DNA Mixture Interpretation:

Principles and Practice in Component Deconvolution and Statistical Analysis

Validation Studies and Preparing Mixture Interpretation Guidelines

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Overview

- **Know Your System (KYS)**
- **Quantitation**
- **Degradation/Inhibition**
- **PCR**
- **STR-Artifacts vs. DNA**
- **Validation Studies- Precision, Sensitivity, Balance, Stutter and Mixture Ratios**
- **Intro to Statistics**

Validation Studies to Establish Parameters

- **Precision-Allele sizes, ranges and standard deviations.**
- **Sensitivity-minimum and maximum thresholds.**
- **Balance-Within and across loci. Allelic and locus drop out.**
- **Stutter-@ each locus, for each allele.**
- **Mixture Ratios for detection of minor.**

Precision

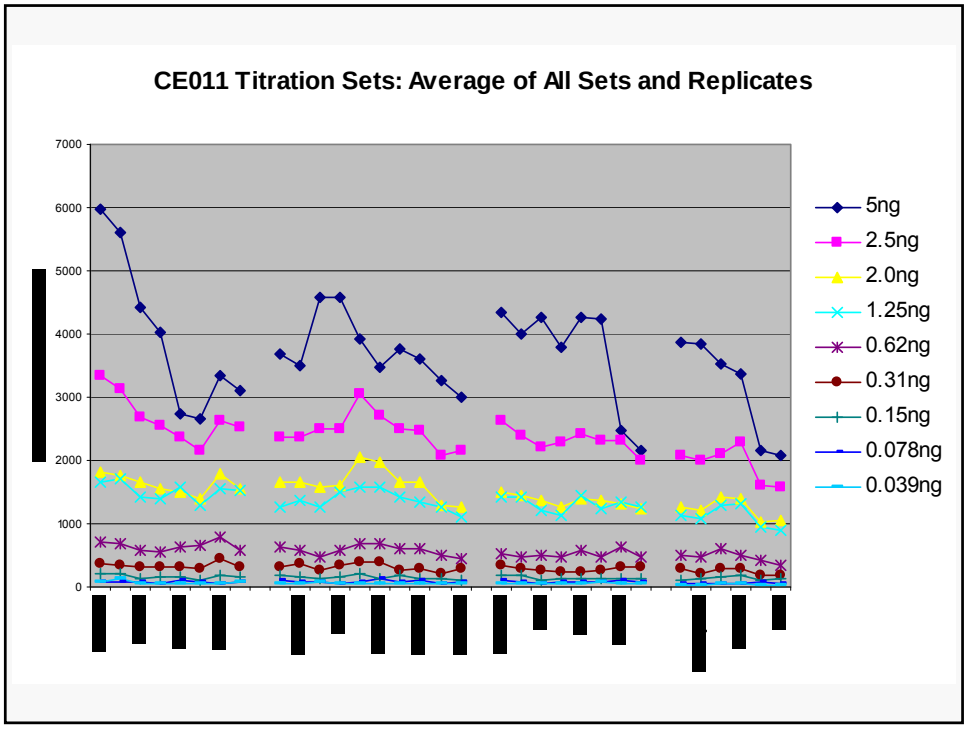
- Validation
  - Repeat injections of allelic ladder and positive control
  - Assess 250 bp peaks for all data within and across runs.
  - Calculate values for Min., Max., Average, SD, Range of +/- 3 SD and bin sizes.
- Guideline
  - Precision check of run data against established ranges for positive control and 250 bp for each instrument.
  - Allelic designations for variants must be made with caution at larger loci.
  - If in region of imprecision one may call with 22.X designation if 1 bp resolution cannot be established (e.g. 22.2 vs. 22.3).

SENSITIVITY

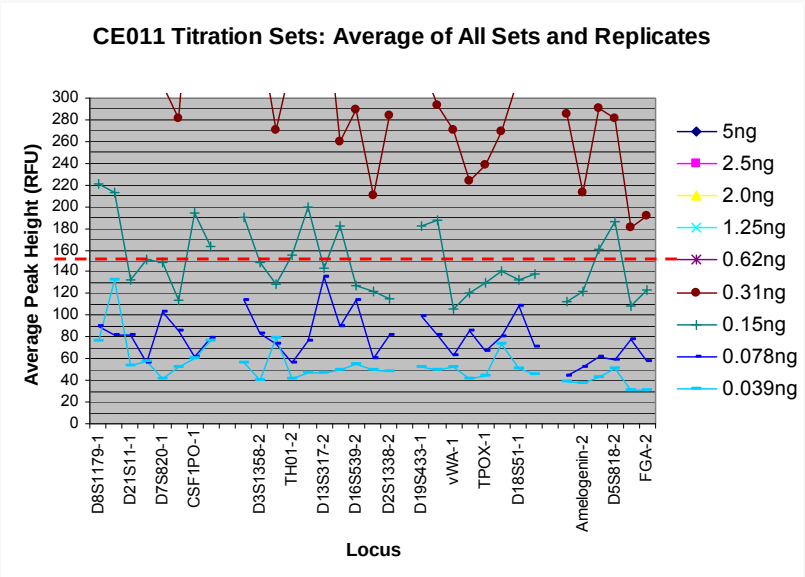
- **Minimum Threshold (Noise Threshold)**
  - **noise=baseline and background**
  - **Reporting threshold usually 3x noise level**
- **Maximum Threshold**
  - **Pull up, Nonspecific product, stutter, balance**
  - **Saturation effects of overloading the CCD camera can interfere with evaluation of stutter and other artifacts.**
  - **Usually at approximately 8100 rfu on CE instruments**
  - **Note that pull up occurs prior to full saturation (dependent on matrix/spectral)**

Sensitivity Studies and Thresholds

- Validation
  - Titration series of range of target DNA in ng (5, 2.5 ------- 0.039) -use @ least two sources
  - Evaluation of rfu values for all targets (both at low end and for high end saturation)
  - Evaluation of signal-to- noise
  - Noise comprised of baseline and background effects
- Guideline
  - Establish criteria for a reporting threshold (RT) and noise threshold (NT)
  - RT set to obtain a partial profile @ 0.15ng and to be at 3X of established noise
  - Peaks above RT have met criteria to be given an allele designation and accompanying statistic (to type 0.25 ng of DNA)

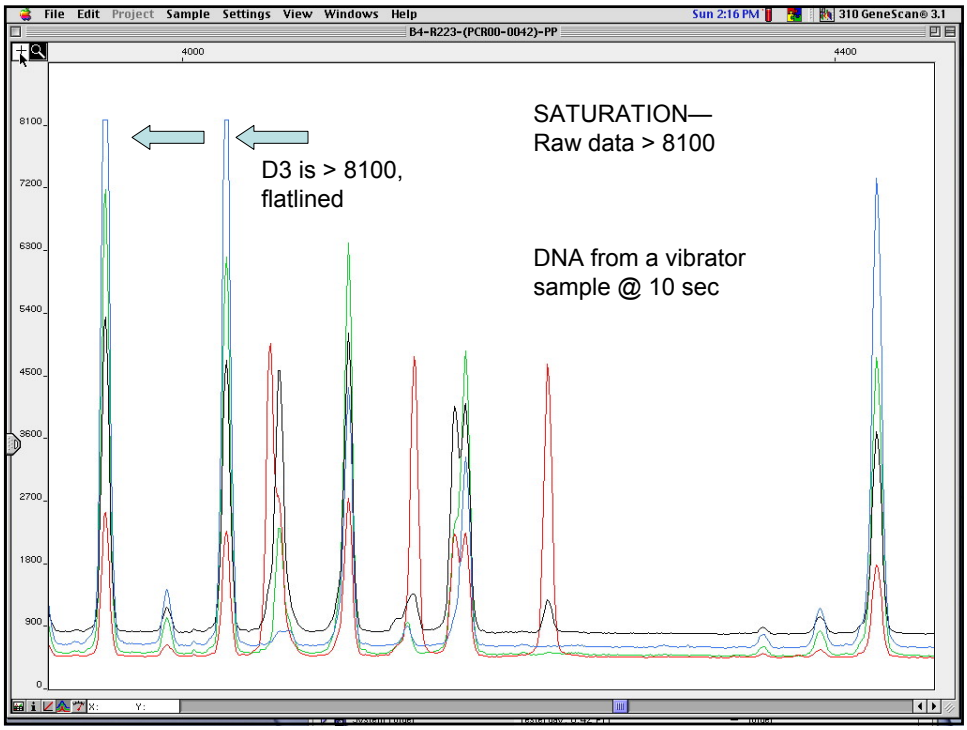


Reporting Threshold set at Red Line for partial profile @ 0.15ng & 3X noise (baseline + background)

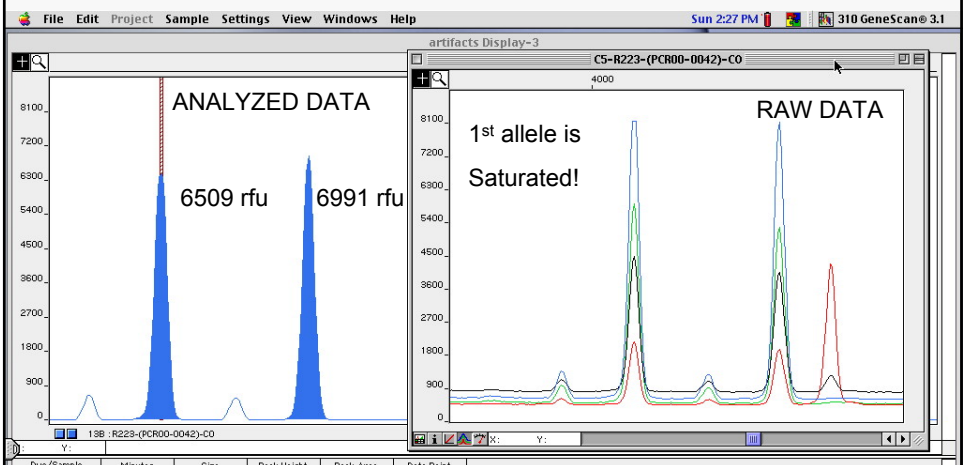


Epg of noise

Link to Noise PDF



Saturated Data- Maximum Thresholds



Dye/Sample Peak	Minutes	Size	Peak Height	Peak Area	Data Point
13B, 1	12.14	55.37	83	596	3309
13B, 2	12.16	55.80	90	1034	3314
13B, 3	14.61	123.66	666	4458	3984
13B, 4	14.75	127.77	6509	51340	4022
13B, 5	14.89	131.91	593	4128	4060
13B, 6	15.03	136.10	6991	50041	4098
13B, 7	18.65	249.36	153	1480	5085
13B, 8	18.77	253.39	2975	32396	5119

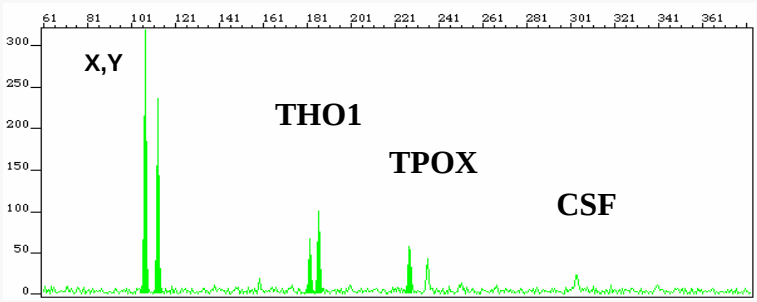
Analyzed data versus Raw Data-

Peak heights may not be illustrative of raw data after being analyzed

Gray Zone
Peaks-Artifact or Real?

- How to utilize data contained in the zone between the NT and RT?
  - NT set to exclude any spurious artifacts from baseline/background and peaks below the NT are not assessed
  - Gray zone-ensure all peaks are assessed and edit out artifacts and mark only if DNA (i.e. exclude pull up, spikes, dyes, etc.)
- Note potential alleles below threshold (pabt) with an asterisk
- Data used for comprehensive view of questioned profile(s)
- Used for overall interpretation of exclusion or inclusion
- No statistic performed at a locus if included with asterisk (pabt)

Degradation/Inhibition and Potential Alleles Below Threshold



Partial profile with 1 allele called and the other below reporting threshold

Pabt's—no alleles called-drop out possible

Potential Alleles Below Threshold (PABT)

EPG of data with PABT's

- Link to Set A
- Link to Set B

Interpretation Guideline-- Excerpt for PABT's

Potential alleles below threshold

Peaks below threshold but above the noise threshold which have been evaluated and determined not to be artifactual are designated with an asterix (*). This designates a potential allele below threshold. This asterix (*) is reflected in the DNA STR report but within the context of the DNA case file the potential allele below threshold also gets the designation of the potential allele call in parenthesis e.g., (#).

Potential alleles below threshold may be observed in limited samples, degraded and inhibited samples and in both single and mixed stains.

Peaks below threshold will be assessed to support the inclusion and/or exclusion of an individual(s). Any locus/loci that is utilized for an inclusion based on the individuals allele(s) falling in a peak below threshold position would not be used in the statistical calculation.

Peaks below threshold also give indication of more than one source or the indication of male DNA contributing to the biological stain. In the DNA STR report writing the following guidelines are used:

Incorporate the following statement when all potential alleles are below threshold for a second contributor:
The DNA profile obtained from the [Questioned item] *indicated the presence of a mixture of DNA from more than one source…*

Incorporate the following statement when at least one of the second contributor's alleles are above threshold:
The DNA profile obtained from the [Questioned item] *is a mixture of DNA from at least two (applicable # of individuals)…*

Stochastic Effects

- Note that stochastic effects are expected with lower targets of DNA (e.g. partial profiles, imbalance, drop out) and need to be addressed.
- These effects can be accounted for by using all of the information available for a comprehensive view and comparative analyses of exclusion or inclusion.
- Conservatively, no statistic would be reported for data below the RT. Hence no weight or significance in a court of law.
- No need to consider this information inconclusive. Know your system and how DNA behaves to not have false exclusions or inclusions.

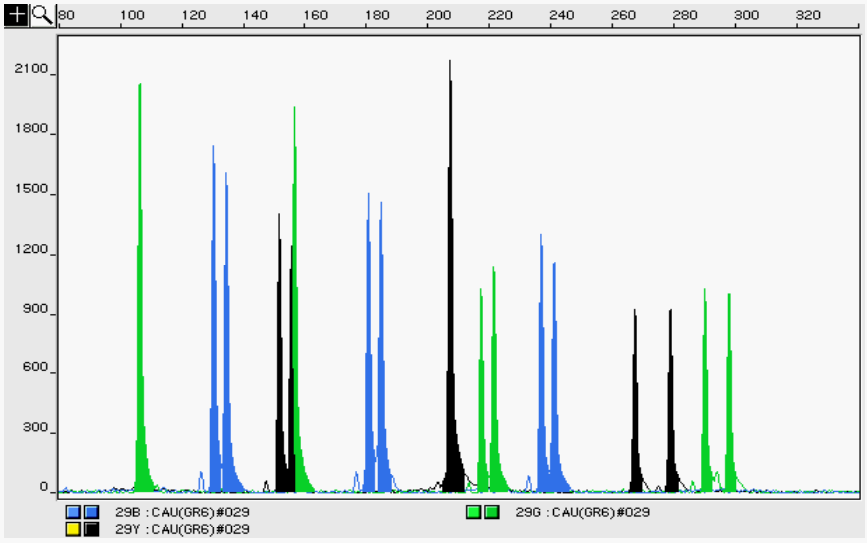
Interpretation Guideline Excerpt -use all information—COMPREHENSIVE PROFILE

Each locus must be analyzed individually to differentiate and quantify peaks in order to distinguish the profiles observed in the mixture. However, the combined profile can be utilized to provide a comprehensive view of the mixture possibilities and ratios of major to minor components.

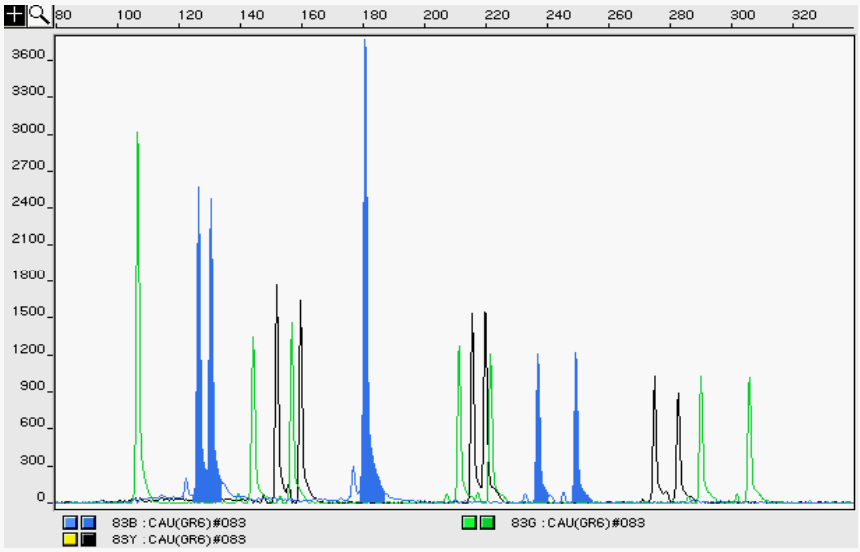
Allele Balance

- Within and Across Loci
  - Sister Allele Balance-measure differences and use + 3 SD
  - Across same and other colors
    -Individual (i.e.Blue, Green, Yellow, Red)- measure relative to highest locus as a percentage
    -Overall (i.e. Blue relative to others and so on).
- Kit performance assessed by examination of positive controls
  - ~ 70 % Rule to indicate mixture
  - Guide to evaluate if minor components of a mixture have dropped out due to degradation or inhibition or kit balance expectations.

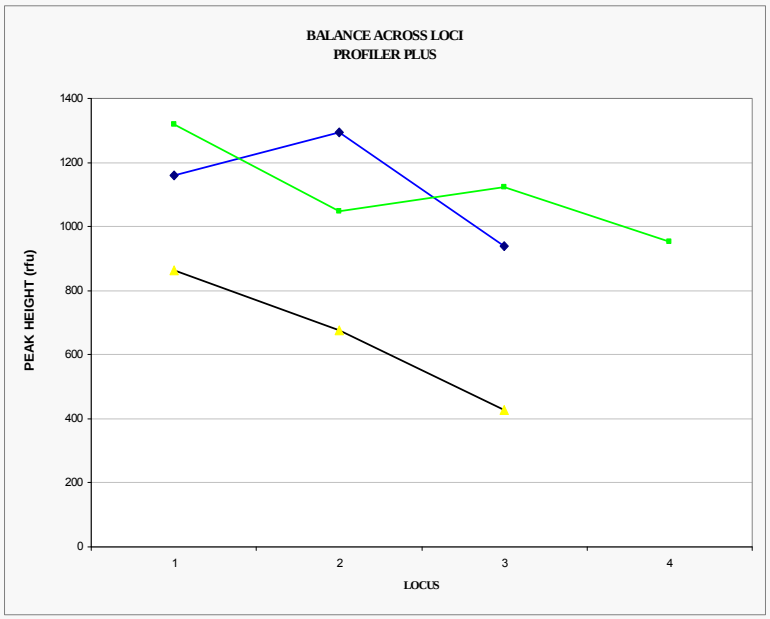
Good Balancing of Loci



Poor Balancing of Loci



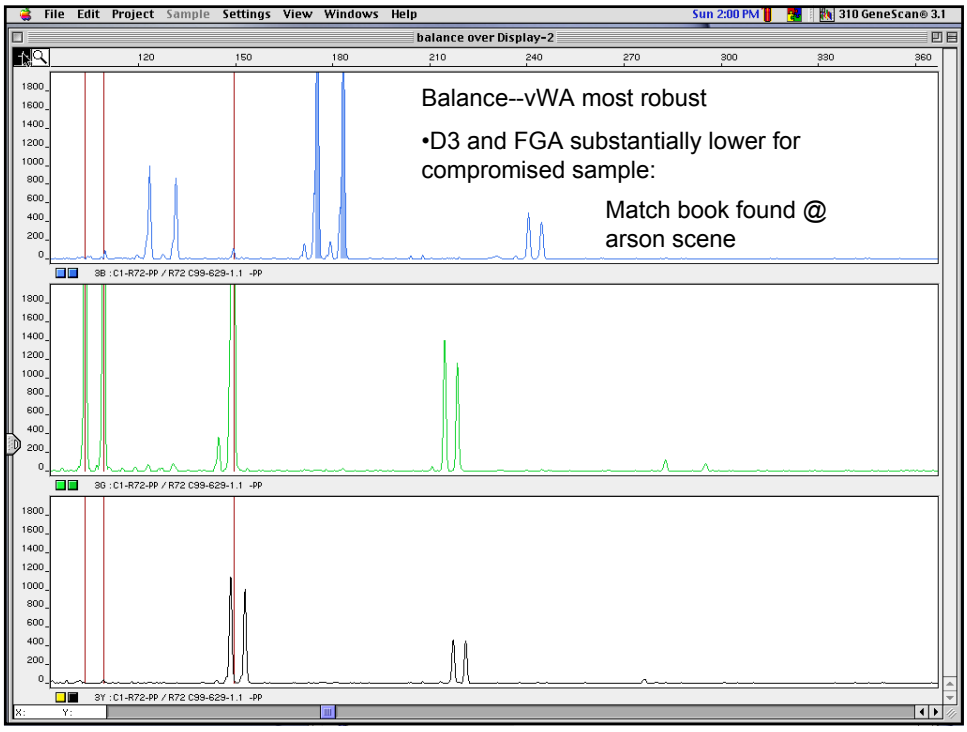
Graph of Balance Across Loci



KEY
Blue
1-D3
2-vWA
3-FGA

Green
1-AMEL
2-D8
3-D21
4-D18

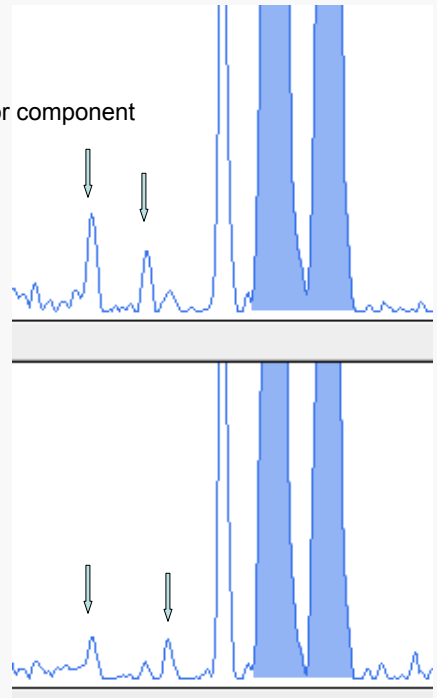
Yellow
1-D5
2-D13
3-D7



vWA-

• most robust locus

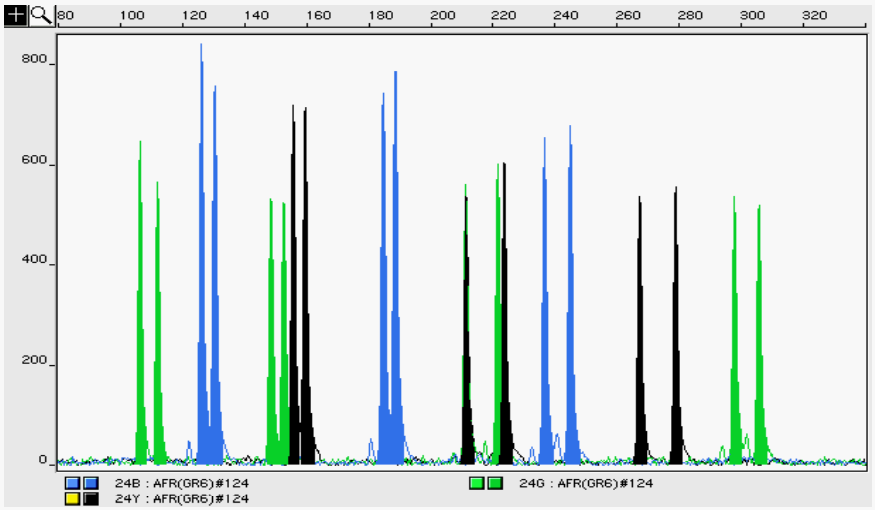
•Only data for minor component



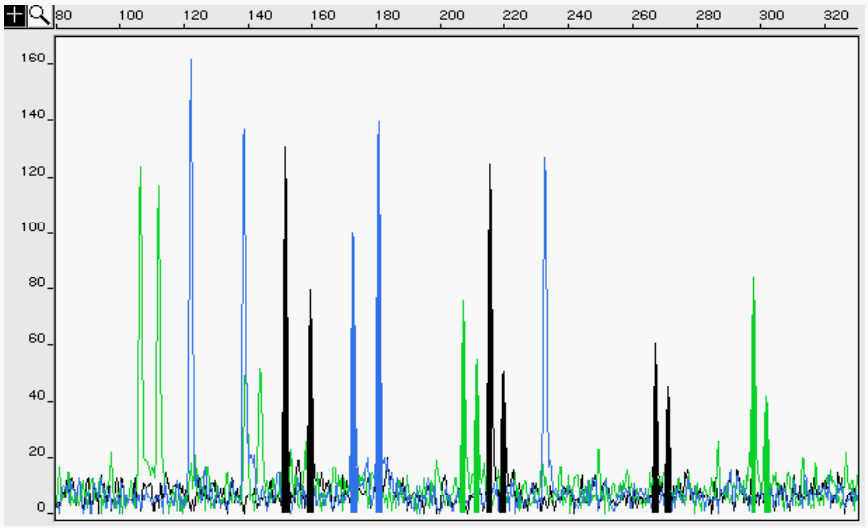
SWAB of Ground

KNIFE

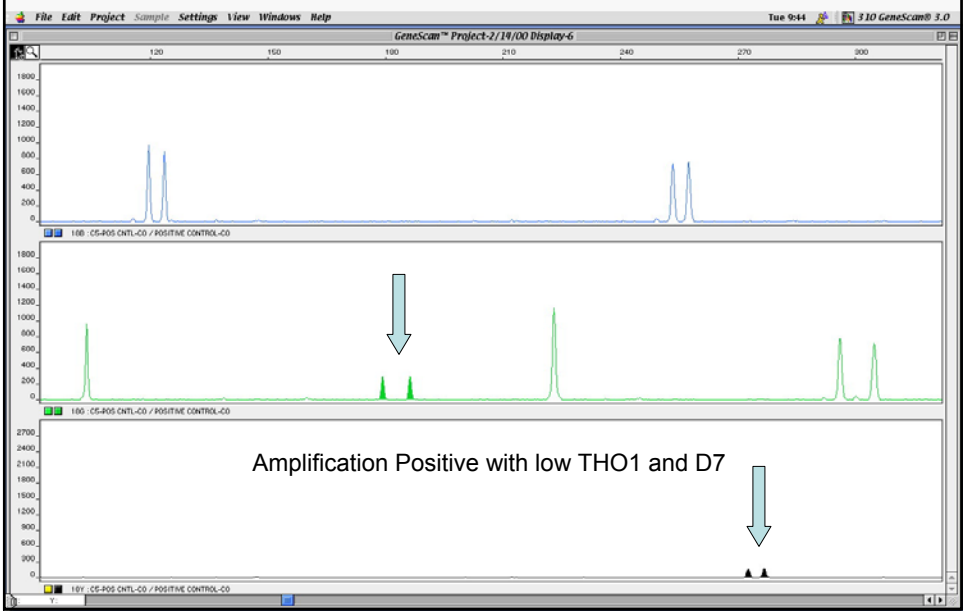
Good Balancing of Alleles

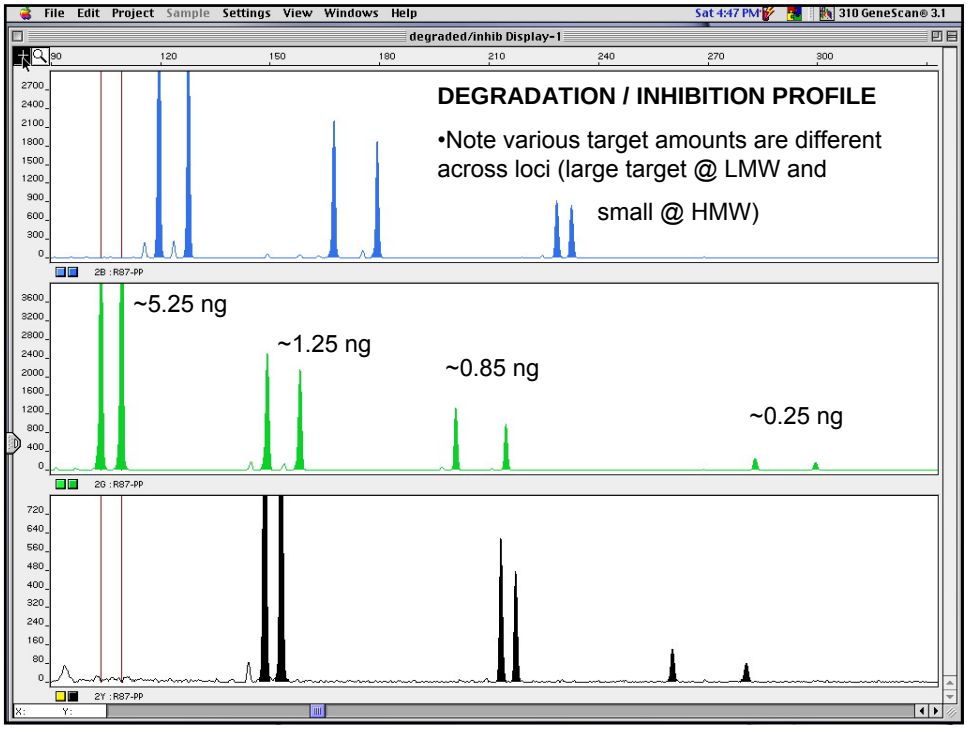


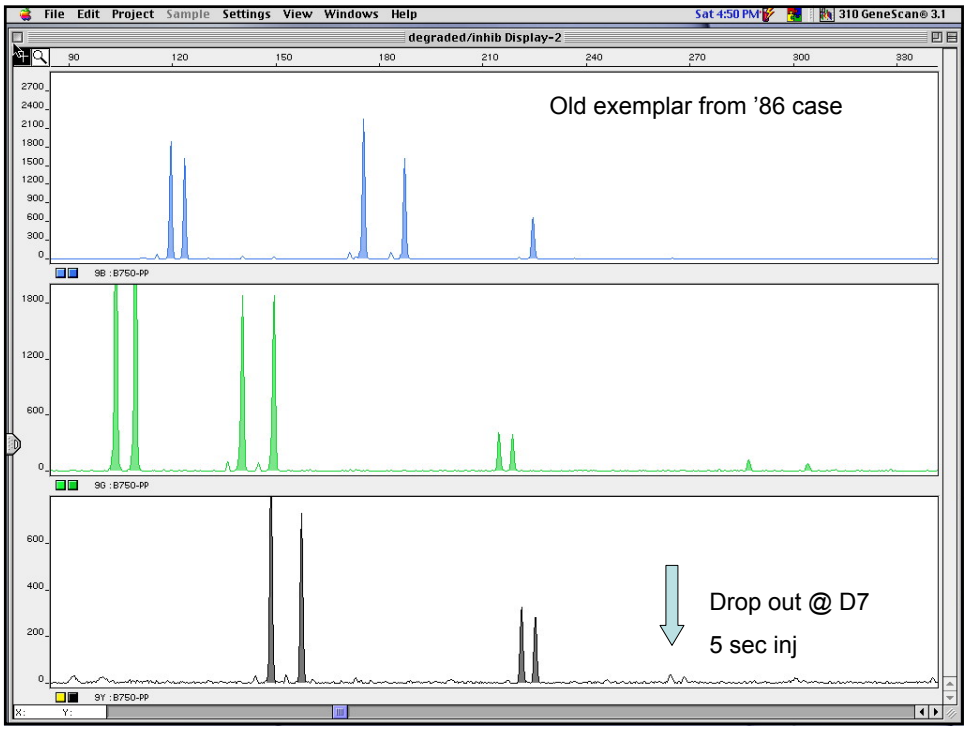
Poor Balancing of Alleles



KIT PRIMERS




File Edit Project Sample Settings View Windows Help
Sat 4:47 PM
310 GeneScan® 3.1
degraded/inhib Display-1
DEGRADATION / INHIBITION PROFILE
•Note various target amounts are different across loci (large target @ LMW and small @ HMW)
~5.25 ng
~1.25 ng
~0.85 ng
~0.25 ng


File Edit Project Sample Settings View Windows Help
Sat 4:50 PM
310 GeneScan® 3.1
degraded/inhib Display-2
Old exemplar from '86 case
Drop out @ D7
5 sec inj

Statements to Incorporate into Interpretation Guidelines

Scientific explanations may include factors such as inhibition, degradation or low template DNA that may affect allele balance within and across loci that can lead to allelic/locus dropout.

Compromised samples may yield an insufficient amount of DNA for analysis at one or more loci and those loci may not be utilized for interpretation.

Note: Allelic dropout may occur in degraded/inhibited samples and the possibility of a second allele should be considered.

Interpretation Guideline Excerpt - Results Options

Interpretation of Single Source DNA-STR Profiles	***Match (inclusion):*** **Single Source DNA Samples:** The donor of the known sample (K) can be included as a source of the questioned sample (Q) when there are no forensically significant differences between the allele designations obtained from these samples. The Random Match Probability Estimate (RMPE) will be calculated. The most conservative probability estimate determined will be reported. **Partial Profile (at a locus):** If one allele is identified and a second potential allele is below threshold (*), the identified allele will be used for comparison purposes first and if concordance is established the peak below threshold will be assessed to support the inclusion and/or exclusion of an individual(s). Any locus/loci that is utilized for an inclusion based on the individuals allele(s) falling in a peak below threshold position will not be used in the statistical calculation. ***Note: Allelic dropout may occur in degraded/inhibited samples and the possibility of a second allele should be considered.*** ***Non-match (exclusion):*** If a single allele peak does not match at any locus, and there is no scientific explanation for the non-match, then the donor of the known sample (K) can be excluded as a source of the questioned sample (Q). ***Note: Tri-allelic patterns have been observed in single source samples and there is a known phenomenon where different tissues from the same individual may or may not exhibit the tri-allelic pattern.*** ***Insufficient:*** If insufficient DNA is obtained at a particular locus or all loci tested, that is the peaks are too weak to identify, the data may not support an inclusion or exclusion. This data will be considered insufficient and will not be utilized for comparative analysis. ***Inconclusive:*** If the DNA STR data does not support an inclusion or exclusion the comparison will be deemed inconclusive and reported as such. ***No Results:*** No results, including potential alleles below threshold, were obtained for a particular locus / loci with the sample.

Y Filer Kit Balance-Interpretation Guidelines

- The validation binder summarizes the balance across the Yfiler™ system. The graphs depict the overall megaplex balance. The Yfiler™ kit did not exhibit consistency from sample to sample. Balance across loci was variable within each color and did not exhibit a general trend from color to color.
- Given the inconsistency in balance across loci, data must be assessed carefully to interpret profiles which may be indicative of degradation and/or inhibition. Caution must be exercised and data scrutinized if a particular locus exhibits imbalance relative to other loci in the multiplex system. It is recommended to be conservative, due to imbalance issues, by only using data present to interpret minor components of a mixture (e.g., there may not be a reasonable scientific explanation, based on expected kit performance, for the absence of an allele(s) at a locus due to degradation and/or inhibition).

Y Filer Kit -Interpretation Guidelines

- **Insufficient**

  Due to insufficient DNA, minor components may not be detectable at one or more loci. If the minor component result is not available at a locus, the peaks are too weak to report, or the minor alleles may be masked by the main allele, then a conservative interpretation must be made at that locus. The data will be evaluated cautiously. For comparative analysis, alleles present in the K but not in the Q may ***not*** be considered exclusionary for samples exhibiting degradation, inhibition or those samples known to contain minimal template DNA.

  Y-STR balance across loci is inconsistent and some loci may not contain the full complement of information from the minor component(s) in a mixture. Peak height information may not allow the analyst to have a reasonable scientific expectation of data present or absent at a locus. In such cases, that particular locus may contain insufficient data for comparative analyses and will be considered inconclusive for interpretive purposes. The other loci containing sufficient interpretable data may be used to make a comprehensive interpretation of the Q to the K.

Stutter Studies

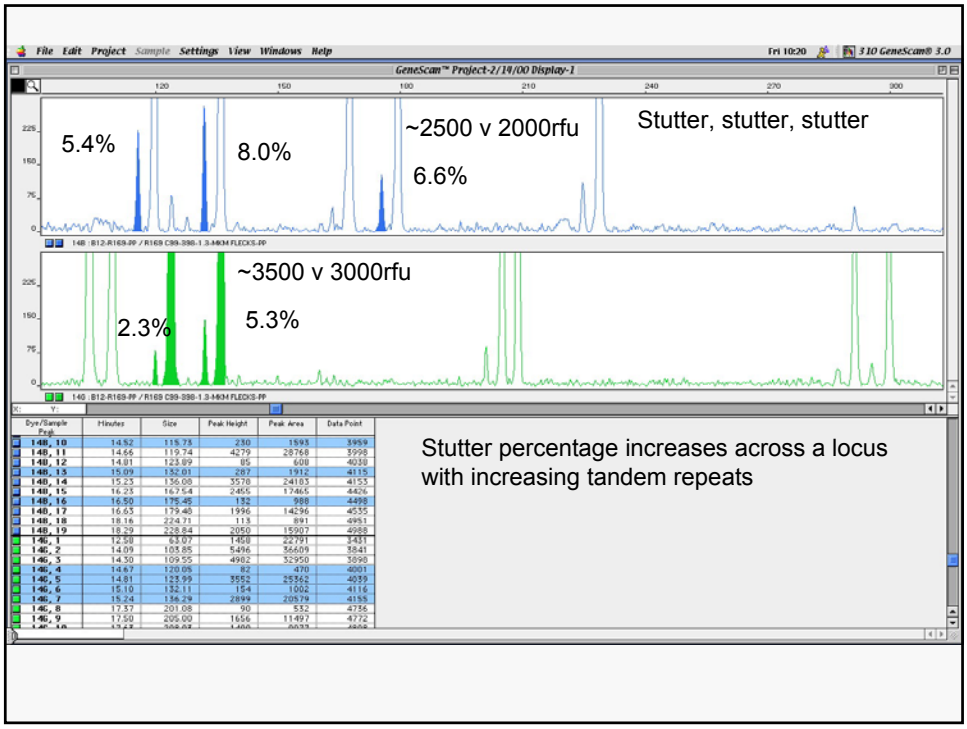
- Calculate stutter percentages from a database of single source samples.
- Useful to have at least 5 data points for most alleles at a locus.
- Average, min., max., SD and + 3 SD values determined for each allele and for locus.

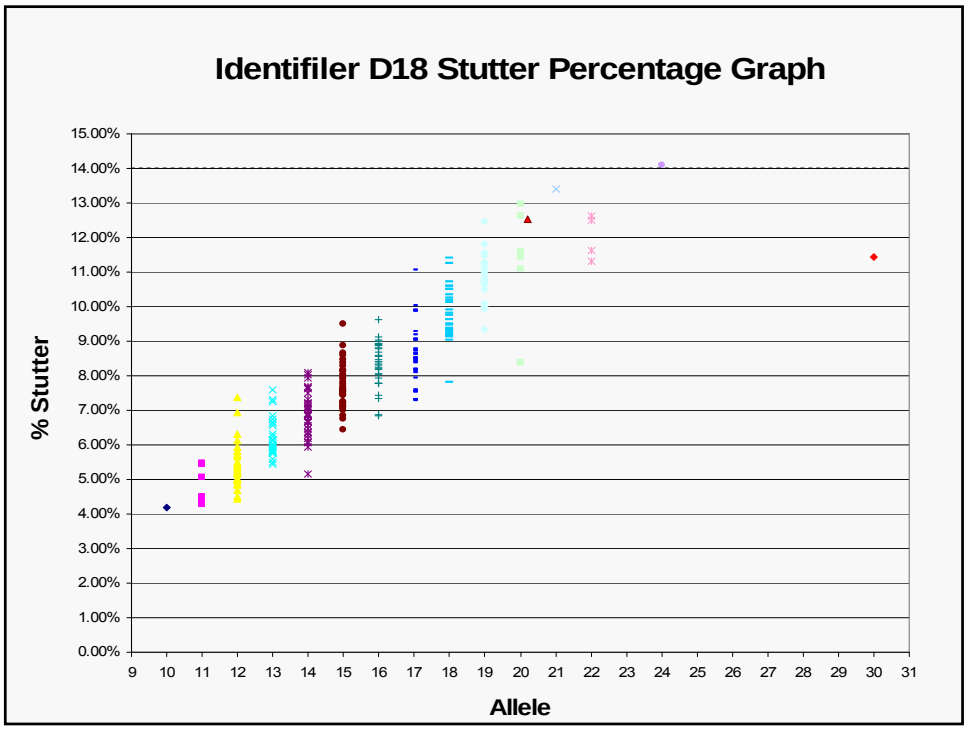
- Incorporate stutter filters into software program for each locus.
- As necessary, refer to stutter binder information for that specific allele for a better assessment of the data in question.

Stutter Percentages input into software program to create filters for the entire locus……

This value represents the average plus 3 SD for all allelic data at that locus.

Identifiler Stutter Percentages	
BLUE	
D8	8.53
D21	8.49
D7	7.86
CSF1PO	7.29
GREEN	
D3	10.26
TH01	4.40
D13	7.91
D16	8.86
D2	11.11
YELLOW	
D19	11.78
vWA	11.32
TPOX	5.07
D18	14.01
RED	
D5	7.89
FGA	11.93


5.4%
8.0%
~2500 v 2000rfu
6.6%
Stutter, stutter, stutter
~3500 v 3000rfu
2.3%
5.3%
Stutter percentage increases across a locus with increasing tandem repeats


Identifiler D18 Stutter Percentage Graph
% Stutter
Allele
15.00%
14.00%
13.00%
12.00%
11.00%
10.00%
9.00%
8.00%
7.00%
6.00%
5.00%
4.00%
3.00%
2.00%
1.00%
0.00%
9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31

Identifiler D18S51

Allele	Data Points	Average	Min	Max	S.D.	(-3) S.D.	(+3) S.D.
10	1	4.18%	4.18%	4.18%	0.00%	4.18%	4.18%
11	6	4.85%	4.27%	5.48%	0.54%	3.23%	6.48%
12	30	5.44%	4.42%	7.37%	0.65%	3.49%	7.38%
13	26	6.24%	5.43%	7.60%	0.57%	4.54%	7.93%
14	36	6.89%	5.14%	8.10%	0.63%	5.01%	8.76%
15	36	7.70%	6.45%	9.51%	0.65%	5.75%	9.65%
16	30	8.31%	6.85%	9.61%	0.65%	6.36%	10.26%
17	38	8.62%	7.28%	11.08%	0.86%	6.03%	11.21%
18	27	9.83%	7.81%	11.40%	0.78%	7.49%	12.16%
19	21	10.88%	9.35%	12.48%	0.70%	8.79%	12.97%
20	7	11.37%	8.38%	12.98%	1.48%	6.92%	15.82%
20.2	1	12.54%	12.54%	12.54%	0.00%	12.54%	12.54%
21	1	13.40%	13.40%	13.40%	0.00%	13.40%	13.40%
22	4	12.02%	11.32%	12.63%	0.64%	10.10%	13.94%
24	1	14.11%	14.11%	14.11%	0.00%	14.11%	14.11%
30	1	11.45%	11.45%	11.45%	0.00%	11.45%	11.45%
ALL ALLELES	266	8.02%	4.18%	14.11%	2.00%	2.04%	14.01%

Interpretation Guideline Excerpt - for Stutter

Summary of Stutter Evaluation

IF…..	THEN…..
Stutter % is < Average % Stutter + 3 S.D. (Locus)	GeneMapper™ *ID* probably filtered it out; if not, manually edit it out
Stutter % is > Average % Stutter + 3 S.D. (Locus)	Check stutter data for allele in question; may be a mixture
Stutter % is > Average % Stutter + 3 S.D. (Locus) but < Average % Stutter + 3 S.D. (Allele)	Assess peak and manually edit out as stutter, if appropriate
Stutter % is > Average % Stutter + 3 S.D. (Locus) and > Average % Stutter + 3 S.D. (Allele)	Check % stutter range (minimum and maximum) and evaluate whether the peak should be edited out or indicative of an allele of a mixture. If indicative of an allele: > reporting threshold: report with allelic designation < reporting threshold, used in the comprehensive interpretation. An individual may be included based on the inclusion in a stutter position.

The overall DNA profile must be evaluated for the presence of a mixture of DNA. Single source profiles may exhibit stutter, n-4 & n+4, and/ or elevated stutter which should be edited out. Caution should be exercised with this interpretation.

Stutter peaks will be assessed for the inclusion and/ or exclusion of an individual(s). Any locus/loci that is utilized for an inclusion based on the individual's allele(s) falling in a stutter position, which is below threshold, would not be used in the statistical calculation.

N + 4 Stutter

- Working with John Butler to get data and characterize for the community.
- Our experience has been ~ 2 % (PP/CO on a 310 with Mac GS/GT).
- Exhibited at larger targets (i.e. low molecular weight loci with high signals).
- Depends on thresholds set and if it is below or in baseline area.

Summary of Stutter

- Assess peak according to locus information
- Evaluate peak relative to allele information as necessary
- Err on the side of caution in not labelling an artifact as DNA (Type I Error).
- Note: there is a zone of uncertainty that can exist below the stutter filter and DNA may be masked.
- Do not exclude based on DNA that can be masked by stutter but no statistical inference should be applied (used for comprehensive view of inclusion/exclusion but no weight or significance attributed to that locus).

Mixture Ratio's

- **Two sets of mixtures**
  - Most shared alleles (@ 12/15 loci)
  - Least shared alleles (@ 7/15 loci)
  - Ratios prepared to contain 2ng/10ul
  - 1:1, 1:5, 1:10, 1:15, 1:20 and vice-versa
- **Detection of minor contributor established above RT and NT.**
- Partial profiles for some loci @ 1:10 and greater

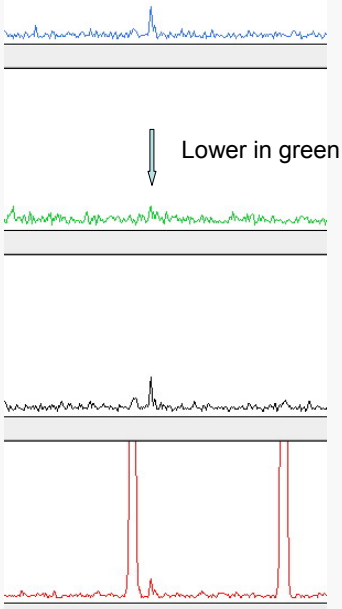
ARTIFACTS

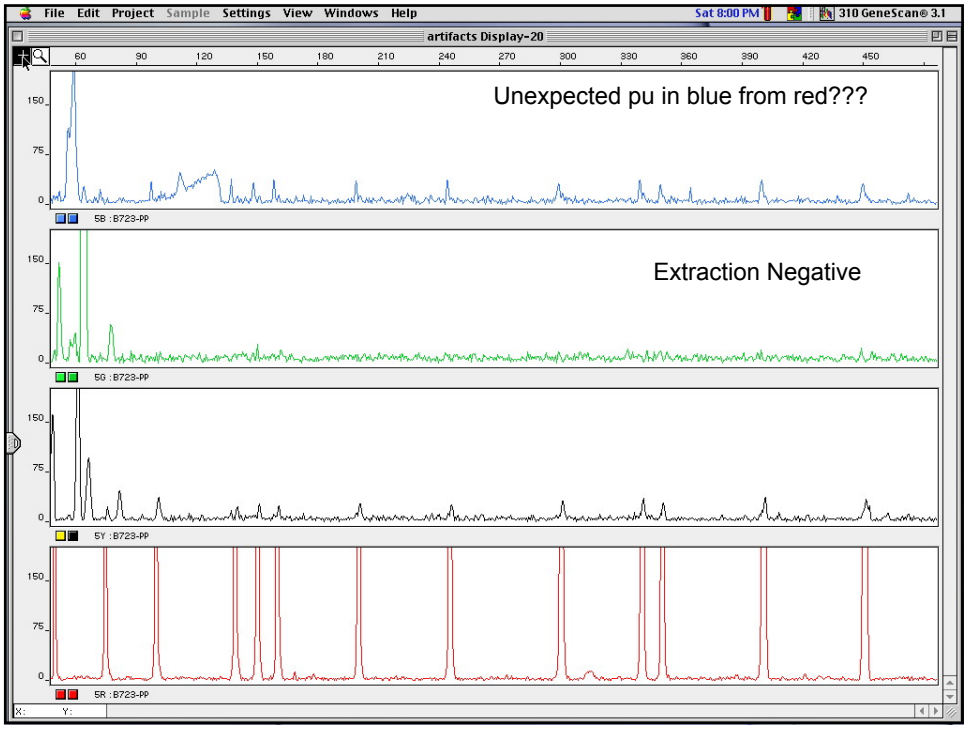
- **PCR**
  - **Stutter**
  - **Non-specific product**
  - **Split peaks**
  - **Y primer site mutation**
- **STR**
  - **Pull Up (Bleed through)**
  - **Spikes**
  - **Dye Blobs**
- **STR runs**
  - **Bubbles**
  - **Waves**
  - **Mis-shapen peaks**

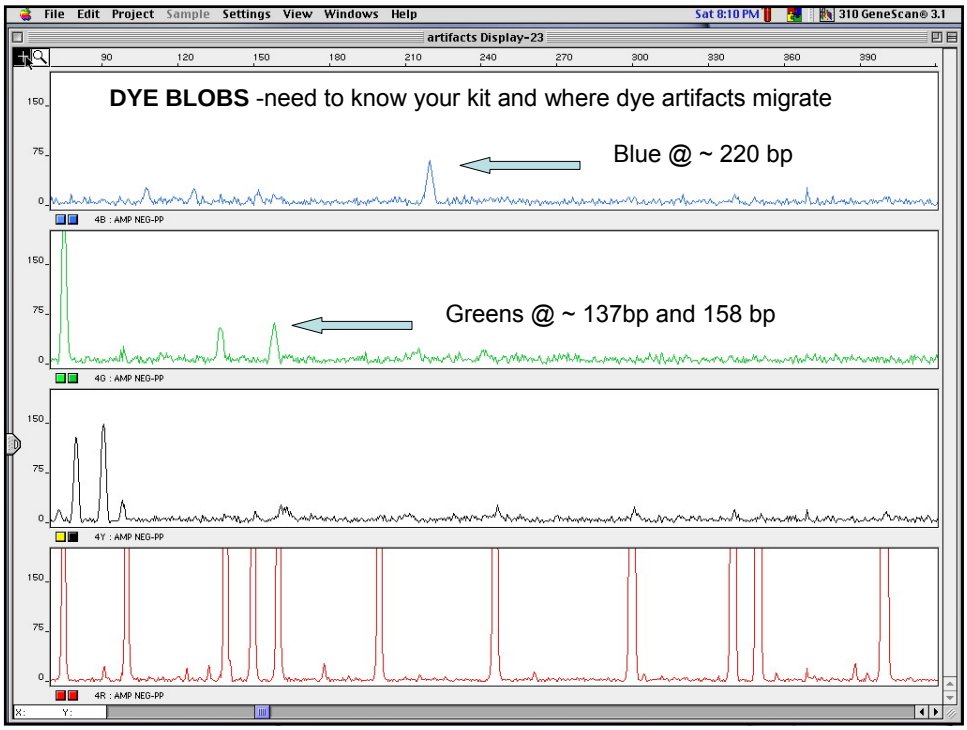
Artifacts

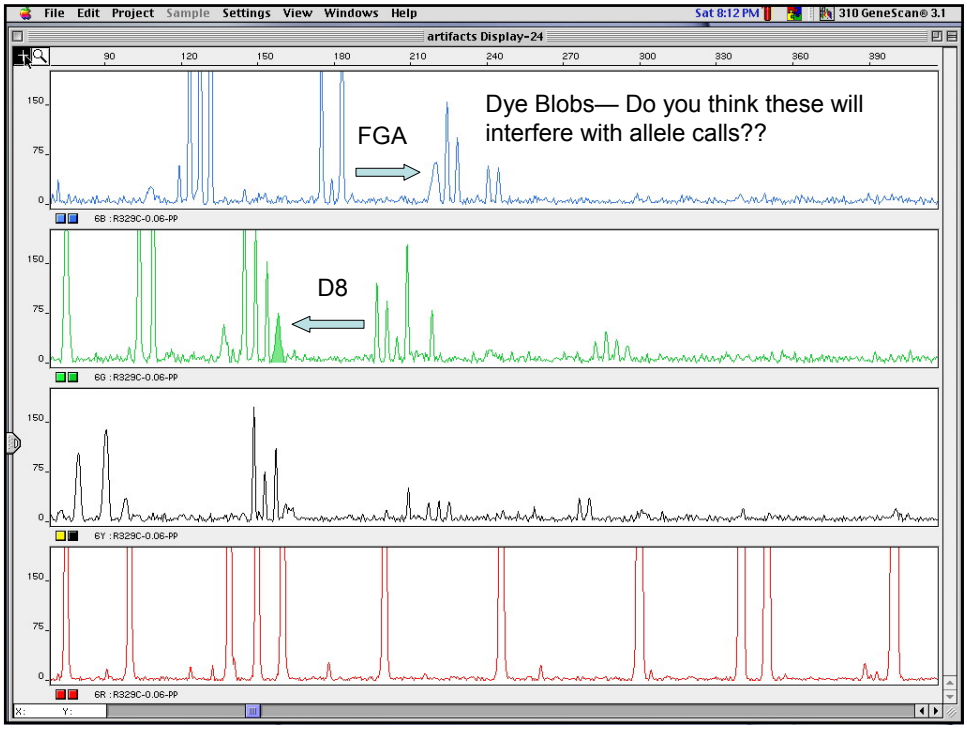
- Recognize, define, identify, characterize and edit out accordingly
  - Pull Up/Bleed through
  - Raised Baseline
  - Stutter Peaks
  - Split Peaks (-A)
  - Spikes
  - Shoulders
  - Dye Blobs

Spike-typical of urea crystal




File Edit Project Sample Settings View Windows Help
Sat 8:00 PM
310 GeneScan® 3.1
artifacts Display-20
Unexpected pu in blue from red???
Extraction Negative
5B : B723-PP
5G : B723-PP
5Y : B723-PP
5R : B723-PP


File Edit Project Sample Settings View Windows Help
Sat 8:10 PM
310 GeneScan® 3.1
artifacts Display-23
DYE BLOBS -need to know your kit and where dye artifacts migrate
Blue @ ~ 220 bp
Greens @ ~ 137bp and 158 bp
4B : AMP NEG-PP
4G : AMP NEG-PP
4Y : AMP NEG-PP
4R : AMP NEG-PP

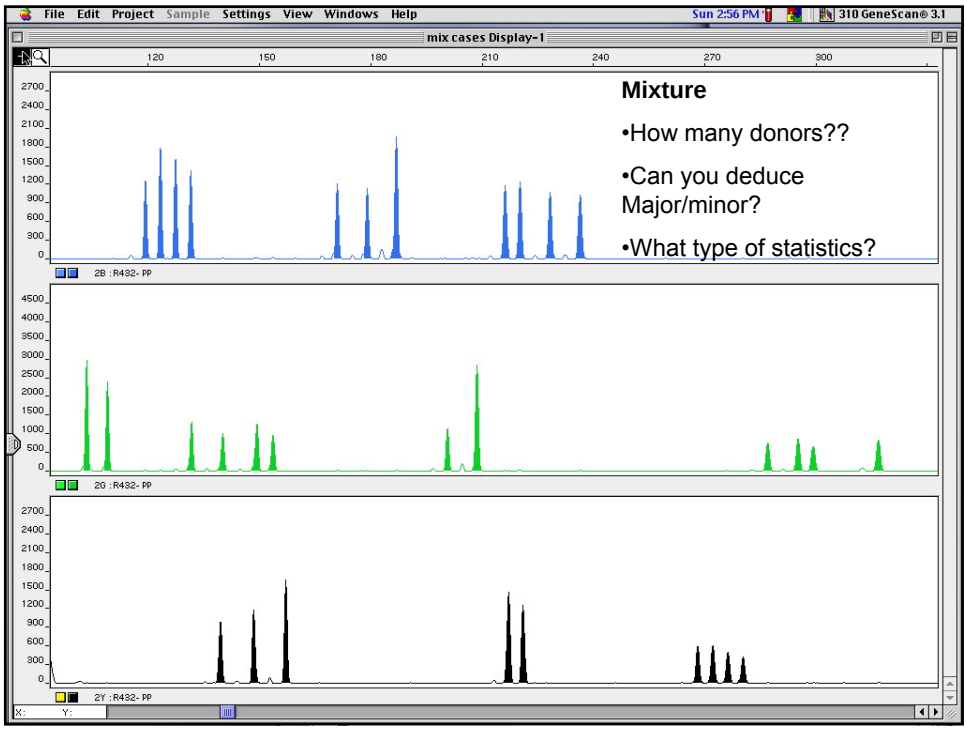


Assessing a Profile

- Is it a mixture?
- How many individuals?
  - Assumption of only 2 (e.g. intimate sample: if one is victim, deduce suspect using obligate alleles-foreign to V)
  - More than one source or @ least X individuals (if greater than 2)
    - Based only on called alleles (not pabt's)
- Is there a major and a minor (s)?

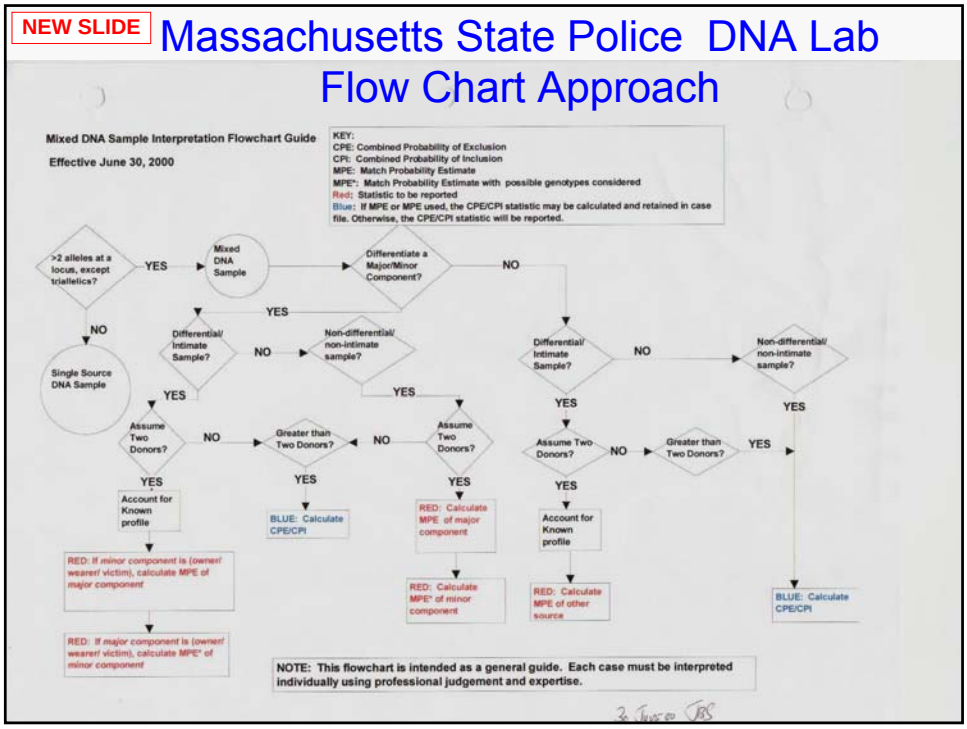
Statistical Calculations

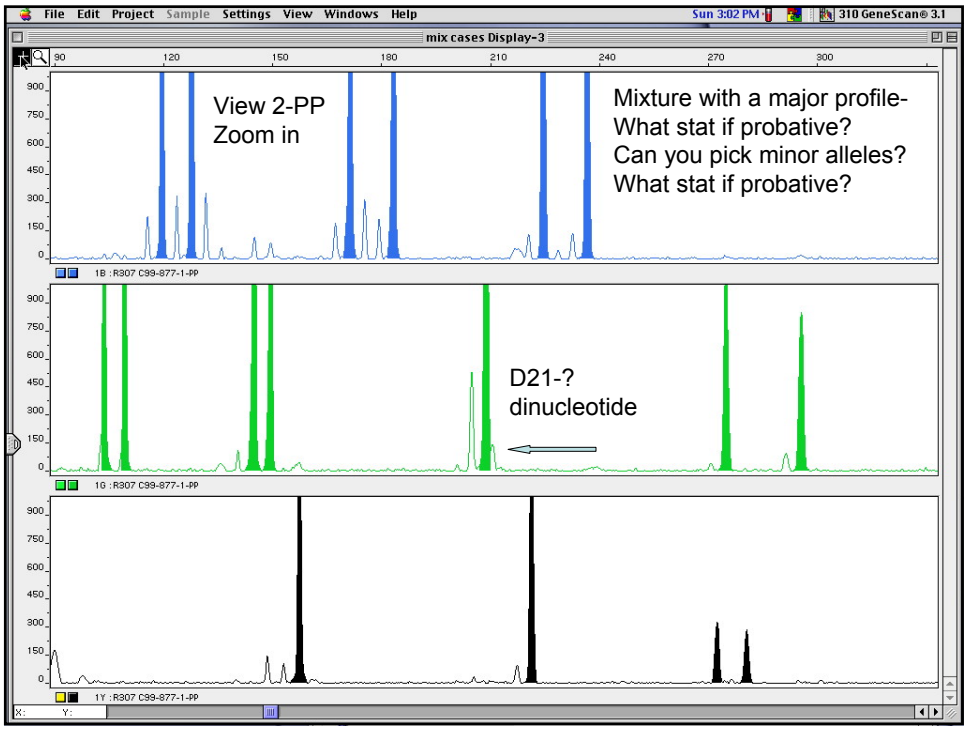
- Random Match Probability Estimate (RMPE)
  - Single source or major in mixture with a single genotype at every locus—product rule
- Modification of RMPE-increase discrimination potential
  - Probative minor profile in mix of 2 donors
  - Use obligate alleles and PH information to consider potential genotypes at the locus (additive at the locus and then product rule)
- Probability of Exclusion (combined CPE)
  - Conservative for mix of 2 (no major/minor) or more donors
  - Use only at loci where all alleles are accounted for (no *)



NEW SLIDE

Massachusetts State Police DNA Lab Flow Chart Approach



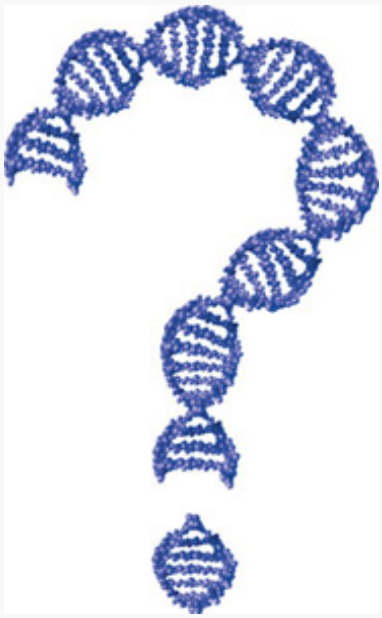


Elements of Laboratory-Specific Mixture Interpretation Guidelines

- Introduction
- Mixture Evidence Profile Evaluation
  - Signal saturation
  - Alleles in stutter product positions (using stutter %)
  - Balance within a locus (using heterozygote PHRs)
  - Balance across loci
- Interpretation Possibilities (with examples)
  - Match, non-match, insufficient, inconclusive
- Worked statistical examples
- Use of IF/THEN tables and flow charts

Acknowledgements

- AAFS
- John Butler
- MA State Police Mixture/Interpretation training with JBS, Dr. George Carmody & Ray Wickenheiser
- Graphs-Dr. Sandra Haddad
- All DNA analysts working with difficult mixtures



Validation Studies

- **Database**
  - **Caucasians**
  - **African Americans**
  - **Hispanics**

Stutter Studies

- **Variants**
  - **Detection of New Alleles**
  - **'Far Out' Variant**
- **Balancing**
  - **Loci**
  - **Alleles**

Precision

- Establish instrument is performing properly to designate allele categories.
- Address temperature fluctuations and oven door issues prior to running other validation studies.
- General rule-the standard deviation of measurements for any allele should not exceed 0.15 bp so the window bin of +/- 3 SD for sizing is less than 1 bp (3 x .15 = .45 x 2 = 0.9 bp)
- Software usually establishes the bin around the ladder size as +/- 0.5 bp) of the average size of that fragment in the ladder.

250 Base Pair Precision Data Check

3130xl (CE004) (Ned)- Estimation of 250 Base Pair Range

Approx. +3 standard deviation = **246.45**

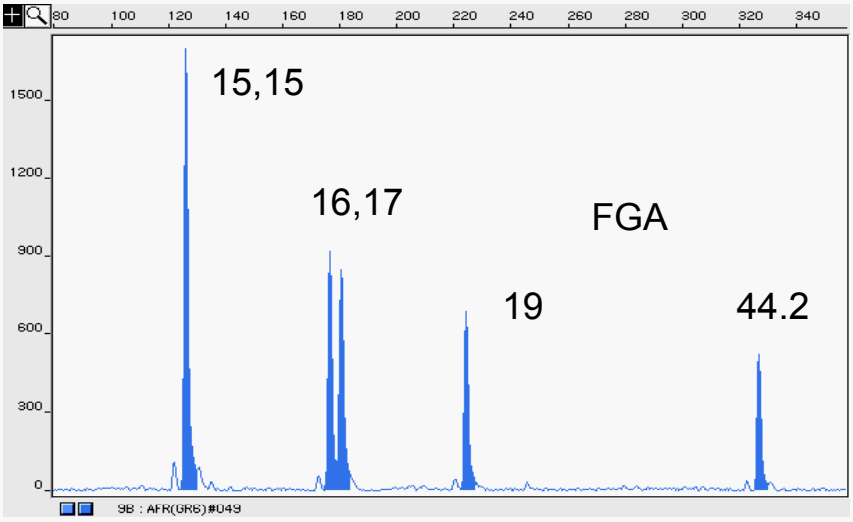
Approximate average value = **246.11**

Approx. –3 standard deviation = **245.77**

Amplification Positive Precision Data Check

BLUE		Average Fragment Range (bp)		
Locus	Allele	Average Size (bp)	-3 Standard Deviations	+3 Standard Deviations
D8S1179	13	143.88	143.60	144.15
D21S11	30	208.06	207.86	208.26
D7S820	10	270.98	270.67	271.30
D7S820	11	275.08	274.73	275.42
CSF1PO	10	321.43	320.83	322.03
CSF1PO	12	329.63	329.02	330.25

‘Far Out’ Variant



MATCH PROBABILITY ESTIMATE

Match Probability Estimate (MPE)

- RMP-random match probability for a single source (SS) sample or Major in a mixture

- Statistic calculated as 1 genotype @ each locus
- If minor component is deduced to 1 GT at each
- Use product rule—
  - Allele frequencies to determine GT frequencies to cross multiply to obtain the profile frequency (MPE)
  - Homozygote GT frequency: $p^2 + p(1-p)\theta$
  - Heterozygote GT frequency: $2pq$

Modified MPE

- Modified MPE-combination of one or more possible GT's at a locus are added together to obtain the genotype frequency
  - Increases the discrimination potential
  - Minor is probative and assumption of 2 donors
  - Utilize peak height information
  - Use 'obligate' allele of minor component
  - After addition @ locus, use product rule across loci
  - Should use for intimate samples and may use for other scenarios, as applicable

Mixture profile having alleles > instrument specific rfu threshold and assuming two donors (including the victim/source individual):

The data must support the assumption that there are only 2 contributors to the DNA mixture. The assume two contributors is reserved for intimate swabs e.g., vaginal swabs, swabs taken from a person e.g., breast swabs, hand swabs. *Alternative scenarios may be considered at the Technical Leader's and/or DNA Supervisor's discretion. Approval will be documented in the case file.*

Differentiate the contributor profiles and account for the victim/source using peak heights and mixture ratios, if possible. For example, use the non-sperm fraction as a reference to account for the carry-over profile in the sperm fraction. After assessment of the victim/source profile, if a single allele peak in the questioned sample (Q) fraction does not match the known sample (K) at any locus and there is no scientific explanation for the non-match, then the donor of the known sample (K) can be excluded as a source of the questioned sample (Q). Utilize the mixture interpretation worksheet Appendix A to document calculations/evaluation. This worksheet is also available electronically in the current worksheets folder.
If there is a match at all loci a match is declared and a random match probability estimate may be calculated.

If it is not possible to deduce out at all loci, the combination of all possible genotypes at the unconvoluted locus could be included in the statistical calculation, alternatively this locus could be dropped from the statistical calculation but it must still include the individual as a contributor.

Example of a Mixture Worksheet to deduce potential genotypes of a minor contributor

Locus	Allele	Peak height	Possible Component profiles giving rise to observed mixture		Comments	Non Sperm Fraction profile	Possible genotypes

Reporting Template Excerpt

The DNA profile obtained from the [Questioned Item] is a mixture of DNA consistent with DNA profiles from [Victim and Exemplar]. Assuming 1) two contributors and 2) [victim's name] is one of the contributors; [Exemplar] is included as the other potential contributor.

Combined Probability of Exclusion

- CPE-conservative calculation of the estimated proportion of individuals that can be excluded as a contributor to a mixed stain
- Mixture of more than 2 individuals
- 1:1 ratio where major/minor cannot be deduced
- MUST have all alleles accounted for to employ at a locus (no potential alleles below threshold)
- Convert to CPI (inclusion)

Interpretation—Example of Calculation (1/4)

Combined Probability of Exclusion (CPE)	This statistical approach involves the calculation of the exclusion probability for a profile. The combined probability of exclusion (CPE) provides a calculation of the estimated proportion of individuals from a defined population group that can be excluded as a contributor to an observed DNA mixture.
CPE Example	The probability of exclusion at a single locus is as follows:
(P)	*The combined frequency of alleles detected (P)*
	•P = frequency of allele 1 + frequency of allele 2 + frequency of allele 3,…….N
Example: *(P)*	***Example:***
	Calculation based on the detection of 3 alleles at the FGA locus (alleles detected-21, 22, 23) with frequencies of 0.187, 0.182 and 0.156 respectively. P = 0.187 + 0.182 + 0.156 P = 0.525

Interpretation—Example of Calculation (2/4)

(Q)	*The combined frequency of alleles not detected (Q)*
	Q=1-P
Example: *(Q)*	***Example:***
	Q = 1- 0.525 Q = 0.475
(PE)	*The probability of exclusion (PE) is as follows:* $PE = Q^2 + 2Q(1-Q)$
Example: *(PE)*	***Example:***
	$PE = (0.475)^2 + 2(0.475)(1-0.475)$ PE = 0.724
	Therefore, 72.4 % of the population can be excluded as contributors to the evidentiary sample based on the data from the FGA locus.

Interpretation—Example of Calculation (3/4)

(CPE)	*Combined Probability of Exclusion (CPE) across many STR loci is as follows:*
	$CPE = 1 - (1\text{-}PE_i)\ (1\text{-}PE_j)\ (1\text{-}PE_k) \ldots (1\text{-}PE_n)$
Example: ***(CPE)***	***Example:***
	For 13 STR loci the CPE could be calculated from the PE at each of the loci to yield a result similar to the following:
	$CPE = 1 - (1\text{-}0.434)\ (1\text{-}0.803)\ (1\text{-}0.724) \ldots (1\text{-}PE_n)$ $CPE = 1 - (8.48 \times 10^{-8})$ $CPE = 0.9999999152$
	Therefore, 99.99999152 % of the population can be excluded as contributors to the evidentiary sample based on the data from all loci.

Interpretation—Example of Calculation (4/4)

(CPI)	*Combined Probability of Inclusion (CPI) can be calculated from the combined probability of exclusion as follows:*
	$CPI = 1 - CPE$
Example: (CPI)	***Example:***
	$CPI = 1 - 0.9999999152$ $CPI = 0.0000000848$
	Therefore, 0.00000848 % of the population can be included as contributors to the evidentiary sample based on the data from all loci.
	Likewise, the expected probability of inclusion in the evidentiary sample is less than 1 in 11.79 million individuals.

Mixture profile having alleles > instrument specific rfu threshold with more than 2 donors

If a mixture profile has more than 4 alleles at a locus a mixture of more than 2 people can be assumed. If no major profile can be determined the overall profile must be assessed for the inclusion or exclusion of an individual. If an allele present in the (K) fraction is not accounted for in the (Q) fraction, and there is no scientific explanation for the missing allele(s), then the donor of the known sample (K) can be excluded as a source of the questioned sample (Q). Scientific explanations may include factors such as inhibition, degradation or low template DNA that may affect allele balance within and across loci that can lead to allelic/locus dropout. Compromised samples may yield an insufficient amount of DNA for analysis at one or more loci and those loci may not be utilized for interpretation. This type of mixture would involve a CPE/CPI calculation at those loci where the individual's alleles are called and all alleles are accounted for at that locus (e.g., no alleles below threshold). Degradation and inhibition must be taken into account as well as those loci where an individual may be adventitiously included in the alleles present. These loci should not be included in the statistical calculation. Caution should be exercised when interpreting these samples.