DNA Mixture Interpretation:
Principles and Practice in Component Deconvolution and Statistical Analysis

Testing of Mixture Software Programs

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NIST

Outline

- NEST work at Marshall University

- Some mixture deconvolution tools

- NIST experiments

NEST

- NIJ Expert Systems Testbed

- Evaluate Expert Systems
  - Single-source samples initially; then mixed specimens
  - GeneMapper™ *ID* Software v. 3.2 (GMID)
  - GMID-X
  - TrueAllele® System 2
  - FSS-i[3]

- Workshops and Training Sessions

- Summarize Features and Limitations

- http://forensics.marshall.edu

PENDULUM (i-STReam)

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

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- FSS-i[3]

- GMID output table required

- Primary purpose is to use the heterozygote balance and mixture proportion guidelines to eliminate unreasonable genotype combinations

Bill M, et al. PENDULUM: a guideline-based approach to the interpretation of STR mixtures. *Forensic Sci Int.* 2005:181-189.

i-STReam Calculations

Heterozygote balance:

$$Hb_3 = \frac{\phi_1}{\phi_2} \quad \boxed{0.6 < Hb < 1.66}$$

Mixture proportion (Mx):
The ratio of the major and minor contributors
Variance between loci ± 0.35

Step 1: List all of the possible genotype combinations without considering peak data

Step 2: Average Mx calculated for whole profile

Step 3: Genotypes evaluated on per locus basis with respect to Hb
0.6 < Hb < 1.66 are retained

Step 4: Mx calculated independently for all loci; must be within ± 0.35 of profile avg

Step 5: Only those genotypes that pass the Hb and Mx are listed as possible genotypes. If Multiple alleles possible, F designations

Step 6: Analyst reviews genotypes

Bill M, et al. PENDULUM: a guideline-based approach to the interpretation of STR mixtures. *Forensic Sci Int.* 2005:181-189.
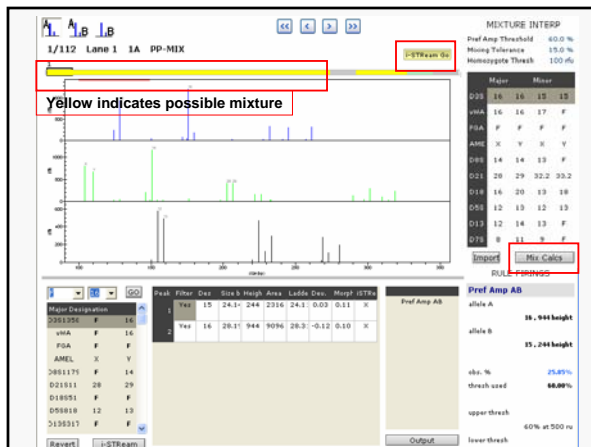

Yellow indicates possible mixture

i-STReam Summary Sheet

i-STRess vs. i-STReam

Least-Square Deconvolution

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

This study utilized the Web-LSD available at https://lsd.lit.net/

Wang T, Xue N, Birdwell J. Least-square deconvolution: a framework for interpreting short tandem repeat mixtures. *J Forensic Sci.* 2006:51(6):1284-1297.

Least-Square Deconvolution

- Considered a filter
  - Gives most likely genotype combinations

- Only inputs are allele designation and peak height/area

- Before LSD calculation, proper allele calls required
  - Input GMID output table or enter manually
  - no artifacts

- Looks at each locus separately

- Calculates best-fit mass proportions and error residuals for all possible genotype combinations

- LDS results reviewed by analyst who then applies heuristic guidelines to create final profiles

Wang T, Xue N, Birdwell J. Least-square deconvolution: a framework for interpreting short tandem repeat mixtures. *J Forensic Sci.* 2006:51(6):1284-1297.

LSD Output

- 3- and 4-allele loci:
  - Small fitting error
  - Mass ratio constant across all loci
    - Error ± 0.35
    - Subjective

- 2-allele loci:
  - Math begins to fail
  - Mass proportion ratio comparable
  - May need to keep all possibilities

LSD Final Output

Locus	Alleles in the mixture	Peak Data	Genotype of Person 1	Genotype of Person 2	Mass Ratio
D3S1358	3	13 3352 / 16 3660 / 17 2208	16,17	13,16	1.0:1.5
VWA	2	15 872 / 17 2834	15,15	17,17	1.0:3.2
FGA	4	19 1239 / 20 519 / 21 1513 / 22 508	20,22	19,21	1.0:2.8
Amelogenin	4	X 2272 / Y 469 / Y 1513 / Y 508	x,y	x,x	1.0:1.92
D8S1179	3	12 3372 / 14 3595 / 15 2757	15,15	12,14	1.0:2.5
D21S11	3	27 3092 / 28 1899 / 31.2 4333	28,31.2	27,31.2	1.0:1.7
D18S51	3	12 1525 / 15 1584 / 16 423	16,16	12,15	1.0:7.3
D5S818	1	11 3468	11,11	11,11	1:1
D13S317	2	11 7486 / 12 3645	11,11	11,12	1.0:1.9

Person 1 = minor contributor

Person 2 = major contributor

DNA_DataAnalysis

- U.S. Army Criminal Investigation Laboratory (USACIL)

- Developed by Tom Overson

- Mouse-driven program that was written in Visual Basic and runs in Microsoft Excel 2003

- NOT an expert system – DNA data interpretation tool to aid analysts
  - Check controls, ladders
  - Matching
  - Statistics
    - Frequency, LR, PI
  - Mixture Interpretation

- Requires proper allele calls and output table from GMID
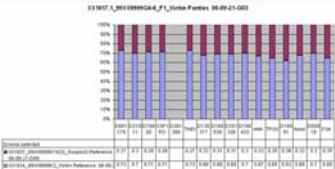
DNA_DataAnalysis: Mixture Calculations

- Mixture proportion (p)
  - Fairly stable across all loci in a sample

- Peak height ratio (phr)
  - Peak height ratios for a locus want to be one

List of possible genotype combinations without references applied

2 or 3 Component Mixture Interpretation Tool

Experiment 1 – MIX05 Data Mixture Deconvolution

Case #	Parts Female	Parts Male	Unique Profiles	Challenges
1	3	1	---	---
2	1	3	---	---
3	1	1	Male missing X (only Y)	Mixtures at or near 1:1 are hard to separate
4	7	1	Male trialletic at TPOX	Trialilele and low minor contributor

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Materials and Methods

- Several STR Kits:
  - SGM+, Profiler Plus, Identifiler, COfiler, Powerplex 16

- 3130xl

- Data already collected and profiles in GMID v3.2

- Mixture deconvolution tools:
  - i-STReam
  - LSD

i-STReam Results

1:1 ratio has lowest accuracy

Case 1
3 female : 1 male

Case 2
1 female : 3 male

Case 3
1 female : 1 male

Case 4
7 female : 1 male

1:3 ratios have highest accuracy

i-STReam stand-alone version

MIX05 i-STReam Results

- ☐ % Genotypes Called
- ■ % Accuracy

Percentage (y-axis: 0–110)
Case Number (x-axis: 1, 2, 3, 4, Overall)

Case #	Genotypes Called	Accuracy
1	74	100
2	82	100
3	69	83
4	68	98
Overall	73	95

LSD Results

MIX05 Least-Square Deconvolution Results

Case 2 best results

Case 3 worst results

4-allele loci most accurate

Overall 84% accurate

- ☐ Total
- ■ 2-Allele
- ☐ 3-Allele
- ☐ 4-Allele

Percent Called Correct (y-axis: 0–110)
Case Number (x-axis: 1, 2, 3, 4)

Case #	% Genotype Correct	% 2-Allele Correct	% 3-Allele Correct	% 4-Allele Correct
1	81	79	77	97
2	96	100	92	100
3	70	53	71	90
4	88	88	82	100

Experiment 2 – Replicates and Ratios
Mixture Deconvolution

Materials and Methods

- Identifiler, COfiler, Profiler Plus
  – Profiler Plus always had opposite major and minor contributor profiles compared to Identifiler and COfiler

- 1:2, 1:3, 1:5, and 1:8 mixture ratios
  – Created mixtures from population plates
  – 1:2 and 1:5 all male genotypes
  – 1:3 and 1:8 one female and one male

Mixture Ratio	Contributor	[DNA] (pg)
1:2	Major	667
	Minor	333
1:3	Major	750
	Minor	250
1:5	Major	833
	Minor	167
1:8	Major	889
	Minor	111

Materials and Methods

- 6-7 amplification replicates per ratio per kit
  – PCR variation

- How do the deconvolution tools handle this variation?
  – Different results for the same mixture?
  – Incorrect calls?

Some i-STReam Observations…

- GeneMapper ID filters set at zero to allow all alleles into FSS-i[3]
  – Some minor alleles filtered out as stutter and not called

Some i-STReam Observations…

- Some incorrect calls
  - Incorrect calls can be explained by variation in peak height ratios
  - 26 / 4080 alleles

- Very conservative
  - F designations allow the program to not make a definite allele call

Total i-STReam Results

Overall i-STReam Results from Replicate and Ratio Study

Total # Alleles	% 4-Allele	% 3-Allele	% 2-Allele	% 1-Allele
4080	25	33	38	4

i-STReam Results According to Mixture Ratio

i-STReam Results According to Mixture Ratio

- **1:2 ratio** worst results
56% called and correct allele calls

- **1:3 ratio** best results
78% called and correct allele calls

i-STReam Results According to STR Kit

i-STReam Replicate and Ratio Results According to STR Kit

Identifiler				
Total # Alleles	% 4.Allele	% 3.Allele	% 2.Allele	% 1.Allele
2952	29	33	33	5

Profiler Plus				
Total # Alleles	% 4.Allele	% 3.Allele	% 2.Allele	% 1.Allele
1000	35	25	35	5

COfiler				
Total # Alleles	% 4.Allele	% 3.Allele	% 2.Allele	% 1.Allele
728	0	43	57	0

PCR Variation and Incorrect Calls

- 26 incorrect calls out of 4080 alleles

- Plotted peak height ratios for replicates
  - According to kit and ratio
  - Amplification variation

Identifiler 1 : 2 Ratio

Genotypes:
Major: 11,11
Minor: 10,12

Most Common
i-STReam Call:
Major: F,F
Minor: F,F

Locus CSF

1a	1.3 : 2.8 : 1.0	
2a	1.5 : 3.1 : 1.0	
3a	1.4 : 3.4 : 1.0	
4a	1.7 : 3.6 : 1.0	10,11 11,12
5a	1.1 : 2.4 : 1.0	
6a	1.1 : 2.5 : 1.0	
7a	1.8 : 4.1 : 1.0	10,11 11,12

Replicate 1a

Incorrect call once 11 passes certain height threshold

Locus TH01

COfiler 1 : 5 Ratio

806 RFUs
7

123 RFUs
6

175

Replicate 3b

1b	1.0 : 5.2
2b	1.0 : 3.6
3b	1.0 : 6.6
4b	1.0 : 4.9
5b	1.0 : 11.3
6b	1.0 : 6.8
7b	1.0 : 6.1

Genotypes:
Major: 7,7
Minor: 6,7

7,7   6,6

Average Ratio:
1.0 : 6.4 ± 2.4

Most Common
i-STReam Call:
Major: 7,7
Minor: 6,F

Stutter Problems

- Stutter introduces problems, lower ratios (1:8)
  - Stutter peaks may be higher than allele peaks
  - Peak Heights:
    - 9 = 252 RFUs
    - 11 = 201 RFUs
    - 12 = 225 RFUs
- International Society of Forensic Genetics (ISFG) Recommendation:
  - When minor alleles same size as stutter, they are indistinguishable
  - Must include both in LR

10

Stutter   Minor alleles
9   11   12

320   330
size(bp)

Identifiler Locus CSF

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

Stutter Problems

- Stutter introduces problems, lower ratios (1:8)
  - If minor allele in stutter position, may cause heterozygote peak imbalance and genotype will not pass the pref amp rule (Hb)
  - F Designations

22

25

24

20

240

Peak Heights of Minor Alleles:
20 = 317 RFUs
24 = 589 RFUs

60% of allele 24 is 353 RFUs

Identifiler 1:8 Locus FGA

Conclusions

- LSD about 84% accuracy

- i-STReam above 95% accuracy

- DNA_DataAnalysis has more user interaction with data

- Amplification variability can lead to different and/or incorrect calls

- Only certain mixture ratios are solvable
    - Window of opportunity between 1:3 and <1:8
    - Influence calculations

- Optimization of program parameters very important
    - Filter settings, threshold settings, etc.

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