

**Purpose**

To examine the sensitivity and reproducibility of STRmix and TrueAllele Casework when testing challenging samples. Part II of this study focuses on the following sample types: 3-person mixtures created in-house that were mixed at multiple ratios and amplified at multiple template quantities.

**Methodology and Results**

See the folder “3PM\_STRmix\_TrueAllele\_Val” for copies of the electronic files used in support of this study.

**System versions and settings**

Except where noted below, see Part I for the STRmix and TrueAllele Casework versions and settings.

- STRmix Interpretations were performed using version 1.07.49 and the allele variance calculated by ESR using the Model Maker module incorporated into version 2.0.

**3-person mixtures**

- Sets of three-person mixtures with no degradation were prepared from previously extracted DNA.
- Mixtures were prepared at four ratios:

1 : 1 : 1  
 4.5 : 4.5 : 1  
 6 : 3 : 1  
 8 : 1 : 1

- PCR amplifications of the three-person mixtures used three separate total template ng: 1.5, 0.75, and 0.375. All amplifications were done in duplicate using the AmpFISTR Identifiler Plus (Life Technologies, Thermo Fisher) multiplex and the Applied Biosystems GeneAmp PCR System 9700 thermal cycler (Life Technologies, Thermo Fisher) using 9600 emulation mode under standard manufacturer conditions. Detection was by Applied Biosystems 3130XL Genetic Analyzer (Life Technologies, Thermo Fisher) with 5-second injections at 3 kV. Where appropriate, fsa files fragment sizing and allele calling were performed with GeneMapper ID v3.2 (Life Technologies, Thermo Fisher).
- Full sets were prepared by the Fresno (FR), Richmond (BK), Riverside (RI), and Sacramento (SC) BFS laboratories. See the notes in Appendix II, supplemental pages...

- Fresno (FR): II 2 to II 9
- Richmond (BK): II 10 to II 40
- Riverside (RI): II 41 to II 41
- Sacramento (SC): II 42 to II 58

NOTE: The Richmond set was not processed through TrueAllele.

- 3-person mixture injections used for study:
  - Fresno (FR)
    - Run folder
      - RF\_U6\_R323\_040713\_JU
    - Control injections used for study
      - Allelic ladder
        - H04\_Ladder.fsa
      - Positive amplification control
        - B01\_PC.fsa
      - Negative amplification control
        - C01\_NC.2.fsa
    - References
      - Donor 1 - Male

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*12/30/14*

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*pp 1-47*  
*12/25/15*

- D01\_1.2.fsa
- Donor 2 - Male
- E01\_2.2.fsa
- Donor 3 - Female
- F01\_3.fsa
- 1-1-1 injections used for study
- 0.375 pg
  - Amp1: H03\_0.375\_A1\_1-1-1.2.fsa
  - Amp2: A04\_0.375\_A2\_1-1-1.2.fsa
- 0.75 pg
  - Amp1: G02\_0.75\_A1\_1-1-1.2.fsa
  - Amp2: H02\_0.75\_A2\_1-1-1.fsa
- 1.5 pg
  - Amp1: G01\_1.5\_A1\_1-1-1.2.fsa
  - Amp2: H01\_1.5\_A2\_1-1-1.2.fsa
- 4.5-4.5-1 injections used for study
- 0.375 pg
  - Amp1: D04\_0.375\_A1\_4.5-4.5-1.fsa
  - Amp2: E04\_0.375\_A2\_4.5-4.5-1.2.fsa
- 0.75 pg
  - Amp1: D03\_0.75\_A1\_4.5-4.5-1.fsa
  - Amp2: E03\_0.75\_A2\_4.5-4.5-1.2.fsa
- 1.5 pg
  - Amp1: C02\_1.5\_A1\_4.5-4.5-1.2.fsa
  - Amp2: D02\_1.5\_A2\_4.5-4.5-1.fsa
- 6-3-1 injections used for study
- 0.375 pg
  - Amp1: F04\_0.375\_A1\_6-3-1.fsa
  - Amp2: G04\_0.375\_A2\_6-3-1.fsa
- 0.75 pg
  - Amp1: F03\_0.75\_A1\_6-3-1.fsa
  - Amp2: G03\_0.75\_A2\_6-3-1.fsa
- 1.5 pg
  - Amp1: E02\_1.5\_A1\_6-3-1.fsa
  - Amp2: F02\_1.5\_A2\_6-3-1.fsa
- 8-1-1 injections used for study
- 0.375 pg
  - Amp1: B04\_0.375\_A1\_8-1-1.fsa
  - Amp2: C04\_0.375\_A2\_8-1-1.fsa
- 0.75 pg
  - Amp1: B03\_0.75\_A1\_8-1-1.fsa
  - Amp2: C03\_0.75\_A2\_8-1-1.2.fsa
- 1.5 pg
  - Amp1: A02\_1.5\_A1\_8-1-1.fsa
  - Amp2: B02\_1.5\_A2\_8-1-1.2.fsa
- o Richmond (BK)
- Run folders
  - Run 1: RF\_24MCE105\_030113
  - Run 2: RF\_24MCE107\_030513
    - Unless noted, all fsa files came from this run folder.
- Control injections used for study
- Allelic ladder
  - Run 1: C03\_26MCE105IP\_ALLELIC\_LADDER\_005\_24MCE105\_030113.fsa
  - Run 2: A01\_26MCE107IP\_ALLELIC\_LADDER\_001\_24MCE107\_030513.fsa
- Positive amplification control
  - Run 1: B03\_26MCE105IP\_POS\_CON\_003\_24MCE105\_030113.fsa

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*12/30/14*

- Run 2: G04\_26MCE107IP\_POS\_CON\_014\_24MCE107\_030513.fsa  
Negative amplification control
- Run 1: D03\_26MCE105IP\_NEG\_CON\_007\_24MCE105\_030113.fsa
- Run 2: H04\_26MCE107IP\_NEG\_CON\_016\_24MCE107\_030513.fsa

References

- Donor 1 - Female
  - F04\_BKTA3PMSSBCIP\_6454\_012\_24MCE107\_030513.fsa
- Donor 2 - Male
  - C04\_BKTA3PMSSBCIP\_5809\_006\_24MCE107\_030513.fsa
- Donor 3 - Male
  - E04\_BKTA3PMSSBCIP\_5920\_010\_24MCE107\_030513.fsa

1-1-1 injections used for study

- 0.375 pg
  - Amp1: B03\_BKTA3PMSSBCIP\_I2\_003\_24MCE107\_030513.fsa
  - Amp2: D03\_BKTA3PMSSBCIP\_I3\_007\_24MCE107\_030513.fsa

- 0.75 pg
  - Amp1: C02\_BKTA3PMSSBCIP\_E2\_006\_24MCE107\_030513.fsa
  - Amp2: D02\_BKTA3PMSSBCIP\_E3\_008\_24MCE107\_030513.fsa

- 1.5 pg
  - Amp1: B01\_BKTA3PMSSBCIP\_A2\_003\_24MCE107\_030513.fsa
  - Amp2: C01\_BKTA3PMSSBCIP\_A3\_005\_24MCE107\_030513.fsa

4.5-4.5-1 injections used for study

- 0.375 pg
  - Amp1: G03\_BKTA3PMSSBCIP\_K2\_013\_24MCE107\_030513.fsa
  - Amp2: H03\_BKTA3PMSSBCIP\_K3\_015\_24MCE107\_030513.fsa

- 0.75 pg
  - Amp1: G02\_BKTA3PMSSBCIP\_G2\_014\_24MCE107\_030513.fsa
  - Amp2: H02\_BKTA3PMSSBCIP\_G3\_016\_24MCE107\_030513.fsa

- 1.5 pg
  - Amp1: F01\_BKTA3PMSSBCIP\_C2\_011\_24MCE107\_030513.fsa
  - Amp2: G01\_BKTA3PMSSBCIP\_C3\_013\_24MCE107\_030513.fsa

6-3-1 injections used for study

- 0.375 pg
  - Amp1: A04\_BKTA3PMSSBCIP\_L2\_002\_24MCE107\_030513.fsa
  - Amp2: B04\_BKTA3PMSSBCIP\_L3\_004\_24MCE107\_030513.fsa

- 0.75 pg
  - Amp1: A02\_BKTA3PMSSBCIP\_H\_002\_24MCE105\_030113.fsa
    - Run 1
  - Amp2: A03\_BKTA3PMSSBCIP\_H2\_001\_24MCE107\_030513.fsa

- 1.5 pg
  - Amp1: H01\_BKTA3PMSSBCIP\_D2\_015\_24MCE107\_030513.fsa
  - Amp2: A02\_BKTA3PMSSBCIP\_D3\_002\_24MCE107\_030513.fsa

8-1-1 injections used for study

- 0.375 pg
  - Amp1: E03\_BKTA3PMSSBCIP\_J2\_009\_24MCE107\_030513.fsa
  - Amp2: F03\_BKTA3PMSSBCIP\_J3\_011\_24MCE107\_030513.fsa

**NOTE: The 0.75 pg and 1.5 ng amps were not interpreted due to excessive artifacts.**

o Riverside (RI)

- Run folder
  - RF\_CE20\_052413\_RUN\_313\_DJG

Control injections used for study

- Allelic ladder
  - H04\_LADDER\_004\_CE20\_052413\_RUN\_313\_DJG.fsa
- Positive amplification control
  - C12\_9947A\_003\_CE20\_052413\_RUN\_313\_DJG.2.fsa
- Negative amplification control

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*12/30/14*

- B12\_AMP\_NEG\_002\_CE20\_052413\_RUN\_313\_DJG.fsa

References

- Donor 1 - Male
- Donor 2 - Female
- Donor 3 – Female

Note: All references for this set were manually entered based upon profiles supplied by the submitting laboratory.

1-1-1 injections used for study

0.375 pg

- Amp1: E01\_C\_1G\_1K\_1C\_001\_CE20\_052413\_RUN\_313\_DJG.fsa
- Amp2: F01\_C\_1G\_1K\_1C\_002\_CE20\_052413\_RUN\_313\_DJG.fsa

0.75 pg

- Amp1: C01\_B\_1G\_1K\_1C\_003\_CE20\_052413\_RUN\_313\_DJG.2.fsa
- Amp2: D01\_B\_1G\_1K\_1C\_004\_CE20\_052413\_RUN\_313\_DJG.fsa

1.5 pg

- Amp1: A01\_A\_1G\_1K\_1C\_001\_CE20\_052413\_RUN\_313\_DJG.2.fsa
- Amp2: B01\_A\_1G\_1K\_1C\_002\_CE20\_052413\_RUN\_313\_DJG.fsa

4.5-4.5-1 injections used for study

0.375 pg

- Amp1: F04\_C\_4G\_4K\_1C\_002\_CE20\_052413\_RUN\_313\_DJG.fsa
- Amp2: G04\_C\_4G\_4K\_1C\_003\_CE20\_052413\_RUN\_313\_DJG.fsa

0.75 pg

- Amp1: D04\_B\_4G\_4K\_1C\_004\_CE20\_052413\_RUN\_313\_DJG.fsa
- Amp2: E04\_B\_4G\_4K\_1C\_001\_CE20\_052413\_RUN\_313\_DJG.fsa

1.5 pg

- Amp1: B04\_A\_4G\_4K\_1C\_002\_CE20\_052413\_RUN\_313\_DJG.2.fsa
- Amp2: C04\_A\_4G\_4K\_1C\_003\_CE20\_052413\_RUN\_313\_DJG.fsa

6-3-1 injections used for study

0.375 pg

- Amp1: C06\_C\_6G\_3K\_1C\_003\_CE20\_052413\_RUN\_313\_DJG.fsa
- Amp2: D06\_C\_6G\_3K\_1C\_004\_CE20\_052413\_RUN\_313\_DJG.2.fsa

0.75 pg

- Amp1: A06\_B\_6G\_3K\_1C\_001\_CE20\_052413\_RUN\_313\_DJG.2.fsa
- Amp2: B06\_B\_6G\_3K\_1C\_002\_CE20\_052413\_RUN\_313\_DJG.fsa

1.5 pg

- Amp1: G05\_A\_6G\_3K\_1C\_003\_CE20\_052413\_RUN\_313\_DJG.fsa
- Amp2: H05\_A\_6G\_3K\_1C\_004\_CE20\_052413\_RUN\_313\_DJG.fsa

8-1-1 injections used for study

0.375 pg

- Amp1: B03\_C\_8G\_1K\_1C\_002\_CE20\_052413\_RUN\_313\_DJG.2.fsa
- Amp2: C03\_C\_8G\_1K\_1C\_003\_CE20\_052413\_RUN\_313\_DJG.fsa

0.75 pg

- Amp1: A03\_B\_8G\_1K\_1C\_001\_CE20\_052413\_RUN\_313\_DJG.2.fsa
- Amp2: G02\_B\_8G\_1K\_1C\_003\_CE20\_052413\_RUN\_313\_DJG.fsa

1.5 pg

- Amp1: E02\_A\_8G\_1K\_1C\_001\_CE20\_052413\_RUN\_313\_DJG.fsa
- Amp2: F02\_A\_8G\_1K\_1C\_002\_CE20\_052413\_RUN\_313\_DJG.fsa

o Sacramento (SC)

Run folder

- Run 1: RF\_6MCE022-13\_030713
  - Unless noted, all fsa files came from this run folder.
- Run 2: RF\_6MCE024-13\_031113

Control injections used for study

Allelic ladder

- Run 1: A04\_Ladder\_001\_6MCE022-13\_030713.fsa
- Run 2: A01\_Ladder\_001\_6MCE024-13\_031113

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*12/30/14*

Positive amplification control

- Run 1: H04\_3PM\_PCR-positive\_004\_6MCE022-13\_030713.fsa
- Run 2: D01\_3PM\_PCR-positive\_004\_6MCE024-13\_031113

Negative amplification control

- Run 1: B05\_3PM\_PCR-negative\_002\_6MCE022-13\_030713.fsa
- Run 2: E01\_3PM\_PCR-negative\_001\_6MCE024-13\_031113

References

Donor 1 - Female

- B01\_3PM\_QC1480\_002\_6MCE022-13\_030713.fsa

Donor 2 - Female

- C01\_3PM\_QC1489\_003\_6MCE022-13\_030713.fsa

Donor 3 - Male

- D01\_3PM\_QC1497\_004\_6MCE022-13\_030713.2.fsa

1-1-1 injections used for study

0.375 pg

- Amp1: B01\_3PM\_1-1-1-(0.375-ng)-(1)\_002\_6MCE024-13\_031113.fsa
  - Run 2
- Amp2: C01\_3PM\_1-1-1-(0.375-ng)-(2)\_003\_6MCE024-13\_031113.fsa
  - Run 2

0.75 pg

- Amp1: F02\_3PM\_1-1-1-(0.75-ng)-(1)\_002\_6MCE022-13\_030713.fsa
- Amp2: G02\_3PM\_1-1-1-(0.75-ng)-(2)\_003\_6MCE022-13\_030713.fsa

1.5 pg

- Amp1: E01\_3PM\_1-1-1-(1.5-ng)-(1)\_001\_6MCE022-13\_030713.fsa
- Amp2: F01\_3PM\_1-1-1-(1.5-ng)-(2)\_002\_6MCE022-13\_030713.2.fsa

4.5-4.5-1 injections used for study

0.375 pg

- Amp1: D04\_3PM\_4.5-4.5-1-(0.375-ng)-(1)\_004\_6MCE022-13\_030713.fsa
- Amp2: E04\_3PM\_4.5-4.5-1-(0.375-ng)-(2)\_001\_6MCE022-13\_030713.fsa

0.75 pg

- Amp1: C03\_3PM\_4.5-4.5-1-(0.75-ng)-(1)\_003\_6MCE022-13\_030713.fsa
- Amp2: D03\_3PM\_4.5-4.5-1-(0.75-ng)-(2)\_004\_6MCE022-13\_030713.fsa

1.5 pg

- Amp1: B02\_3PM\_4.5-4.5-1-(1.5-ng)-(1)\_002\_6MCE022-13\_030713.fsa
- Amp2: C02\_3PM\_4.5-4.5-1-(1.5-ng)-(2)\_003\_6MCE022-13\_030713.2.fsa

6-3-1 injections used for study

0.375 pg

- Amp1: F04\_3PM\_6-3-1-(0.375-ng)-(1)\_002\_6MCE022-13\_030713.fsa
- Amp2: G04\_3PM\_6-3-1-(0.375-ng)-(2)\_003\_6MCE022-13\_030713.fsa

0.75 pg

- Amp1: E03\_3PM\_6-3-1-(0.75-ng)-(1)\_001\_6MCE022-13\_030713.fsa
- Amp2: F03\_3PM\_6-3-1-(0.75-ng)-(2)\_002\_6MCE022-13\_030713.fsa

1.5 pg

- Amp1: D02\_3PM\_6-3-1-(1.5-ng)-(1)\_004\_6MCE022-13\_030713.2.fsa
- Amp2: E02\_3PM\_6-3-1-(1.5-ng)-(2)\_001\_6MCE022-13\_030713.fsa

8-1-1 injections used for study

0.375 pg

- Amp1: B04\_3PM\_8-1-1-(0.375-ng)-(1)\_002\_6MCE022-13\_030713.2.fsa
- Amp2: C04\_3PM\_8-1-1-(0.375-ng)-(2)\_003\_6MCE022-13\_030713.fsa

0.75 pg

- Amp1: H02\_3PM\_8-1-1-(0.75-ng)-(1)\_004\_6MCE022-13\_030713.2.fsa
- Amp2: B03\_3PM\_8-1-1-(0.75-ng)-(2)\_002\_6MCE022-13\_030713.fsa

1.5 pg

- Amp1: G01\_3PM\_8-1-1-(1.5-ng)-(1)\_003\_6MCE022-13\_030713.2.fsa
- Amp2: H01\_3PM\_8-1-1-(1.5-ng)-(2)\_004\_6MCE022-13\_030713.fsa

Creation of mixture interpretation input files

- See Part I for a description of the preparation of mixture interpretation input files.
- Import file names:

FR

- Evidence import file: 3PM\_FRammend2\_STRmix.txt  
Note: Some runs may have been performed with import files 3PM\_FR\_STRmix.txt or 3PM\_FRammended\_STRmix.txt. As described later in this document, these files were found to have profiles that needed additional edits. Input file 3PM\_FRammend2\_STRmix.txt is the final version.
- Reference import file: 3PM\_FR\_STRmix\_R.txt

BK

- Evidence import file: 3PM\_BKsc1(ammended)\_STRmix.txt  
Note: Some runs may have been performed with import file 3PM\_BKsc1\_STRmix.txt. As described later in this document, this file was found to have one profile that needed additional edits. Input file 3PM\_BKsc1(ammended)\_STRmix.txt is the final version.
- Reference import file: 3PM\_BKsc\_STRmix\_R.txt

RI

- Evidence import file: 3PM\_RI\_STRmix\_Renamed.txt  
Note: Sample files names were adjusted after export from GMID so that the two amps could be distinguished from one another.
- Reference import file: None (RI supplied the profiles in text form only)

SC

- Evidence import file: 3PM\_SC1\_STRmix (8-1-1 corr.).txt  
3PM\_SC2\_STRmix.txt  
Note: Some runs may have been performed with import file 3PM\_SC1\_STRmix.txt. As described later in this document, this file was found to have one profile that needed an additional edit. Input file 3PM\_SC1\_STRmix (8-1-1 corr.).txt is the final version.
- Reference import file: 3PM\_SC1\_STRmix\_R.txt

Interpretations

- STRmix and TrueAllele Casework interpretations were performed either in duplicate or 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.)
  - Note: The entirety of the Fresno set was run for a fourth joint interpretation because of identical results between the first two interpretations.
- See Part I for the interpretation settings.
  - Note: There was no systematic assessment of the TrueAllele chains for Part II.
- Summary: Number of interpretations per mixture and interpretation system

Lab	Mixture	Total ng	1st Amp Interpretations		2nd Amp Interpretations		Joint Interpretations	
			STRmix	TAC	STRmix	TAC	STRmix	TAC
FR	1-1-1	1.5	3	3	3	3	3	4
		0.75	3	3	3	3	3	4
		0.375	3	3	3	3	3	4
	4.5-4.5-1	1.5	2	3	2	3	2	4
		0.75	2	3	2	3	2	4

*SPM*  
*12/30/14*

		0.375	2	3	2	3	2	4
	6-3-1	1.5	3	3	3	3	3	4
		0.75	3	3	3	3	3	4
		0.375	3	3	3	3	3	4
	8-1-1	1.5	3	3	3	3	3	4
		0.75	3	3	3	3	3	4
		0.375	3	3	3	3	3	4
BK	1-1-1	1.5	2	N/A	2	N/A	2	N/A
		0.75	2	N/A	2	N/A	2	N/A
		0.375	2	N/A	2	N/A	2	N/A
	4.5-4.5-1	1.5	2	N/A	2	N/A	2	N/A
		0.75	2	N/A	2	N/A	2	N/A
		0.375	2	N/A	2	N/A	2	N/A
	6-3-1	1.5	2	N/A	2	N/A	2	N/A
		0.75	2	N/A	2	N/A	2	N/A
		0.375	2	N/A	2	N/A	2	N/A
	8-1-1	1.5	N/A	N/A	N/A	N/A	N/A	N/A
		0.75	N/A	N/A	N/A	N/A	N/A	N/A
		0.375	2	N/A	2	N/A	2	N/A
RI	1-1-1	1.5	2	2	2	1	2	2
		0.75	2	2	2	1	2	2
		0.375	2	2	2	1	2	2
	4.5-4.5-1	1.5	2	2	2	1	2	2
		0.75	2	2	2	1	2	2
		0.375	2	2	2	1	2	2
	6-3-1	1.5	2	2	2	1	2	2
		0.75	2	2	2	1	2	2
		0.375	2	2	2	1	2	2
	8-1-1	1.5	2	2	2	1	2	2
		0.75	2	2	2	1	2	2
		0.375	2	2	2	1	2	2
SC	1-1-1	1.5	2	2	2	1	2	2
		0.75	2	2	2	1	2	2
		0.375	2	2	2	1	2	2
	4.5-4.5-1	1.5	2	2	2	1	2	2
		0.75	2	2	2	1	2	2
		0.375	2	2	2	1	2	2
	6-3-1	1.5	2	2	2	1	2	2
		0.75	2	2	2	1	2	2
		0.375	2	2	2	1	2	2
	8-1-1	1.5	2	2	2	1	2	2
		0.75	2	2	2	1	2	2
		0.375	2	2	2	1	2	2

Likelihood Ratios

- See Part I for the calculation methodology and parameters.

Graphs and tables for the Sensitivity and Precision studies

- Mixture weights and population-specific LRs for each comparison were imported into the following spreadsheets
  - STRmix\_Graphs\_Data (spm 07.10.2014).xslm
  - TAC\_Graphs\_Data (spm 07.10.2014).xslm

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*12/30/14*

**SENSITIVITY: Graphing the 3-person mixtures**

- Harmonic means ( $LR_H$ ) were calculated for each comparison.
- The results were plotted as the...
  - ng of the contributor (calculated as total template \* assumed Mx) on the x-axis; and
  - $\log_{10} LR_H$  on the y-axis.

Note: Since you can't calculate a log of LR = 0, those values were assigned the value of -15.

Note: For the Richmond set, the observed mixture proportions quite obviously deviated from the expected mixture proportions. To get a better estimate of the proportion of each contributor's DNA within the total mixture template DNA, I used STRmix to run a joint interpretation of Amp2\_1-1-1\_1.5.csv and Amp3\_1-1-1\_1.5.csv, assuming contributors 2 and 3. The weight assigned to the remaining contributor's genotype was 1.0 at each locus. Rounding the STRmix proportions to the first decimal place, the mixture proportions were...

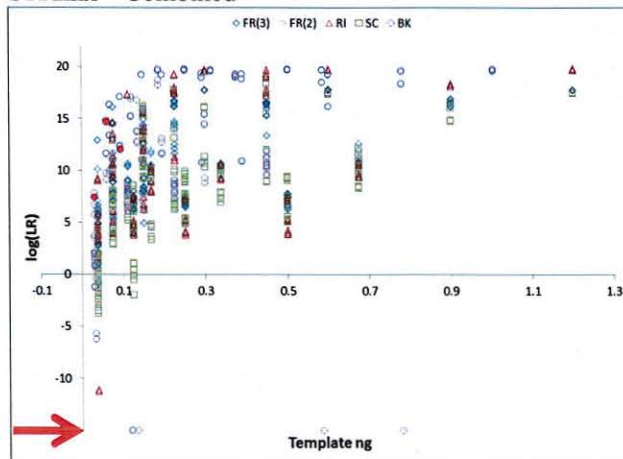
Contributor	Expected	Observed (STRmix)
1	0.3333	0.4
2	0.3333	0.3
3	0.3333	0.3

Based upon the STRmix estimates, which appeared consistent with a visual assessment, ng estimates for the Richmond mixture contributors were calculated.

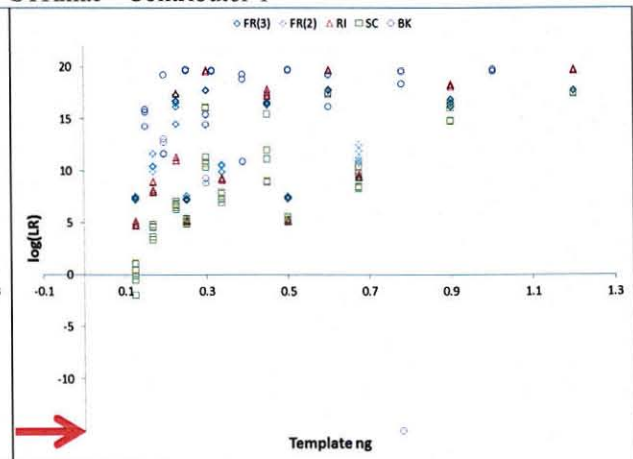
Ratios (Intended)	Ratios (adjusted)	Ratios (normalized)	Template: 1.5 amp	Template: 0.75 amp	Template: 0.375 amp
1	1.2	0.4	0.6	0.3	0.15
1	0.9	0.3	0.45	0.23	0.11
1	0.9	0.3	0.45	0.23	0.11
4.5	5.4	0.52	0.78	0.39	0.20
4.5	4.1	0.39	0.59	0.29	0.15
1	0.9	0.087	0.13	0.065	0.033
6	7.2	0.667	1	0.5	0.25
3	2.7	0.250	0.38	0.19	0.094
1	0.9	0.083	0.13	0.063	0.031
8	9.6	0.84	1.3	0.63	0.32
1	0.9	0.079	0.12	0.059	0.030
1	0.9	0.079	0.12	0.059	0.030

- All ratios, template amounts, and interpretation types (i.e., single-amp or joint-amp)

STRmix – Combined



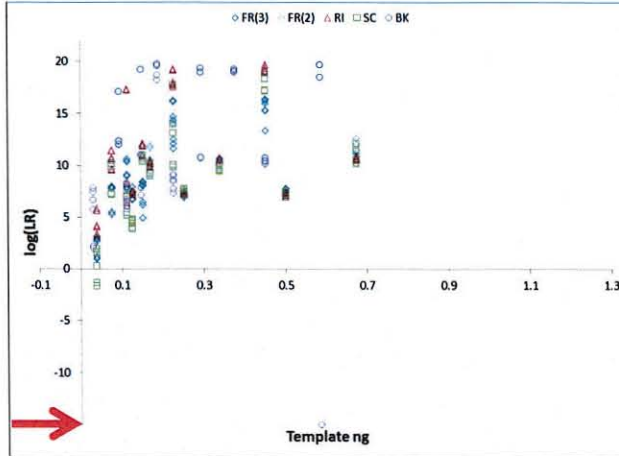
STRmix – Contributor 1



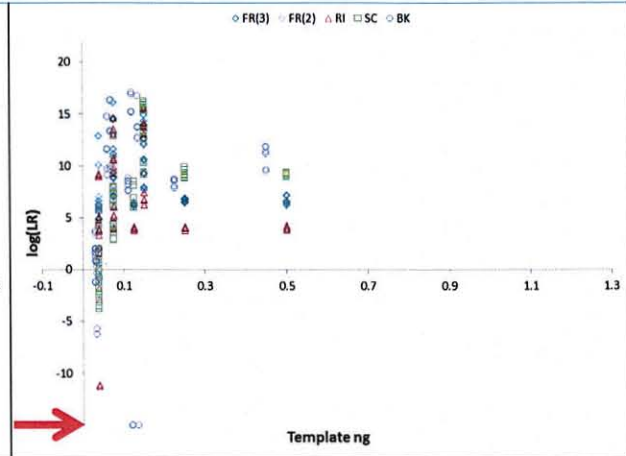
*Handwritten signature and date:*  
12/30/14



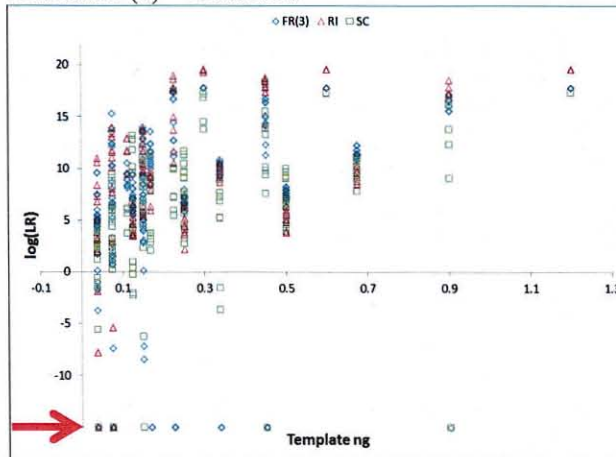
STRmix – Contributor 2



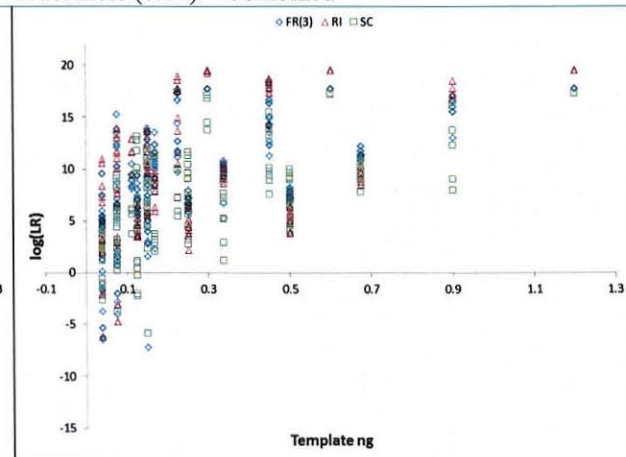
STRmix – Contributor 3



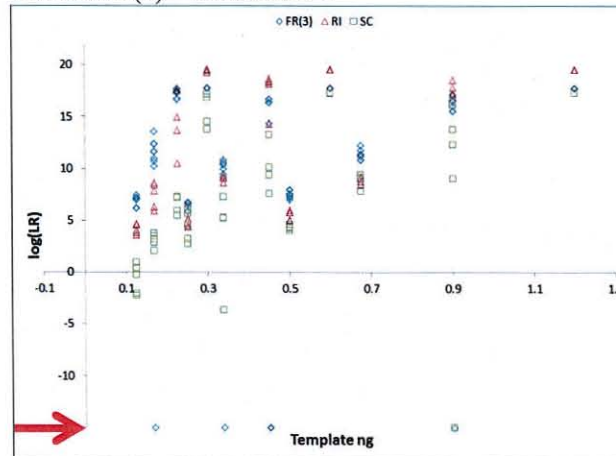
TrueAllele (0) – Combined



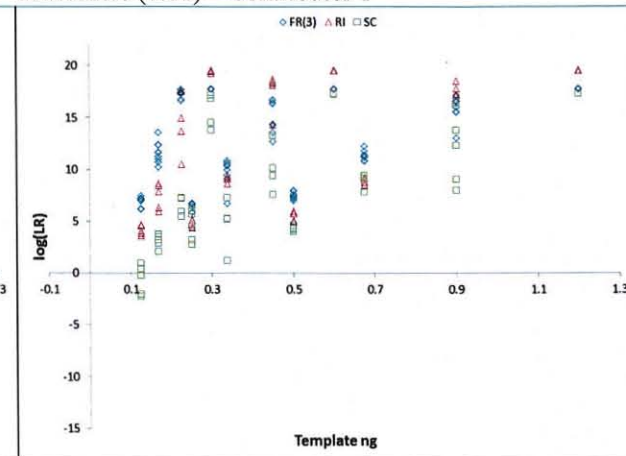
TrueAllele (0.01) – Combined



TrueAllele (0) – Contributor 1

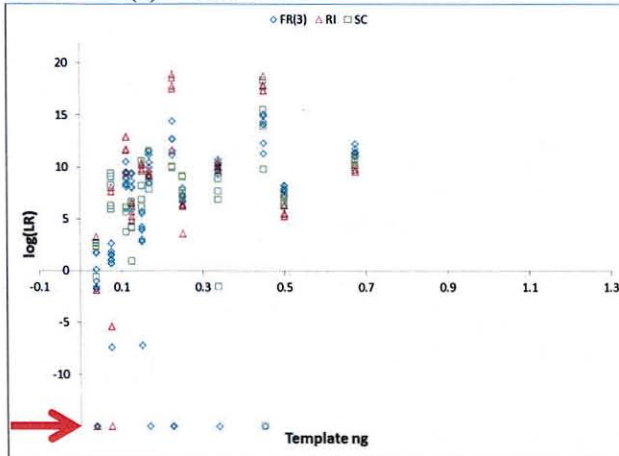


TrueAllele (0.01) – Contributor 1

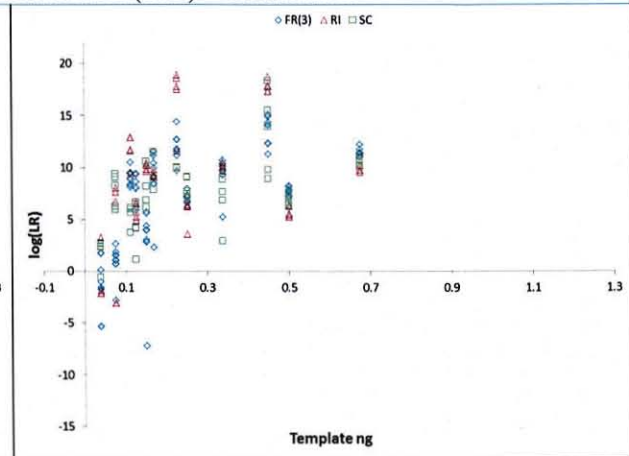


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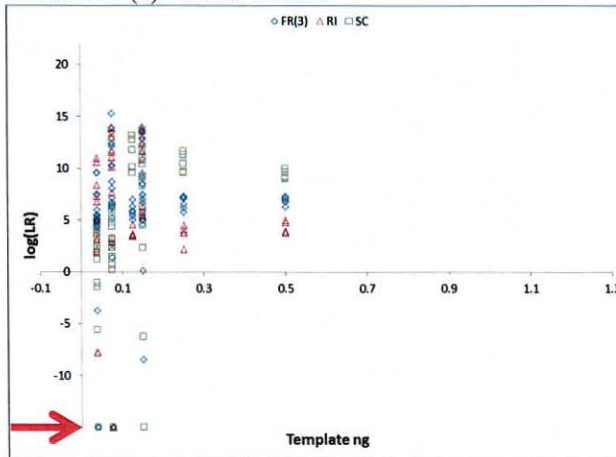
TrueAllele (0) – Contributor 2



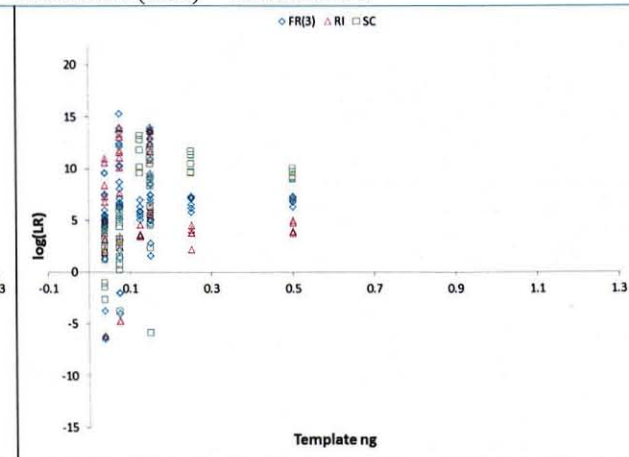
TrueAllele (0.01) – Contributor 2



TrueAllele (0) – Contributor 3

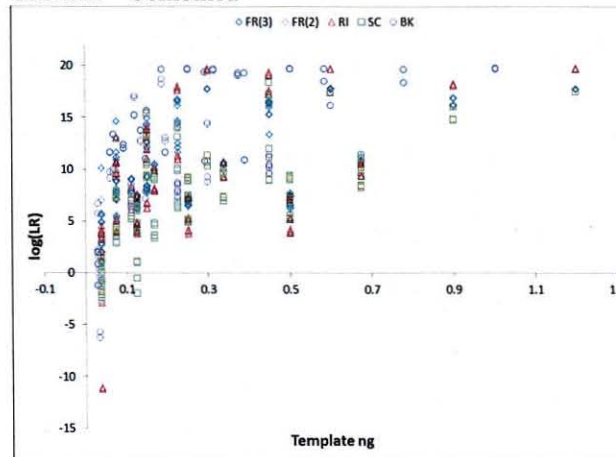


TrueAllele (0.01) – Contributor 3

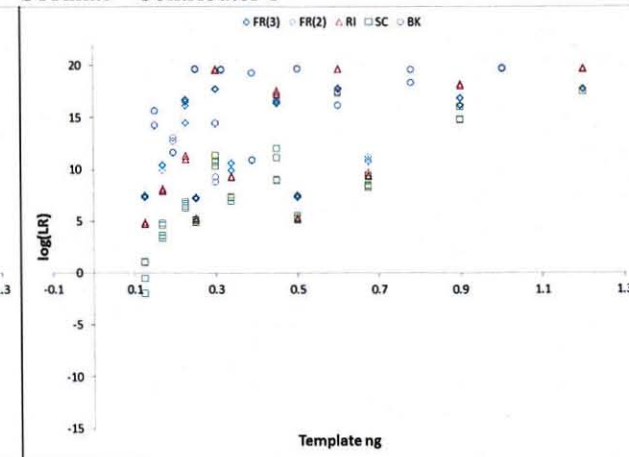


- All ratios and template amounts for single-amp interpretations (i.e., joint-amps removed)

STRmix – Combined

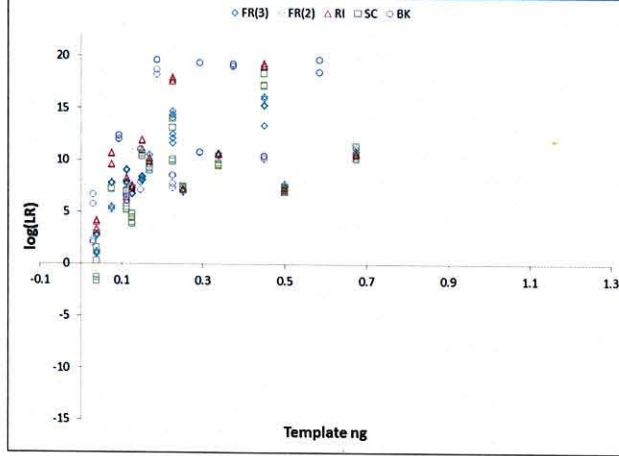


STRmix – Contributor 1

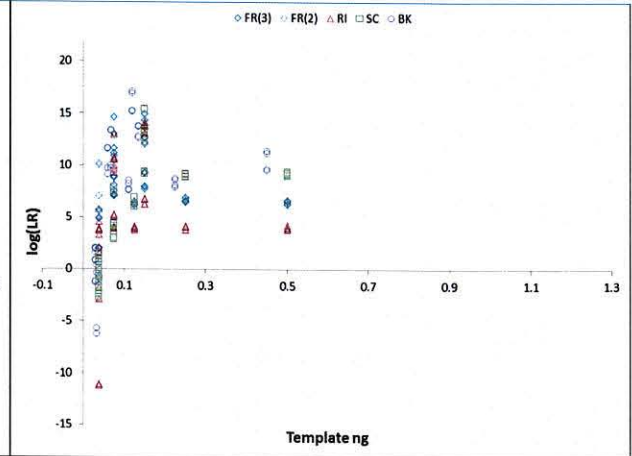


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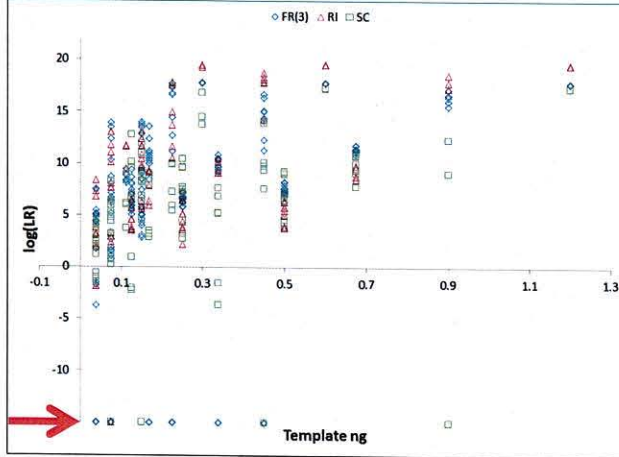
STRmix – Contributor 2



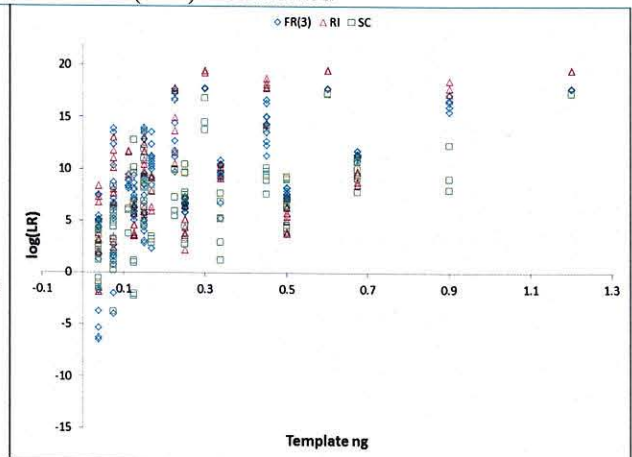
STRmix – Contributor 3



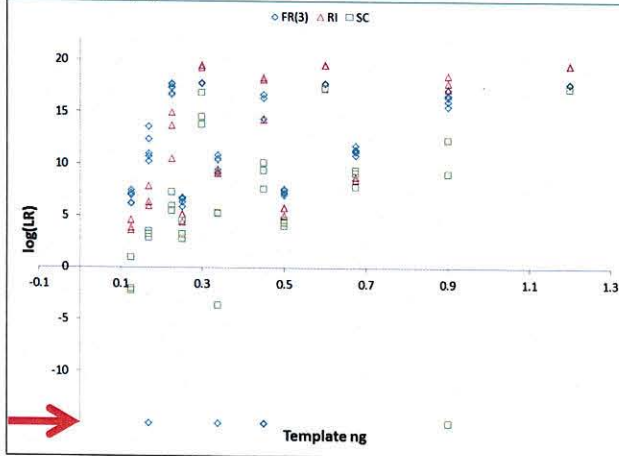
TrueAllele (0) – Combined



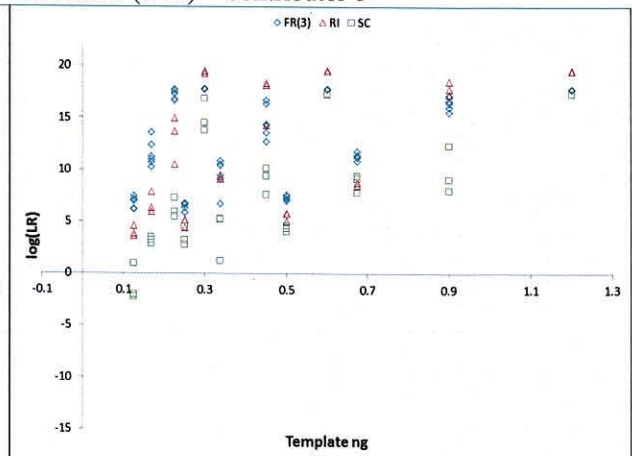
TrueAllele (0.01) – Combined



TrueAllele (0) – Contributor 1

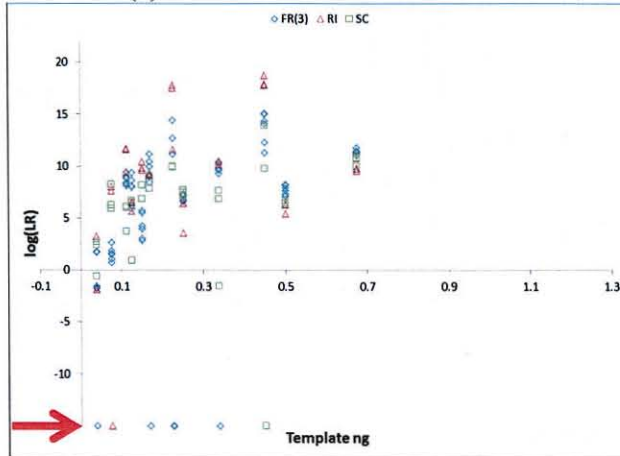


TrueAllele (0.01) – Contributor 1

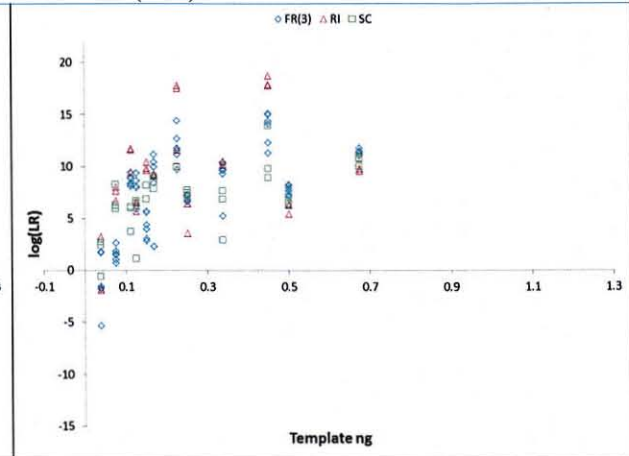


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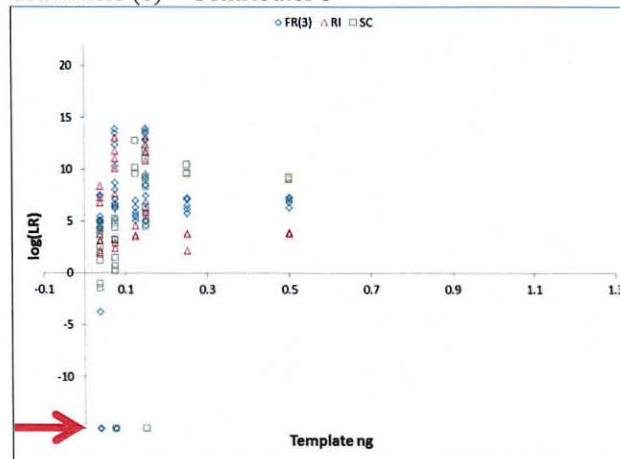
TrueAllele (0) – Contributor 2



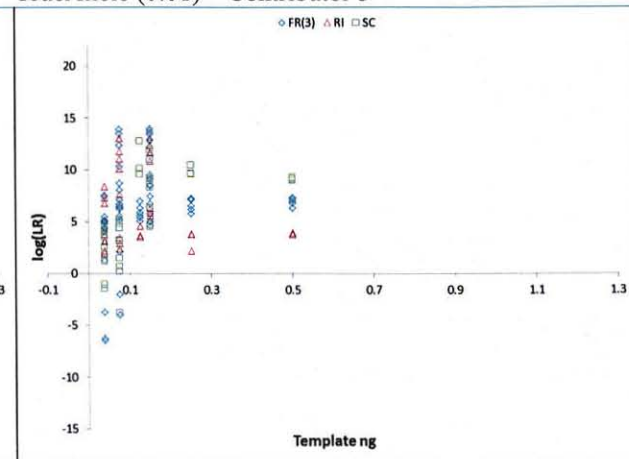
TrueAllele (0.01) – Contributor 2



TrueAllele (0) – Contributor 3

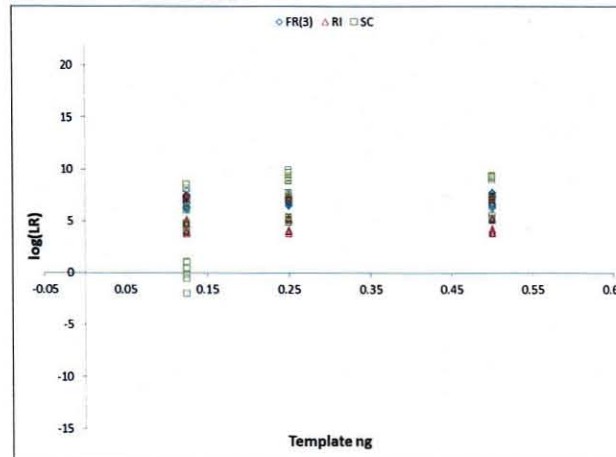


TrueAllele (0.01) – Contributor 3

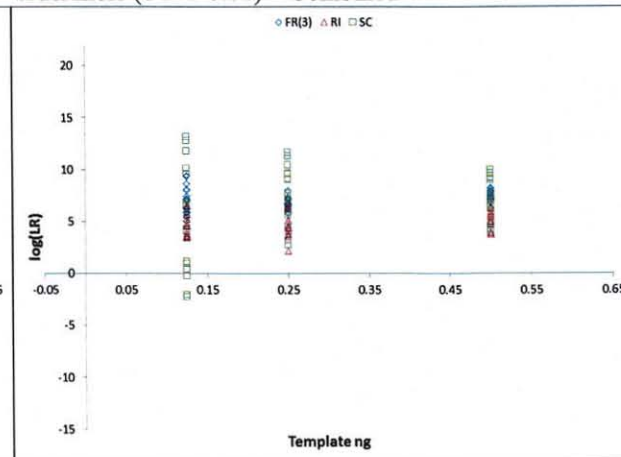


- 1-1-1 mixtures for all template amounts and interpretation types (i.e., single amp or joint amp)  
Note: The graphs below exclude the BK set, thus providing a more direct comparison between the two typing systems.

STRmix – Combined

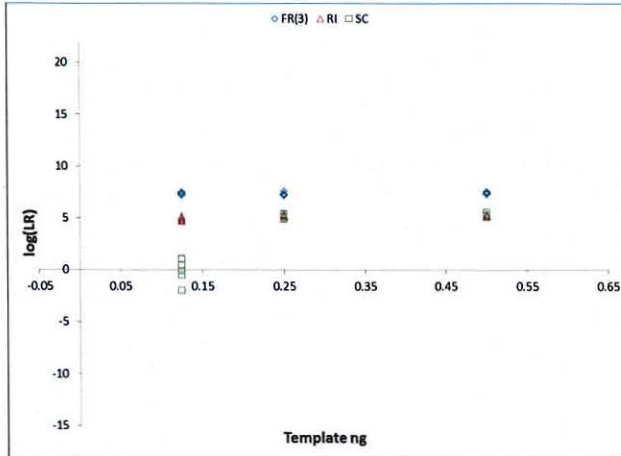


TrueAllele (0 and 0.01) – Combined

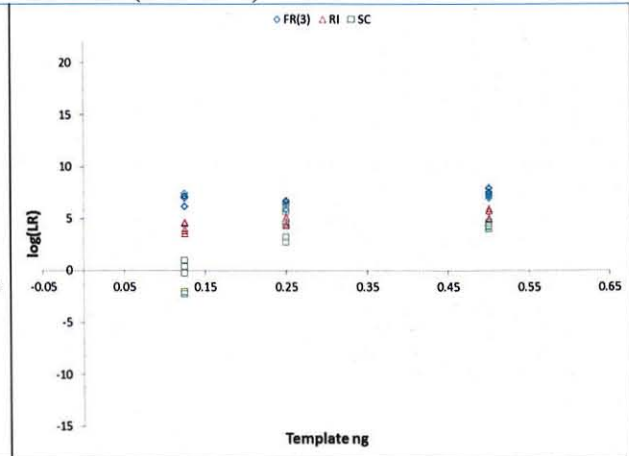


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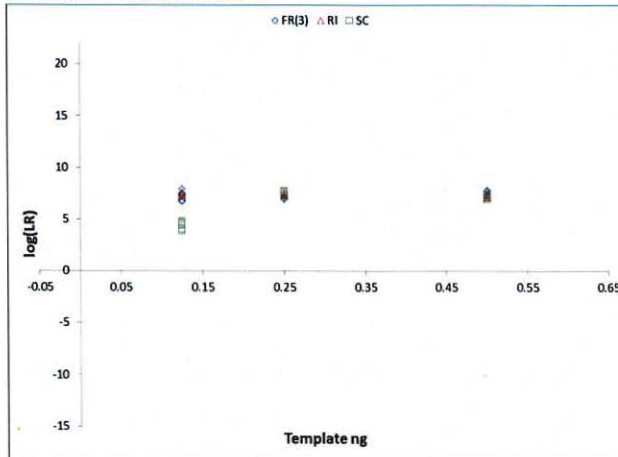
STRmix – Contributor 1



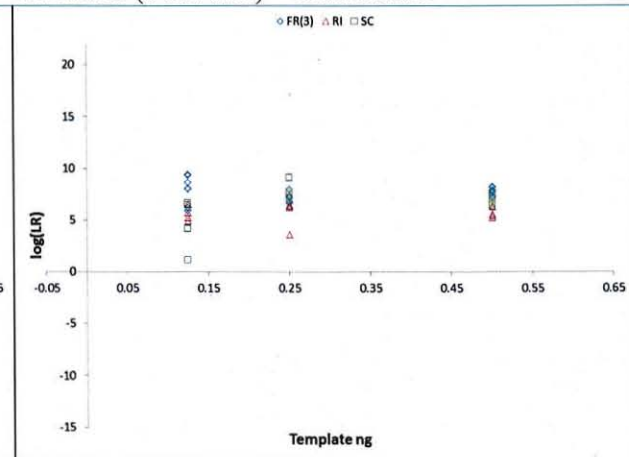
TrueAllele (0 and 0.01) – Contributor 1



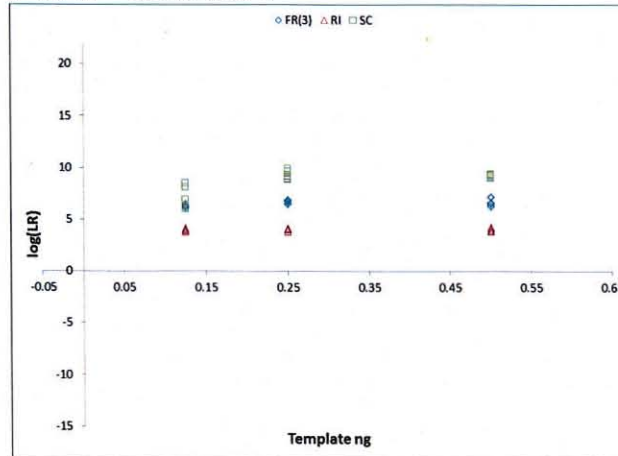
STRmix – Contributor 2



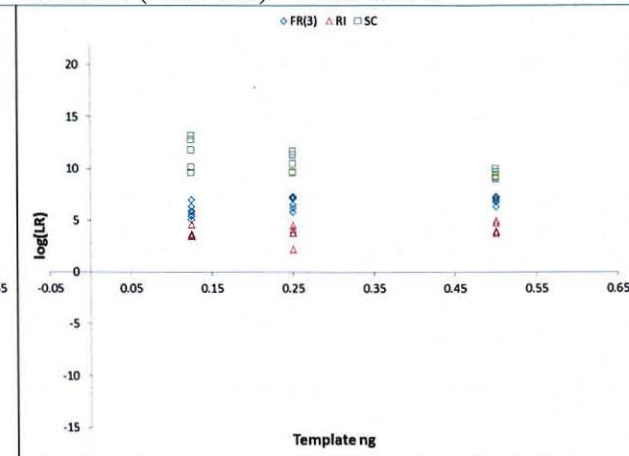
TrueAllele (0 and 0.01) – Contributor 2



STRmix – Contributor 3



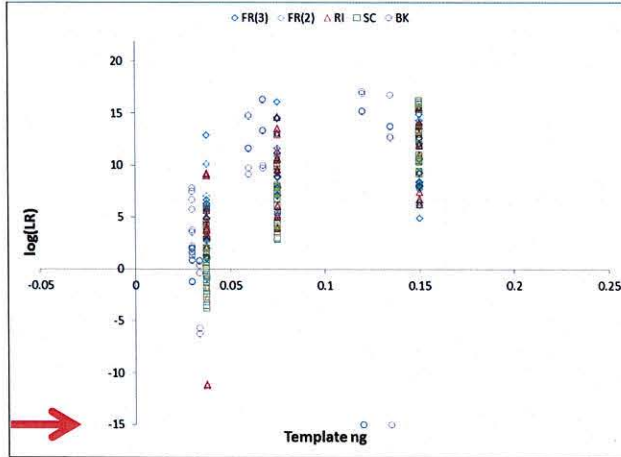
TrueAllele (0 and 0.01) – Contributor 3



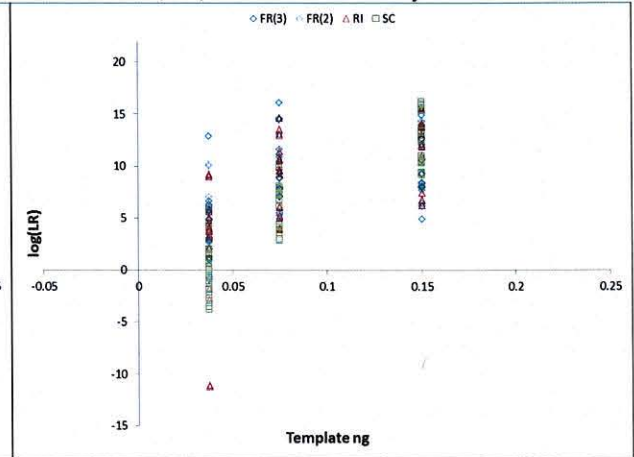
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- Contributors 2 and 3 for results where they each represented 1/10th of the total template DNA
    - Contributor 2: 8-1-1 mixtures
    - Contributor 3: 4.5-4.5-1, 6-6-1, and 8-1-1 mixtures
- All template amounts and interpretation types (i.e., single amp or joint amp)

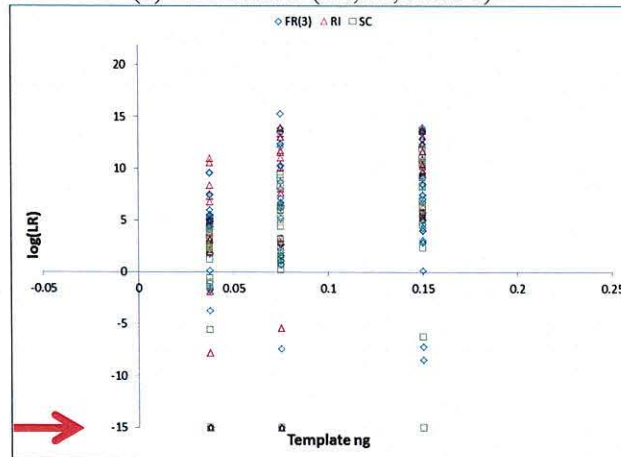
STRmix – All studies



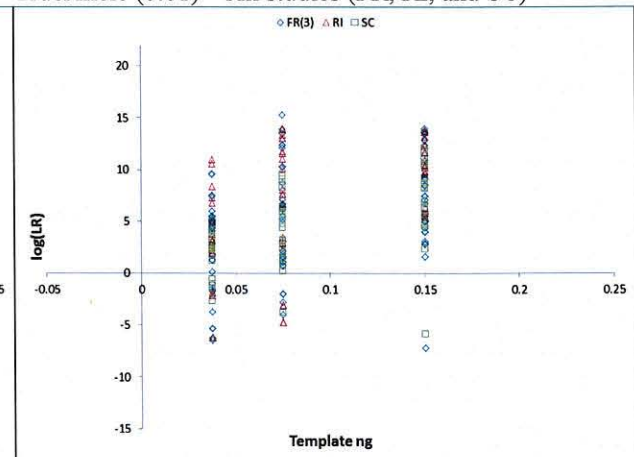
STRmix – FR, RI, and SC studies only



TrueAllele (0) – All studies (FR, RI, and SC)



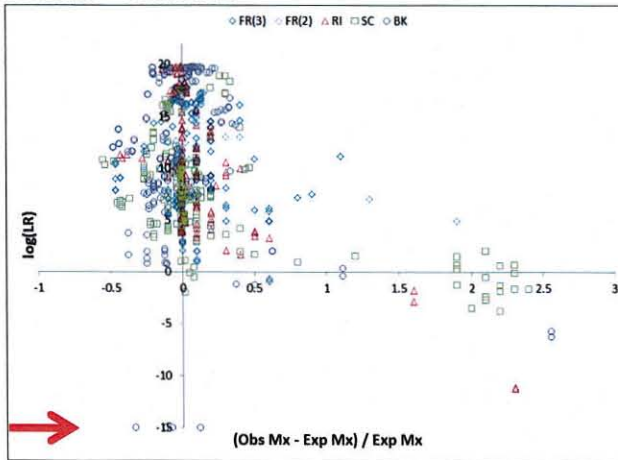
TrueAllele (0.01) – All studies (FR, RI, and SC)



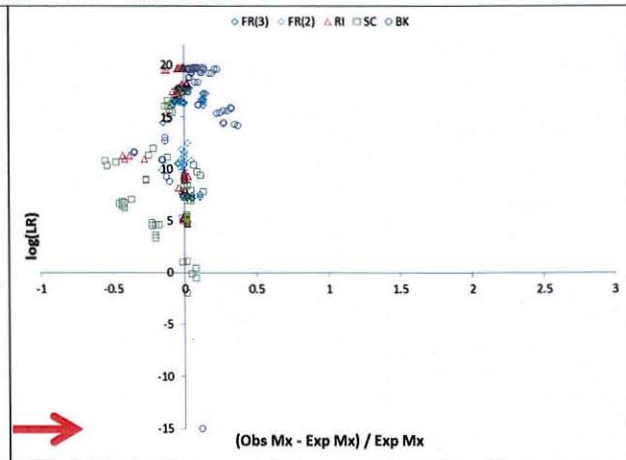
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- Sensitivity as a function of how much the system's estimated Mx deviated from the expected Mx. All ratios, template amounts, and interpretation types (i.e., single-amp or joint-amp).

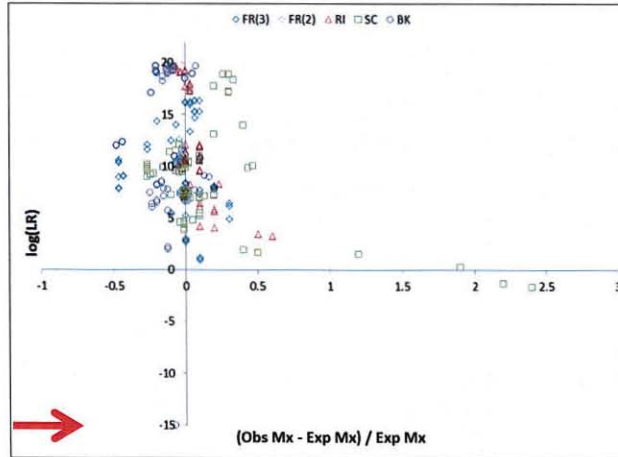
STRmix – Combined



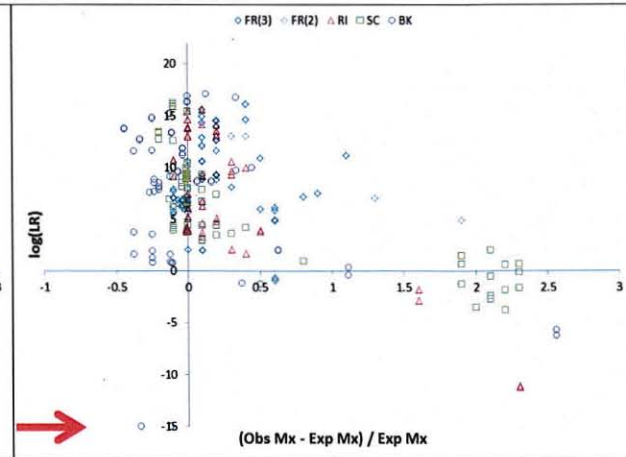
STRmix – Contributor 1



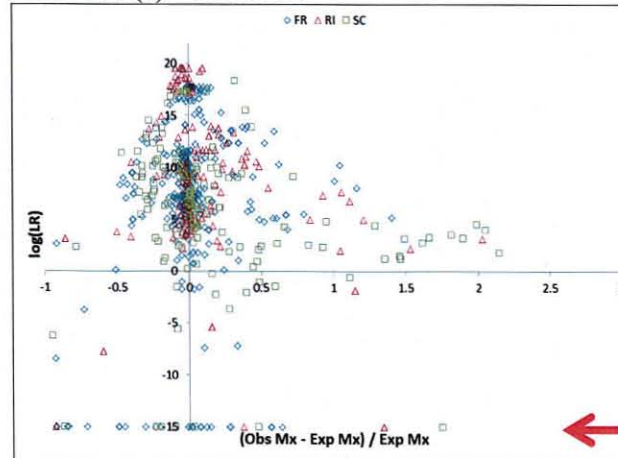
STRmix – Contributor 2



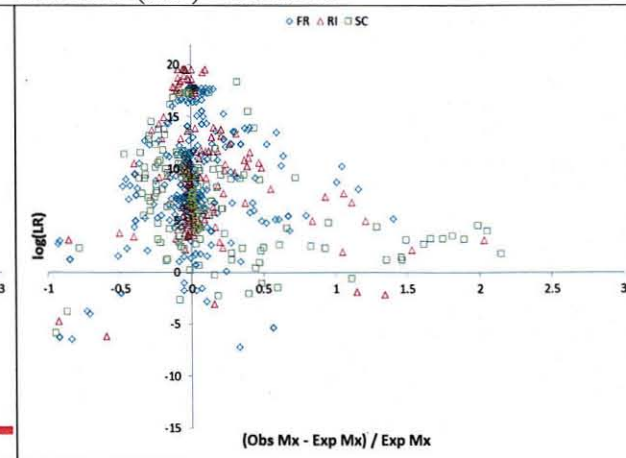
STRmix – Contributor 3



TrueAllele (0) – Combined

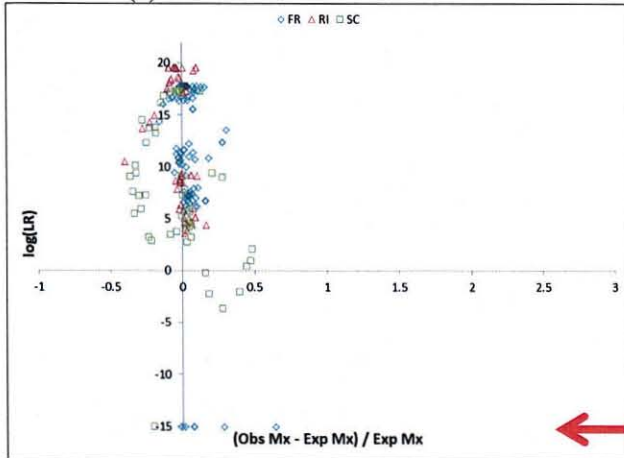


TrueAllele (0.01) – Combined

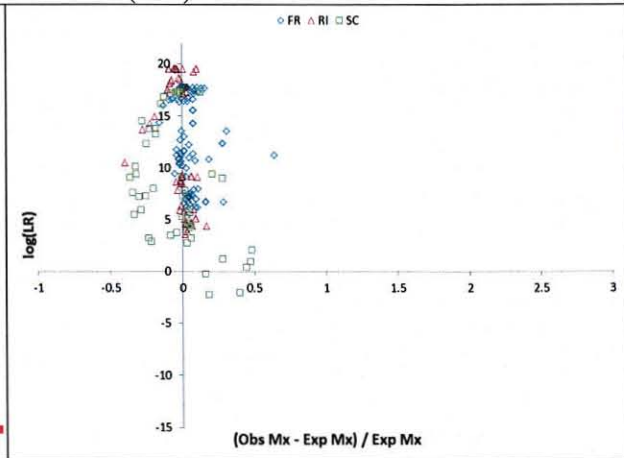


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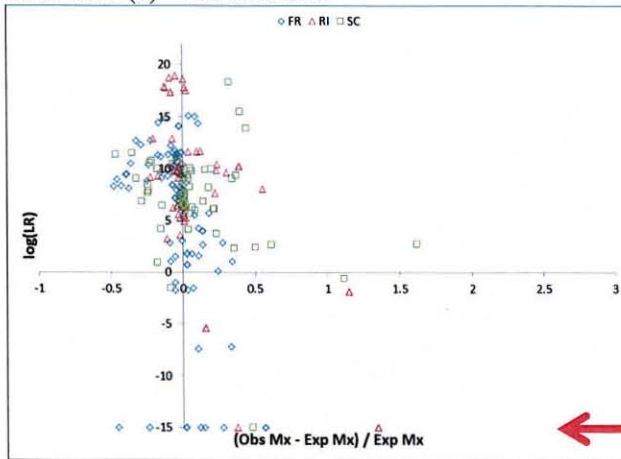
TrueAllele (0) – Contributor 1



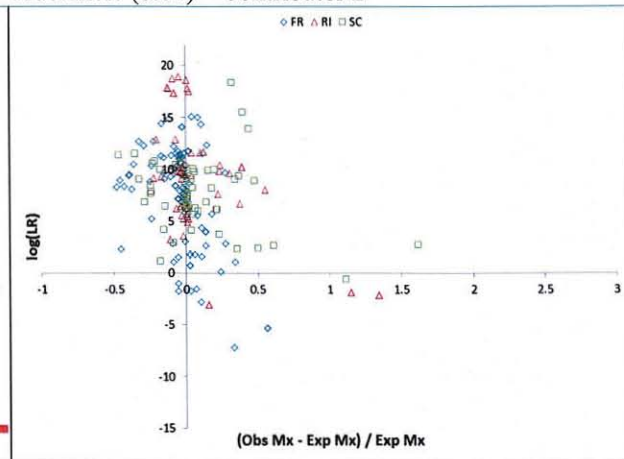
TrueAllele (0.01) – Contributor 1



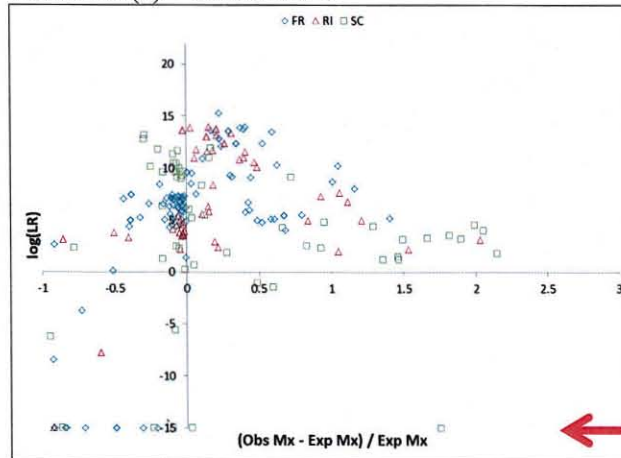
TrueAllele (0) – Contributor 2



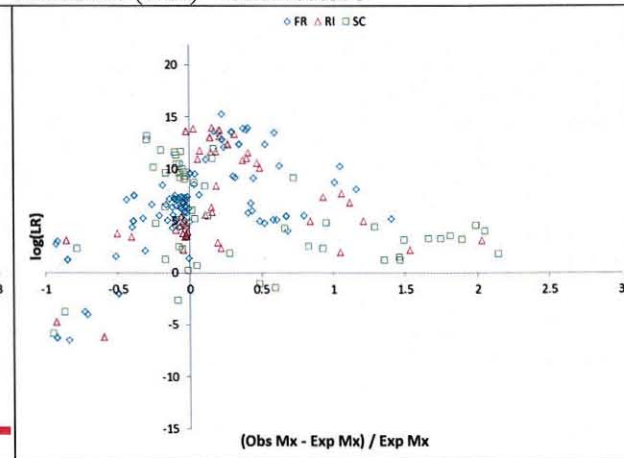
TrueAllele (0.01) – Contributor 2



TrueAllele (0) – Contributor 3



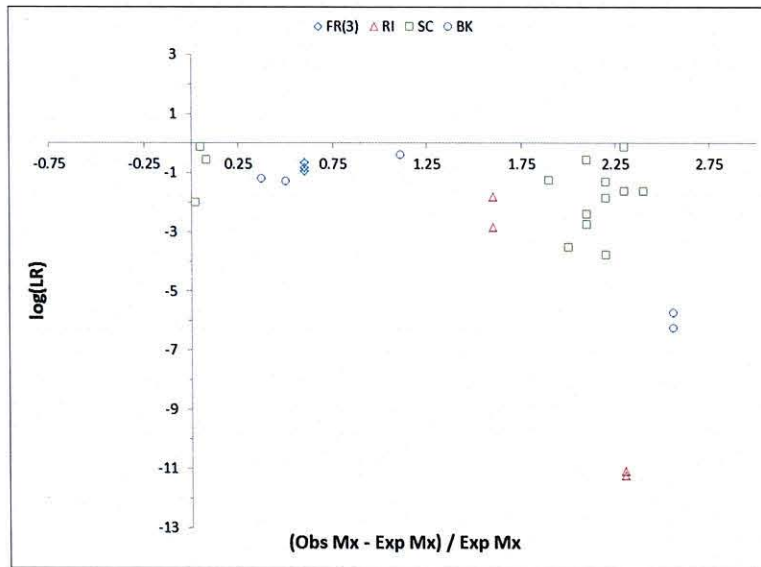
TrueAllele (0.01) – Contributor 3



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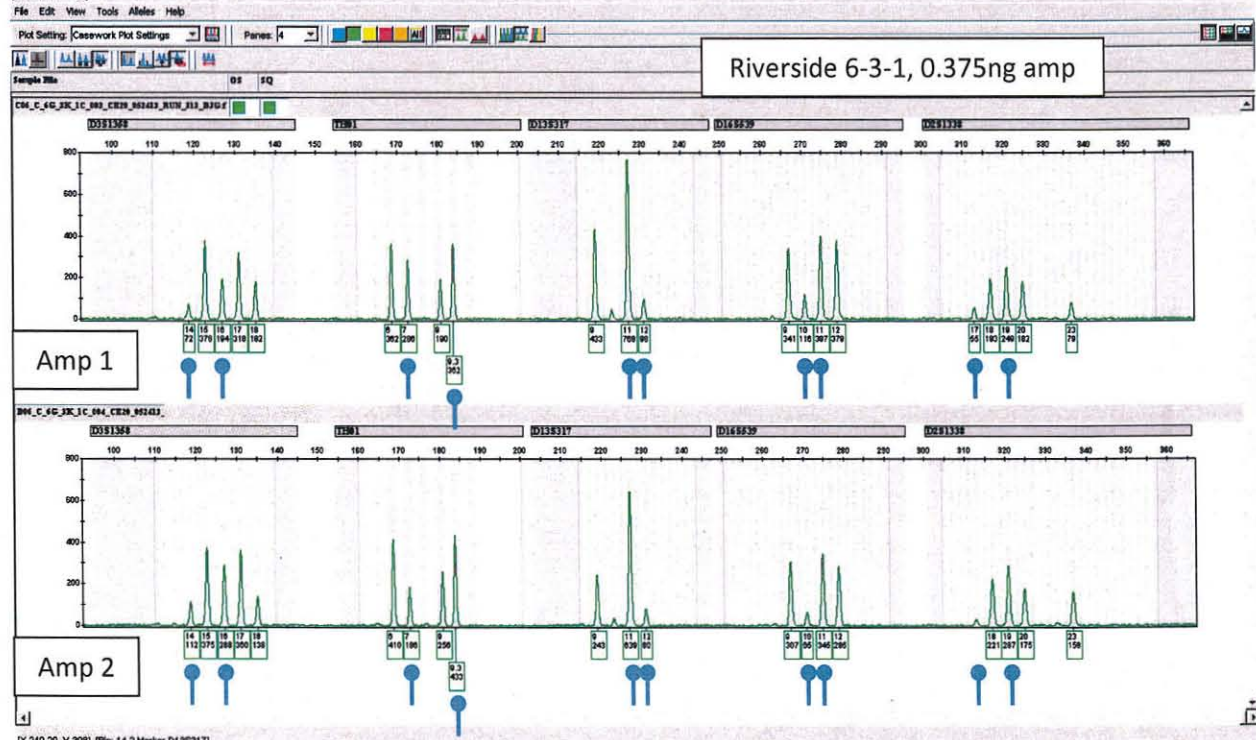
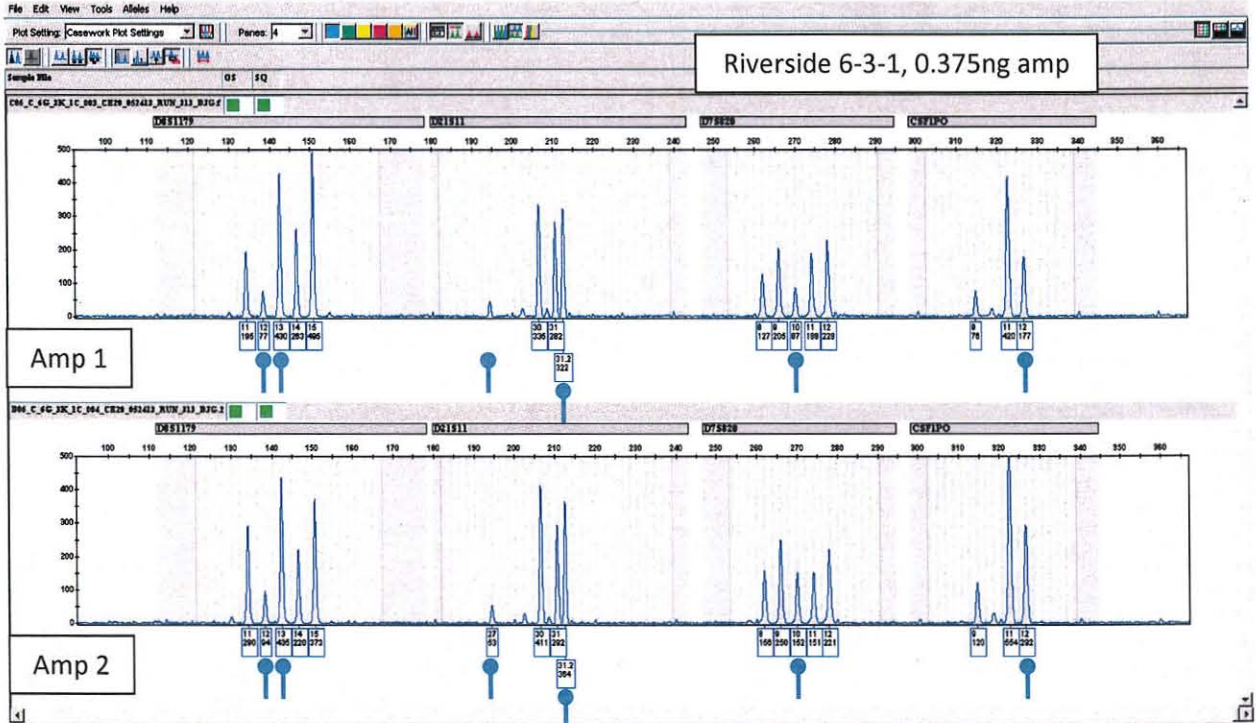
- A closer examination of the STRmix comparisons with  $-\log(\text{LR})$



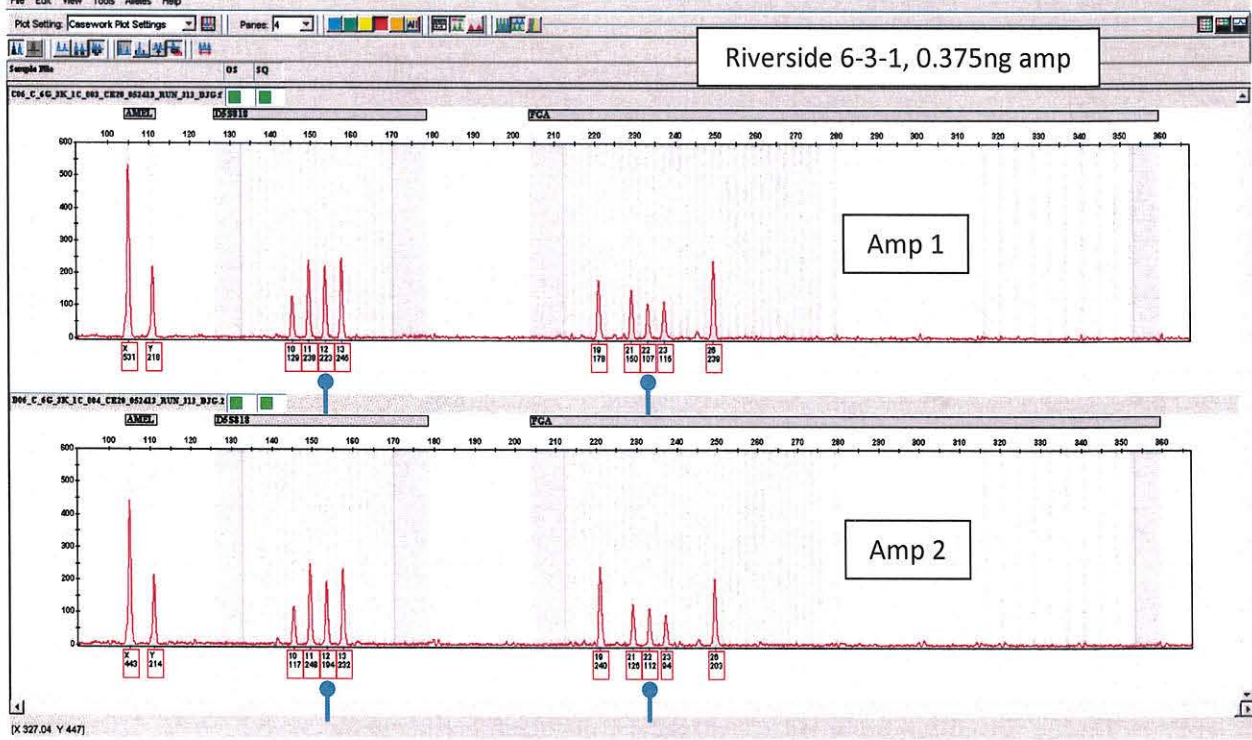
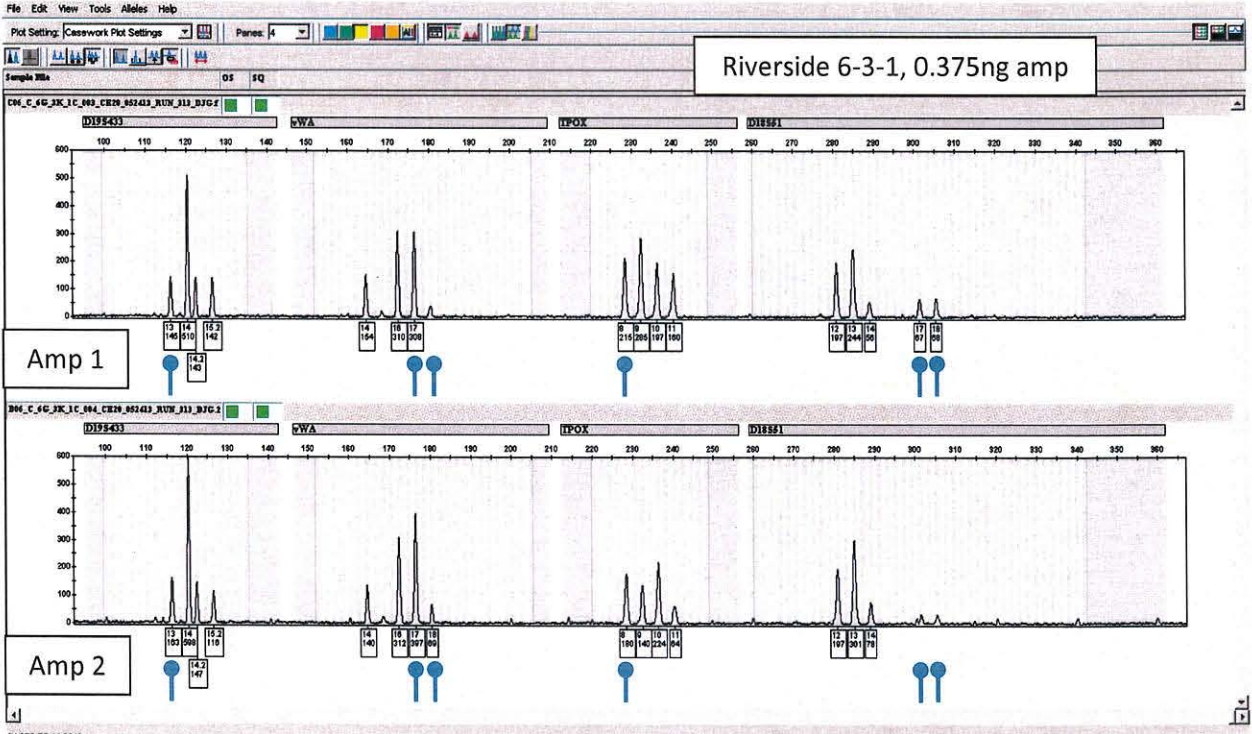
- The LR = 0 results from the BK sample set (not included in the graph above) were all due to interpretations that exceeded the Java cap for iterations. These are discussed in a separate validation study. All such mixtures gave LR > 1 when the ratio of MCMC burnin to readout accepts was adjusted to keep the readout iterations below the Java cap. However, those LR = 0 values are retained for the sensitivity percentages in Part II of the 3-person mixture study.
- Of the remaining interpretations with  $-\log(\text{LR})$ ...
  - All are from the 0.375 ng amps of seven mixtures (see Appendix I, pp. 32-47 for plots).
  - With the exception of the SC\_1-1-1 mixture, all are for a donor who contributed  $\leq 10\%$  of the total template DNA.
  - The most negative log(LR) values came from interpretations with comparatively large deviations from the expected Mx.
  - All 1-1-1, 4.5-4.5-1, and 8-1-1 mixtures with  $-\log(\text{LR})$  had no more than four alleles at a locus, even when comparing across amplifications.
  - The 6-3-1 mixtures from all four studies had at least one amplification where STRmix gave a  $-\log(\text{LR})$ .
    - In each case, the joint amplifications appear to have overcome the difficulties STRmix had with interpreting one or both of the 6-3-1 mixtures individually.
    - Amp 1 of the FR mixture gave  $-\log(\text{LR})$ , and it had no more than four alleles at a locus. Amp 2, on the other hand, had up to six alleles at a locus
    - The BK, RI, and SC mixtures had no more than five alleles at a locus.
    - The RI mixture gave the lowest  $-\log(\text{LR})$ . See below for the plots, where the labels indicate the allele positions for the 10% contributor. While both RI amplifications individually gave a  $-\log(\text{LR})$  and deviated from the expected mixture proportions, the joint interpretation gave a strongly  $+\log(\text{LR})$  and aligned well with the expected mixture proportions:

Amp and Interp	Log(LR) Harmonic	Mx from STRmix			Mx Rel. Dev.  obs - exp  / exp
		Donor 1	Donor 2	Donor 3	
1.2a	9.27	59%	31%	11%	0.1
1.2b	9.16	58%	31%	11%	0.1
1a	-11.22	34%	33%	33%	2.3
1b	-11.08	35%	33%	33%	2.3
2a	-2.84	37%	37%	26%	1.6
2b	-1.80	43%	31%	26%	1.6

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▪ Summary table: Comparisons with  $-\log(\text{LR})$ .

Study	Mix	Amp, Interp	Donor	Log(LR) Harmonic	Mx from STRmix			Mx Rel. Dev. $\frac{ \text{obs} - \text{exp} }{\text{exp}}$	Detected Alleles	Max alleles per Locus
					Donor 1	Donor 2	Donor 3			
FR	6-3-1	1a	3F	-0.82	68%	16%	16%	0.6	21	4
FR	6-3-1	1b	3F	-0.65	68%	16%	16%	0.6	21	4
FR	6-3-1	1c	3F	-0.94	51%	33%	16%	0.6	21	4
BKsc1 <sup>†</sup>	4.5-4.5-1	1a	3M	-6.24	34%	33%	32%	2.6	19	4
BKsc1 <sup>†</sup>	4.5-4.5-1	1b	3M	-5.74	34%	33%	32%	2.6	19	4
BKsc1 <sup>†</sup>	4.5-4.5-1	2b	3M	-0.38	45%	36%	19%	1.1	21	4
BKsc1 <sup>†</sup>	6-3-1	1a	3M	-1.27	76%	13%	12%	0.5	23	5
BKsc1 <sup>†</sup>	6-3-1	1b	3M	-1.18	76%	13%	11%	0.38	23	5
RI	6-3-1	1a	3F	-11.22	34%	33%	33%	2.3	28	5
RI	6-3-1	1b	3F	-11.08	35%	33%	33%	2.3	28	5
RI	6-3-1	2a	3F	-2.84	37%	37%	26%	1.6	27	5
RI	6-3-1	2b	3F	-1.80	43%	31%	26%	1.6	27	5
SC	1-1-1	1.2a	1F	-0.12	35%	33%	32%	0.05	22*	4
SC	1-1-1	1a	1F	-1.99	34%	33%	33%	0.02	13	4
SC	1-1-1	1b	1F	-0.55	36%	35%	29%	0.08	13	4
SC	4.5-4.5-1	1.2a	3M	-3.53	37%	33%	30%	2	20	4
SC	4.5-4.5-1	1.2b	3M	-3.78	35%	33%	32%	2.2	20	4
SC	4.5-4.5-1	1a	3M	-2.40	36%	33%	31%	2.1	20	4
SC	4.5-4.5-1	1b	3M	-2.74	35%	34%	31%	2.1	20	4
SC	4.5-4.5-1	2a	3M	-0.55	36%	33%	31%	2.1	20	4
SC	4.5-4.5-1	2b	3M	-1.24	36%	35%	29%	1.9	20	4
SC	6-3-1	1a	3M	-1.64	34%	33%	33%	2.3	22	5
SC	6-3-1	1b	3M	-1.86	35%	33%	32%	2.2	22	5
SC	6-3-1	2a	3M	-0.14	34%	33%	33%	2.3	22	5
SC	8-1-1	1a	2F	-1.63	37%	34%	29%	2.4	15	4
SC	8-1-1	1b	2F	-1.30	36%	32%	32%	2.2	15	4

\* Between them, these two amps had 22 of contributor 1F's alleles, but only 12 of those alleles were observed  $\geq 50$  rfu in both amps.

<sup>†</sup> Mx comparisons were made to the adjusted BK mixture proportions previously noted.

- Overall, STRmix appears to have increased difficulty defining the genotypes of very low-level contributors. Contributing factors appear to be...
  - Increased stochastic variation (especially in the 6-3-1 mixtures);
  - Reduced number of detected alleles for the low-level contributor(s), for example the 13 of 30 possible alleles detected for Contributor 1 in the SC\_1-1-1 mixture; and
  - Difficulty in assessing the mixture proportions when there are few/no loci with more than  $2*(N - 1)$  alleles, where N is the number of contributors. In such cases, STRmix appears to be biased toward even mixture proportions.

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SENSITIVITY: Summary Tables

- All contributors, ratios, and template amounts:

All interpretation types (i.e., single-amp or joint-amp)

$LR_H$	STRmix (V2.0 variance)		TAC	
	All	No BK	0 minimum	0.01 minimum
Total	909	729	684	684
$LR_H = 0$	0.55%	0.00%	5.56%	0.00%
$0 < LR_H < 1$	2.86%	2.88%	3.65%	5.12%
$LR_H < 1$	3.41%	2.88%	9.21%	5.12%

Separate interpretations of Amp 1 and amp 2:

$LR_H$	STRmix (V2.0 variance)		TAC	
	All	No BK	0 minimum	0.01 minimum
Total	606	486	432	432
$LR_H = 0$	0.00%	0.00%	5.56%	0.00%
$0 < LR_H < 1$	3.80%	3.70%	3.01%	3.94%
$LR_H < 1$	3.80%	3.70%	8.56%	3.94%

Joint interpretations of Amp 1 and amp 2:

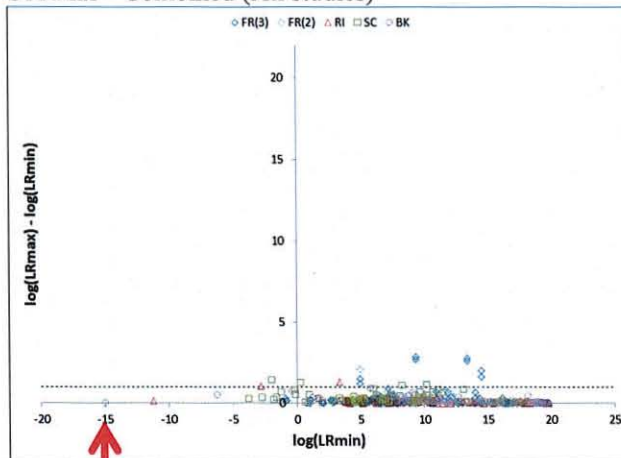
$LR_H$	STRmix (V2.0 variance)		TAC	
	All	No BK	0 minimum	0.01 minimum
Total	303	243	252	252
$LR_H = 0$	1.65%	0.00%	5.56%	0.00%
$0 < LR_H < 1$	0.99%	1.23%	4.76%	7.14%
$LR_H < 1$	2.64%	1.23%	10.32%	7.14%

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*12/30/14*

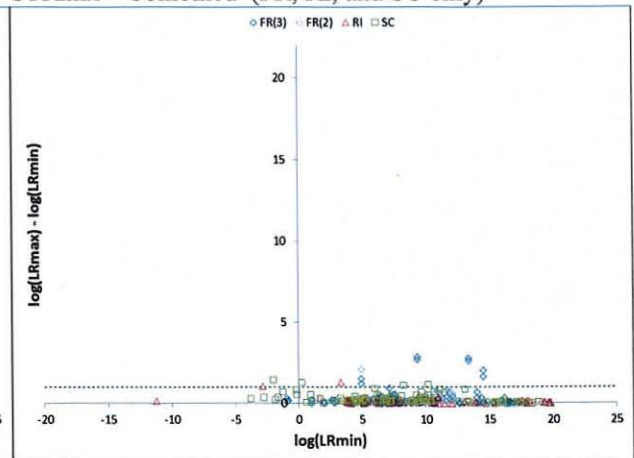
**PRECISION: Graphing the 3-person mixture**

- Mixture weights and population-specific LRs for each comparison were imported into the following spreadsheets
  - STRmix\_Graphs\_Data (spm 07.10.2014).xslm
  - TAC\_Graphs\_Data (spm 07.10.2014).xslm
- $LR_H$  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  $\Delta \log LR_H$  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  $-15$ .
- All ratios, template amounts, and interpretation types (i.e., single-amp or joint-amp)  
Note: SC comparisons that involved  $LR = 0$  are not all visible in the graphs below, because the y-axis values are off scale (in the 30s). Those data sets with  $LR = 0$  values are marked with an arrow.

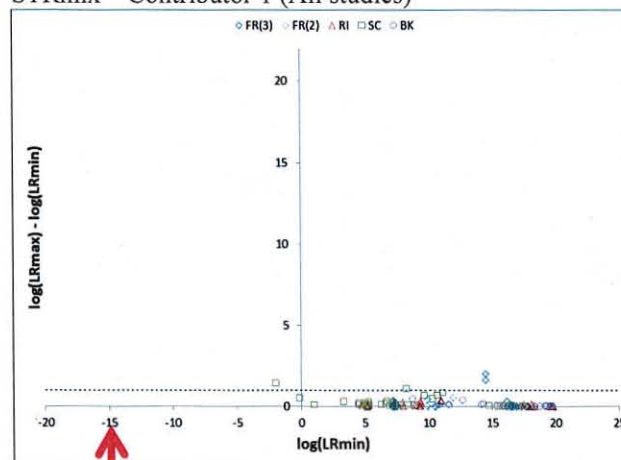
STRmix – Combined (All studies)



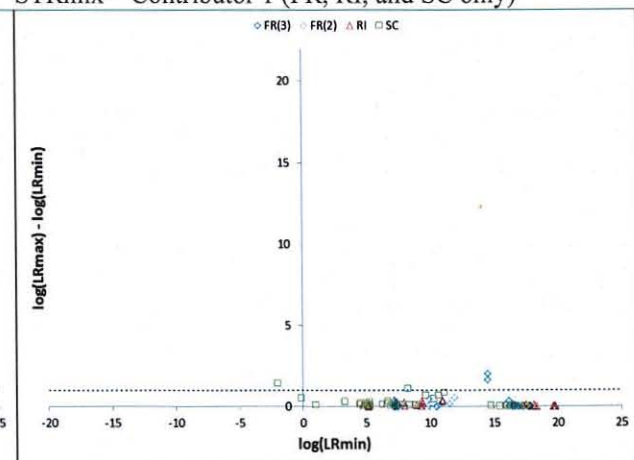
STRmix – Combined (FR, RI, and SC only)



STRmix – Contributor 1 (All studies)

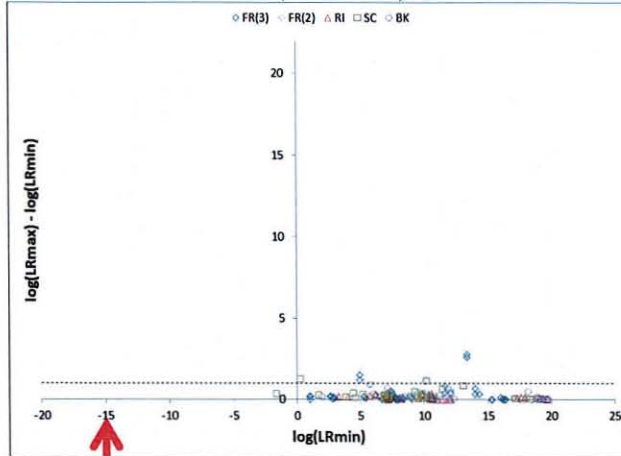


STRmix – Contributor 1 (FR, RI, and SC only)

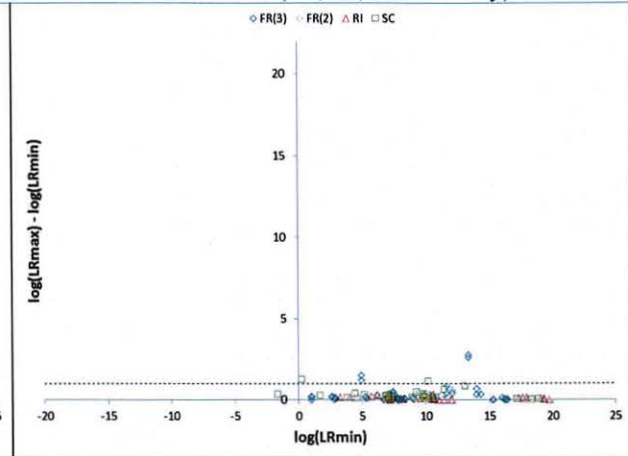


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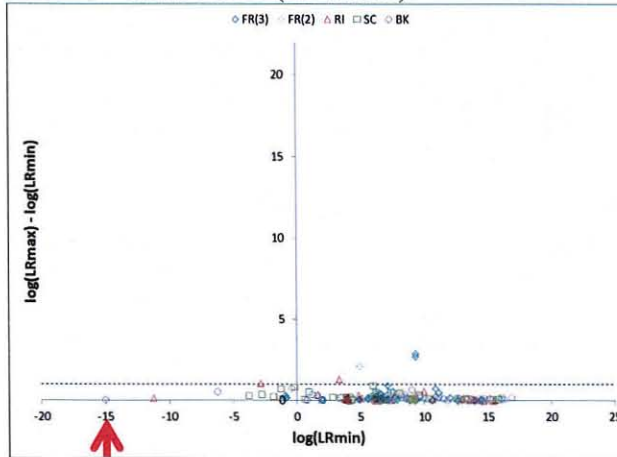
STRmix – Contributor 2 (All studies)



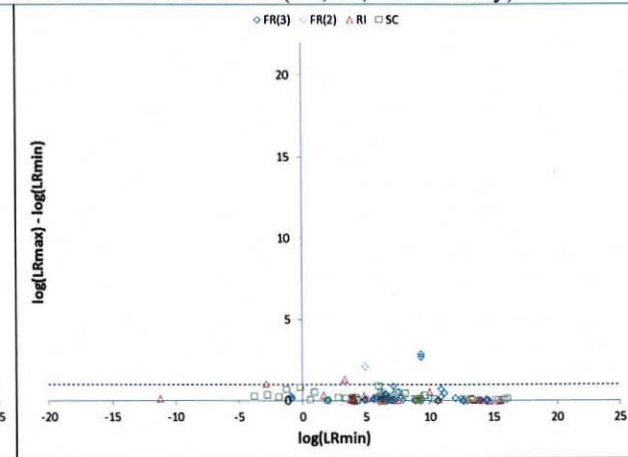
STRmix – Contributor 2 (FR, RI, and SC only)



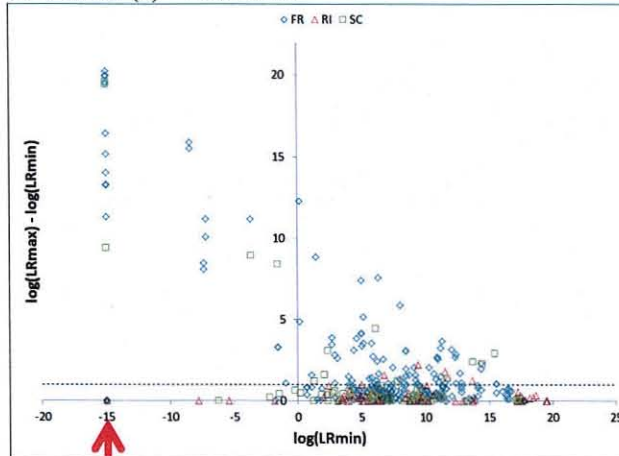
STRmix – Contributor 3 (All studies)



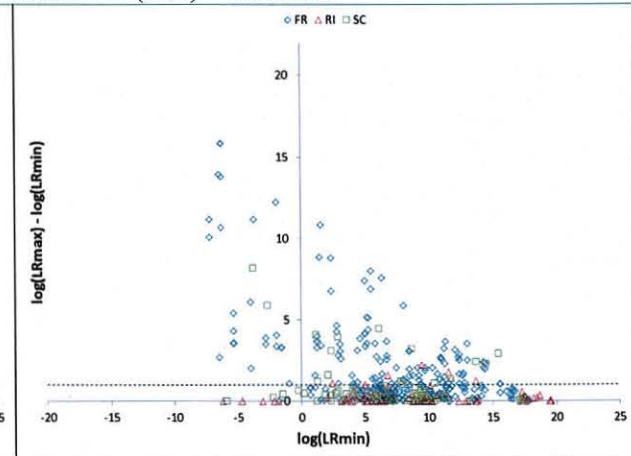
STRmix – Contributor 3 (FR, RI, and SC only)



TrueAllele (0) – Combined

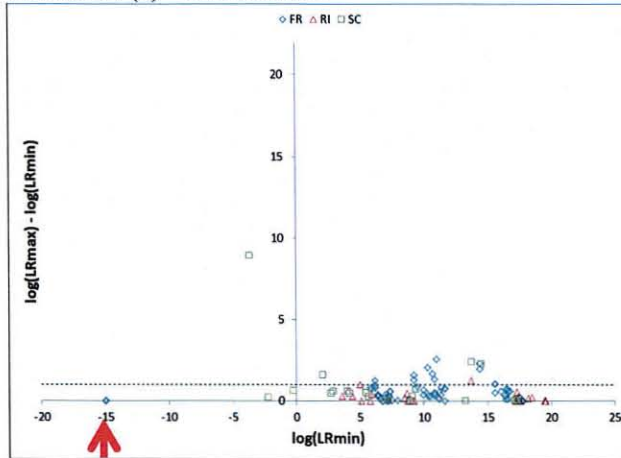


TrueAllele (0.01) – Combined

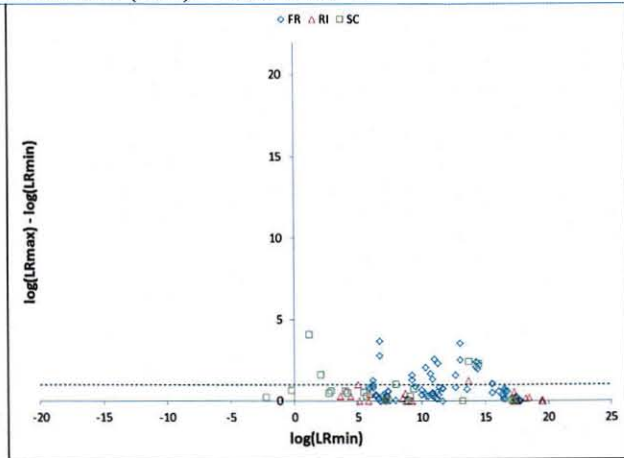


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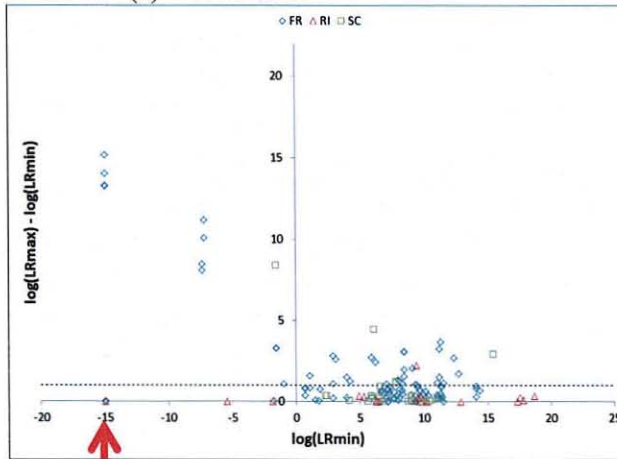
TrueAllele (0) – Contributor 1



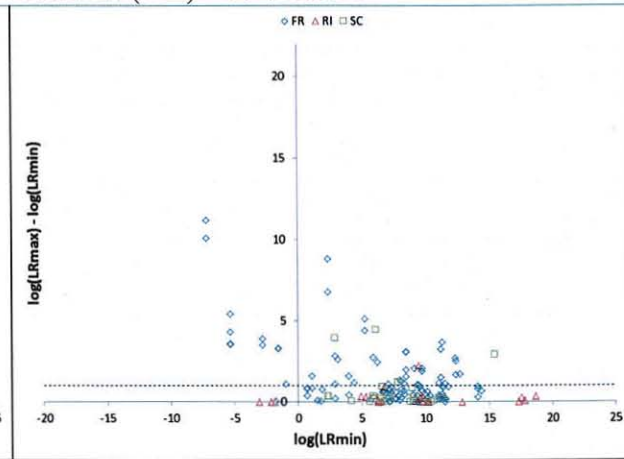
TrueAllele (0.01) – Contributor 1



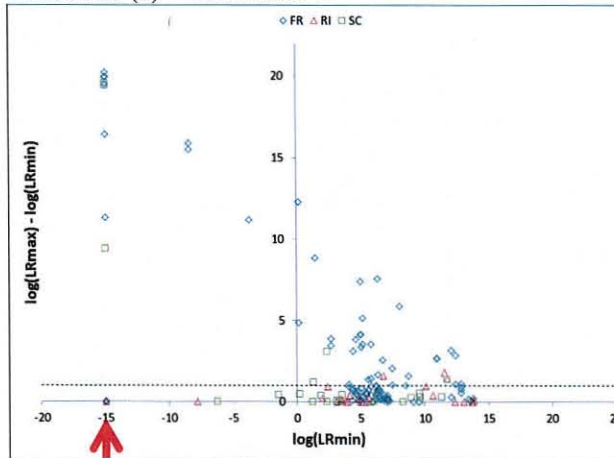
TrueAllele (0) – Contributor 2



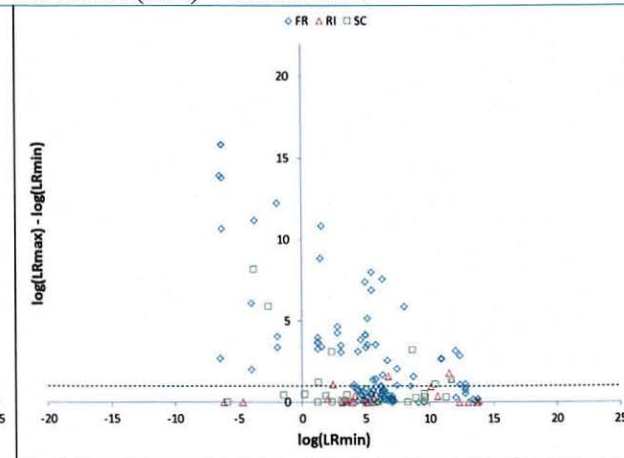
TrueAllele (0.01) – Contributor 2



TrueAllele (0) – Contributor 3



TrueAllele (0.01) – Contributor 3



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12/30/14



PRECISION: Summary Tables

- Note: The maximum deviation doesn't include those pairwise comparisons where one of the two was arbitrarily assigned a -15 log value. The absolute values of those  $\Delta \log LR_H$  are somewhat meaningless.
- All contributors, ratios, and template amounts:

All interpretation types (i.e., single-amp or joint-amp)

$\Delta \log LR_H$	STRmix (V2.0 variance)		TAC	
	All	No BK	0 minimum	0.01 minimum
Total	576	486	468	468
Min	0.00E+00	1.09E-04	0.00E+00	0.00E+00
Max (no LR=0)	2.87E+00	2.87E+00	1.59E+01	1.58E+01
% > 0.3 (i.e., 2X)	16.15%	15.43%	57.05%	58.33%
% > 1 (i.e., 10X)	3.13%	3.09%	29.70%	29.91%

Separate interpretations of Amp 1 and amp 2

$\Delta \log LR_H$	STRmix (V2.0 variance)		TAC	
	All	No BK	0 minimum	0.01 minimum
Total	384	324	288	288
Min	1.06E-04	1.09E-04	0.00E+00	0.00E+00
Max (no LR=0)	2.87E+00	2.87E+00	1.12E+01	1.39E+01
% > 0.3 (i.e., 2X)	17.71%	17.28%	60.07%	62.50%
% > 1 (i.e., 10X)	3.39%	4.01%	30.21%	31.25%

Joint interpretations of Amp 1 and amp 2

$\Delta \log LR_H$	STRmix (V2.0 variance)		TAC	
	All	No BK	0 minimum	0.01 minimum
Total	192	162	180	180
Min	0.00E+00	2.31E-04	0.00E+00	0.00E+00
Max (no LR=0)	1.52E+00	1.52E+00	1.59E+01	1.58E+01
% > 0.3 (i.e., 2X)	13.02%	11.73%	52.22%	51.67%
% > 1 (i.e., 10X)	2.60%	1.23%	28.89%	27.78%

- When  $\Delta \log LR_H = 0$ : STRmix
  - The only instances where  $\Delta \log LR_H = 0$  were from the SC comparisons that were  $LR_H = 0$  for >1 comparison of a like kind. As noted previously, these were due to the number of readout iterations exceeding the Java cap.
  - STRmix did not give identical LRs across multiple interpretations for three-person mixtures, even when the contributor was assigned one genotype with 100% weight. This is not unexpected. Since STRmix applies the full Balding and Nichols theta approach, accounting for all contributors alleles in the mixture, the adjusted allele probabilities will vary with the different genotype combinations in each comparison.
- When  $\Delta \log LR_H = 0$ : TrueAllele
  - Overall, 47 pairs of requests had at least one contributor with identical LRs (i.e.,  $\Delta \log LR_H = 0$ .) This includes comparisons between FR joint interpretations (1.2a and 1.2b) that were not used for the tables above.
  - TrueAllele is expected to give identical  $LR_H$  across multiple interpretations whenever a contributor is assigned 100% weight to one genotype. This is because TrueAllele applies the Balding and Nichols theta approach only to the alleles of the one contributor being compared. By applying formulae NRC II 4.10a and 4.10b (recommendation 4.2), the other contributors have no influence on allele probabilities, and therefore genotype probabilities can match exactly.
    - 15 pairs were only identical for contributor 1. They were all the major contributor to an 8-1-1 mixture.

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