DNA Interpretation Workshop 1

Mixture Examples:
Clayton et al. 1998 rules

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ISFG Pre-Conference Workshop
Melbourne, Australia
September 2-3, 2013

Presentation Outline

- History & Background on DNA Mixtures
- ISFG 2006 Recommendations
- Clayton et al. 1998 Steps
- Examples
- SWGDAM Guidelines

Final version of this presentation will be available at:
http://www.cstl.nist.gov/strbase/NISTpub.htm

A Brief History of DNA Mixtures (1)

- **1991**– Ian Evett article (with single-locus RFLP probes)
- **1995** – Mixtures presented in OJ Simpson trial
- **1996** – 9plex STR kits (Profiler Plus, PowerPlex 1.1)
- **1997** – Weir et al using Likelihood Ratios (LRs) for mixture statistics
- **1998** – **Clayton et al (FSS) DNA mixture deconvolution**
- **2000** – initial SWGDAM Interpretation Guidelines published
- **2000** – Combined Probability of Inclusion (CPI) statistic is allowed by DNA Advisory Board and pushed by the FBI
- **2000** – 16plex STR kits (PP16 and Identifiler)
- **2005** – NIST Interlaboratory Mixture Study **(MIX05) finds extensive variation in laboratory approaches**

A Brief History of DNA Mixtures (2)

- **2006** – ISFG Mixture Recommendations published emphasizing that LRs are a better method over CPI
- **2007** – informal SWGDAM study finds most labs doing 2-person mixtures (committee begins writing guidelines)
- **2008** – NIJ study shows value of DNA in burglary cases and more touch DNA samples with complex mixtures begin being processed
- **2010** – SWGDAM Interpretation Guidelines emphasize need for statistics and stochastic thresholds with CPI; probabilistic genotyping approach is mentioned
- **2012** – ISFG publishes LR with probability of dropout to cope with potential of allele dropout
- ***Present*** – a number of software programs exist to help with calculations but no universal approach exists

Statistical Approaches with Mixtures

See Ladd *et al.* (2001) *Croat Med J.* 42:244-246; SWGDAM (2010) section 5

1. **Random Match Probability (after inferring genotypes of contributors)** – Separate major and minor components into individual profiles and compute the random match probability estimate as if a component was from a single source

2. **Combined Probability of Exclusion/Inclusion** – CPE/CPI (RMNE) – Calculation of the probability that a random (unrelated) person would be excluded/included as a contributor to the observed DNA mixture

   RMNE = Random Man Not Excluded (same as CPI)
   CPE = Combined Probability of Exclusion (CPE = 1 – CPI)
   CPI = Combined Probability of Inclusion (CPI = 1 – CPE)

3. **Likelihood Ratio (LR)** – Compares the probability of observing the mixture data under two alternative hypotheses; in its simplest form LR = 1/RMP

$$LR = \frac{\Pr(E \mid H_1)}{\Pr(E \mid H_2)}$$

DAB Recommendations on Statistics

February 23, 2000
Forensic Sci. Comm. 2(3); available on-line at
http://www.fbi.gov/hq/lab/fsc/backissu/july2000/dnastat.htm

"The DAB finds either one or both PE or LR calculations acceptable and strongly recommends that one or both calculations be carried out whenever feasible and a mixture is indicated"

- Probability of exclusion (PE)
  - Devlin, B. (1993) Forensic inference from genetic markers. *Statistical Methods in Medical Research*, 2, 241–262.
- Likelihood ratios (LR)
  - Evett, I. W. and Weir, B. S. (1998) *Interpreting DNA Evidence*. Sinauer, Sunderland, Massachusetts.

NIST Interlaboratory Studies on Mixtures

- 1997 - Mixed Stain Study 1 (MSS1)
- 1999 – MSS2
- 2001 – MSS3 (five 2-person and one 3-person mixture)
- **2005 – MIX05** (supplied data only with four 2-person mixtures)
- **2013 – another study to evaluate current variation in mixture interpretation**

Download .fsa data files from (5 case scenarios):
http://www.cstl.nist.gov/strbase/interlab/MIX13.htm

The Forensic Institute

Forensic e-Symposium™
The Forensic Institute

April 14, 2005

"If you show 10 colleagues a mixture, you will probably end up with 10 different answers."
- Dr. Peter Gill

"Don't do mixture interpretation unless you have to"
- Dr. Peter Gill (1998)

Elements of DNA Mixture Interpretation

Principles (theory)
↓
Protocols (validation)
↓
Practice (training & experience)

Consistency across analysts

ISFG Recommendations
SWGDAM Guidelines
⇣
Your Laboratory SOPs
⇕
Training within Your Laboratory

Periodic training will aid accuracy and efficiency within your laboratory.

Available for download from the ISFG Website:
http://www.isfg.org/Publication;Gill2006


Available online at www.sciencedirect.com
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Forensic Science International 160 (2006) 90–101
Forensic Science International
www.elsevier.com/locate/forsciint

DNA commission of the International Society of Forensic Genetics: Recommendations on the interpretation of mixtures

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Our discussions have highlighted a significant need for continuing education and research into this area.

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Received 4 April 2006; accepted 10 April 2006
Available online 5 June 2006

Gill *et al.* (2006) DNA Commission of the International Society of Forensic Genetics: Recommendations on the interpretation of mixtures. *Forensic Sci. Int.* 160: 90-101

Responses to ISFG DNA Commission Mixture Recommendations

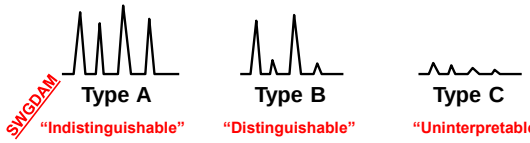
- UK Response
  - Gill *et al.* (2008) *FSI Genetics* 2(1): 76–82
- **German Stain Commission**
  - Schneider *et al.* (2006) *Rechtsmedizin* 16:401-404 (German version)
  - Schneider *et al.* (2009) *Int. J. Legal Med.* 123: 1-5 (English version)
- ENFSI Policy Statement
  - Morling *et al.* (2007) *FSI Genetics* 1(3):291–292
- New Zealand/Australia Support Statement
  - Stringer *et al.* (2009) *FSI Genetics* 3(2):144-145
- **SWGDAM** – Interpretation Guidelines
  - Approved Jan 2010 and released April 2010 on FBI website

German Mixture Classification Scheme

Schneider *et al.* (2009) *Int. J. Legal Med.* 123: 1-5

(German Stain Commission, 2006):

- **Type A**: no obvious major contributor, no evidence of stochastic effects
- **Type B**: clearly distinguishable major and minor contributors; consistent peak height ratios of ***approximately 4:1*** (major to minor component) for all heterozygous systems, no stochastic effects
- **Type C**: mixtures without major contributor(s), evidence for stochastic effects



ISFG Recommendations on Mixture Interpretation

http://www.isfg.org/Publication;Gill2006

1. The likelihood ratio (LR) is the preferred statistical method for mixtures over RMNE
2. Scientists should be trained in and use LRs
3. Methods to calculate LRs of mixtures are cited
4. Follow Clayton et al. (1998) guidelines when deducing component genotypes
5. Prosecution determines H_p and defense determines H_d and multiple propositions may be evaluated
6. When minor alleles are the same size as stutters of major alleles, then they are indistinguishable
7. Allele dropout to explain evidence can only be used with low signal data
8. No statistical interpretation should be performed on alleles below threshold
9. Stochastic effects limit usefulness of heterozygote balance and mixture proportion estimates with low level DNA

Gill *et al.* (2006) DNA Commission of the International Society of Forensic Genetics: Recommendations on the interpretation of mixtures. *Forensic Sci. Int.* 160: 90-101

Steps in the Interpretation of Mixtures
(Clayton *et al.* 1998)

Step #1 Identify the Presence of a Mixture

Step #2 Designate Allele Peaks

Step #3 Identify the Number of Potential Contributors

Step #4 Estimate the Relative Ratio of the Individuals Contributing to the Mixture

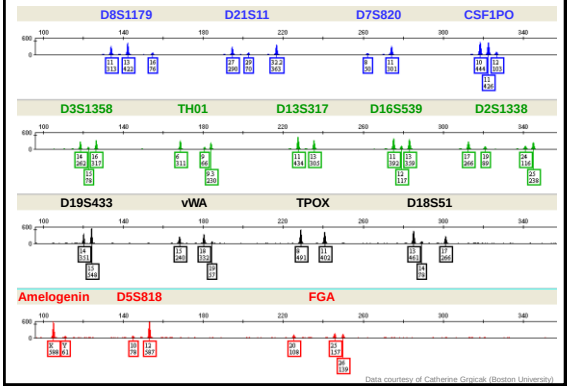
Step #5 Consider All Possible Genotype Combinations

Step #6 Compare Reference Samples

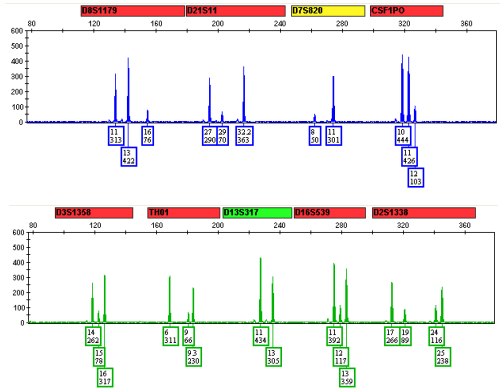

Analysis and interpretation of mixed forensic stains using DNA STR profiling

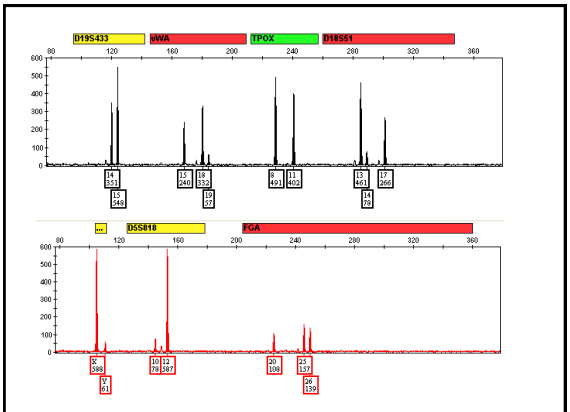
Clayton *et al.* (1998) *Forensic Sci. Int.* 91:55-70

Example Mixture (Identifiler data)



Data courtesy of Catherine Grgicak (Boston University)





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

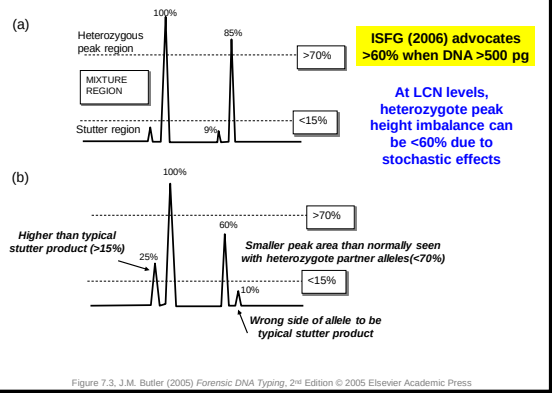
- Examine the number of peaks present in a locus
  - More than 2 peaks at a locus (except for tri-allelic patterns at perhaps one of the loci examined)
- Examine relative peak heights
  - Heterozygote peak imbalance <60%
  - Peak at stutter position >15%
- Consider all loci tested

Is a DNA Profile Consistent with Being a Mixture?

From J.M. Butler (2005) *Forensic DNA Typing, 2nd Edition*, pp. 156-157

If the answer to any one of the following three questions is yes, then the DNA profile may very well have resulted from a mixed sample:

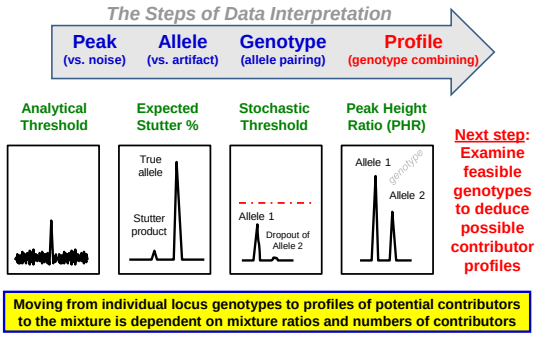
- Do any of the loci show more than two peaks in the expected allele size range?
- Is there a severe peak height imbalance between heterozygous alleles at a locus?
- Does the stutter product appear abnormally high (e.g., >15-20%)?



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

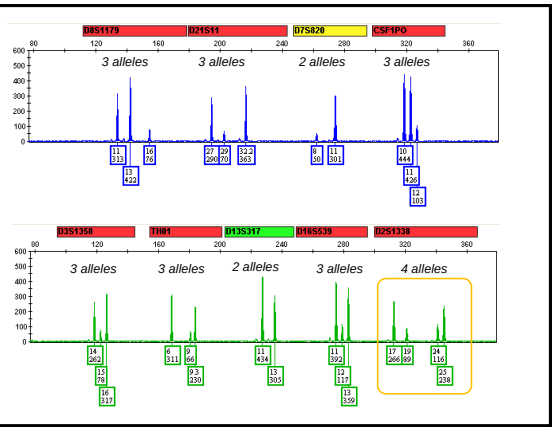
Data Interpretation Steps



Moving from individual locus genotypes to profiles of potential contributors to the mixture is dependent on mixture ratios and numbers of 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



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

David R. Paoletti,[1] M.S.; Travis E. Doom,[1,2] Ph.D.; Carissa M. Krane,[3] Ph.D.; Michael L. Raymer,[1,2] Ph.D.; and Dan E. Krane,[4] Ph.D.

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)

Follow-on Article by Buckleton *et al.*

ELSEVIER
Available online at www.sciencedirect.com
ScienceDirect
Forensic Science International: Genetics 1 (2007) 20–28
FSI GENETICS
www.elsevier.com/locate/fsig

Towards understanding the effect of uncertainty in the number of contributors to DNA stains

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Received 31 May 2006; received in revised form 12 September 2006; accepted 13 September 2006

Abstract

DNA evidence recovered from a scene or collected in relation to a case is generally declared as a mixture when more than two alleles are observed at several loci. However, in principle, all DNA profiles may be considered to be potentially mixtures, even those that show not more than two alleles at any locus. When using a likelihood ratio approach to the interpretation of mixed DNA profiles it is necessary to postulate the number of potential contributors. However, this number is never known with certainty. The possibility of a, say three-person mixture, presenting four or fewer peaks at each locus of the CODIS set was explored by Paoletti et al. [D.R. Paoletti, T.E. Doom, C.M. Krane, M.L. Raymer, D.E. Krane, Empirical analysis of the STR profiles resulting from conceptual mixtures, J. Forensic Sci. 50 (2005) 1361–1366]. In this work we extend this analysis to consider the profiler plus and SGM plus multiplices. We begin the assessment of the risk associated with current practice in the calculation of LR's. We open the discussion of possible ways to surmount this ambiguity.

Two-Person Mixtures for Simulated Profiles:

Probability by Locus of A Particular Number of Alleles Being Observed

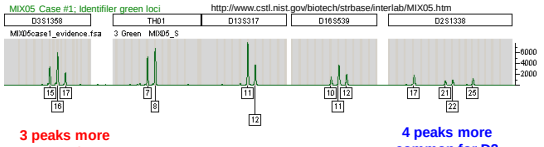
Table 1
The probability of observing a given number of alleles in a two-person mixtures for simulated profiles at the SGM^{+TM} loci

Loci	No. of alleles			
	1	2	3	4
D3	0.011	0.240	0.559	0.190
vWA	0.008	0.194	0.548	0.250
D16	0.016	0.287	0.533	0.164
D2	0.003	0.094	0.462	0.441
D8	0.011	0.194	0.521	0.274
D21	0.007	0.147	0.505	0.341
D18	0.003	0.095	0.472	0.430
D19	0.020	0.261	0.516	0.203
THO	0.016	0.271	0.547	0.166
FGA	0.003	0.116	0.500	0.381

Buckleton et al. (2007) Towards understanding the effect of uncertainty in the number of contributors to DNA stains. *FSI Genetics* 1:20-28

Levels of Locus Heterozygosity Impact Number of Alleles Observed in Mixtures

Loci	No. of alleles			
	1	2	3	4
D3	0.011	0.240	0.559	0.190
vWA	0.008	0.194	0.548	0.250
D16	0.016	0.287	0.533	0.164
D2	0.003	0.094	0.462	0.441



3 peaks more common for D3

4 peaks more common for D2

Three-Person Mixtures for Simulated Profiles:

Probability by Locus of A Particular Number of Alleles Being Observed

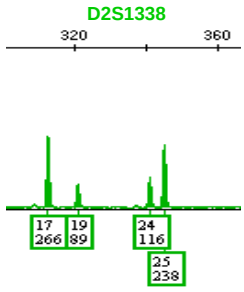
Table 2
The probability of observing a given number of alleles in a three-person mixtures for simulated profiles at the SGM^{+TM} loci

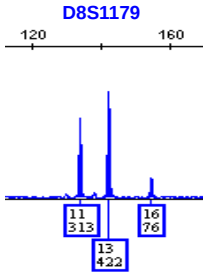
Loci	No. of alleles showing					
	1	2	3	4	5	6
D3	0.000	0.053	0.366	0.463	0.115	0.002
vWA	0.000	0.037	0.285	0.468	0.194	0.016
D16	0.001	0.086	0.397	0.411	0.100	0.005
D2	0.000	0.008	0.104	0.385	0.393	0.110
D8	0.001	0.041	0.258	0.436	0.236	0.029
D21	0.000	0.023	0.192	0.428	0.302	0.055
D18	0.000	0.007	0.109	0.392	0.396	0.096
D19	0.003	0.078	0.352	0.401	0.152	0.014
THO	0.001	0.074	0.395	0.439	0.088	0.002
FGA	0.000	0.012	0.144	0.424	0.346	0.074

Buckleton et al. (2007) Towards understanding the effect of uncertainty in the number of contributors to DNA stains. *FSI Genetics* 1:20-28

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…





Step #5: Consider All Possible Genotype Combinations

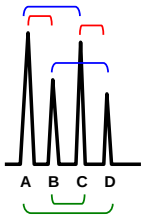
Table 3
Pairwise combinations of two, three and four alleles

Four alleles (a,b,c,d)		Three alleles (a,b,c)		Two alleles (a,b)	
a,b	c,d	a,a	b,c	a,a	a,b
a,c	b,d	b,b	a,c	a,b	a,b
a,d	b,c	c,c	a,b	a,a	b,b
c,d	**a,b**	a,b	a,c	a,b	b,b
b,d	**a,c**	b,c	a,c	**a,b**	**a,a**
b,c	**a,d**	a,b	b,c	**b,b**	**a,a**
		b,c	**a,a**	**b,b**	**a,b**
		a,c	**b,b**		
		a,b	**c,c**		
		a,c	**a,b**		
		a,c	**b,c**		
		b,c	**a,b**		

Key: bold entries represent reciprocal combinations.

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

Considering Genotype Combinations



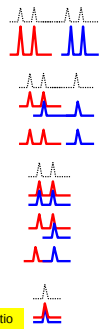
AC
BD
AB
CD
BC
AD

Depends on PHR

Peak Height Ratios (PHR)
Minimum Peak Height (mPH)
Proportion (p) or mixture proportion (M_x)

Possible Genotype Combinations

See Butler, J.M. (2005) *Forensic DNA Typing, 2nd Edition*, pp. 156-157



Four Peaks **(4 allele loci)**
- heterozygote + heterozygote, no overlapping alleles (genotypes are unique)

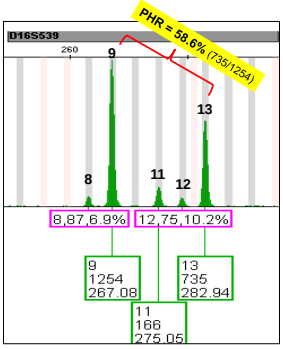
Three Peaks **(3 allele loci)**
- heterozygote + heterozygote, one overlapping allele
- heterozygote + homozygote, no overlapping alleles (genotypes are unique)

Two Peaks **(2 allele loci)**
- heterozygote + heterozygote, two overlapping alleles (genotypes are identical)
- heterozygote + homozygote, one overlapping allele
- homozygote + homozygote, no overlapping alleles (genotypes are unique)

Single Peak **(1 allele loci)**
- homozygote + homozygote, overlapping allele (genotypes are identical)

May also have to consider the stutter position(s) depending on the mixture ratio

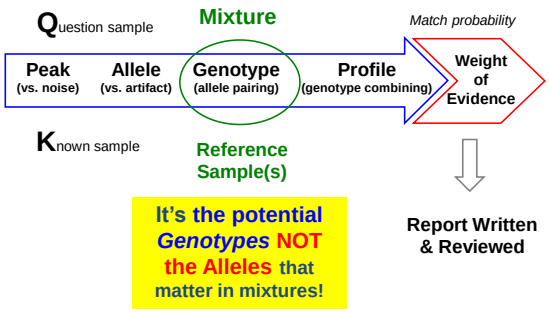
Example (a different profile): D16S539



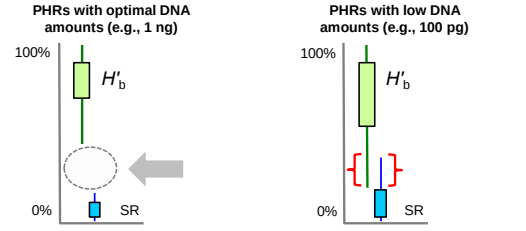
PHR = peak height ratio; also known as heterozygote balance (Hb)

Some Observations:
- Depending on expected PHR, alleles 9 and 13 may or may not be associated into a genotype (<60%)
- Allele 11 could be paired with 8, 9, 12, or 13 or itself (11,11 homozygote) depending on stochastic threshold
- Alleles 8 and 12 could be stutter products or possibly be paired with allele 11

Steps in DNA Interpretation



Impact of Template DNA Amount on Variation in Peak Height Ratio



This gap between the stutter ratio (SR) and the heterozygote balance (H_b) is what enables mixture deconvolution through assuming restricted genotype combinations

The overlap that occurs between H'_b and SR with low level DNA (e.g., minor components in mixture results) creates greater uncertainty in reliably associating alleles into genotypes

ISFG (2006) Table 2

Table 2
Assessment of major (*ab*)/minor (*cd*) genotypes of a mixture of two contributors relative to $\hat{M}_x$ and H_b calculated using $\phi_a = 1200$ rfu, $\phi_b = 100$ rfu, $\phi_c = 400$ rfu, $\phi_d = 380$ rfu, where rfu is relative fluorescence units (allele peak height)

Genotypes		M_x|major, minor genotypes	Heterozygous balance		Comment
Major	Minor		$H_{b\ major}$	$H_{b\ minor}$	
ab	*cd*	0.70	0.9	0.9	Passes H_b, $\hat{M}_x$
ac	*bd*	0.53	0.3	0.3	Fails H_b
ad	*bc*	0.51	0.3	0.3	Fails H_b
cd	*ab*	0.30	0.9	0.9	Fails $\hat{M}_x$
bd	*ac*	0.48	0.3	0.3	Fails H_b
bc	*ad*	0.49	0.3	0.3	Fails H_b

Gill *et al.* (2006) DNA Commission of the International Society of Forensic Genetics: Recommendations on the interpretation of mixtures. *Forensic Sci. Int.* 160: 90-101

The Defense Hypothesis will include all possible combinations

Table 1
Evaluation of $\Pr(E|H_d)$; two person mixture with four discrete alleles present

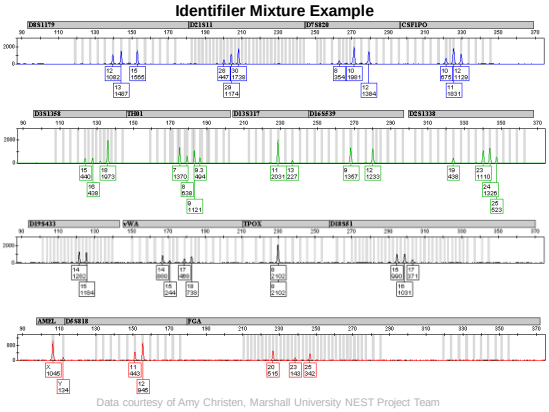
Individual 1	Individual 2	Genotype probability
ab	*cd*	$4p_ap_bp_cp_d$
ac	*bd*	$4p_ap_bp_cp_d$
ad	*bc*	$4p_ap_bp_cp_d$
cd	*ab*	$4p_ap_bp_cp_d$
bd	*ac*	$4p_ap_bp_cp_d$
bc	*ad*	$4p_ap_bp_cp_d$
Sum		$24p_ap_bp_cp_d$

Gill *et al.* (2006) DNA Commission of the International Society of Forensic Genetics: Recommendations on the interpretation of mixtures. *Forensic Sci. Int.* 160: 90-101

Step #6: Compare Reference Samples

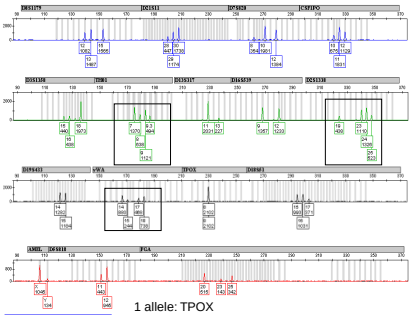
- If there is a suspect, a laboratory must ultimately decide to include or exclude him…
- **If no suspect is available for comparison, does your laboratory still work the case?** (Isn't this a primary purpose of the national DNA database?)
- Victim samples can be helpful to eliminate their allele contributions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

Identifiler Mixture Example



Data courtesy of Amy Christen, Marshall University NEST Project Team

Profile Overview



Evaluation Notes:

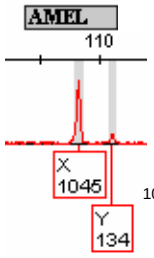
1. Loci seen with 1,2,3,&4 alleles (**a mixture** with at least 2 contributors)
2. Imbalance at amelogenin (female & male mixture with female as major)
3. Decent overall signal with D8 in ~1500 RFU (out of stochastic range)
4. Large MW loci have decent signal with D18 in ~1000 RFU range (degradation unlikely)
5. Ratio of **major to minor around 3:1** (from amelogenin X/Y ratios)

1045/134 = 7.8
~3 female (X,X):
1 male (X,Y)

1 allele: TPOX
2 alleles: D19, D5, D13, D16
3 alleles: D8, D21, D7, CSF, D3, D18, FGA
4 alleles: TH01, D2, VWA

Amelogenin Ratio

In many cases, amelogenin provides a helpful guide to assessing the mixture ratio



Female/Male ratio = X:X / X:Y

X/3 = 1045/3 = 348

348/134 = 2.6 (closest to 3 parts female to 1 part male)

1045/134 = 7.80

Chart of Expected Ratios

F:M	Chr ratio
1:1	3X:1Y
2:1	5X:1Y
3:1	7X:1Y
4:1	9X:1Y

1045/134 = 7.8
~3 female (X,X):
1 male (X,Y)

Potential problems with X or Y amplicon deletions

Anomalous Amelogenin Alleles

http://www.cstl.nist.gov/biotech/strbase/Amelogenin.htm

- **Males possessing only a single X amelogenin amplicon (Y null)** - *a male DNA sample will falsely look like a female DNA sample*:
  - Santos et al. (1998) reported a rare deletion of the amelogenin gene on the Y-chromosome
  - Y-STR typing can be performed to verify that other portions of the Y-chromosome are present
- **Males possessing only a single Y amelogenin amplicon (X null)**:
  - Shewale et al. (2000) observed loss of the X chromosome amplicon in three our of almost 7,000 males examined
  - while this phenomenon should not result in a gender misclassification (as the Y null situation might), its occurrence can impact the expected X and Y amplicon ratios in a mixture (see NIST MIX05 interlab study, case #3)

Running reference samples from suspect and/or victim may help discover potential amelogenin anomalies

Population Database Used for STR Allele Frequencies

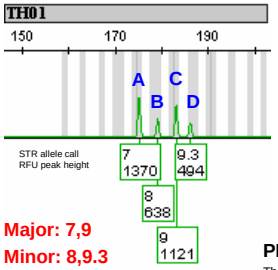
- U.S. population data contained in J.M. Butler (2005) *Forensic DNA Typing, 2nd Edition*, Appendix II (pp. 577-583)
- Published in Butler *et al.* (2003) *J. Forensic Sci.* 48(4): 908-911
- Available at http://www.cstl.nist.gov/strbase/NISTpop.htm
- Will focus on Caucasians for simplicity

TH01

Allele	Caucasian N = 302	African-American N = 258	Hispanic N = 140
5	0.00166*	0.00388*	
6	0.23179	0.12403	0.21429
7	0.19040	0.42054	0.27857
8	0.08444	0.19380	0.09643
9	0.11424	0.15116	0.15000
9.3	0.36755	0.10465	0.24643
10	0.00828	0.00194*	0.01429*
11	0.00166*		

Remember that different population databases will have different allele frequencies because they are based on different samples

4 Allele Locus: TH01 — Stats



Allele	Frequency
7	0.190
8	0.084
9	0.114
9.3	0.368

$PI = (P_A + P_B + P_C + P_D)^2$

$= (0.190 + 0.084 + 0.114 + 0.368)^2$

$= (0.756)^2$

$= 0.572$

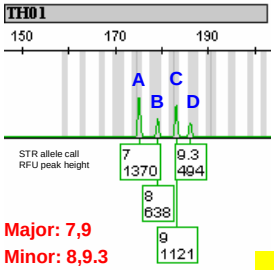
Major: 7,9
Minor: 8,9.3

PE = 1 – PI = 1 – 0.572 = 0.428

Thus ~43% of Caucasian population can be excluded from contributing to this mixture (primarily because allele 6 is missing)

Four Peaks **(4 allele loci)**
heterozygote + heterozygote, no overlapping alleles (genotypes are unique)

4 Allele Locus: TH01 — PHRs



Consider all possible combinations:

B/A = 638/1370 = 0.466

B/C = 638/1121 = 0.569

C/A = 1121/1370 = 0.818 **major**

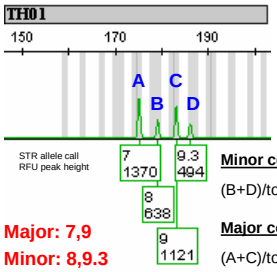
D/B = 494/648 = 0.774 **minor**

Major: 7,9
Minor: 8,9.3

All other combinations <0.60
(60% heterozygote Peak Height Ratio)

Four Peaks **(4 allele loci)**
heterozygote + heterozygote, no overlapping alleles (genotypes are unique)

4 Allele Locus: TH01 — Mix Ratio



Total of all peak heights
= 1370 + 638 + 1121 + 494
= 3623 RFUs

Minor component:
(B+D)/total = (638+494)/3623 = **0.312**

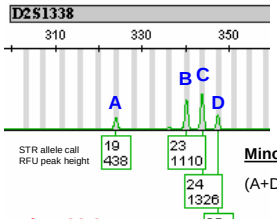
Major component:
(A+C)/total = (1370+1121)/3623 = **0.688**

Major: 7,9
Minor: 8,9.3

Close to the ~3:1 predicted by amelogenin X/Y allele ratio – thus major component = female

Four Peaks **(4 allele loci)**
heterozygote + heterozygote, no overlapping alleles (genotypes are unique)

4 Allele Locus: D2S1338 — Mix Ratio



Total of all peak heights
= 438 + 1110 + 1326 + 523
= 3397 RFUs

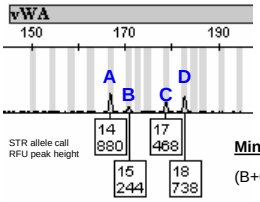
Minor component:
(A+D)/total = (438+523)/3397 = **0.283**

Major component:
(B+C)/total = (1110+1326)/3397 = **0.717**

Major: 23,24
Minor: 19,25

Four Peaks **(4 allele loci)**
heterozygote + heterozygote, no overlapping alleles (genotypes are unique)

4 Allele Locus: vWA — Mix Ratio



Total of all peak heights
= 880 + 244 + 468 + 738
= 2330 RFUs

Minor component:
(B+C)/total = (244+468)/2330 = **0.306**

Major component:
(A+D)/total = (880+738)/2330 = **0.694**

Major: 14,18
Minor: 15,17

Four Peaks **(4 allele loci)**
heterozygote + heterozygote, no overlapping alleles (genotypes are unique)

Forensic Sci. Int. 2005;148(2-3): 181-189

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PENDULUM—a guideline-based approach to the interpretation of STR mixtures

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Received 7 January 2004; received in revised form 28 May 2004; accepted 1 June 2004

J Forensic Sci. 2006; 51(6):1284-1297

J Forensic Sci. November 2006, Vol. 51, No. 6
doi:10.1111/j.1556-4029.2006.00268.x
Available online at: www.blackwell-synergy.com

Available for use over internet at **https://lsd.lit.net/**

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Least-Square Deconvolution: A Framework for Interpreting Short Tandem Repeat Mixtures*

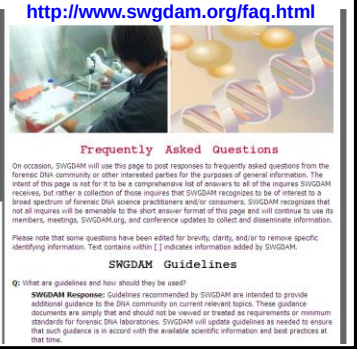
Overview of the SWGDAM 2010 Interp Guidelines

1. Preliminary evaluation of data – **is something a peak and is the analysis method working properly?**
2. Allele designation – **calling peaks as alleles**
3. Interpretation of DNA typing results – **using the allele information to make a determination about the sample**
   1. Non-allelic peaks
   2. Application of peak height thresholds to allelic peaks
   3. Peak height ratio
   4. Number of contributors to a DNA profile
   5. Interpretation of DNA typing results for mixed samples
   6. Comparison of DNA typing results
4. Statistical analysis of DNA typing results – **assessing the meaning (rarity) of a match**

Other supportive material: statistical formulae, references, and glossary

SWGDAM Website

http://www.swgdam.org/faq.html



Q: What are guidelines and how should they be used?

SWGDAM Response: Guidelines recommended by SWGDAM are intended to provide additional guidance to the DNA community on current relevant topics. These guidance documents are simply that and should not be viewed or treated as requirements or minimum standards for forensic DNA laboratories. SWGDAM will update guidelines as needed to ensure that such guidance is in accord with the available scientific information and best practices at that time.

http://www.swgdam.org/faq.html

Q: Within many of the SWGDAM guidelines the statement is made that these guidelines are not intended to be used retroactively. What is the intent of this "retroactive" statement?

SWGDAM Response: SWGDAM includes a "retroactive" statement with the intent that the revised guidance be applied prospectively and not retroactively. **With the underlying assumption that work (validation, training, analysis, interpretation) performed prior to the issuance of the revisions was appropriate and scientifically valid**, revision of the applicable guidelines is not intended to invalidate or call into question the previous work.

http://www.swgdam.org/faq.html

Q: Are the 2010 SWGDAM Interpretation Guidelines applicable to all DNA mixtures?

SWGDAM Response: **These guidelines were written with single-source samples and two-person mixtures in mind**, and are not intended to replace a laboratory's previously validated mixture interpretation guidelines and/or policy. The *basic concepts* outlined in the 2010 SWGDAM Mixture Interpretation Guidelines hold true as they relate to DNA mixtures of three or more contributors, low-level DNA samples, and mixtures containing biologically related individuals. However, **there are nuances and limitations to the interpretation of these more complex mixtures, which are not fully explored in the 2010 guidelines**. The Autosomal STR Interpretation Committee is tasked with reviewing and revising these SWGDAM guidelines. Laboratories are encouraged to perform additional validation studies of complex mixtures to further their understanding of the issues related to these challenging samples.

http://www.swgdam.org/faq.html

Many Labs are in the Process of Changing their Protocols

Perhaps lowering the expected peak height ratio (PHR) from 70% down to 55% when interpreting DNA mixtures?

Your Laboratory Interpretation Protocols should be developed from data

Standard Operating Procedures (SOPs)

Validation studies | **Literature** | **Experience**

SWGDAM Guidelines (2010) Introduction: *…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.*

Acknowledgments

$ National Institute of Justice and NIST OLES
John Buckleton and co-presenters

Slides and Discussions on DNA Mixtures
Mike Coble (NIST Applied Genetics Group)
Robin Cotton & Catherine Grgicak (Boston U.)
Bruce Heidebrecht (Maryland State Police)
Charlotte Word (consultant)

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Final version of this presentation will be available at:
http://www.cstl.nist.gov/strbase/NISTpub.htm