Purpose

To examine the sensitivity and reproducibility of STRmix and TrueAllele Casework when testing challenging samples. Part I of this study focuses on the following sample types: 2-person and 3-person mixtures created in-house that have differential degradation as an element of complexity.

Methodology and Results

System versions and settings

STRmix (ESR)

- STRmix is a stand-alone program written in Java (Oracle) for Windows. Prior to use, laboratory-specific parameters were established for the Identifiler Plus/3130XL combination of multiplex amplification kit and detection platform. These parameters included a regression analysis of inter-allelic stutter levels at each locus, the detection system's signal saturation point, probability of drop-in, allele amplification variance for a given template quantity, and inter-locus amplification variance. The first two parameters were assessed outside of the software with Excel. The latter two parameters were assessed through the STRmix Model Maker module and, with additional calculations in Excel for Model Maker version 2.0.
- Interpretations were performed using version 1.0.7.49.
- Model Maker version 1.0.7.49 was used to establish the inter-locus amp variance.
- Unless noted otherwise, studies were tested using two separate allele amplification variance settings. One was from the Cal DOJ analysis using Model Maker version 1.0.7.49. The other was calculated for Cal DOJ by ESR using the Model Maker approach incorporated into the commercial version 2.0.

TrueAllele Casework (Cybergenetics)

- TrueAllele Casework is a software and hardware system. The software employs the MATLAB programming language. Hardware includes a Linux-based server with a Macintosh interface. Additional PC workstations have been added to the system. All settings were established by Cybergenetics with reference to a set of sample files supplied by Cal DOJ.
- Analyses for this study were performed using an HP ProLiant ML350 G6 with 8 parallel processors; server version 3.25.444.1; VUIer version 3.3.4723.1 (27-Sep-2012); and Analyze build 290 (24-Sep-2012).

Preparation of sample sets was performed by Mavis Date-Chong (Book nos. 495, 504, and Appendix pages 1-10 of these notes.)

DNA preparation

- Liquid whole blood was obtained from a female contributor (profile QCA018).
- Liquid semen was obtained from two male contributors (profiles QCA108 and QCA100).
- DNA was obtained from these samples through a ProK/SDS digestion, phenol/chloroform/isoamyl alcohol extraction, and Centricon YM-30 (Millipore) concentration into Tris-EDTA (TE^{-4}) buffer. For the semen samples, DTT was added to the digestion buffer to aid in sperm cell lysis.

DNase1 digestion

- To examine the effect of degradation on each program's ability to detect a contributor, a degradation series was prepared using DNase1 (Invitrogen, Life Technologies) and the DNA extracted from male contributor QCA108. Following an approach previously described (Swango et al. Forensic Sci. Int. 158 (2006) 14–26), a series of digests representing a range of degradation levels was selected from two sets of multiple time points and enzyme concentrations (no digestion; 1.7×10^{-4} U/ng, digested for 6, 12, 20, 30, and 55 minutes; 3.3×10^{-4} U/ng, digested for 6, 12, 30, and 55 minutes.)
    - DNA concentrations were determined by a qPCR assay (Hudlow et al., Forensic Sci. Int.: Genetics 2 (2008) 108-125) that utilizes the TH01 STR locus as a target sequence to assess total human DNA. The TH01 amplicon size of approximately 170–190 bp is in the middle of the molecular weight range for most multiplexes. The assay also includes a 67 bp target sequence flanking the

CSF1PO STR locus. The ratio of the results from these two targets was one measure used to assess degradation when selecting the time points noted above.

- Because the qPCR TH01 target is a mid-range STR locus, it is expected that the degraded contributor will present as the majority contributor at low molecular weight loci and as the minority contributor at high molecular weight loci. When the genotypes of the contributor are known, this would be observed as a decrease in the degraded DNA donor's mixture proportion (Gill et al. Forensic Sci. Int. 91 (1998) 41-53) as the molecular weight of the loci increases. The slope of the linear regression line for the Mx values across loci is another measure used to assess degradation in these test samples.

Mixture preparation

- Two-person differential degradation mixtures (Book no. 504) were created as a 1:1 combination of R1 (intact DNA) and R2 (intact or degraded DNA).
- A three-person differential degradation mixture was created as a 6:3:1 combination of, respectively, R3 (intact DNA), R2 (degraded DNA; 3.3×10^{-4} U/ng, digested for 6 minutes.), and R1 (intact DNA).

PCR amplifications and capillary electrophoresis

- The two-person and three-person differential degradation samples were amplified using 1 ng of total template DNA. Amplifications were done in duplicate using the AmpFlSTR Identifiler Plus multiplex and the Applied Biosystems GeneAmp PCR System 9700 thermal cycler using 9600 emulation mode under standard manufacturer conditions. Detection was by Applied Biosystems 3130XL Genetic Analyzer with 5-second injections at 3 kV. Where appropriate, fsa files fragment sizing and allele calling were performed with GeneMapper ID v3.2.
- 2-person mixture
  Run folder
  - RF_21MCE268_051711_IDPLUS_mixtr-deg

  Injections used for study (first injection for each amped sample); folder "[…]\2PM DifDeg STRmix\2PM DifDeg STRmix Study Samples":
  - H04_LADDER_004_21MCE268_051711_IDPLUS_mixtr-deg.fsa
    - All ladders overlaid in GM: Sizing appeared uniform.
    - I selected a ladder with the high RFU results that appeared to have average sizing.
  - C04_9947A_003_21MCE268_051711_IDPLUS_mixtr-deg.fsa
    - Positive amplification control
  - D04_NC_004_21MCE268_051711_IDPLUS_mixtr-deg.fsa
    - Negative amplification control
  - B01_2_QC108-500pg_002_21MCE268_051711_IDPLUS_mixtr-deg.fsa
    - Male
  - A01_1_QC18-500pg_001_21MCE268_051711_IDPLUS_mixtr-deg.fsa
    - Female
  - E01_5_QC18-QC108_intact_001_21MCE268_051711_IDPLUS_mixtr-deg.fsa
  - F01_6_QC18-QC108_intact_002_21MCE268_051711_IDPLUS_mixtr-deg.fsa
  - A02_8_QC18-QC108Tube5_001_21MCE268_051711_IDPLUS_mixtr-deg.fsa
  - G01_7_QC18-QC108Tube5_003_21MCE268_051711_IDPLUS_mixtr-deg.fsa
  - B02_9_QC18-QC108Tube6_002_21MCE268_051711_IDPLUS_mixtr-deg.fsa
  - C02_10_QC18-QC108Tube6_003_21MCE268_051711_IDPLUS_mixtr-deg.fsa
  - D02_11_QC18-QC108Tube7_004_21MCE268_051711_IDPLUS_mixtr-deg.fsa
  - E02_12_QC18-QC108Tube7_001_21MCE268_051711_IDPLUS_mixtr-deg.fsa
  - F02_13_QC18-QC108Tube8_002_21MCE268_051711_IDPLUS_mixtr-deg.fsa
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  - A04_23_QC18-QC108Tube12_001_21MCE268_051711_IDPLUS_mixtr-deg.fsa
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- B03_17_QC18-QC108Tube14_002_21MCE268_051711_IDPLUS_mixtr-deg.fsa
- C03_18_QC18-QC108Tube14_003_21MCE268_051711_IDPLUS_mixtr-deg.fsa
- F03_21_QC18-QC108Tube16_002_21MCE268_051711_IDPLUS_mixtr-deg.fsa
- G03_22_QC18-QC108Tube16_003_21MCE268_051711_IDPLUS_mixtr-deg.fsa

- 3-person mixture injections used for study (first injection for each amped sample):
  Run Folder
  - RF_23MCE185_121913_CAFDAsmpls

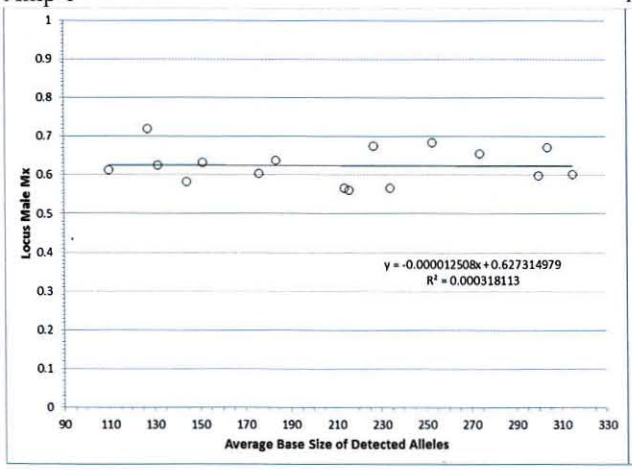
  Injections used for study; folder "[…]\STRmix\3PM DifDeg\3PM_DifDeg_STRmix"
  - D02_LADDER_004.fsa
    - Allelic ladder
  - A01_QC18_R1_001.2.fsa
    - "1" parts contributor, female, intact DNA
  - C01_QC108_R3_003.2.fsa
    - "3" parts contributor, female, degraded DNA
  - D01_QC100_004.2.fsa
    - "6" parts contributor, male, intact DNA
  - H01_PC_004.2.fsa
    - Positive amplification control
  - G01_NC_003.2.fsa
    - Negative amplification control
  - E01_EM1_001.fsa
  - F01_EM1_002.fsa

Evaluation of qPCR degradation ratio and Mx slope: 2-person differential degradation mixtures

- Mx values for the 2-person differential degradation mixtures:
  - The relevant electronic sample files are in the folder "[…]\2PM DifDeg STRmix\2PM_DifDeg_Mx_Estimation"
  - Calculated using spreadsheet "TubeXX.Mx Var (Dil Series).xlsm" (a copy was saved for each amplification.) This worksheet uses the true genotype combinations, allele heights, and Gill et al. residuals calculation to establish, on a per-locus basis, the Mx that yields the lowest residual. Where the residual minimizes, this is the Mx for which the data best fits the genotypes in question. Note: No adjustments for overlapping stutter were made when entering allele heights.
  - Locus values were plotted: x-axis is the locus Mx, y-axis is the average allele size in bases for that locus. (Note: These are based upon the true allele size, not the perceived size affected by mobility modifiers.) The metric for further consideration is the slope of the inter-locus linear regression line. See spreadsheet "TubeXX.DifDeg Male Mx Graph.xls" (a copy was saved for each amplification.)

QC018 + QC108 Intact

Amp 1



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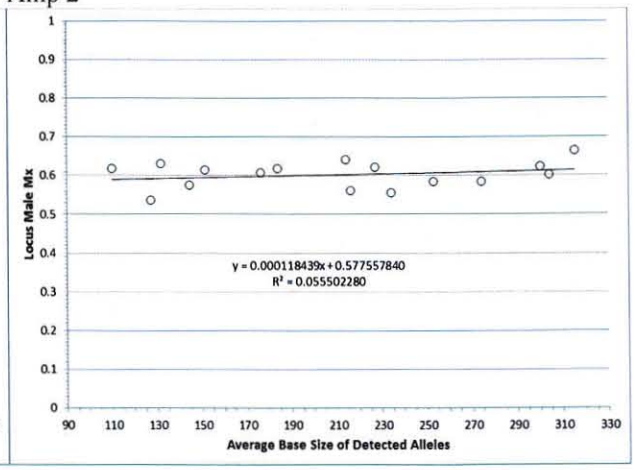

QC018 + QC108 Tube05

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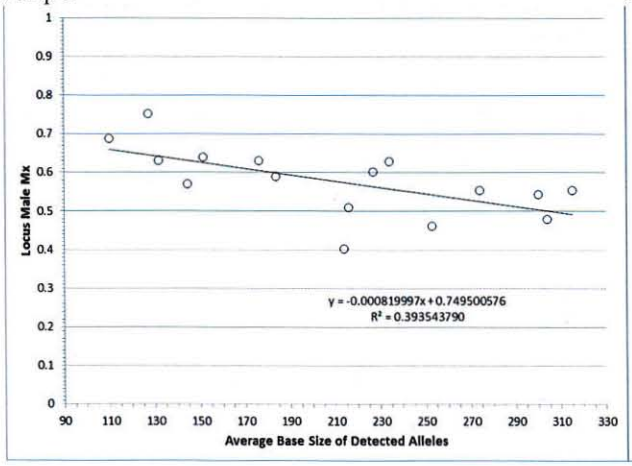

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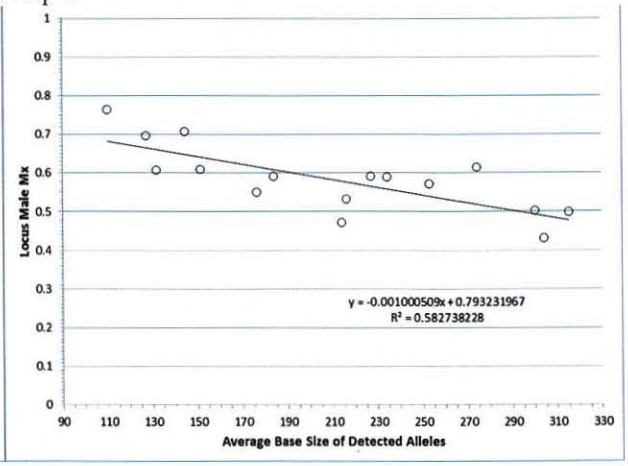

QC018 + QC108 Tube06

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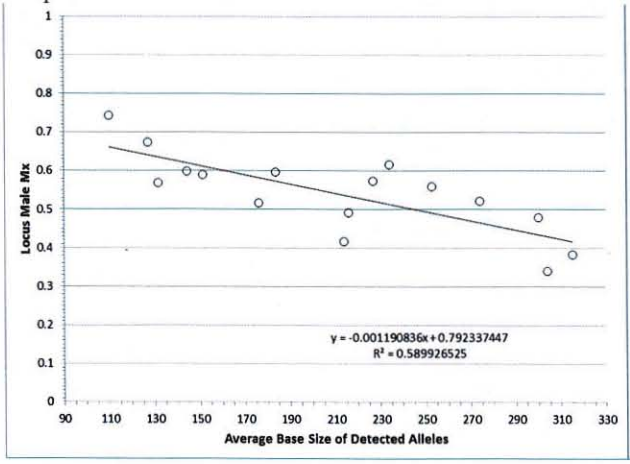

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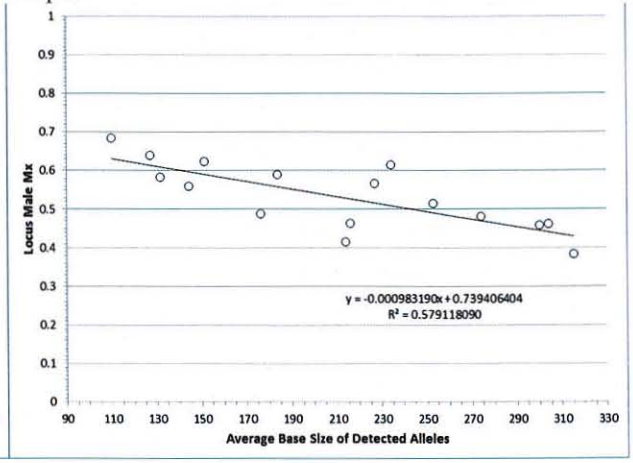

QC018 + QC108 Tube07

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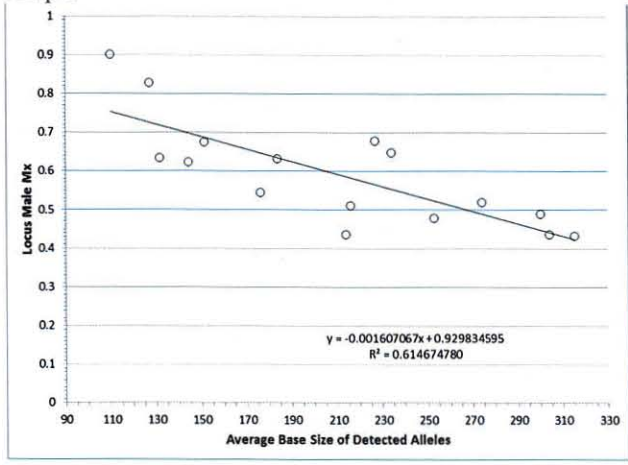

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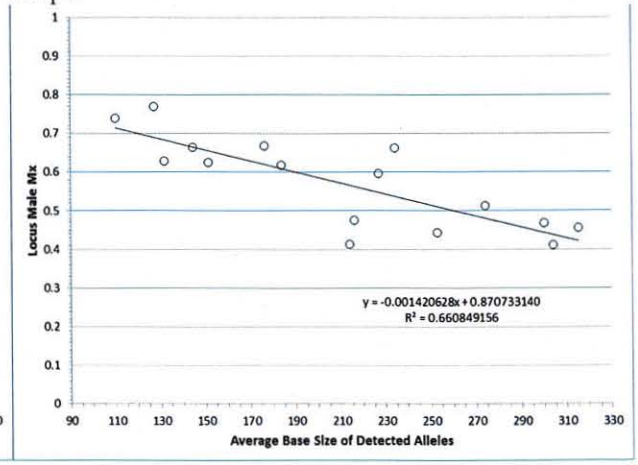

QC018 + QC108 Tube08

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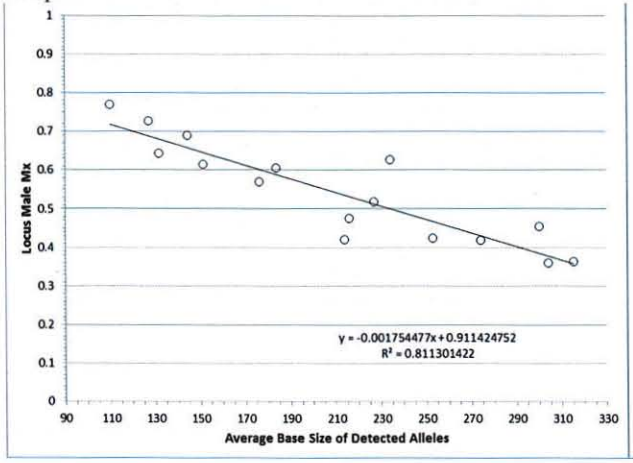

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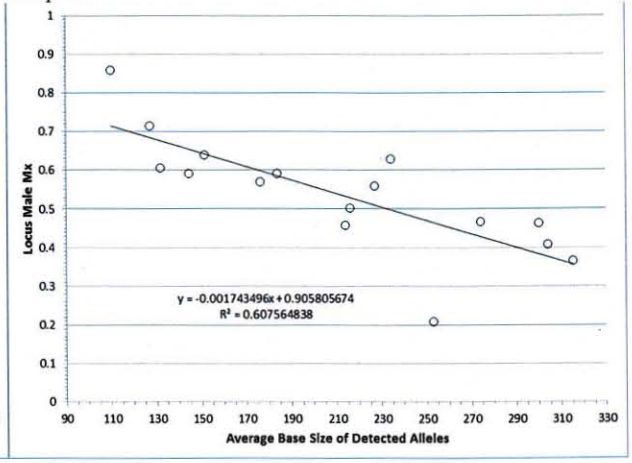

QC018 + QC108 Tube10

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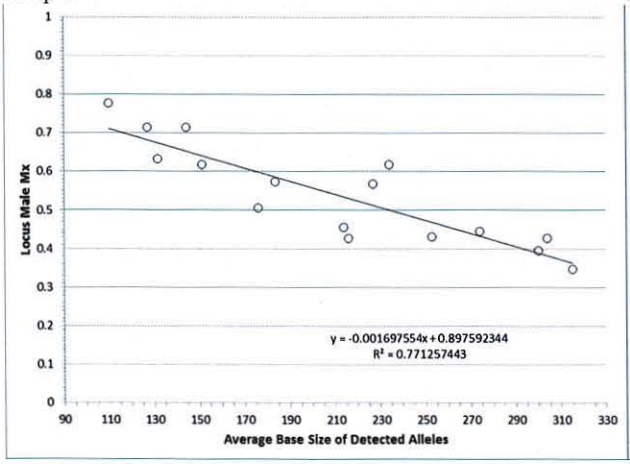

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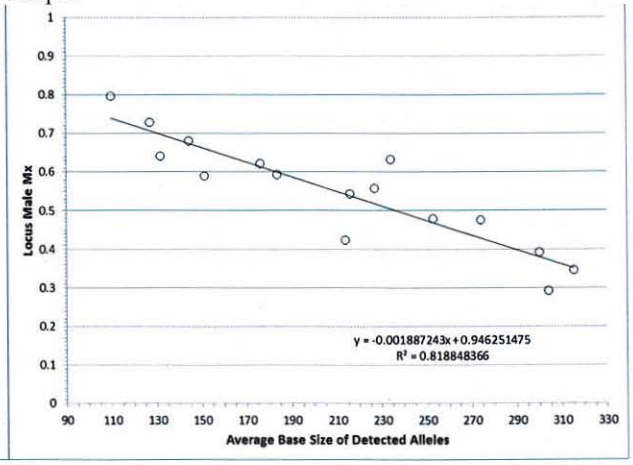

QC018 + QC108 Tube11

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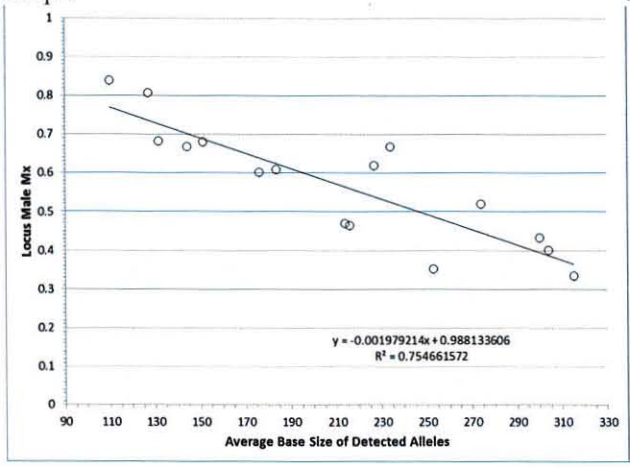

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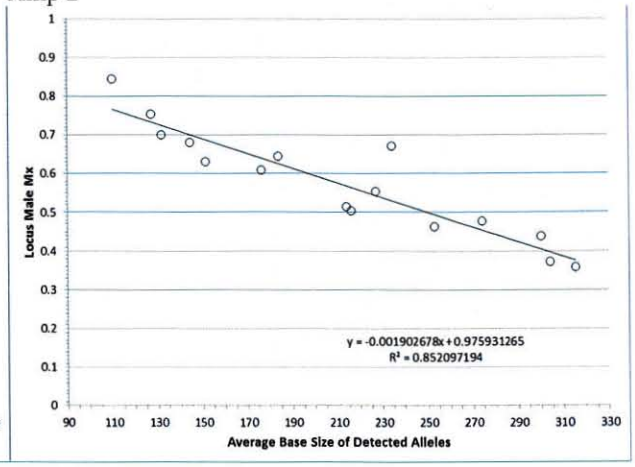

QC018 + QC108 Tube12

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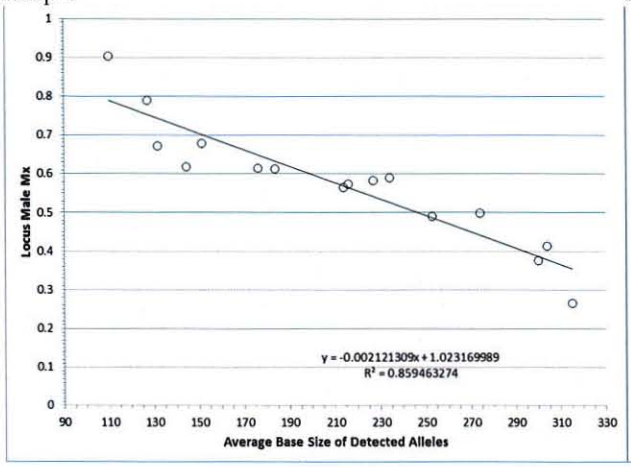

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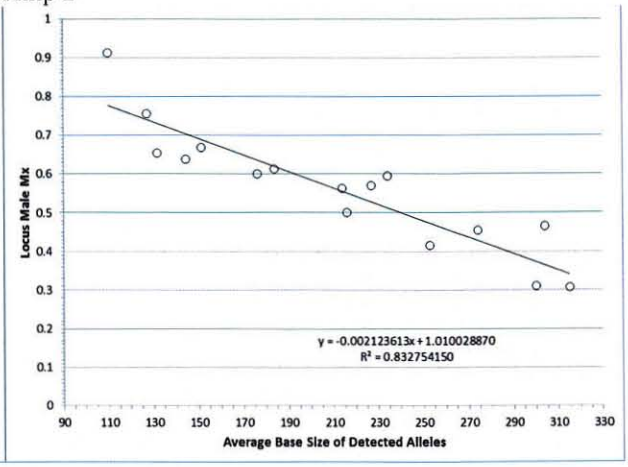

QC018 + QC108 Tube14

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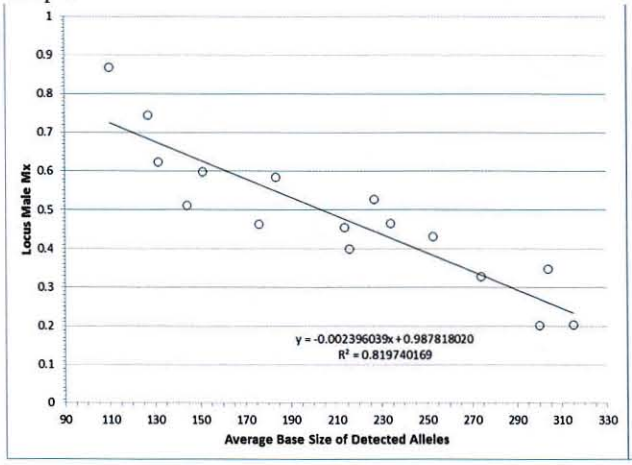

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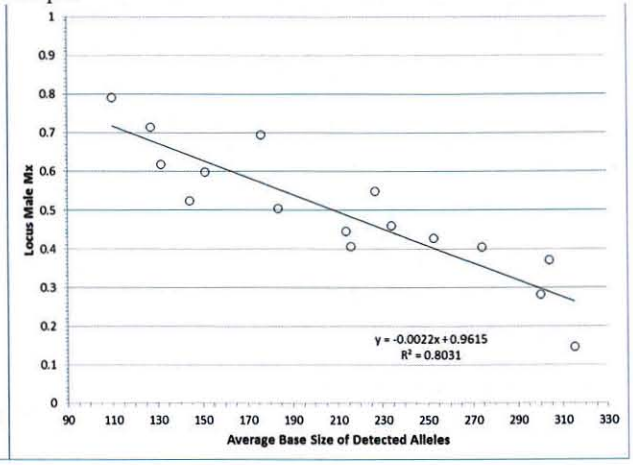
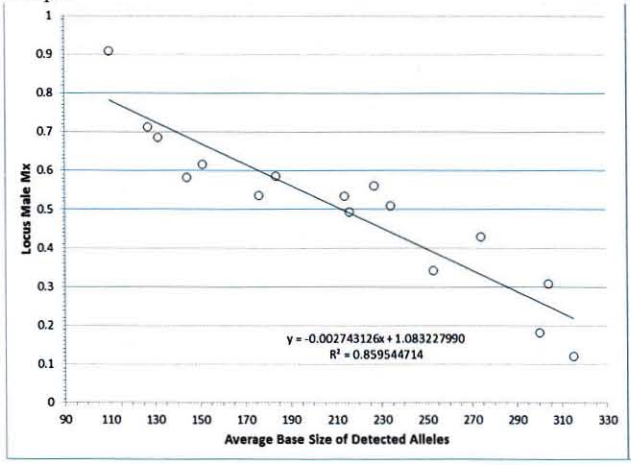


QC018 + QC108 Tube16

Amp 1



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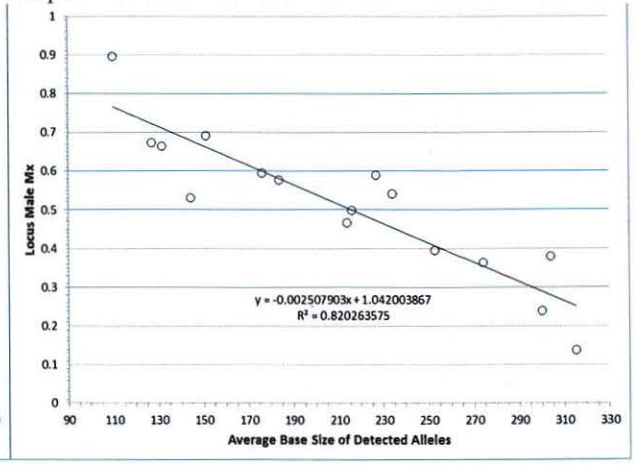
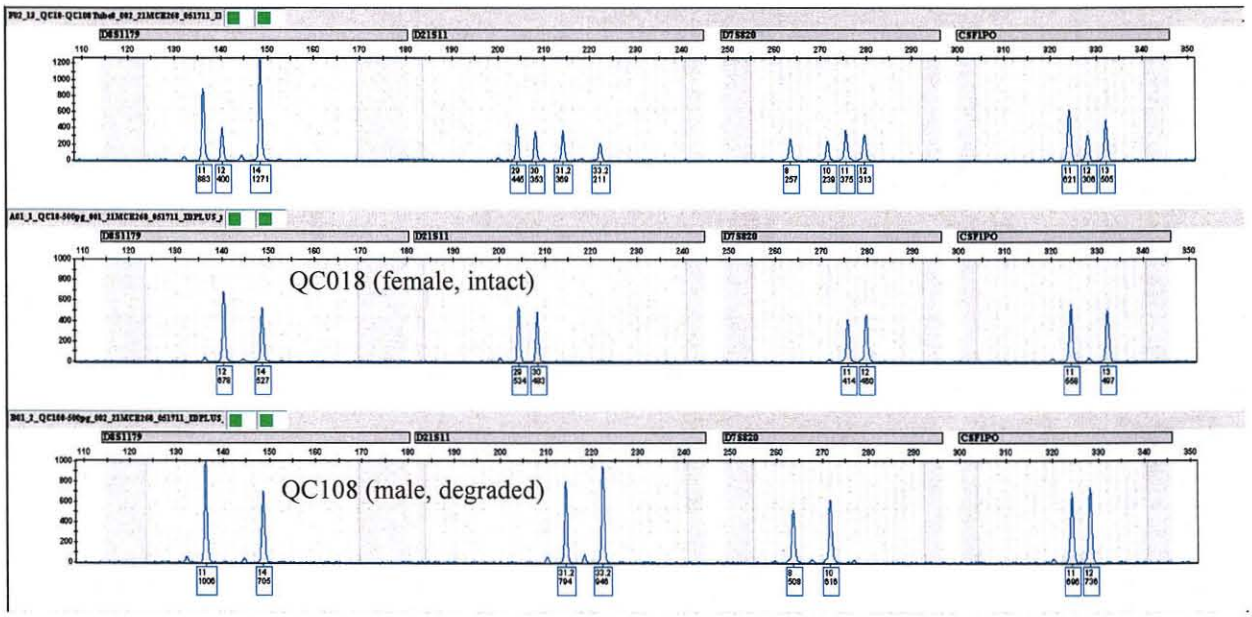
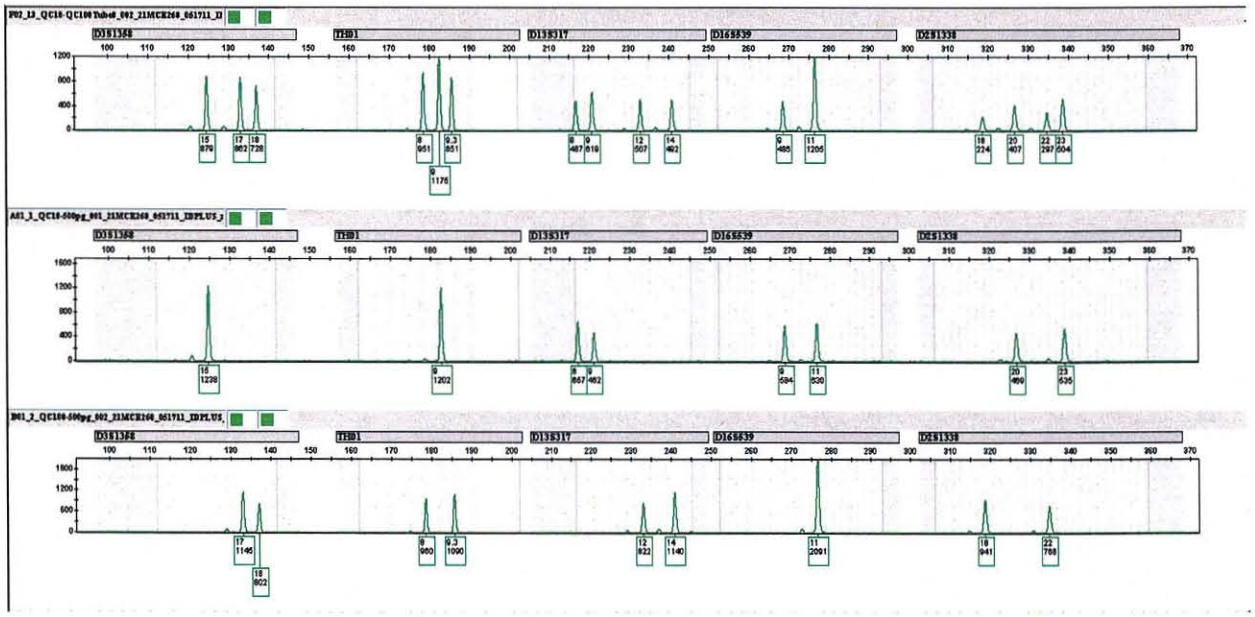
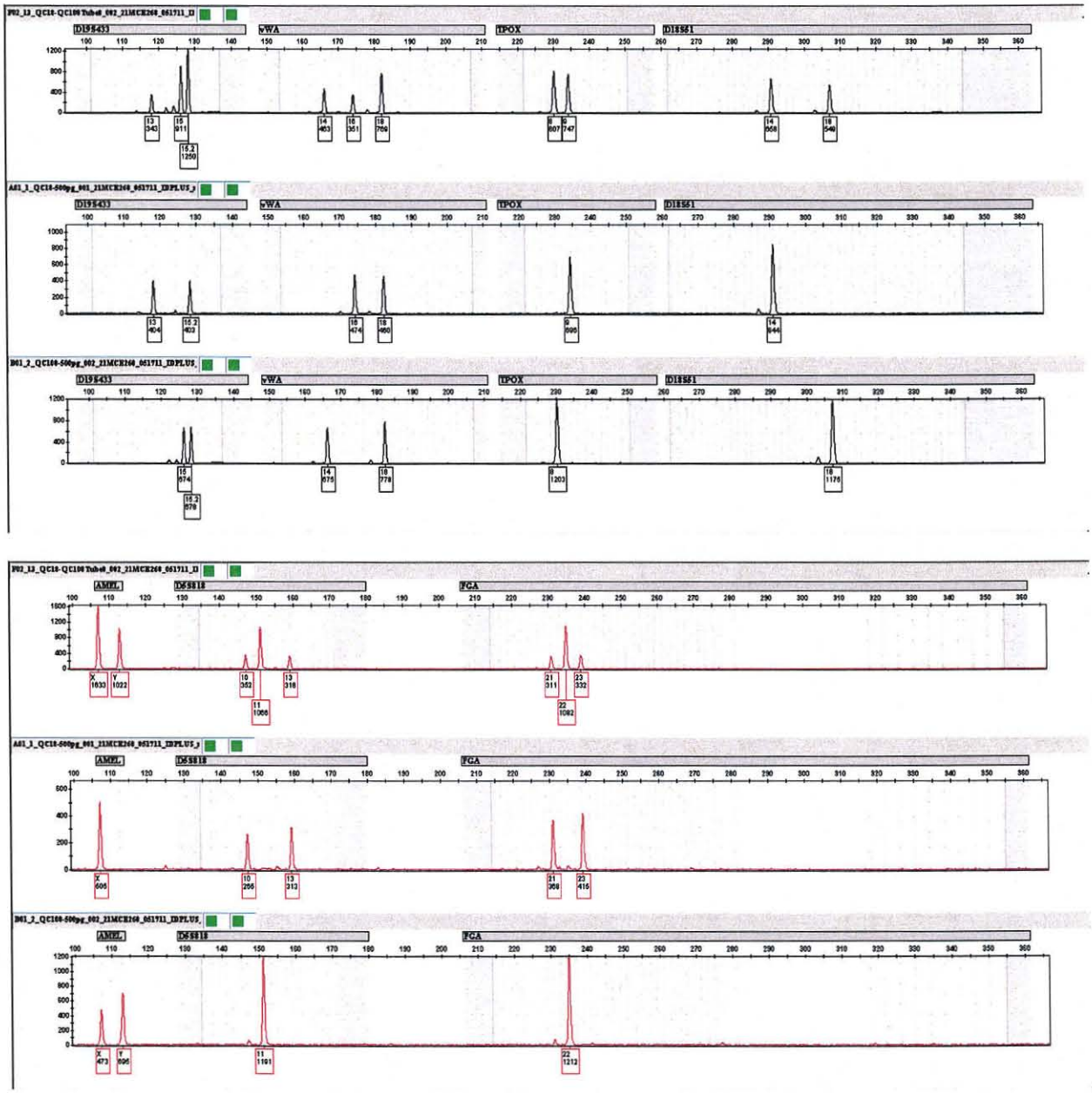


- As demonstrated in the electropherogram for sample QC018 + QC108 Tube08 where the Mx slope for the degraded contributor is –0.0017, the shift in the Mx may not be readily discernible when simply looking at the mixture. It's only when the true profiles are known that the differential degradation becomes apparent.





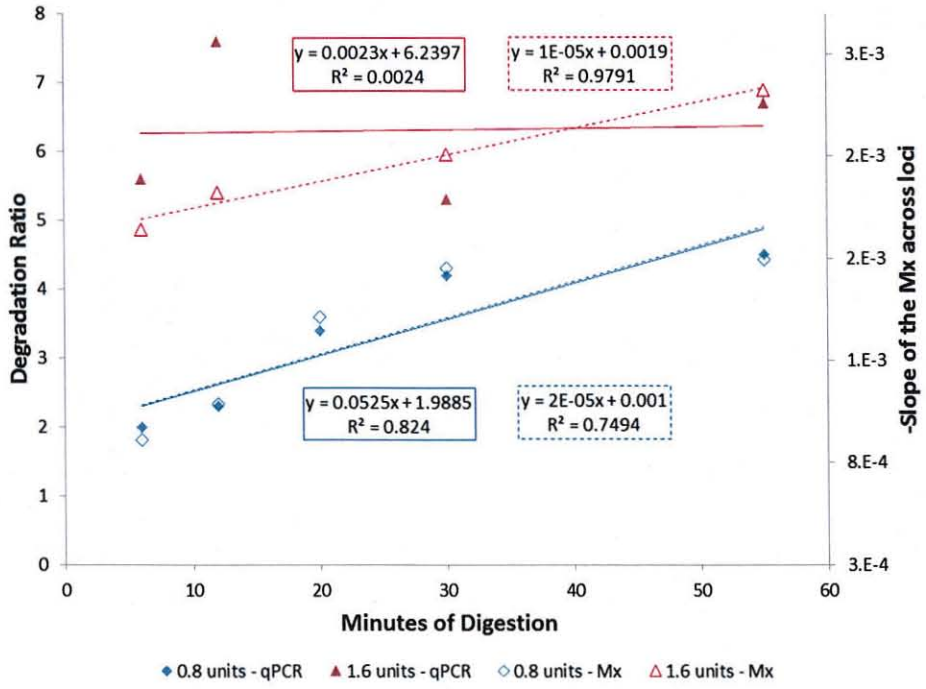

D19S433
vWA
TPOX
D18S51
AMEL
D5S818
FGA

- In order to evaluate the STRmix and TrueAllele 2-person mixture results as a function of degradation, the QC108 qPCR degradation ratio and QC108 Mx slopes were plotted for each combination of DNase1 units and time point.

Sample Name	DNase1			Digestion	qPCR	-Slope *
	uL	units / uL	units	min	deg ratio	average
QC108 Tube05	4	0.2	0.8	6	2	9.1E-04
QC108 Tube06	4	0.2	0.8	12	2.3	1.1E-03
QC108 Tube07	4	0.2	0.8	20	3.4	1.5E-03
QC108 Tube08	4	0.2	0.8	30	4.2	1.7E-03
QC108 Tube10	4	0.2	0.8	55	4.5	1.8E-03
QC108 Tube11	4	0.4	1.6	6	5.6	1.9E-03
QC108 Tube12	4	0.4	1.6	12	7.6	2.1E-03
QC108 Tube14	4	0.4	1.6	30	5.3	2.3E-03
QC108 Tube16	4	0.4	1.6	55	6.7	2.6E-03

*Average for the two amplifications.

- Plotting the 2-person differential degradation data
  - The two y-axes were scaled to promote graphical overlap.
  - Linear regression lines: Solid for qPCR degradation ratios, dashed for Mx slopes.

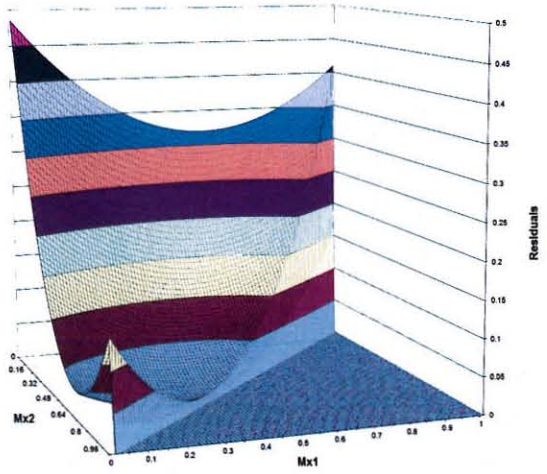


- For the purposes of this study, the slope of the Mx increased consistently as a function of time and/or DNase1 quantities. The qPCR degradation ratio and Mx slope values gave almost identical results for 0.8 units of DNase1. The regression lines overlap and have similar R^2. However, the Mx slope had a much higher correlation to increases in time for 1.6 units. Additionally, the slopes of the 0.8 and 1.6 linear regression lines for the Mx slope values are similar, unlike those of the qPCR degradation ratios.
- Graphing of the STRmix and TrueAllele results will use the Mx slopes for the y-axis.

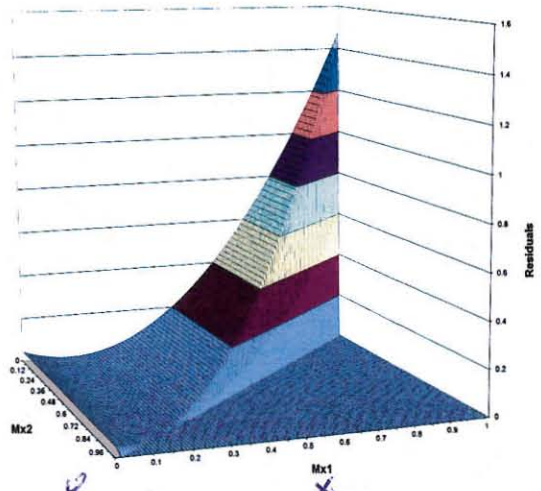
Evaluation of Mx slope: 3-person differential degradation mixture

- Mx values for the 3-person differential degradation mixture:
  - Calculated using spreadsheet "3PM_DifDeg Mx spm 01.24.2014.xls" (a copy was saved for each amplification.) This worksheet uses the true genotype combinations, allele heights, and Gill et al. residuals calculation to establish, on a per-locus basis, the Mx that yields the lowest residual. A 3-dimensional map is created with the Mx for contributor 1 on the x-axis, the Mx for contributor 2 on the y-axis, and the residual on the z-axis. (No graphing is needed for contributor 3, since that Mx becomes fixed once you know the Mx values for contributors 1 and 2.) The bottom of the cavity (or trough for a map where two of the contributors have the same genotype) is the point where the residual has minimized. These are the Mx values for which the data best fits the three genotypes in question. Note: No adjustments for overlapping stutter were made when entering allele heights.
  - Examples of residuals maps:

All genotypes different

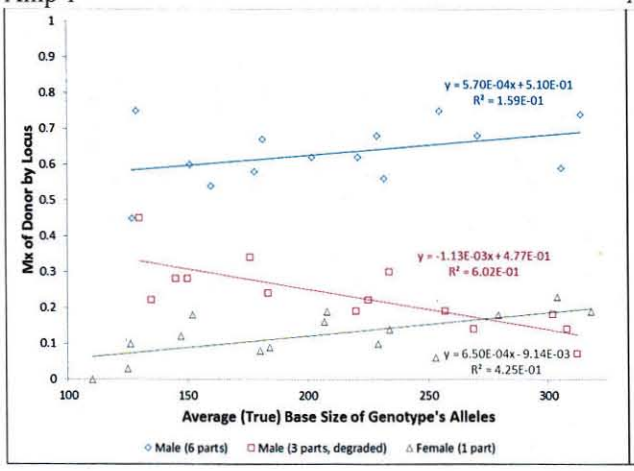


Two genotypes the same, one different

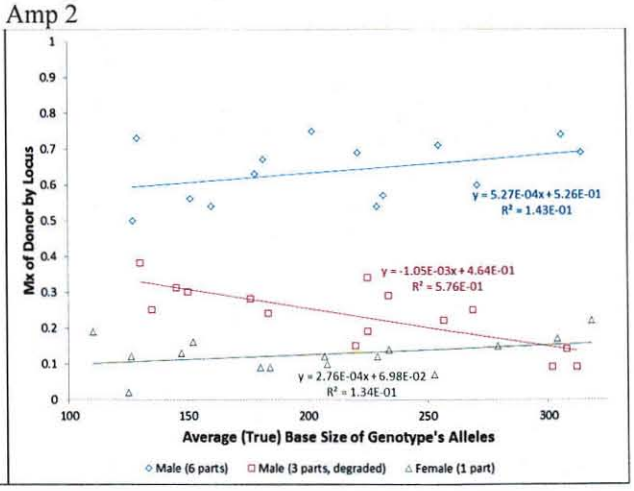


  - Locus Mx values were plotted for each contributor: x-axis is the locus Mx, y-axis is the average allele size in bases for that locus. (Note: These are based upon the true allele size, not the perceived size affected by mobility modifiers.) The metric for further consideration is the slope of the inter-locus linear regression line. See spreadsheet "3PM_DifDeg Mx Summaries spm 01.24.2014.xls".

Amp 1



Amp 2



- Graphing of the STRmix and TrueAllele results will use the Mx slopes for the y-axis.

Creation of mixture interpretation input files

- STRmix – Import files were created as GeneMapper ID exported Genotype tables (txt format) per the STRmix recommendations and settings.
    - Reference import files included the following information: sample name; marker name; and allele designations and the measured size in bases for the genotype's allele peaks.
    - Mixture import files included the following information: sample name; marker name; and allele designations, measured size, and heights (RFU) for all possible allelic and reverse stutter peaks.
    - 50 RFU analytical threshold.
    - Peaks consistent with artifacts other than reverse stutter were electronically deleted prior to the table export.
    - Exported tables did not always maintain the proper locus order. To correct this, the txt files were reordered using the Excel spreadsheet "LocusOrderForExportTXT.xlsm".
    - Evidence import file: 2PM_DifDeg_STRmix.txt
    - Reference import file: 2PM_DefDeg_STRmix_R.txt

- TrueAllele Casework – This system incorporates its own data analysis component that uses the original fsa files for the samples of interest, allelic ladders, and amplification controls. Individual capillary's files were combined into a virtual gel for sizing and allele calls. The resulting information was then uploaded to the server.
    - Mixture settings:
        - "newdd_acquire" set to File mode.
        - Identifiler template
        - ABI3130xl
        - Ladder assignment: closest
        - Size standard
            - 2-person: GS500 (MDC didn't run this using the final lab protocol)
            - 3-person: LIZ600
        - Controls: Names (Ladder, 9947A, NC)
    - Analyze Module
        - All size standard peaks labeled.
        - No rules fired.
        - Gels:
            - 2-person: TACMDCDifDegStudyData.gel
            - 3-person: 3PM_DifDeg_RF.gel
    - Data Module
        - Uploaded gel to world: Rosifume

Interpretations

- STRmix and TrueAllele Casework interpretations were performed in triplicate. Both amplifications were interpreted separately and as a joint interpretation. In some, but not all, instances where likelihood ratios reported by TrueAllele Casework were observed to be identical to the ninth decimal place, an additional interpretation was performed, and one of the duplicated results was dropped from further consideration. Such instances were not limited to major contributors with 100% probability attached to all of their genotypes.
- In general, both systems use similar approaches to mixture interpretation and the calculation of the likelihood ratio. However, they differ in myriad details. Rather than try to limit the differences, the systems were compared "as is". They were, however, interpreted with more MCMC cycles than standard.
- Both systems were interpreted with no assumed contributors. Initial interpretations were based solely upon the mixtures.
- STRmix interpretation:
    - The 2-person differential degradation study was tested twice. Once using the allele variance settings calculated by Cal DOJ using Model Maker version 1.0.7.49, and a second time using the allele variance settings supplied by ESR, calculated using the same Cal DOJ data and the Model Maker version found in STRmix V2.0.

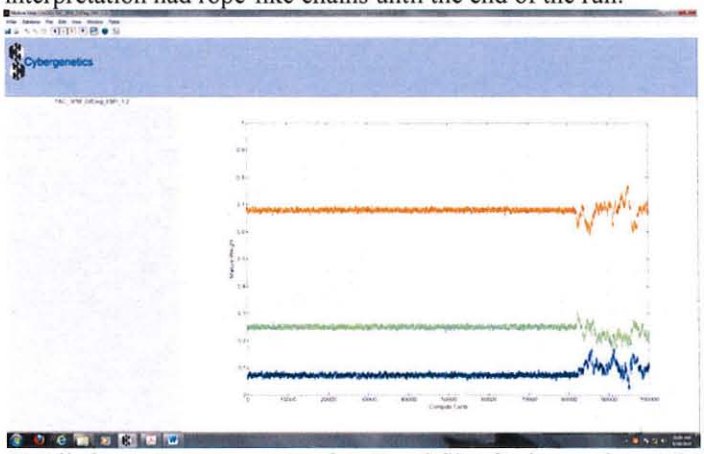
- The 3-person differential degradation sample was only tested using the ESR settings.
- Settings
  - Variance
    - Model Maker V1.0.7.49: 1.62
      For interpretations using this variance, see folder "[…]\2PM DifDeg STRmix\Interp"
    - ESR supplied using approach in Model Maker V2.0: 3.392
      For interpretations using this variance, see folder "[…]\2PM DifDeg STRmix\Interp New Variance"
    - Note: V2.0 Model Maker approximately doubled the allele variance.
  - Detection threshold: 50
  - Stutter: 0.3
  - Degradation: 0.02
  - Drop-in: 0.0
  - Drop-in parameters: 0.0,0.0
  - Clip rate: 0.01
  - Extreme clip: 0.005
  - Saturation: 7500
  - Use ref alleles: N
  - Use locus amp factors: Y
  - # MCMC chains: 4
  - Extended output: N
  - MCMC accepts: 100,000
  - Burnin accepts: 20,000
  - HPD iterations: 1,000
  - Sig value: 99.0
  - Sides: 1
  - Alleles per locus: 10
  - Locus amp variance: 0.022
  - Default kit: IDP_3130XL

- TrueAllele Casework interpretations.
  - Request Module
    - Settings:
      - Process: twounknown
      - Part: evidence
      - Defer: no (see the note below re. the 3-person mixtures)
      - Burnin: 100,000
      - Readout: 100,000
      - Offladder: short
      - Degraded: on
      - Logging: off
      - Sort: off
      - Overwrite: no
    - 2-person mixture requests
      - Request: TAC_MDC_DifDeg2a.req
        - Joint interpretation of two amplifications.
        - Duplicate amplifications joined at the item level, so that they will be based upon one mixture weight.
      - Request: TAC_MDC_DifDeg2a.1.req
        - Additional requests.
        - In most cases, this was just a third request. For Tube14.17.18, a fourth request was performed, because the first two gave identical LRs (they might have randomized from identical seeds.)
      - Request: TAC_MDC_DifDeg3.req

- The interpretation of each amplification was performed separately.
- Request: TAC_MDC_DifDeg3.1.req
  - Additional requests were performed. In most cases, this was just a third request. For Tube12.23, a fourth request was performed, because the first two gave identical LRs (they might have randomized from identical seeds.).
- Request: TAC_MDC_DifDeg3.2.req
  - Additional requests were performed for the second amp.
  - All samples were cycled through prior to starting the second set of request. This was done to avoid duplicate requests getting the same seed.

- 3-person mixture requests
  - Request: TAC_3PM_DifDeg.req
    - Separate and joint interpretations of the two amplifications.
    - NOTE: The Defer setting was "yes" for the duplicate mixture requests. After the requests were initiated, and the first set of mixture requests were running, the second set of requests was activated.
  - Request: TAC_3PM_DifDeg.1.req
    - Additional requests were performed.

- Report Module
  - 2-person mixtures: No overly narrow mixture weights distributions were observed during a review of all interpretations. Some interpretations had the chains switch weights, leading to distributions with large amounts of overlap. Other interpretations had little to no overlap observed.
  - 3-person mixture: With one exception, the interpretations had chains that appear to be reasonable, with two of the contributors swapping higher-lower order. The 3rd joint interpretation had rope-like chains until the end of the run:



  - Detailed reports were exported as Excel files for import into DOJ LR spreadsheets (see below).

Summary: Number of interpretations per mixture and interpretation system

	1st Amp Interpretations		2nd Amp Interpretations		Joint Interpretations	
2-Person Mixtures	STRmix *	TAC	STRmix *	TAC	STRmix *	TAC
QC18-QC108_intact	3	3	3	3	3	3
QC18-QC108Tube5	3	3	3	3	3	3
QC18-QC108Tube6	3	3	3	3	3	3
QC18-QC108Tube7	3	3	3	3	3	3
QC18-QC108Tube8	3	3	3	3	3	3
QC18-QC108Tube10	3	3	3	3	3	3
QC18-QC108Tube11	3	3	3	3	3	3
QC18-QC108Tube12	3	4	3	3	3	3
QC18-QC108Tube14	3	3	3	3	3	4
QC18-QC108Tube16	3	3	3	3	3	3
3-Person Mixture	STRmix	TAC	STRmix	TAC	STRmix	TAC
EM1	3	3	3	3	3	3

*All STRmix interpretations performed twice under separate allele variances.

Likelihood Ratios

- Likelihood ratios were calculated at $\theta = 0.01$ using FBI African American, Caucasian, and Hispanic databases (JFS 1999 44(6); FSC 1999 1(2); FSC 2001 3(3); each adapted as necessary for use in the specific software package, for example by removing "<" and ">" binned alleles.)
- Electronic reports were generated for each system:
  - STRmix "[…]_Results.txt" files that include genotype combinations and associated donor combination weights, but no LRs.
  - TrueAllele "detailed reports" (xls files), with all values to 9 decimal place, for each interpreted contributor to the mixture. The confidence level was set to 1.0 so that 100% of the interpreted genotypes (those assigned any probability by TrueAllele) would be included in the report. Note: In the initial part of the study, separate reports were created for each combination of reference-contributor-population database based upon a manually selected reference-contributor pairing. Later, as allowed by a change in the LR calculation spreadsheet, only a single reference-population was necessary for each contributor. These latter reports were all imported into a single spreadsheet for automated contributor selection by the program (see the discussion below.)
- Electronic reports were imported into an Excel spreadsheet ["STRmix Val. Non-Contrib. (spm 1.18.14).xlt", "TAC Val. Non-Contrib. (spm 4.9.2013).xlt", and "TAC Val. Non-Contrib. (spm 1.18.2014).xlt"]. The January 18, 2014 versions also required the import of a csv file containing a known contributor's profile. Each spreadsheet applies the approach of its respective system to calculate the LR.
  - The TrueAllele reports imported into spreadsheet "TAC Val. Non-Contrib. (spm 4.9.2013).xlt" were based upon a manual selection of the interpreted contributor for each comparison (i.e., whether QC018 was assigned contributor 1 and QC108 was assigned contributor 2, or vice versa, was based upon a manual review of which order gave higher LRs for these two references.)
  - For spreadsheets "STRmix Val. Non-Contrib. (spm 1.18.14).xlt" and "TAC Val. Non-Contrib. (spm 1.18.2014).xlt", the assignment of a particular interpreted contributor to a comparison reference was done automatically based upon a single LR that is a combination of the three population LRs:

- The STRmix spreadsheet uses a LR created from the sums of the three populations numerator and denominator conditional probabilities

$$LR_S = \frac{Num_{AfAm} + Num_{Cauc} + Num_{Hisp}}{Den_{AfAm} + Den_{Cauc} + Den_{Hisp}}$$

This value is also calculated by STRmix as a stratified LR (in this case, all populations would be in equal proportion.) It is noted that STRmix assigns the highest population-specific LR to each comparison regardless of whether or not all population LRs will represent the same interpreted contributor. Therefore, at times the values used for the study at hand may be slightly lower than those observed using the LR calculating function in STRmix. This difference should be limited in scope, since a given reference should only have similar LRs for multiple interpreted contributors when the contributors are present in similar proportions as in, for example, a 1:1 mixture.

- The TrueAllele spreadsheet uses the harmonic mean of the three population LRs:

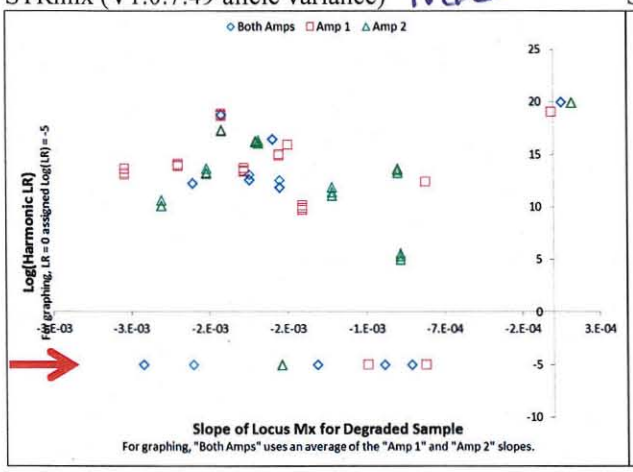
$$LR_H = \frac{3}{\sum_{i=1}^{N} \frac{1}{LR_i}}$$

The harmonic mean is not part of the TrueAllele approach. TrueAllele requires the user to assign a specific reference to a specific interpreted contributor. For some mixtures, this can be a simple matter [e.g., when each reference has LR>>1 for one interpreted contributor and LR<<1 for the other interpreted contributor(s)]. For other mixtures, multiple contributors may give their maximum LR for the same interpreted donor, or a reference may give their maximum population-specific LR in different contributor (as also happens in STRmix; see above.) In TrueAllele, unlike in STRmix, there is no ability to calculate a single LR for multiple references jointly.

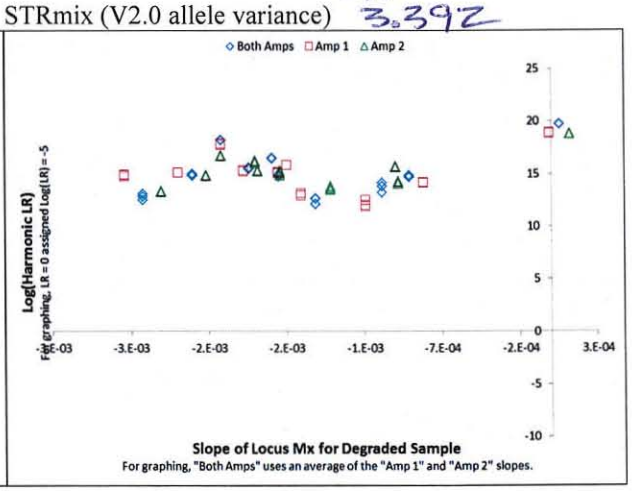
SENSITIVITY: Graphing the 2-person mixtures

- Population-specific LRs for each comparison were imported into the following spreadsheets
  - STRmix 2PM_DifDeg Graphs (spm 06.24.2014).xlsm
  - TAC 2PM_DifDeg 0 and 0.01 Graphs (spm 06.24.2014).xlsm
- Harmonic means (LR_H described above) were calculated for each comparison.
- The results were plotted as the Mx slope on the x-axis and the $\log_{10} LR_H$ on the y-axis.
  - Note: Since you can't calculate a log of LR = 0, those values were assigned the value of –5.
- QC018 (female, intact DNA)

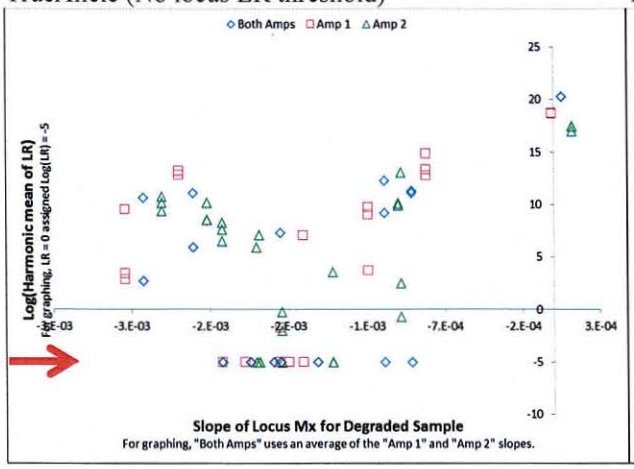
STRmix (V1.0.7.49 allele variance) spm 1.02



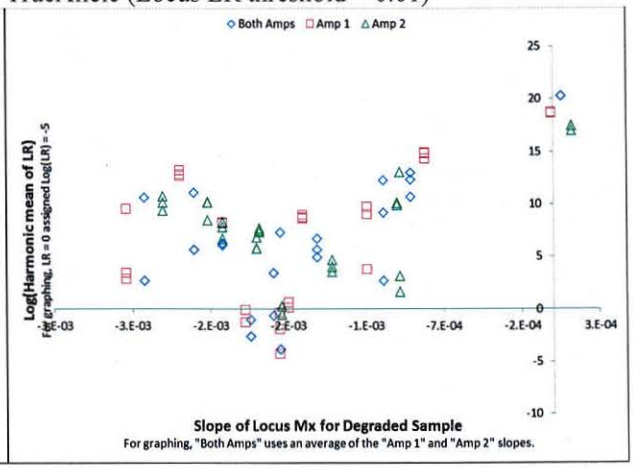
STRmix (V2.0 allele variance) spm 3.39Z



TrueAllele (No locus LR threshold)

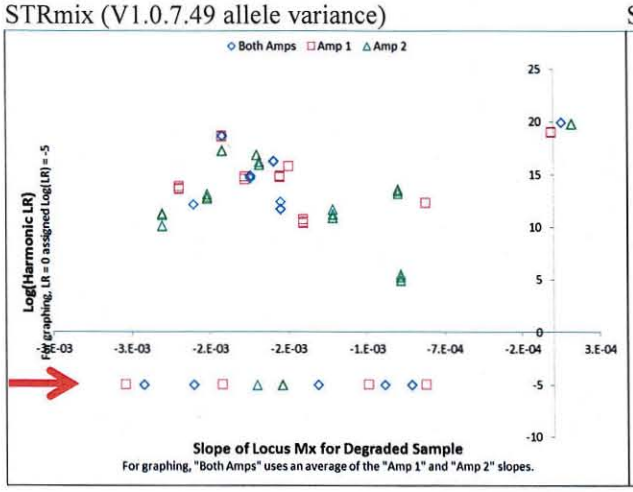


TrueAllele (Locus LR threshold = 0.01)

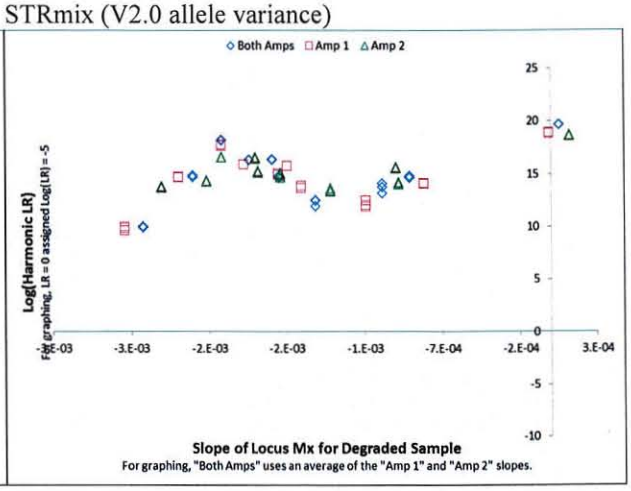


- QC018 (male, degradation series)

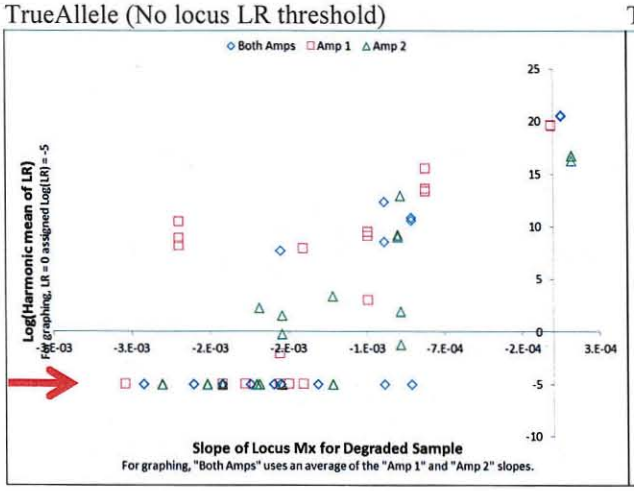
STRmix (V1.0.7.49 allele variance)



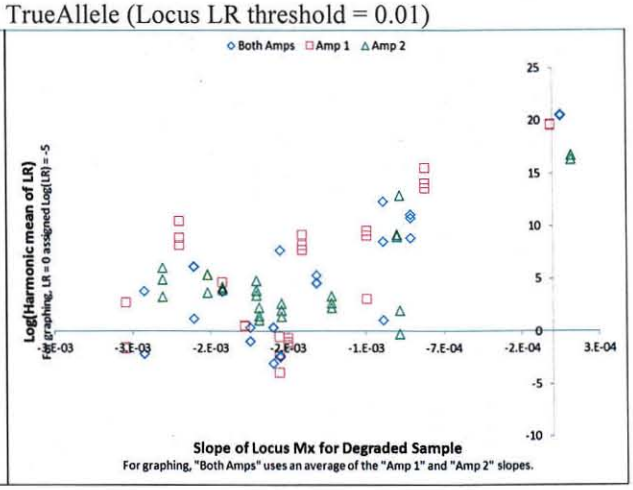
STRmix (V2.0 allele variance)



TrueAllele (No locus LR threshold)



TrueAllele (Locus LR threshold = 0.01)



- As a point of comparison to our current approach, values using MixMaster were plotted in the same way.
  - Only one interpretation was performed for each comparison.
    - MixMaster has a randomization seed, so the results would be identical if started fresh from opening the spreadsheet.
  - Interpretation performed using "MixMaster IDP (Rel. 1.1).xlt"
    - No joint interpretations of the two amps were possible.
    - Note: When MixMaster was run using the default Mx calculated from 4-allele loci, Tube16 amp 1 had a Type I error (false exclusion) at D2S1338 for QC108. The Tube16 amp 1 data included in the plots is based upon a reanalysis using the 3- and 4-allele Mx estimated using "Mx CALculator (Rel. 1.0).xlt". With that Mx, QC108 was properly included. All other amplifications' interpretations are based upon the default Mx.

- The major and minor contributor allowed genotypes were compared to the profiles of QC018 (female, intact) and QC108 (male, degradation series). This comparison was done by examining for simple inclusion/exclusion, as well as by using the average Mx to see which contributor would fit them best.

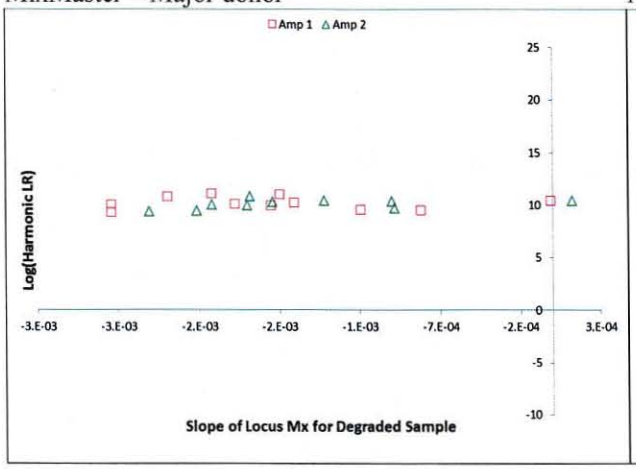
	Amp 1				Amp 2			
Tube	Female		Male		Female		Male	
	Incl/Excl	Ave Mx	Incl/Excl	Ave Mx	Incl/Excl	Ave Mx	Incl/Excl	Ave Mx
Intact	both	minor	both	major	both	minor	both	major
Tube05	both	minor	both	major	both	minor	both	major
Tube06	both	major	both	minor	both	major	both	minor
Tube07	both	minor	both	major	both	minor	both	major
Tube08	both	major	both	minor	both	major	both	minor
Tube10	both	major	both	minor	both	minor	both	major
Tube11	both	minor	both	major	both	minor	both	major
Tube12	both	minor	both	major	both	minor	both	major
Tube14	major	major	minor	minor	major	major	minor	minor
Tube16	major	minor	no	major	major	minor	minor	major
Tube16*	major	minor	minor	major				

*The interpretation using the override Mx from Mx Calculator.

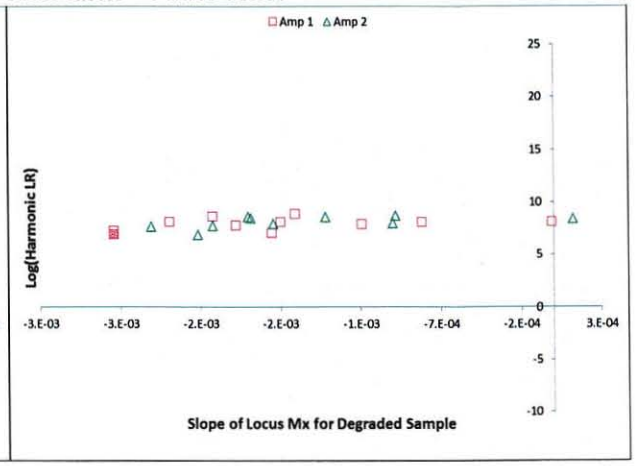
"BOTH" = INCLUDED IN BOTH THE MAJOR AND MINOR DONOR PROFILES. SPM

- The major and minor donor RMPs were calculated in "STRstatID_DOJ_v042612.xlt".
- LRs were calculated as 1/RMP.
- Population-specific LRs for each comparison were entered into the following spreadsheet
  - "MixMaster 2PM_DifDeg Graphs (spm 06.12.2014).xlsm"
- Harmonic means (LR_H described above) were calculated for each comparison.
- The results were plotted as the Mx slope on the x-axis and the $\log_{10} LR_H$ on the y-axis.
  - For Tube 16, the LR_H for both interpretations (standard Mx and Mx Calculator override Mx) were both plotted.
  - **NOTE: The stippled minor donor data point in the Minor donor graph would be LR=0 for QC108, per the discussion above.**

MixMaster – Major donor



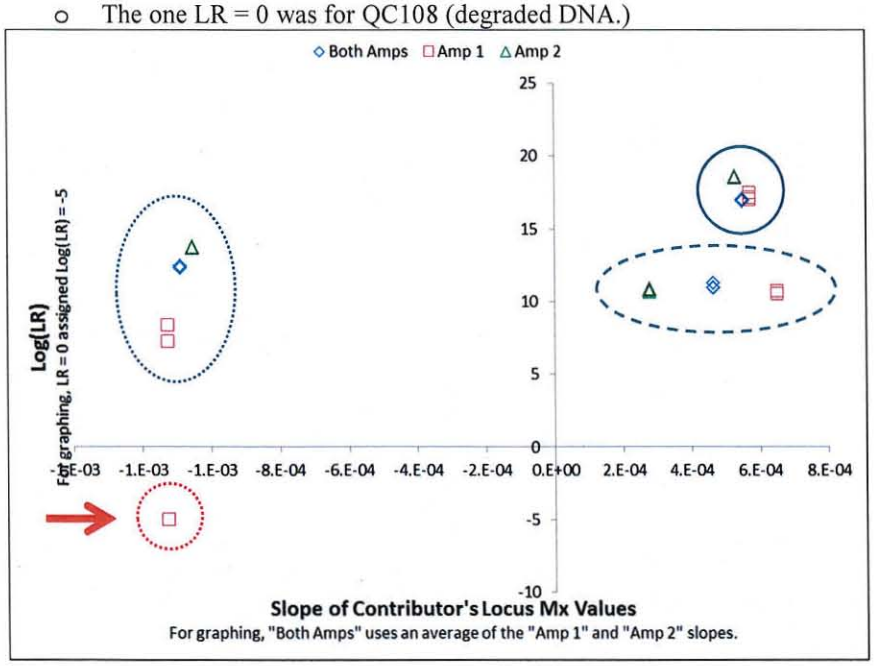
MixMaster – Minor donor



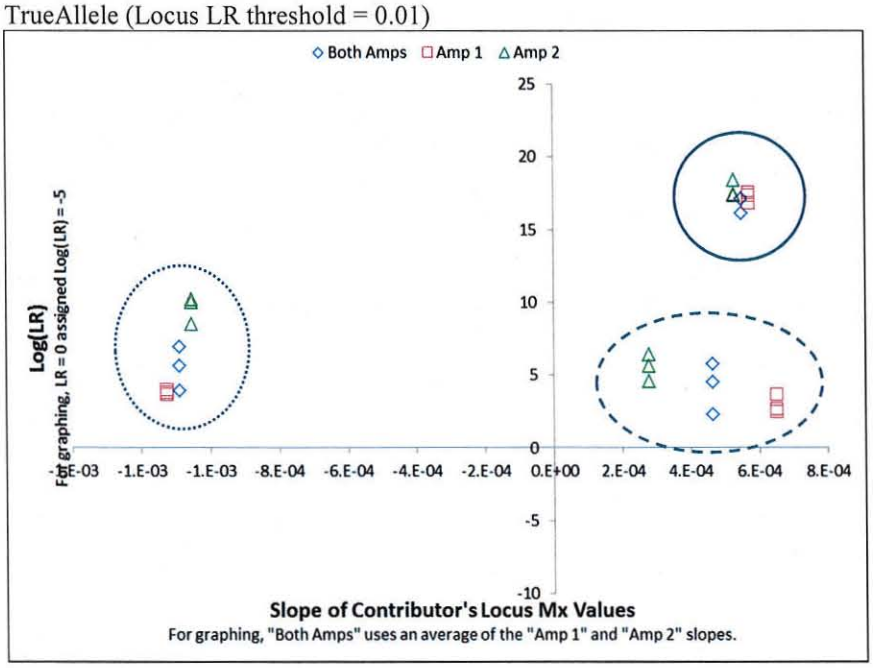
LR_H	2PM
Total (degraded mixtures only)	36
$LR_H = 0$ (standard Mx)	2.78%
$LR_H = 0$ (Tube16 Amp 1 alt. Mx)	0.00%

SENSITIVITY: Graphing the 3-person mixture

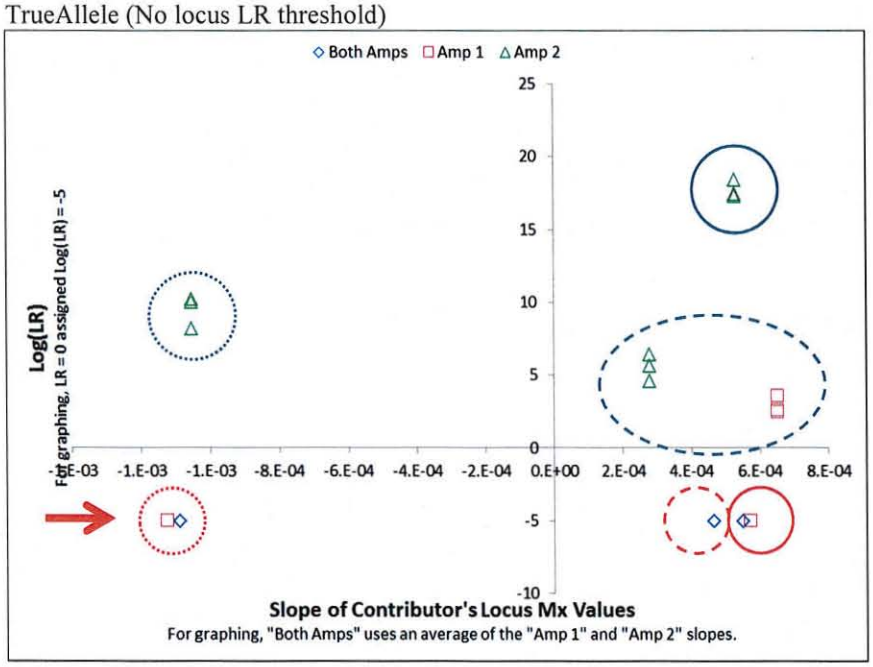
- Population-specific LRs for each comparison were imported into the following spreadsheets
  - STRmix 3PM_DifDeg Graphs (spm 06.24.2014).xlsm
  - TAC 3PM_DifDeg 0 and 0.01 Graphs (spm 06.24.2014).xlsm
- Harmonic means (LR_H described above) were calculated for each comparison.
- The results were plotted as the Mx slope on the x-axis and the $\log_{10} LR_H$ on the y-axis.
  - The results for each contributor are encircled.
  - $LR_H = 0$ was plotted as –5.
- STRmix
  - The one LR = 0 was for QC108 (degraded DNA.)



- TrueAllele (Locus LR threshold = 0.01)



- TrueAllele (No locus LR threshold)



SENSITIVITY: Summary Tables

- Population-specific LRs for each comparison were imported into the following spreadsheets
  - STRmix_Graphs_Data (spm 07.10.2014).xlsm
  - TAC_Graphs_Data (spm 07.10.2014).xlsm
- Notes:
  - These tables do not include the results from the 2-person mixture differential degradations study's "QC18-QC108_intact" mixture.
  - STRmix results using the V1.0.7.49 variance were not included.
- All contributors for 3 replicates each of amp 1, amp 2, and the joint interpretation:

LR_H	STRmix (V2.0 variance)			TAC (0.01 minimum)			TAC (0 minimum)		
	2PM	3PM	Comb.	2PM	3PM	Comb.	2PM	3PM	Comb.
Total	162	27	189	162	27	189	162	27	189
$LR_H = 0$	0.00%	3.70%	0.53%	0.00%	0.00%	0.00%	56.17%	55.56%	56.08%
$0 < LR_H < 1$	0.00%	0.00%	0.00%	17.90%	0.00%	15.34%	4.32%	0.00%	3.70%
$LR_H < 1$	0.00%	3.70%	0.53%	17.90%	0.00%	15.34%	60.49%	55.56%	59.79%

- All contributors for 3 replicates each of amp 1 and amp 2:

LR_H	STRmix (V2.0 variance)			TAC (0.01 minimum)			TAC (0 minimum)		
	2PM	3PM	Comb.	2PM	3PM	Comb.	2PM	3PM	Comb.
Total	108	18	126	108	18	126	108	18	126
$LR_H = 0$	0.00%	5.56%	0.79%	0.00%	0.00%	0.00%	49.07%	33.33%	46.83%
$0 < LR_H < 1$	0.00%	0.00%	0.00%	14.81%	0.00%	12.70%	6.48%	0.00%	5.56%
$LR_H < 1$	0.00%	5.56%	0.79%	14.81%	0.00%	12.70%	55.56%	33.33%	52.38%

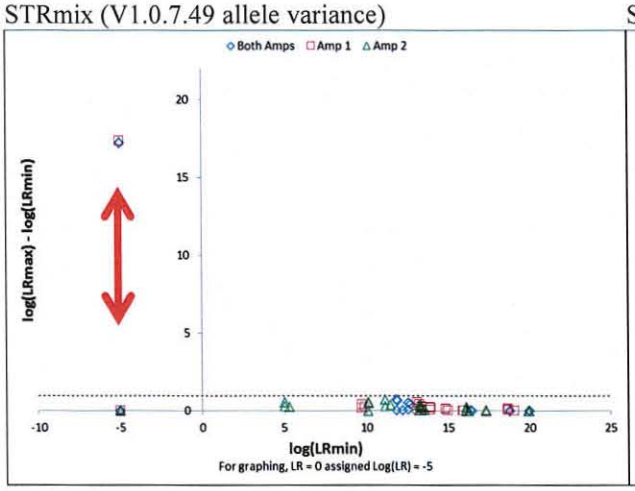
- All contributors for 3 replicates each of the joint interpretations:

LR_H	STRmix (V2.0 variance)			TAC (0.01 minimum)			TAC (0 minimum)		
	2PM	3PM	Comb.	2PM	3PM	Comb.	2PM	3PM	Comb.
Total	54	9	63	54	9	63	54	9	63
$LR_H = 0$	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	70.37%	100.00%	74.60%
$0 < LR_H < 1$	0.00%	0.00%	0.00%	24.07%	0.00%	20.63%	0.00%	0.00%	0.00%
$LR_H < 1$	0.00%	0.00%	0.00%	24.07%	0.00%	20.63%	70.37%	100.00%	74.60%

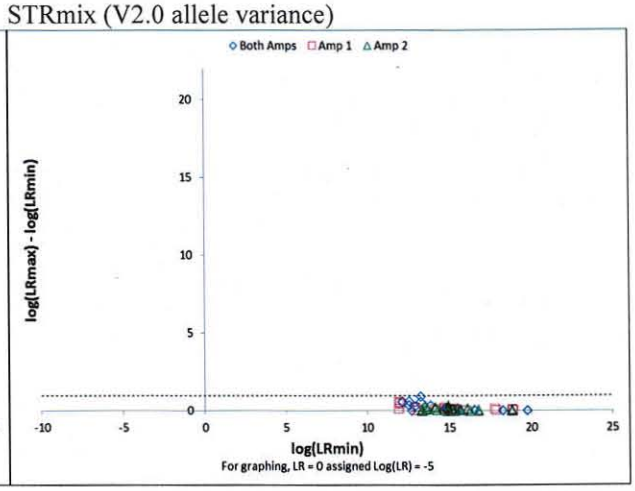
PRECISION: Graphing the 2-person mixtures

- See the following spreadsheets
  - STRmix 2PM_DifDeg Graphs (spm 06.24.2014).xlsm
  - TAC 2PM_DifDeg 0 and 0.01 Graphs (spm 06.24.2014).xlsm
- LR_H within like interpretations were compared in a pairwise manner.
  - E.g., the results of the three joint interpretations for a mixture were compared to each other.
- The results were plotted as the lower of the two $\log_{10} LR_H$ on the x-axis and the absolute value of the difference on the y-axis.
  - The dashed line represents 1 log unit, i.e., a factor of 10 difference.
  - $LR_H = 0$ was plotted as –5.
- QC018 (female, intact DNA)

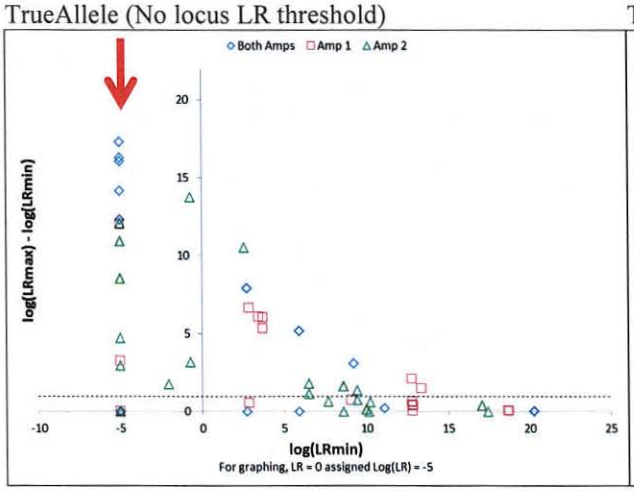
STRmix (V1.0.7.49 allele variance)



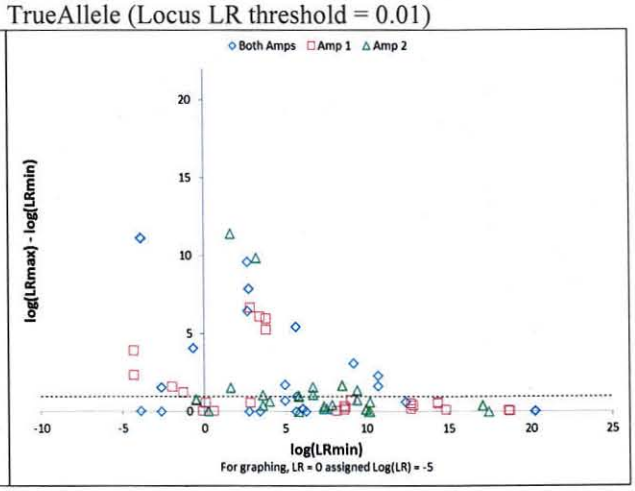
STRmix (V2.0 allele variance)



TrueAllele (No locus LR threshold)

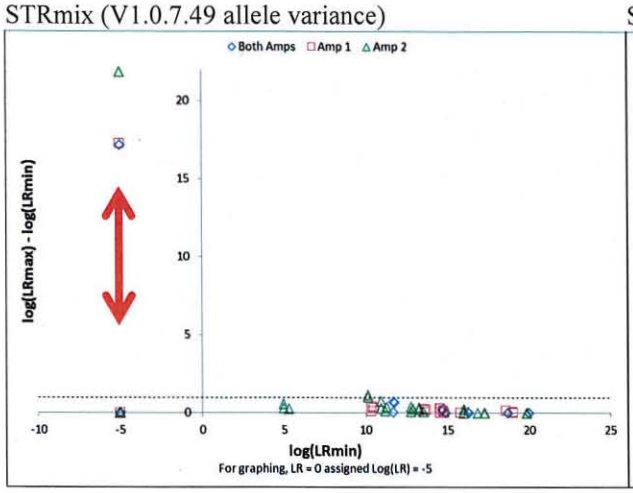


TrueAllele (Locus LR threshold = 0.01)

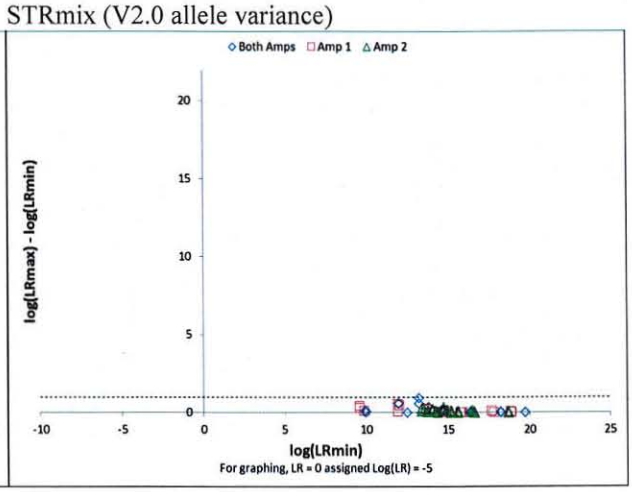


- QC018 (male, degradation series)

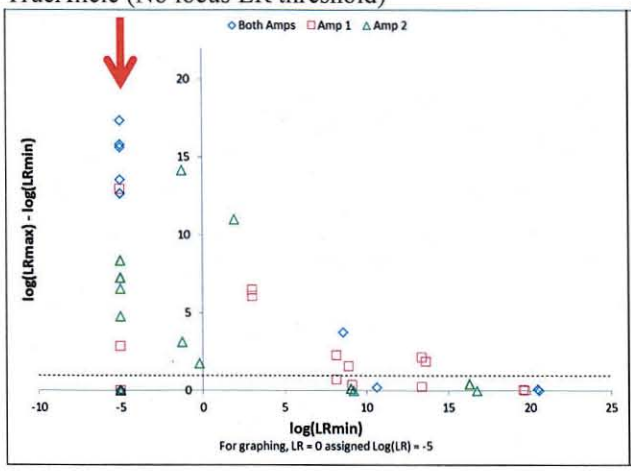
STRmix (V1.0.7.49 allele variance)



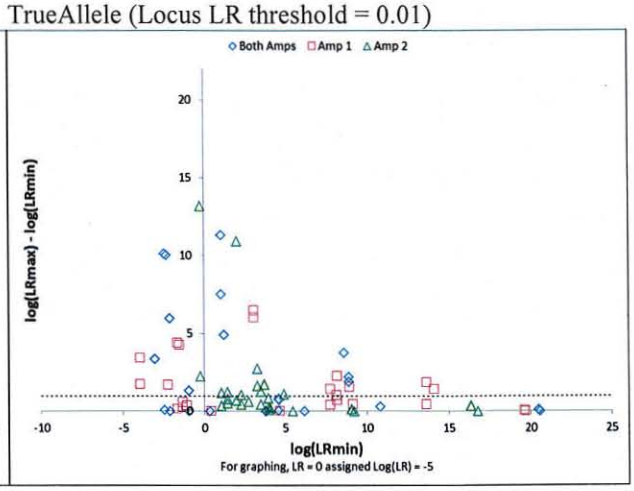
STRmix (V2.0 allele variance)



TrueAllele (No locus LR threshold)



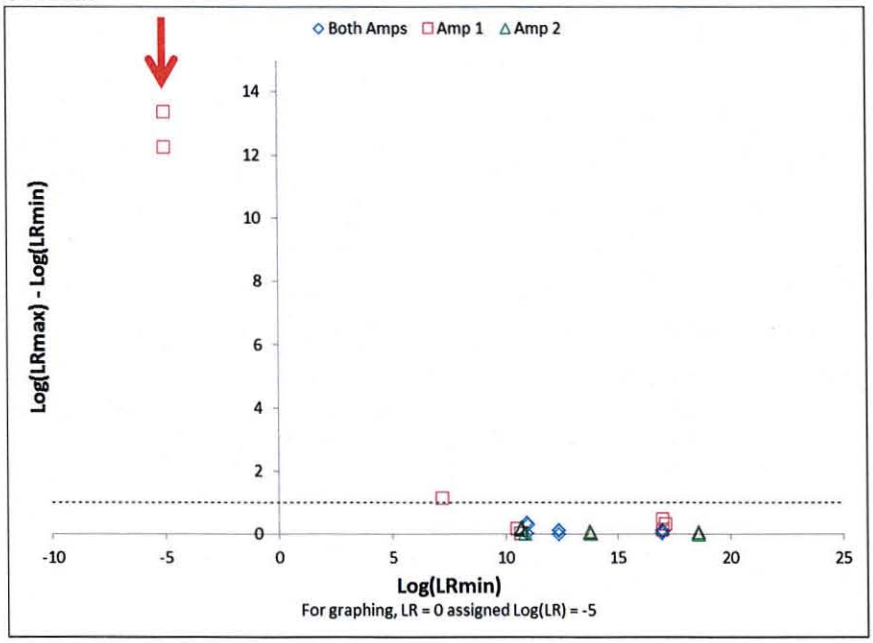
TrueAllele (Locus LR threshold = 0.01)



PRECISION: Graphing the 3-person mixture

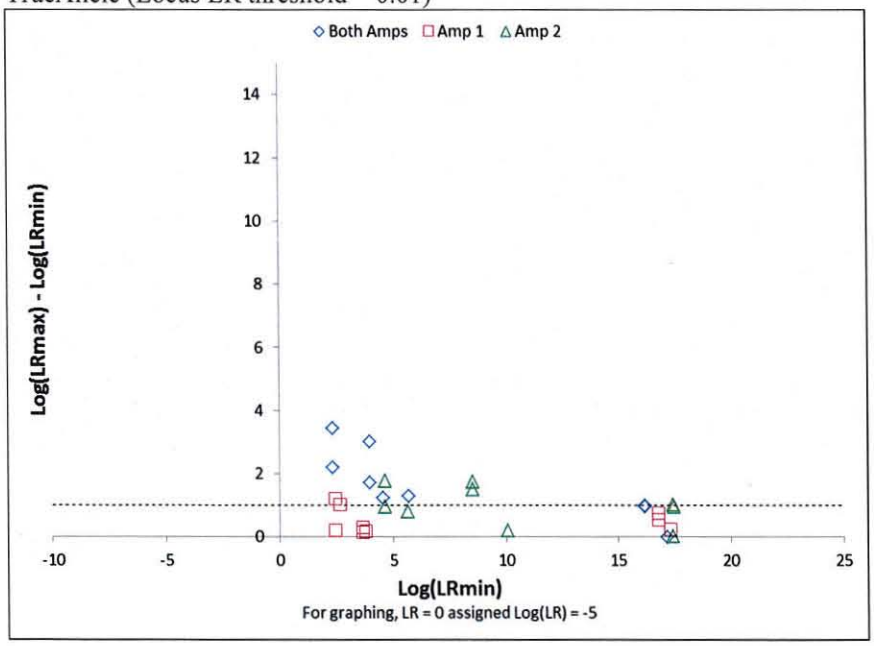
- See the following spreadsheets
  - STRmix 3PM_DifDeg Graphs (spm 06.24.2014).xlsm
  - TAC 3PM_DifDeg 0 and 0.01 Graphs (spm 06.24.2014).xlsm
- LR_H within like interpretations were compared in a pairwise manner.
  - E.g., the results of the three joint interpretations for a mixture were compared to each other.
- The results were plotted as the lower of the two $\log_{10} LR_H$ on the x-axis and the absolute value of the difference on the y-axis.
  - The dashed line represents 1 log unit, i.e., a factor of 10 difference.
  - $LR_H = 0$ was plotted as –5.

- STRmix

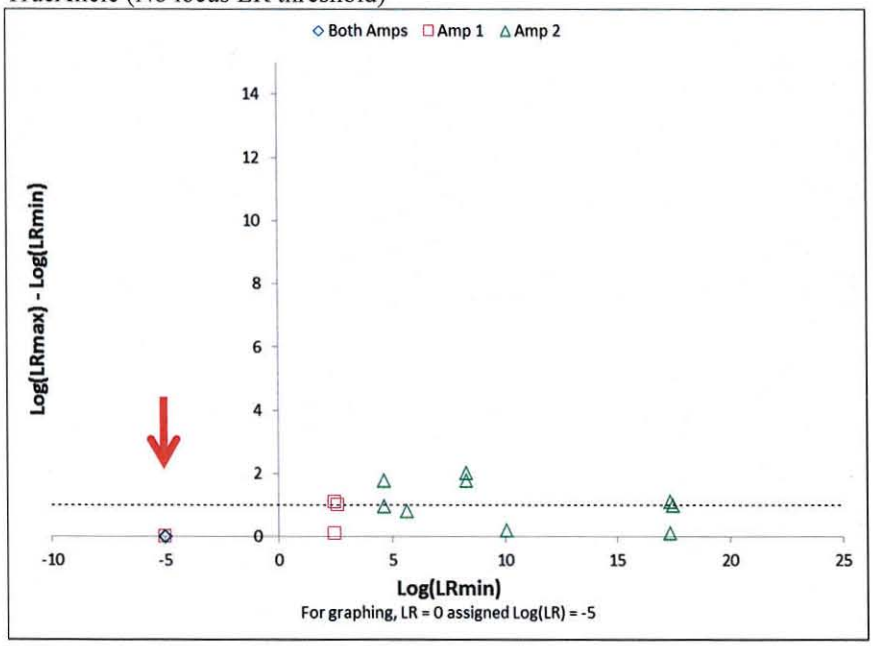


SPM 1/7/16
ALL DONORS SHOWN.
✓ 1/9/16

- TrueAllele (Locus LR threshold = 0.01)



- TrueAllele (No locus LR threshold)



PRECISION: Summary Tables

- See the following spreadsheets
  - STRmix_Graphs_Data (spm 07.10.2014).xlsm
  - TAC_Graphs_Data (spm 07.10.2014).xlsm
- Notes:
  - These tables do not include the results from the 2-person mixture differential degradations study's "QC18-QC108_intact" mixture.
  - STRmix results using the V1.0.7.49 variance were not included.
- All contributors for 3 replicates each of amp 1, amp 2, and the joint interpretation:

ΔLR_H	STRmix (V2.0 variance)			TAC (0.01 minimum)			TAC (0 minimum)		
	2PM	3PM	Comb.	2PM	3PM	Comb.	2PM	3PM	Comb.
Total	162	27	189	162	27	189	162	27	189
Min	2.9E-05	7.3E-03	2.9E-05	0.0E+00	2.2E-02	0.0E+00	0.0E+00	0.0E+00	0.0E+00
Max	9.6E-01	2.3E+01	2.3E+01	1.3E+01	3.4E+00	1.3E+01	2.7E+01	2.0E+00	2.7E+01
% > 1	0.00%	11.11%	1.59%	46.30%	40.74%	45.50%	40.12%	22.22%	37.57%

- All contributors for 3 replicates each of amp 1 and amp 2:

ΔLR_H	STRmix (V2.0 variance)			TAC (0.01 minimum)			TAC (0 minimum)		
	2PM	3PM	Comb.	2PM	3PM	Comb.	2PM	3PM	Comb.
Total	108	18	126	108	18	126	108	18	126
Min	2.9E-05	7.3E-03	2.9E-05	0.0E+00	5.4E-02	0.0E+00	0.0E+00	0.0E+00	0.0E+00
Max	5.6E-01	2.3E+01	2.3E+01	1.3E+01	1.8E+00	1.3E+01	2.3E+01	2.0E+00	2.3E+01
% > 1	0.00%	16.67%	2.38%	40.74%	27.78%	38.89%	43.52%	33.33%	42.06%

- All contributors for 3 replicates each of the joint interpretations:

ΔLR_H	STRmix (V2.0 variance)			TAC (0.01 minimum)			TAC (0 minimum)		
	2PM	3PM	Comb.	2PM	3PM	Comb.	2PM	3PM	Comb.
Total	54	9	63	54	9	63	54	9	63
Min	1.0E-02	1.4E-02	1.0E-02	0.0E+00	2.2E-02	0.0E+00	0.0E+00	0.0E+00	0.0E+00
Max	9.6E-01	3.6E-01	9.6E-01	1.1E+01	3.4E+00	1.1E+01	2.7E+01	0.0E+00	2.7E+01
% > 1	0.00%	0.00%	0.00%	57.41%	66.67%	58.73%	33.33%	0.00%	28.57%

Discussion and Conclusions

Sensitivity was measured as the proportion of comparisons (including each mixture being compared to each contributor) that gave positive $\log(LR_H)$.

MixMaster 1.1 sensitivity was 97.22% for the 2-person mixtures when using the standard 4-allele approach to estimating Mx. The remaining 2.78% was attributed to a single false exclusion (LR = 0) at a single locus for the comparison of the male reference (QC108) to one amplification of the sample with the most degraded male DNA (Tube16 Amp 1). The female profile (QC018) was included in the results for this mixture. When MixMaster was rerun using the Mx Calculator 3 and 4-allele Mx estimate, this comparison was no longer LR = 0.

STRmix sensitivity was 100% for the 2-person mixtures, and 96.3% for the 3-person mixture when using the V2.0 variance supplied by ESR. Overall, STRmix had positive $\log(LR_H)$ in 99.47% of the comparisons. The remaining 0.53% was attributed to a single false exclusion (LR = 0) for the degraded DNA contributor to the 3-person mixture. In nine-total comparisons to this person, the false exclusion occurred in one interpretation of Amp 1. The other two interpretations of Amp 1, all three interpretations of Amp 2, and all three joint interpretations gave positive $\log(LR_H)$. Had the V1.0.7.49 Model Maker variance been the only option, sensitivity would have dropped considerably, as evidence by the false negatives (LR = 0) observed in the graphs.

TrueAllele Casework sensitivity was 82.1% for the 2-person mixtures, and 100% for the 3-person mixture. Overall, TrueAllele had positive $\log(LR_H)$ in 84.66% of the comparisons. However, when the minimum LR was bypassed (i.e., LR = 0 allowed), the sensitivity decreased to 39.51% for the 2-person mixtures, and 44.44% for the 3-person mixture. Overall, when bypassing the minimum locus LR threshold, sensitivity was only 40.21%, with over half (56.08%) of the comparisons giving LR = 0 for at least one locus.

Precision was measured as the proportion of pairwise comparisons, within like-interpretations, that were within one $\log(LR_H)$ unit.

STRmix precision was 100% for the 2-person mixtures, and 88.89% for the 3-person mixture when using the V2.0 variance supplied by ESR. Overall, STRmix had 98.41% of the pairwise comparisons within one log unit. The remaining 1.59% was attributed to the single false exclusion (LR = 0) for the degraded DNA contributor to the 3-person mixture. This one false exclusion was compared to two other interpretations that have positive $\log(LR_H)$.

TrueAllele Casework precision was 53.7% for the 2-person mixtures, and 59.26% for the 3-person mixture. Overall, TrueAllele had 54.5% of the pairwise comparisons within one log unit. Paradoxically, this appeared to improve when the minimum locus LR was bypassed. However, this appears to be an artifact of the increased number of LR = 0. When comparing two false negatives, the LRs are identical, i.e., the LR = 0.

Overall, STRmix had better sensitivity and precision than TrueAllele Casework for the same number of interpretations. (STRmix also outperformed MixMaster (standard settings) for the interpretation of the 2-person set.) The degradation approach in STRmix appeared to better handle the kind of differential degradation highlighted in these samples – where the Mx of the contributors flips in the middle of the locus size (base pair) range.