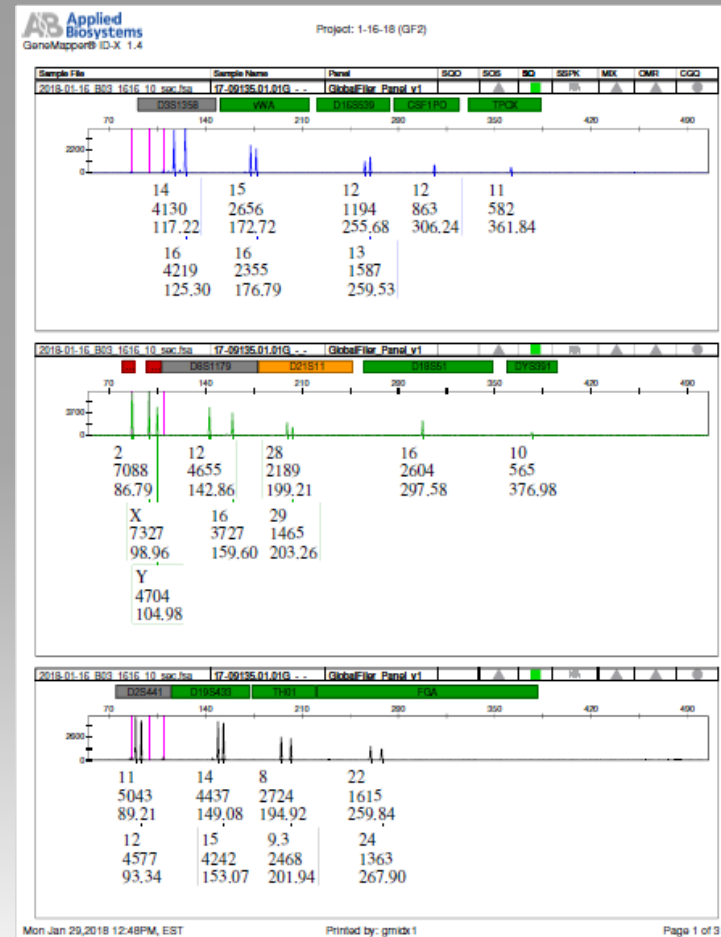
Additional Studies with GlobalFiler and PowerPlex Fusion 6C

- Relationship LR's
  - Family studies

Which LR do we report?

- Factor of N!
  - DNA profile of comparison sample is compared to the mixture as a whole
- 99.0% 1-sided lower Highest Posterior Density (HPD)
  - Confidence interval is applied to the point estimate LR; lower end of the 99% HPD interval is reported

Which LR do we report?

- Unified LR
  - ▫ Takes into account that the unknown contributors in Hp and Hd are made up of both relatives and unrelated individuals
- Stratified LR
  - ▫ A single LR that samples across all populations (Caucasian, African-American, Southeast Hispanic and Southwest Hispanic)

LR (population proportion)	DLI_GlobalFiler_Af Am_FBIextended. csv (0.14)	DLI_GlobalFiler_Ca uc_FBIextended. csv (0.69)	DLI_GlobalFiler_S EHISP_FBIextende d.csv (0.06)	DLI_GlobalFiler_S WHISP_FBIextende d.csv (0.11)	Stratified
Total LR	3.31E20	1.93E19	2.29E20	2.40E21	1.96E19
Sibling	4.54E7	1.92E7	3.77E7	5.67E7	1.93E7
Parent/Child	3.70E11	7.95E10	3.25E11	9.31E11	8.01E10
Half sibs	7.28E14	1.07E14	5.72E14	2.27E15	1.08E14
Grandparent / Grandchild	7.28E14	1.07E14	5.72E14	2.27E15	1.08E14
Uncle or Aunt /Niece or Nephew	7.28E14	1.07E14	5.72E14	2.27E15	1.08E14
First Cousin	1.66E17	1.75E16	1.22E17	6.51E17	1.77E16
Unified	1.77E15	5.77E15	2.56E14	7.17E14	2.02E15

The Verbal Scale- What's this I hear about strength of support?

Sample Verbal Scale

Figure 12 Verbal equivalents for Likelihood Ratio values

Verbal 'equivalent'		Likelihood Ratio
provides extremely strong support		over 1,000,000
provides very strong support		1000-1,000,000
provides strong support	[for H_p]	100-1000
provides moderate support		10-100
provides slight support		1-10
is neutral		1
provides slight support		1-0.1
provides moderate support		0.1-0.01
provides strong support	[against H_p]	0.01-0.001
provides very strong support		0.001-0.000,001
provides extremely strong support		less than 0.000,001

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Flipping the LR

- LR<1
  - A likelihood ratio of 0.00056 was obtained

- Rather than reporting support against the prosecution proposition we can 'flip the LR'

- 1/LR

- Now we can phrase the statement to report strength of support for the defense proposition

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So what happens to the verbal scale?

Figure 12 Verbal equivalents for Likelihood Ratio values

Verbal 'equivalent'		Likelihood Ratio
provides extremely strong support		over 1,000,000
provides very strong support		1000-1,000,000
provides strong support	[for H_p]	100-1000
provides moderate support		10-100
provides slight support		1-10
is neutral		1
provides sli~~~ ~~~~~		1-0.1
provides me~		
provides strong support		~.01-0.001
provides very strong sup~		~ 0.000,001
provides e~		

Reporting LR's favoring Hp vs. Hd

- The DNA profile obtained from the item is approximately 1 trillion times more probable if the sample originated from Joe Suspect and two unknown persons than if it originated from three unknown persons. Therefore, there is extremely strong support that Joe Suspect and two unknown persons contributed to this mixed DNA profile, rather than three unknown persons.

  Favors Hp

- The DNA profile obtained from the item is approximately 1 trillion times more probable if the sample originated from three unknown persons than if it originated from Joe Suspect and two unknown persons. Therefore, there is extremely strong support that three unknown persons contributed to this mixed DNA profile, rather than Joe Suspect and two unknown persons.

  Favors Hd

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Report remarks

- The probabilistic genotyping method utilizes STRmix™, an internally validated expert forensic software. STRmix™ uses a fully continuous approach for DNA profile interpretation. The propositions used to determine the likelihood ratios for this report were calculated from the information available at the time the report was written. Should any additional information become available it may be necessary to reconsider these interpretations. <u>Additional propositions may be considered upon request if instructed to do so prior to testimony.</u>

- The DNA population statistics are estimates with a confidence level of plus or minus a factor of 10. The reported frequency was derived from the Expanded Federal Bureau of Investigation (FBI) DNA population database (2016) for the Caucasian, Southeast Hispanic, Southwest Hispanic and African American/Bahamian/ Jamaican populations. The stratified likelihood ratio, which incorporates all four population groups, was selected for reporting.

Uninformative Range

Implemented upon completion of PowerPlex Fusion 6C validation

- Highest LR observed internally for a known non-contributor (Hd true)
- Database search is an option to further evaluate the quality of the profile

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How about RMP and CPI?

Continue to utilize traditional statistical approaches

- RMP/mRMP
  - Single source
  - Major contributors with inconclusive minors
- CPI
  - Clear two person mixtures with all alleles above STH
- STRmix™
  - Quality of profile
  - No limit to the number of contributors that can be assumed in the software. However, generally mixtures above 4 contributors will not be resolved with the exception of 'perfect' circumstances.

*Caution should be used in all mixture deconvolutions but in particular mixtures of 3 and 4 persons. *All deconvolutions must be checked to ensure they are intuitively correct.*

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Pending validations

- STRmix v2.5
  - In process with GlobalFiler and PowerPlex Fusion 6C
- GeneMapper ID-X vs. GeneMarker HID
- 3130xl vs. 3500
- Additional kits
  - Qiagen 24plex

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Training Program

- Internal training program
  - Lectures
  - Literature Review
  - Testimony observation
  - Oral Competency
  - Mock court
- Implemented 2nd Technical Review
  - To be conducted by a Senior DNA analyst
  - In agreement with conclusions
    - Inconclusive or suitable for STRmix™ analysis
    - Exclusions of reference standards

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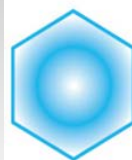
To the court room!

- Four senior DNA analysts have testified

- Two pro se cases

- Five Florida counties

- Reports accepted as evidence without testimony in numerous additional counties/states/countries

- Numerous Depositions/Pre-Trial Hearings

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How do I explain this in court?
Analogies!!!

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Why did I assume a contributor?

- Sexual Assault Kit Example

- Support shown for that individuals inclusion prior to assuming

- Intimate? Previously high statistic? i.e. Major

- Jigsaw Puzzle

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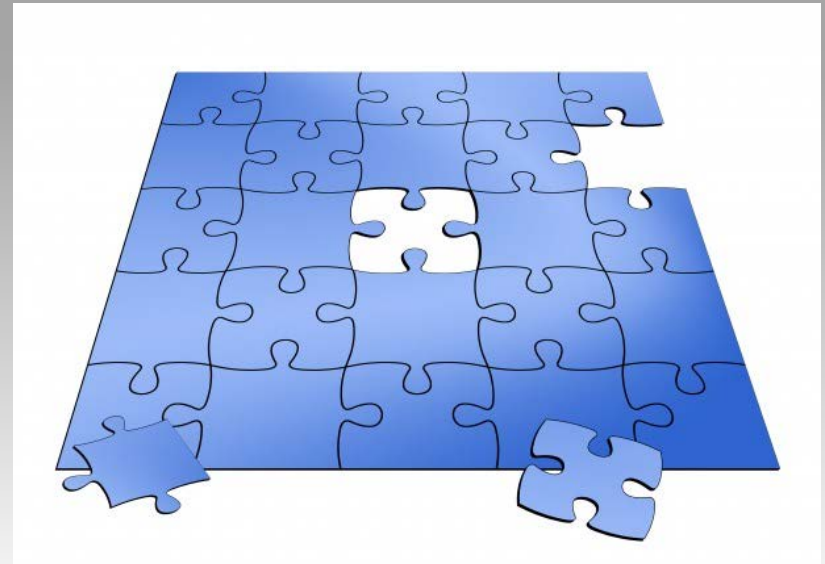
How does MCMC work?

• Game of Hot and Cold

• The true answer is somewhere in this building

• How about those first few guesses?
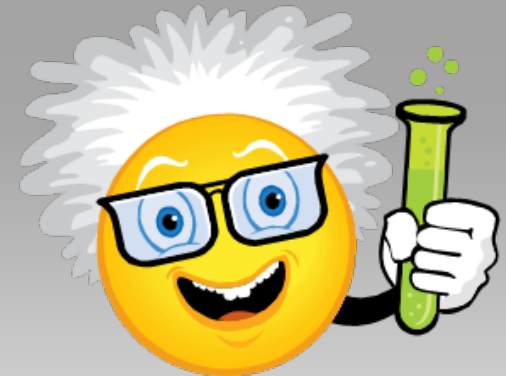
What makes a hot guess?

- Say we make a DNA profile of the bailiff and I…

- What would that look like?

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What does this statistic mean?

- What's in my breakfast?

- What if I knew I had a poached egg?

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Daubert/Frye Challenges

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State of Florida
v
Marc Regisme

The DNA evidence at issue and the law

The DNA results in the attached report are based upon "probabilistic genotyping methods", and provides an opinion that Regisme cannot be ruled out a possible contributor to the DNA found on the gun for which he is charged with possessing. The methodology used to produce the results and subsequent opinion does not meet *Daubert* and therefore is inadmissible.

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State's Response

. The court should take judicial notice of the propriety, relevance, and reliability of a technique ("probabilistic genotypic methods") that has been used in this courtroom previously and is not new or novel, in lieu of the requested *Daubert* hearing. When the scientific foundation for an expert's theory is so common and understood that the proponent can lay the foundation while qualifying the witness as an expert, the court may take judicial notice of the reliability of the knowledge or theory that undergirds the expert's proposed testimony. A preliminary hearing may not even be necessary to establish admissibility. *Owens v. Sylvia*, 838 A.2d 881 (R.I. 2003).

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DAUBERT MOTION DENIED

Here, the Defendant's motion fails to provide record support for a "serious, specified and substantial question as to the continued reliability of the science, theory or methodology". The Court adopts and incorporates by reference the State's Motion to Strike Defense Motion in Limine Re: DNA Evidence filed October 18, 2016. Accordingly, it is;

ORDERED AND ADJUDGED that the Defendant's Motion in Limine RE: DNA Evidence is *DENIED* and the State's Motion to Strike is *GRANTED*.

DONE AND ORDERED in Chambers, at West Palm Beach, Palm Beach County, Florida this 22nd day of November, 2016.

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The evidence does not support a DNA inclusion analysis, but merely makes a probability determination that this DNA may belong to Jordan Finlon, Ahmad Dunbar, or Dwayne Cummings.

Relying upon a probability factor is a novel area of science, and falls belong the required standard practice of DNA analysis and evidence. This evidence places vague and ambiguous evidence before a jury, violating Mr. Cummings rights to Due Process.

State of Florida
v
Dwayne Cummings

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Florida Daubert *or was it Frye…*

Basically made the same arguments but also questioned:

- The expert's qualifications

- Whether the evidence would assist the jury in understanding a fact or issue

- General acceptance

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State's Response

Let's have a hearing!

Judge Rules… Admissible!

more lenient). The Court is of the opinion that <u>Daubert</u> is not more lenient than <u>Frye</u>; instead, it is more rigorous but at the same time more flexible. Regardless of this academic debate, the proposed testimony here satisfies <u>Daubert</u>, and it would also satisfy <u>Frye</u>.

- "It is difficult to image that the U.S. Army and the FBI would employ these tools if there had not been acceptance. Perhaps that is why nearly half of all forensic laboratories in the United States have purchased licenses to use the STRmix software, including the FDLE laboratory." -Judge Hunter W. Carroll

- Also determined the expert was capable.

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Additional challenges

- Orange County, FL- Daubert Hearing (State of Florida vs Ramon A. Mercado) Admissible

- New Mexico Daubert Hearing(United States v Melvin Russell) Admissible

- Minnesota- Frye Mack Hearing(State of Minnesota v Johnny Earl Edwards and Bryston Markeis Hill-Turnipseed) Admissible

- Wyoming- Daubert and Rule 702 (Wyoming vs Bradley Ross Fairbourn) Admissible

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My report is being challenged what do I do?

- Maintain records of pertinent rulings

- Consider issuing an affidavit in support

- Consider recommending an additional expert

- Know your publications!

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Challenge Prep Continued…

- Provide exhibits prior to hearing that you can reference

- Study!

- Review previous motions/rulings/transcripts

- Review training and competency materials

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Challenge Prep Continued…

- Schedule pre-hearing meeting with attorney

- Everything is not up to you- you are not a computer programmer

- Know your current events (STRmix™ website or John Buckleton's website)

- Have the attorney request your presence during defense expert testimony

Where are other laboratories and legal systems at with STRmix™?

- DNA Labs International was the 5th laboratory in the United States to validate STRmix™. (1st private laboratory)
- Erie County, NY/ Michigan State/ SanDiego, CA/ United States Army Criminal Investigation Laboratory (USACIL)/FBI also have STRmix™
- About half of the laboratories in the US are either live with STRmix™ or in the process of validating
- STRmix™ is widely accepted in Australia and New Zealand
- NY/Michigan/Texas - Found admissible
- Probabilistic Genotyping has already been upheld by several courts in cases involving other software; i.e. TrueAllele in Pennsylvania

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Interesting Development Since Releasing for Casework

The family that commits crime together…

- STRmix™ provides a summary of relative LR's

- The following propositions are considered:

Hp: The DNA profile has originated from the POI and N-1 individuals, unrelated to the Database profile

<div align="center">vs</div>

Hd: The DNA profile has originated from a [insert relationship] of the Database profile + N-1 unknown contributors

- No longer requires us to make the unrelated statement when reporting statistics

What happens when the relatives LR does not support our original proposition?

SUMMARY OF LR

LR (population proportion)	FBI-African American-ID+_FINAL.csv (0.14)	FBI-Caucasian-ID+_FINAL.csv (0.69)	FBI-Southwest Hispanic-ID+_FINAL.csv (0.18)		Stratified
Total LR	9.21E13	2.31E11	9.90E11		3.22E11
Sibling	5.63E1	1.97E1	3.04E1		2.32E1
Parent/Child	4.59E5	4.33E4	7.81E4		5.50E4
Half sibs	2.88E8	1.15E7	2.79E7		1.54E7
Grandparent / Grandchild	2.88E8	1.15E7	2.79E7		1.54E7
Uncle or Aunt /Niece or Nephew	2.88E8	1.15E7	2.79E7		1.54E7
First Cousin	3.53E10	6.39E8	1.93E9		8.57E8
Unified	2.19E9	3.81E9	1.53E9		2.80E9

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Familial Mixtures

- Two family units were evaluated
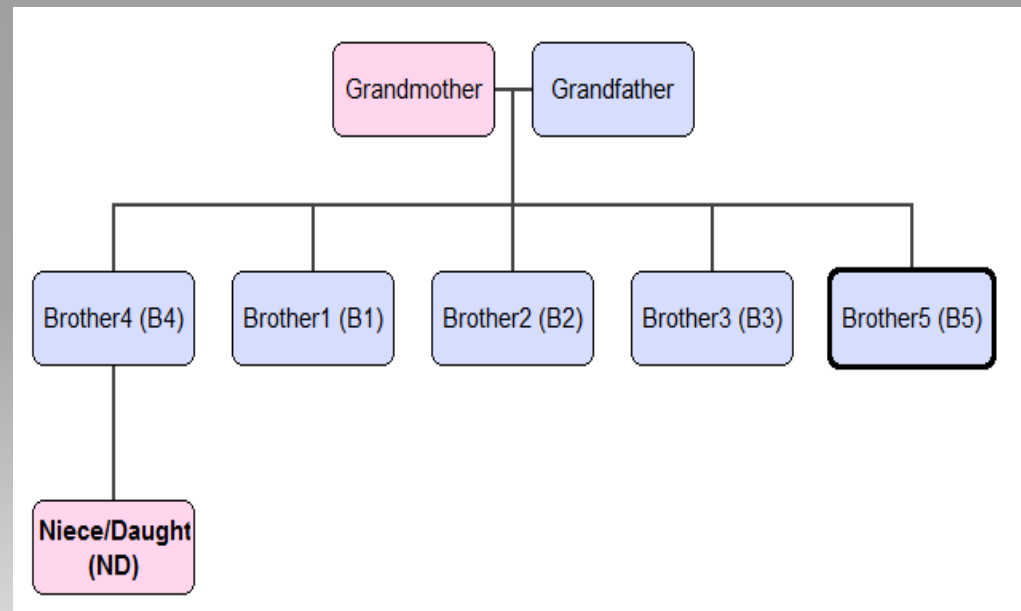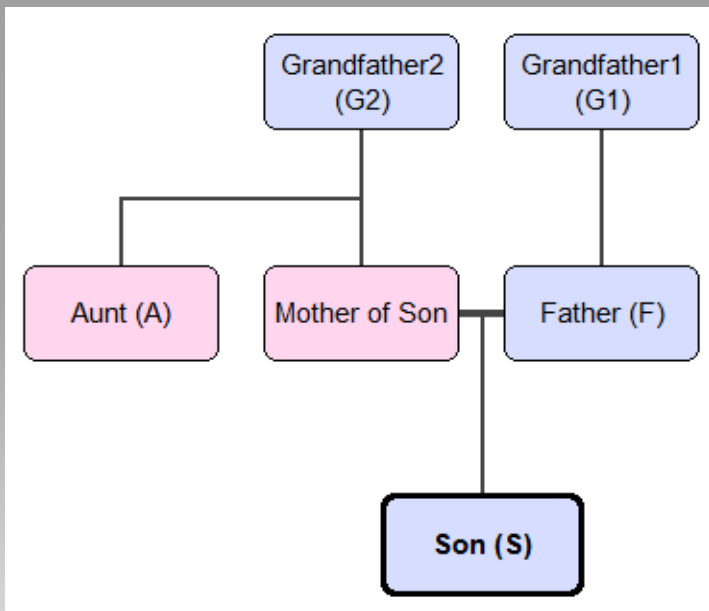
Familial Mixtures

Mixture	Mixture Proportions	Known Contributors	Contributors Analyzed in Hp (POI)
A	1:3	S/F	S/F/G1/A/NC
B	1:1:1	S/F/GF1	S/F/G1/A/NC
C	1:2:5	S/F/G1	S/F/G1/A/NC
D	1:2:3:5	S/F/G1/U1	S/F/G1/A/NC
E	1:1:1:1:1	B1/B2/B3/B4/B5	B1/B2/B3/B4/B5/ND/NC
F	1:3	B4/B5	B3/B4/B5/ND/NC
G	1:2:5	B3/B4/B5	B2/B3/B4/B5/ND/NC
H	1:2:3:5	B3/B4/B5/U1	B2/B3/B4/B5/ND/NC
I	1:2:3	S/G1/G2	S/F/G1/G2/AD/NC
J	1:2:3	B3/U1/U2	B1/B2/B3/B4/B5/ND/NC
K	1:2:3:5	B3/B4/U1/U2	B1/B2/B3/B4/B5/ND/NC

S=Son, F=Father, B=Brother, GF=Grandfather, ND=Niece/Daughter, A=Aunt of Son,
U=Unrelated Individual, NC=Non-Contributors

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Conclusions

- Results of the familial mixtures were compared to non-familial mixtures
- Examining the LR value isn't as meaningful - can be dependent on the quality of the profile and the overall presence of the applicable contributor in that profile
- More effective: evaluate the degree of change in the Unified LR:Relative LR
- Sibling LR's usually most affected: Change in LR should be approximately half the powers of ten from the Unified LR

LR (population proportion)	DLI_GlobalFiler_Af Am_FBIextended. csv (0.14)	DLI_GlobalFiler_Ca uc_FBIextended. csv (0.69)	DLI_GlobalFiler_S EHISP_FBIextende d.csv (0.06)	DLI_GlobalFiler_S WHISP_FBIextende d.csv (0.11)	Stratified
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Unified	1.77E15	3.77E15	2.56E14	7.17E14	2.02E15

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How do I check this?

Sibling LR   Parent/Child LR

LOG(STRATIFIED LR)   LOG(Stratified LR)
LOG(SIBLING LR)   LOG(Parent/Child LR)

• Acceptable ranges should be defined by analyzing mixtures with and without familial components.

• Should be determined as part of your internal validation.

• Report wording defining request for standards should be established.

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Conclusions

- Even when the reported LR is high, family relationship likelihood ratios should always be considered during the evaluation of the results.

- *If applicable to the case, it is recommended that reference standards from any first degree relatives of [Name] be submitted for additional comparisons.*

- Overall, when in doubt standards from any primary relatives should be requested if applicable to the case.
  - Especially if there is a chance that a relative could have come into contact with the evidence

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Thank You!

- Promega

- Forensic Magazine

- Institute of Environmental Science & Research (ESR)

Any questions, sample review requests or for a copy of the presentation please contact:

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