Advanced Topics in STR DNA Analysis

STR Mixture Interpretation

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STR Mixture Interpretation

Outline for This Section

- Challenge of mixture interpretation
- NIST mixture interlaboratory studies
- MIX05 study details and results
- Steps to mixture interpretation
- Software programs

Mixtures: Issues and Challenges

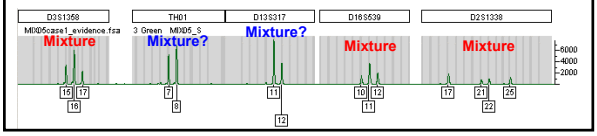
From J.M. Butler (2005) Forensic DNA Typing, 2nd Edition, p. 154

- Mixtures arise when two or more individuals contribute to the sample being tested.
- Mixtures can be challenging to detect and interpret without extensive experience and careful training.
- Differential extraction can help distinguish male and female components of many sexual assault mixtures.

Mixtures: Issues and Challenges

From J.M. Butler (2005) Forensic DNA Typing, 2nd Edition, p. 155

- The probability that a mixture will be detected improves with the use of more loci and genetic markers that have a high incidence of heterozygotes.
- The detectability of multiple DNA sources in a single sample relates to the ratio of DNA present from each source, the specific combinations of genotypes, and the total amount of DNA amplified.
- Some mixtures will not be as easily detectable as other mixtures.



A High Degree of Variability Currently Exists with Mixture Interpretation

- "If you show 10 colleagues a mixture, you will probably end up with 10 different answers"
  - Peter Gill, Human Identification E-Symposium, April 14, 2005
- Interlaboratory studies help to better understand why variability may exist between laboratories

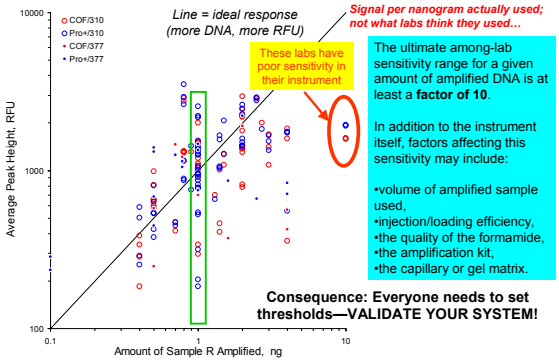
NIST Initiated Interlaboratory Studies

Studies involving STRs	# Labs	Publications
Evaluation of CSF1PO, TPOX, and TH01	34	Kline MC, Duewer DL, Newall P, Redman JW, Reeder DJ, Richard M. (1997) Interlaboratory evaluation of STR triplex CTT. *J. Forensic Sci.* 42: 897-906
Mixed Stain Studies #1 and #2 (Apr–Nov 1997 and Jan–May 1999)	45	Duewer DL, Kline MC, Redman JW, Newall PJ, Reeder DJ. (2001) NIST Mixed Stain Studies #1 and #2: interlaboratory comparison of DNA quantification practice and short tandem repeat multiplex performance with multiple-source samples. *J. Forensic Sci.* 46: 1199-1210
MSS3 **Mixed Stain Study** #3 (Oct 2000-May 2001)	74	Kline, M.C., Duewer, D.L., Redman, J.W., Butler, J.M. (2003) NIST mixed stain study 3: DNA quantitation accuracy and its influence on short tandem repeat multiplex signal intensity. *Anal. Chem.* 75: 2463-2469. Duewer, D.L., Kline, M.C., Redman, J.W., Butler, J.M. (2004) NIST Mixed Stain Study #3: signal intensity balance in commercial short tandem repeat multiplexes, *Anal. Chem.* 76: 6928-6934.
DNA Quantitation Study (Jan-Mar 2004) QS04	80	Kline, M.C., Duewer, D.L., Redman, J.W., Butler, J.M. (2005) Results from the NIST 2004 DNA Quantitation Study, *J. Forensic Sci.* 50(3):571-578
Mixture Interpretation Study (Jan - Aug 2005)	**69**	MIX05 ***Data analysis currently on-going ...***

Overall Lessons Learned from NIST MSS 1,2,&3

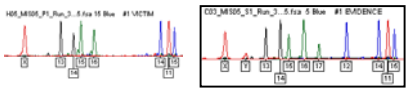
- Laboratories have instruments with different sensitivities
- Different levels of experience and training plays a part in effective mixture interpretation
- Amount of input DNA makes a difference in the ability to detect the minor component (labs that put in "too much" DNA actually detected minor components more frequently)

Amount of MSS3 Control Sample "R" amplified vs. average RFUs



Mixture Interpretation Interlab Study (MIX05)

- **Only involves interpretation of data – to remove instrument detection variability and quantitation accuracy issues**
- **94 labs enrolled** for participation
- **69 labs have returned results** (17 from outside U.S.)
- Four mock cases supplied with "victim" and "evidence" electropherograms (GeneScan .fsa files – that can be converted for Mac or GeneMapper; gel files made available to FMBIO labs)
- Data available with Profiler Plus, COfiler, SGM Plus, PowerPlex 16, Identifiler, PowerPlex 16 BIO (FMBIO) kits



Perpetrator Profile(s) ??

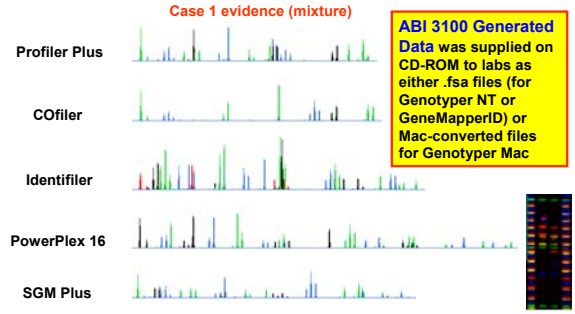
Along with reasons for making calls and any stats that would be reported

MIX05 Study Design and Purpose

- Permit a large number of forensic practioners to **evaluate the same mixture data**
- Provide multiple cases representing a range of mixture scenarios
- Generate data from multiple STR kits on the same mixture samples to compare performance for detecting minor components
- The primary variable should be the laboratory's interpretation guidelines rather than the DNA extraction, PCR amplification, and STR typing instrument sensitivity
- **Are there best practices in the field that can be advocated to others?**

MIX05 Results on Multiple Kits

http://www.cstl.nist.gov/biotech/strbase/interlab/MIX05.htm



ABI 3100 Generated Data was supplied on CD-ROM to labs as either .fsa files (for Genotyper NT or GeneMapperID) or Mac-converted files for Genotyper Mac

FMBIO data was also made available upon request

Value of the MIX05 Study

http://www.cstl.nist.gov/biotech/strbase/interlab/MIX05.htm

- Data sets exist with multiple mixture scenarios and a variety of STR kits that **can be used for training purposes**
- A wide variety of approaches to mixture interpretation have been applied on the **same data sets evaluated as part of a single study**
- **Interpretation guidelines from many laboratories are being compared to one another for the first time in an effort to determine challenges facing future efforts to develop "expert systems" for automated mixture interpretation**
- **We are exploring the challenges of supplying a common data set to a number of forensic laboratories** (e.g., if a standard reference data set was ever desired for evaluating expert systems)

Requests for Participants in MIX05

Mixtures representing four different case scenarios have been generated at NIST with multiple STR kits and provided to laboratories as electropherograms.

We would like to receive the following information:

1) **Report the results as though they were from a real case** including whether a statistical value would be attached to the results. *Please summarize the perpetrator(s) alleles in each "case" as they might be presented in court—along with an appropriate statistic (if warranted by your laboratory standard operating procedure) and the source of the allele frequencies used to make the calculation.* Please indicate which kit(s) were used to solve each case.

2) **Estimate the ratio for samples present in the evidence mixture** and how this estimate was determined.

3) **Provide a copy of your laboratory mixture interpretation guidelines** and a brief explanation as to why conclusions were reached in each scenario

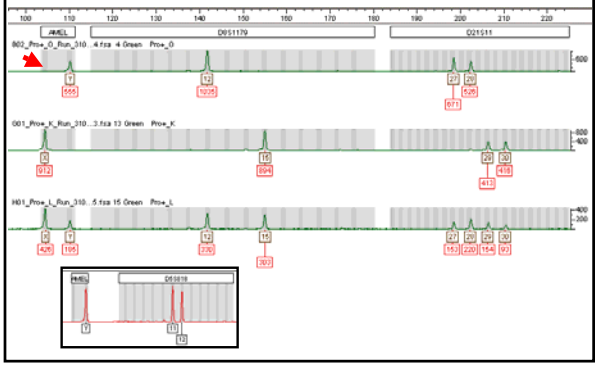
MIX05 Case Scenarios

Genomic DNA samples with specific allele combinations ("evidence") were mixed in the following ratios:

	#alleles		#loci with #alleles				
	N all	N unq	N 1	N 2	N 3	N 4	N 5
Case #1 – victim is major contributor (3F:1M)	39	26	2	6	5	2	0
Case #2 – perpetrator is major contributor (1F:3M)	55	52	0	1	4	10	0
Case #3 – balanced mixture (1F:1M) • Male lacked amelogenin X	48	37	0	3	8	4	0
Case #4 – more extreme mixture (7F:1M) • Male contained tri-allelic pattern at TPOX	50	42	0	3	7	4	1

Female victim DNA profile was supplied for each case

Amelogenin X allele is missing in male perpetrator DNA sample for MIX05 Case #3



Summary of MIX05 Responses

94 labs enrolled for participation

69 labs returned results (17 from outside U.S.)

50 labs made allele calls
39 labs estimated ratios
29 labs provided stats

STR kit results used
- **34 ProfilerPlus/COfiler**
- **10 PowerPlex 16**
- **7 PP16 BIO**
- **5 Identifiler**
- **2 SGM Plus**
- **1 All ABI kit data**
- **9 Various combinations**

When is a Sample a Potential Mixture?

According to several MIX05 participant interpretation guidelines

- Number of Observed Peaks
  - Greater than two peaks at a locus
  - More than two alleles are present at two or more loci, although three banded patterns can occur
  - Presence of 3 alleles at a single locus within a profile
  - 4 peaked patterns (if observed at any locus), 3 peaked patterns (if observed at two or more loci), significant imbalances (peak height ratios <60%) of alleles for a heterozygous genotype at two ore more loci with the exception of low template amplifications, which should be interpreted with caution
- Imbalance of heterozygote alleles
  - thresholds range from 50-70%
- Stutter above expected levels
  - generally 15-20%

Summary of Some MIX05 Reported Results

CASE #2		D3S1358	VWA	FGA	AMEL	D8S1179	D21S11	D18S51	D5S818	D13S317	D7S820	D16S539	TH01	TPOX	CSF1PO
True Pop	Z779619	15,15	15,15	20,24	X,Y	11,13	28,32.2	17,18	8,13	12,14	8,10	10,11	7,9.3	9,10	7,10
LabID	Kit Used														
16	ProPlus/Cofiler	--	--	--	--	--	--	--	--	--	--	--	--	--	--
6	ProPlus/Cofiler	15	15	20,24	X,Y	11,13	28,32.2	17,18	8,13	12,14	8,10	10,11	7,9.3	9,10	7,10
91	SGM Plus	15	15	20,24	X,Y	11,13	28,32.2	17,18				10,11	7,9.3		
46	PP16	--	--	--	--	--	--	--	--	--	--	--	--	--	--
37	ProPlus/Cofiler	--	15	20	X,Y	13	28,32.2	17,18	8,13	12,14	8,10	10,11	7,9.3	9,10	7,10
2	PP16	15	15,15	20,24	X,Y	11,13	28,32.2	17,18	8,13	INC	8,10	10,11	7,9.3	9,10	7,10
13	PP16 & Identifiler	15	15	20,24	--	11,13	28,32.2	17,18	8,13	12,14	8,10	10,11	7,9.3	9,10	7,10
34	ProPlus/Cofiler	15	15	20,24	--	11,13	28,32.2	17,18	8,13	12,14	8,10	10,11	7,9.3	9,10	7,10
70	Identifiler	15	15	20,24	X,Y	11,13	28,32.2	17,18	8,13	12,14	8,10	10,11	7,9.3	9,10	7,10
55	ProPlus/Cofiler	15	15	20,24	--	11,13	28,32.2	17,18	8,13	12,14	8,10	10,11	7,9.3	9,10	7,10
21	ProPlus/Cofiler	15,15	15,15	20,24	X,Y	11,13	28,32.2	17,18	8,13	12,14	8,10	10,11	7,9.3	9,10	7,10
73	ProPlus/Cofiler	15,15	15,15	20,24	X,Y	11,13	28,32.2	17,18	8,13	12,14	8,10	10,11	7,9.3	9,10	7,10
29	Identifiler	15	15	20,24	X,Y	11,13	28,32.2	17,18	8,13	12,14	8,10	10,11	7,9.3	9,10	7,10
64	All Kits	15,15	15,15	20,24	X,Y	11,13	28,32.2	17,18	8,13	12,14	8,10	10,11	7,9.3	9,10	7,10
90	ProPlus/Cofiler	15	15	20,24	X,Y	11,13	28,32.2	17,18	8,13	12,14	8,10	10,11	7,9.3	9,10	7,10
9	ProPlus/Cofiler	15	15	20,24	X,Y	11,13	28,32.2	17,18	8,13	12,14	8,10	10,11	7,9.3	9,10	7,10
4	ProPlus/Cofiler	15	15	20,24	X,Y	11,13	28,32.2	17,18	8,13	12,14	8,10	10,11	7,9.3	9,10	7,10
33	ProPlus/Cofiler	--	--	--	--	--	--	--	--	--	--	--	--	--	--
12	ProPlus/Cofiler	15	15	20,24	X,Y	11,13	28,32.2	17,18	8,13	12,14	8,10	10,11	7,9.3	9,10	7,10
67	PP16	15	15,16	20,24	X,Y	11,13	28,32.2	17,18	8,13	12,14	8,10	10,11	7,9.3	9,10	7,10
86	ProPlus/Cofiler	15,15	15,15	20,24	--	11,13	28,32.2	17,18	8,13	12,14	8,10	10,11	7,9.3	9,10	7,10
79	ProPlus/Cofiler	15,15	15,15	20,24	--	11,13	28,32.2	17,18	8,13	12,14	8,10	10,11	7,9.3	9,10	7,10
77	Identifiler	--	--	--	--	--	--	--	--	--	--	--	--	--	--
60	PP16	15	15	20,24	X,Y	11,13	28,32.2	17,18	8,13	12,14	8,10	10,11	7,9.3	9,10	7,10
61	Identifiler	--	--	--	--	--	--	--	--	--	--	--	--	--	--

Most calls were correct (when they were made)

Some Mixture Ratios Reported in MIX05

Many labs do not routinely report the estimated ratio of mixture components

LabID	Case1 (F:M)	Case2 (M:F)	Case3 (M:F)	Case4 (F:M)
13	2	5	<2	10
34	1.8--3.6	3.9--6.7	1.6--1.8	6.2--7.6
70				
55	68%:32%	85%:15%	64%:36%	
21				
73	2:1	6:1	2:1	not determined
29				
54	2:1	6:1	2:1	6:1
90	male23-39%	not determined	male64-71%	
9	3 or 4:1	4 or 5:1	1.4:1	~10:1
4	10:1	6:1	1:1	not determined
33	male60-78%	male80-90%	male58-71%	victim86%
12	male25%	male85%	male40-45%	unknown10%
67	1:2.3	6.4:1	2:1	1:6.8
86	2:1	6-6.5:1	1.6-2:1	4-4.5:1
79	~3:1 to ~2:1	~6:1 to ~4:1	~2:1*	a lot of victim
77				
60	2:1	5:1	2:1	10:1
61				

Some Reported Stats for MIX05 Case #1

		Case1		
LabID	Kits Used	Caucasians	African Americans	Hispanics
77	Identifiler	PE calculated	PE calculated	PE calculated
73	ProPlus/Cofiler	none provided	none provided	none provided
4	ProPlus/Cofiler	none provided	none provided	none provided
12	ProPlus/Cofiler	none provided	none provided	none provided
29	Identifiler	none provided	none provided	none provided
90	ProPlus/Cofiler	1.18E+15	2.13E+14	3.09E+15
34	ProPlus/Cofiler	2.40E+11	7.00E+09	9.80E+10
46	PP16	5.60E+09	3.80E+11	none provided
33	ProPlus/Cofiler	2.94E+08	1.12E+08	1.74E+09
6	ProPlus/Cofiler	40,000,000	3,500,000	280,000,000
9	ProPlus/Cofiler	1.14E+07	1.97E+07	1.54E+08
61	Identifiler	1.50E+06	260,000	2.40E+07
79	ProPlus/Cofiler	930,000	47,900	1,350,000
16	ProPlus/Cofiler	434,600	31,710	399,100

Some Differences in Reporting Statistics

		Case1		
LabID	Kits Used	Caucasians	African Americans	Hispanics
90	ProPlus/Cofiler	1.18E+15	2.13E+14	3.09E+15
34	ProPlus/Cofiler	2.40E+11	7.00E+09	9.80E+10
33	ProPlus/Cofiler	2.94E+08	1.12E+08	1.74E+09
6	ProPlus/Cofiler	40,000,000	3,500,000	280,000,000
9	ProPlus/Cofiler	1.14E+07	1.97E+07	1.54E+08
79	ProPlus/Cofiler	930,000	47,900	1,350,000
16	ProPlus/Cofiler	434,600	31,710	399,100

Remember that these labs are interpreting the same MIX05 electropherograms

Questions

- Do you look at the evidence data first without considering the suspect's profile?
- Do you have a decision point whereby you consider a mixture too complicated and do not try to solve it? If so, is the case declared inconclusive?
- Should two amplifications be done – e.g., one at 1 ng to type the major component and one at higher concentration to move the minor component out of the low-copy number regime?

Two Parts to Mixture Interpretation

- **Deduction of alleles present in the evidence** (compared to victim and suspect profiles)
- **Providing some kind of statistical answer** regarding the weight of the evidence
  - An ISFG DNA Commission (Peter Gill, Bruce Weir, Charles Brenner, etc.) is evaluating the statistical approaches to mixture interpretation and will make recommendations soon

Steps in the interpretation of mixtures

(Clayton *et al.* *Forensic Sci. Int.* 1998; 91:55-70)

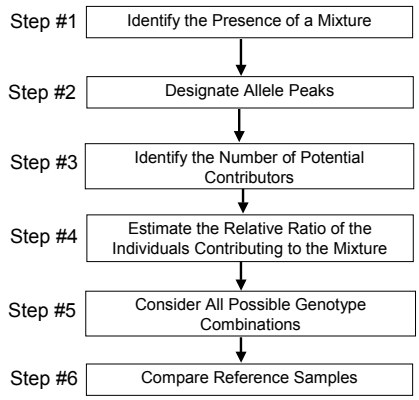


Figure 7.4, J.M. Butler (2005) *Forensic DNA Typing*, 2nd Edition © 2005 Elsevier Academic Press

Step #1: Is a Mixture Present in an Evidentiary Sample?

- Examine the number of peaks present in a locus
  - More than 2 peaks at a locus (except for tri-allelic patterns at perhaps one of the loci examined)
- Examine relative peak heights
  - Heterozygote peak imbalance <60%
  - Peak at stutter position >15%
- Consider all loci tested

Step #2: Designate Allele Peaks

- Use regular data interpretation rules to decipher between true alleles and artifacts
- Use stutter filters to eliminate stutter products from consideration (although stutter may hide some of minor component alleles at some loci)
- Consider heterozygote peak heights that are highly imbalanced (<60%) as possibly coming from two different contributors

Step #3: Identifying the Potential Number of Contributors

- **Important for some statistical calculations**
- Typically if 2, 3, or 4 alleles then 2 contributors
- If 5 or 6 alleles per locus then 3 contributors
- If >6 alleles in a single locus, then >4 contributors
- JFS Nov 2005 paper by Forensic Bioinformatics on number of possible contributors
  - Relies on maximum allele count alone
  - Does not take into account peak height information

Forensic Bioinformatics Article

http://www.bioforensics.com/articles/empirical_mixtures.pdf


J Forensic Sci, Nov. 2005, Vol. 50, No. 6
Paper ID JFS2004475
Available online at: www.astm.org

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Empirical Analysis of the STR Profiles Resulting from Conceptual Mixtures

TABLE 2—*Count and percent of three-person mixtures in which a particular number of unique alleles was the maximum observed across all loci, both for the original and randomized individuals*[a].

Unique Alleles	Count	Percent (%)
2	0	0.00%
3	78	0.00%
4	4,967,034	3.39%
5	93,037,010	63.49%
6	48,532,037	33.12%

Using 959 complete 13-locus STR profiles from FBI dataset

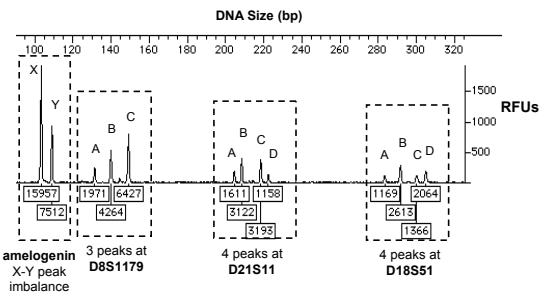
146,536,159 possible combinations **with 3-person mixtures**

3.39 % (4,967,034 combinations) would only show a maximum of four alleles (i.e., appear based on maximum allele count alone to be a 2-person mixture)

Step #4: Estimation of Relative Ratios for Major and Minor Components to a Mixture

- Mixture studies with known samples have shown that the mixture ratio between loci is fairly well preserved during PCR amplification
- Thus it is generally thought that the peak heights (areas) of alleles present in an electropherogram can be related back to the initial component concentrations
- Start with loci possessing 4 alleles…

Example Data from 2-Person Mixture



Peak areas (rather than heights) are listed under each allele

Figure 7.6, J.M. Butler (2005) *Forensic DNA Typing*, 2nd Edition © 2005 Elsevier Academic Press

Step #5: Consider All Possible Genotype Combinations

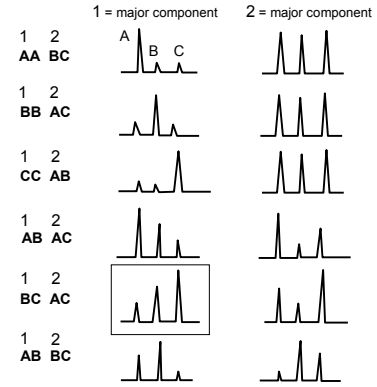


Figure 7.7, J.M. Butler (2005) *Forensic DNA Typing*, 2nd Edition © 2005 Elsevier Academic Press

Step #6: Compare Reference Samples

- If there is a suspect, a laboratory must ultimately decide to include or exclude him…
- If no suspect is available for comparison, does your laboratory still work the case? (Isn't this a primary purpose of the national DNA database?)
- Victim samples can be helpful to eliminate their allele contributions to intimate evidentiary samples and thus help deduce the perpetrator

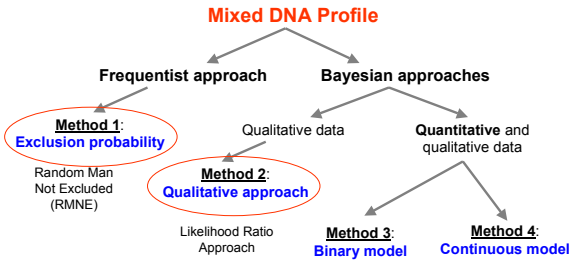
Mixture Interpretation in the Low-Copy Number Regime

- If 500 pg of total DNA is the amount inputted for PCR amplification, then in a 1:10 mixture the minor component is present in <50 pg amount and susceptible to stochastic (selected) amplification
- I would recommend amplifying mixture again using a higher total amount of DNA (if available)
  - e.g., 5 ng so that a 1:10 minor component is now at 500 pg
  - Yes, the major component will be overloaded…
- Use caution in interpreting LCN minor components

Two Parts to Mixture Interpretation

- **Deduction of alleles present in the evidence** (compared to victim and suspect profiles)
- **Providing some kind of statistical answer** regarding the weight of the evidence
  - An ISFG DNA Commission (Peter Gill, Bruce Weir, Charles Brenner, etc.) is evaluating the statistical approaches to mixture interpretation and will make recommendations soon

Approaches to Statistical Evaluation of Mixture Results



An ISFG DNA Commission chaired by Peter Gill will comment on these four methods for statistical mixture interpretation.

Figure 7.1 from Tim Clayton and John Buckleton, Chapter 7 "Mixtures" in *Forensic DNA Evidence Interpretation* (2005) CRC Press

Additional Thoughts on Mixtures

From J.M. Butler (2005) Forensic DNA Typing, 2nd Edition, p. 166

- Some forensic DNA laboratories may decide not to go through the trouble of fully deciphering the genotype possibilities and assigning them to the major and minor contributors.
- An easier approach is to simply include or exclude a suspect's DNA profile from the crime scene mixture profile. If all of the alleles from a suspect's DNA profile are represented in the crime scene mixture, then the suspect cannot be excluded as contributing to the crime scene stain.
- Likewise, the alleles in a victim's DNA profile could be subtracted out of the mixture profile to simplify the alleles that need to be present in the perpetrator's DNA profile.

Software Programs Under Development for Mixture Deconvolution

- Linear Mixture Analysis (LMA)
  - Part of TrueAllele system developed by Mark Perlin and Cybergenetics
  - Perlin, M. W. and Szabady, B. (2001) Linear mixture analysis: a mathematical approach to resolving mixed DNA samples. *J.Forensic Sci.* 46(6): 1372-1378
- Least Squares Deconvolution (LSD)
  - Described by T. Wang (University of Tennessee) at Oct 2002 Promega meeting
  - Available for use at **https://lsd.lit.net/**
- PENDULUM
  - **Part of FSS i-3 software suite**
  - Bill, M., Gill, P., Curran, J., Clayton, T., Pinchin, R., Healy, M., and Buckleton, J. (2005) PENDULUM-a guideline-based approach to the interpretation of STR mixtures. *Forensic Sci.Int.* 148(2-3): 181-189

NIST Software Programs to Aid Mixture Work

Excel-based programs developed by David Duewer (NIST)

- **mixSTR** *(developed at request of Palm Beach Sheriff's Office)*
  - Does not interpret data (relies on user inputted alleles following STR data review)
  - Aids in the organization of STR mixture information
  - Considers only the presence/absence of alleles (no peak heights used)
- **Virtual MixtureMaker** *(developed to aid MIX05 sample selection)*
  - Creates mixture combinations through pairwise comparisons of input STR profiles
  - Returns information on the number of loci possessing 0,1,2,3,4,5, or 6 alleles in each 2-person mixture (also reports number of loci in each sample with 0,1,2, or 3 alleles)
  - Useful for selection of samples in mixture or validation studies with various degrees of overlapping alleles in combined STR profiles
  - Useful in checking for potentially related individuals in a population database

Programs can be downloaded from NIST STRBase web site:
http://www.cstl.nist.gov/div831/strbase/software.htm

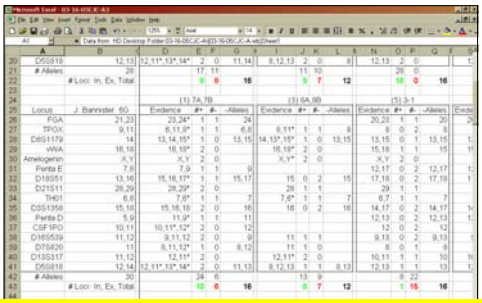
mixSTR Program

Comparisons are made between

- suspect and evidence (S/E) alleles,
- suspect and suspect (S/S) alleles **(to look for potential close relatives),**
- evidence and other evidence (E/E) sample(s) alleles **(to see how various evidentiary samples compare to one another),** and
- controls to evidence (C/E) and controls to suspect (C/S) alleles **(as a quality control contamination check).**

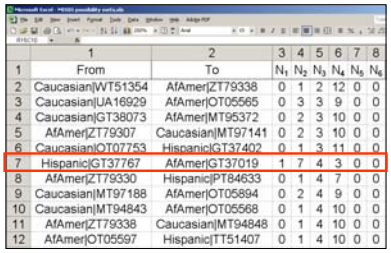
mixSTR S/E output

Data from Palm Beach County Sheriff's Office Case Supplied by Catherine Cothran



Example of suspect to evidence (S/E) comparisons made in this case. Note that the suspect is 21,23 at FGA while the evidence contains 23,24* (* indicates that allele 24 is a minor component). Thus this suspect has allele 23 in common and is missing allele 24 in the evidence.

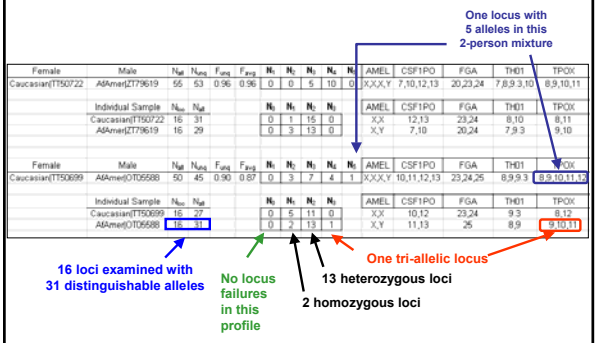
Virtual MixtureMaker Output



	1	2	3	4	5	6	7	8
1	From	To	N_1	N_2	N_3	N_4	N_5	N_6
2	Caucasian|WT51354	AfAmer|ZT79338	0	1	2	12	0	0
3	Caucasian|JA16929	AfAmer|OT05565	0	3	3	9	0	0
4	Caucasian|GT38073	AfAmer|MT95372	0	2	3	10	0	0
5	AfAmer|ZT79307	Caucasian|MT97141	0	2	3	10	0	0
6	Caucasian|OT07753	Hispanic|GT37402	0	1	3	11	0	0
7	Hispanic|GT37767	AfAmer|GT37019	1	7	4	3	0	0
8	AfAmer|ZT79330	Hispanic|PT84633	0	1	4	7	0	0
9	Caucasian|MT97188	AfAmer|OT05894	0	2	4	9	0	0
10	Caucasian|MT94843	AfAmer|OT05568	0	1	4	10	0	0
11	AfAmer|ZT79338	Caucasian|MT94848	0	1	4	10	0	0
12	AfAmer|OT05597	Hispanic|TT51407	0	1	4	10	0	0

When the STR profiles for these two individuals are combined to create a 2-person mixture, the mixture profile will contain 1 locus with a single allele, 7 loci with two alleles, 4 loci with three alleles, and 3 loci with four alleles (and no loci with 5 or 6 alleles, which is only possible if one or both samples possess tri-allelic patterns at the same STR locus).

Virtual MixtureMaker Output



Conclusions

- We plan to develop training information based on lessons learned from the MIX05 study.
- We intend to create other useful software tools like ***mixSTR*** and ***Virtual MixtureMaker*** to increase mixture interpretation capabilities of the forensic DNA typing community.

Some Final Thoughts…

- It is of the highest importance in the art of detection to be able to recognize out of a number of facts, which are incidental and which vital. Otherwise your energy and attention must be dissipated instead of being concentrated (Sherlock Holmes, *The Reigate Puzzle*).
- "**Don't do mixture interpretation unless you have to**" (Peter Gill, Forensic Science Service, 1998).

Acknowledgments

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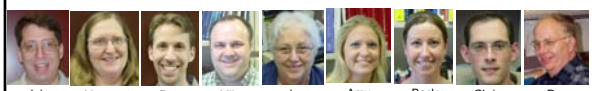

John Butler | Margaret Kline | Pete Vallone | Mike Coble | Jan Redman | Amy Decker | Becky Hill | Chris DeAngelis | Dave Duewer

Role in MIX05

- Margaret Kline (sample prep, running study)
- John Butler (study design and data review)
- Becky Hill (GeneMapper*ID* data review)
- Jan Redman (Access database entry, shipping)
- Dave Duewer (*Virtual MixtureMaker* to aid sample selection; **mixSTR program**)
- Chris Tomsey & Frank Krist (FMBIO Mac data)
- Kermit Channel & Mary Robnett (FMBIO NT data)

The many forensic scientists and their supervisors who took time out of their busy schedules to examine the MIX05 data provided as part of this interlaboratory study