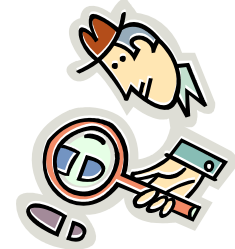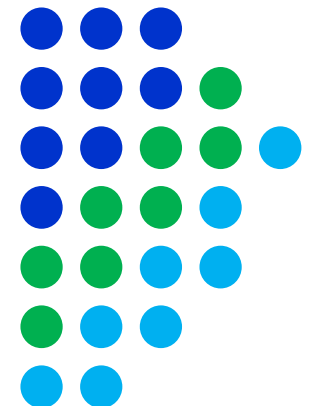Mitchell M. Holland, Ph.D.

Professor, Biochem & MolBio
Former Director, Forensic Science Program
Eberly College of Science
Penn State University, University Park, PA

MaSTR™: interpretation of STR mixtures associated with differentially degraded DNA

FRNSC 821 Class: Advanced Forensic MolBio

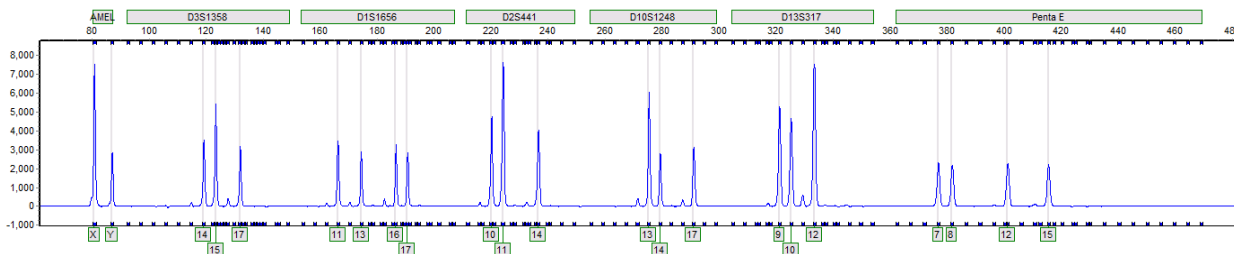https://sites.psu.edu/hollandresearch/

Teresa Tiedge (NC State U, PhD Program)

Abby Bender (TX DPS, Austin)

Sidney Gaston-Sanchez (AFDIL)
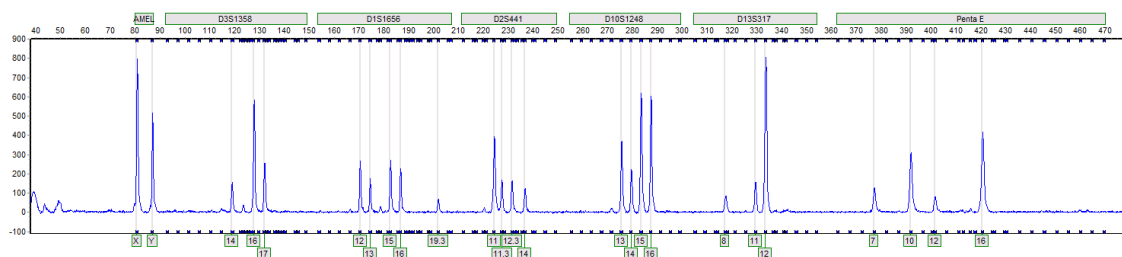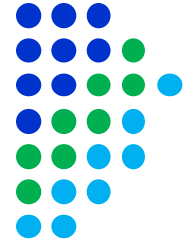
2-Person Mixtures

Manuscript Submitted

Sofia Canlas (MA State Police)

Grace Marino (Alabama)

3 and 4-Person Mixtures

Manuscript In Preparation

Research paper

Internal validation of STRmix™ for the interpretation of single source and mixed DNA profiles

Tamyra R. Moretti[a,*], Rebecca S. Just[a], Susannah C. Kehl[b], Leah E. Willis[a], John S. Buckleton[c,d], Jo-Anne Bright[c], Duncan A. Taylor[e,f], Anthony J. Onorato[a]

[a] DNA Support Unit, Federal Bureau of Investigation Laboratory, 2501 Investigation Parkway, Quantico, VA 22135, USA
[b] Biometrics Analysis Section, Federal Bureau of Investigation Laboratory, 2501 Investigation Parkway, Quantico, VA 22135, USA
[c] Institute of Environmental Science and Research, Private Bag 92021, Auckland 1025, New Zealand
[d] National Institute of Standards and Technology, 100 Bureau Drive, Gaithersburg, MD 20899, USA
[e] Forensic Science South Australia, 21 Divett Place, Adelaide, SA 5000, Australia
[f] School of Biological Sciences, Flinders University, GPO Box 2100 Adelaide, SA, 5001 Australia

The DNA samples in these studies were not degraded in a controlled manner or …

Research paper

Systematic evaluation of STRmix™ performance on degraded DNA profile data

Kyle R. Duke*, Steven P. Myers

California Department of Justice Bureau of Forensic Services Jan Bashinski DNA Laboratory, 1001 W Cutting Boulevard, Richmond, CA, 94804, United States

… controlled differential degradation was assessed on *in-silico* mixtures

Degradation of forensic DNA profiles

Jo-Anne Bright[a,b*], Duncan Taylor[c], James M. Curran[b] and John S. Buckleton[a]

[a]ESR, Private Bag 92021, Auckland 1025, New Zealand; [b]Department of Statistics, University of Auckland, Private Bag 92019, Auckland 1025, New Zealand; [c]Forensic Science South Australia, 21 Divett Place, SA 5000, Australia

Experimental Design

M = Male
F = Female

P = Pristine
250 = 250 bp
150 = 150 bp

3 = 0.1, 0.25 and 0.5 ng input amounts

|	Ratio: 1:1		Ratio: 1:3		Ratio: 1:6		Ratio: 1:10		TOTAL
	DNA Input: 0.1, 0.25 or 0.5 ngs		DNA Input: 0.1, 0.25 or 0.5 ngs		DNA Input: 0.1, 0.25 or 0.5 ngs		DNA Input: 0.1, 0.25 or 0.5 ngs		
M1:F1 M1 was always the major (except for 1:1 ratios) and was associated with the first degradation status	P:P	3	P:P	3	P:P	3	P:P	3	12
	P:250	3	P:250	3	P:250	3	P:250	3	12
	P:150	3	P:150	3	P:150	3	P:150	3	12
	250:250	3	250:250	3	250:250	3	250:250	3	12
	150:250	3	150:250	3	150:250	3	150:250	3	12
	150:150	3	150:150	3	150:150	3	150:150	3	12
								Total	72
F2:M2 F2 was always the major (except for 1:1 ratios) and was associated with the first degradation status	P:P	3	P:P	3	P:P	3	P:P	3	12
	P:250	3	P:250	3	P:250	3	P:250	3	12
	P:150	3	P:150	3	P:150	3	P:150	3	12
	250:250	3	250:250	3	250:250	3	250:250	3	12
	150:250	3	150:250	3	150:250	3	150:250	3	12
	150:150	3	150:150	3	150:150	3	150:150	3	12
								Total	72
								GRAND TOTAL	144

Shearing the DNA

~10 ng/uL DNA mechanically sheared with a Covaris S220

Peak incident power (w) of 75, 200 cycles per burst, and 510 seconds of treatment time for the 150 bp samples or 160 seconds of treatment time for the 250 bp samples
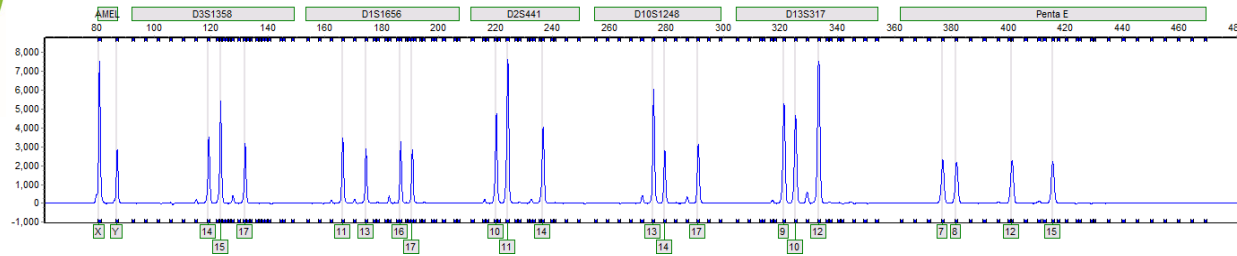
An aliquot of each sheared sample was run on a Bioanalyzer 2100 (Agilent) with a high sensitivity chip
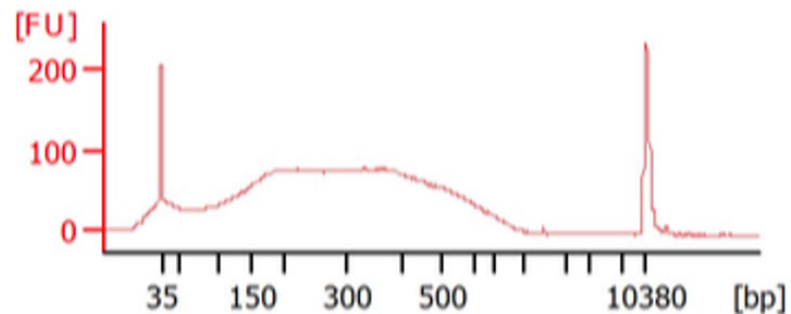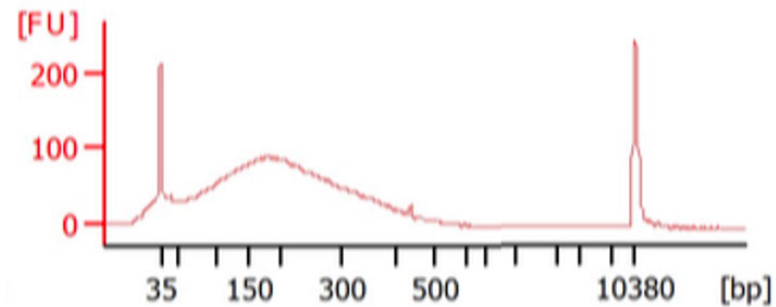
Sample extracts were quantified with Quantifiler™ HP before and after shearing
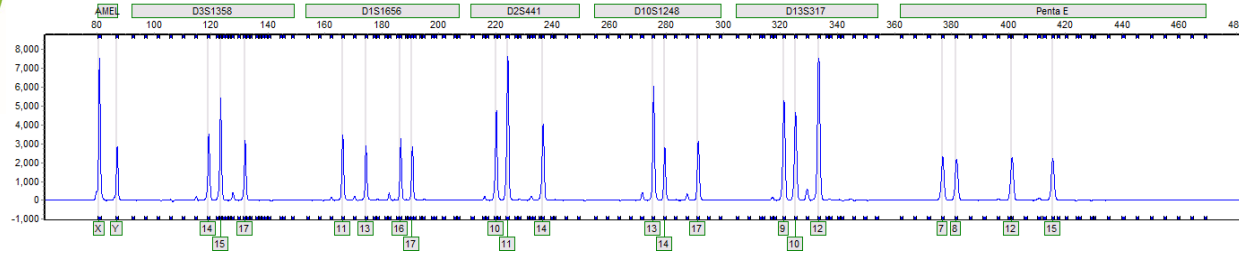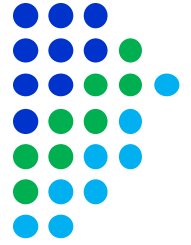
Sample extracts were amplified with Fusion 6C (Promega), run on a 3130xl CE (ThermoFisher), and the data manually analyzed using GeneMarker® HID (v2.9.0, SoftGenetics)
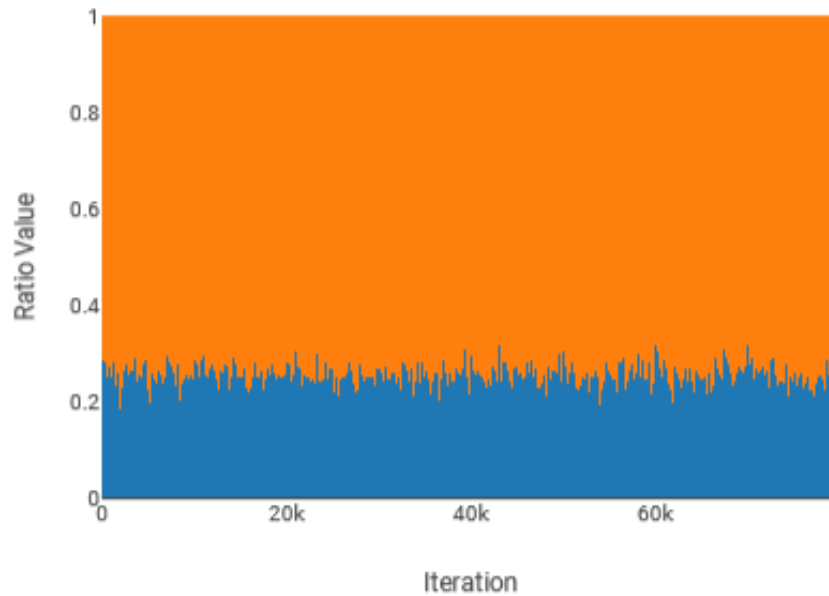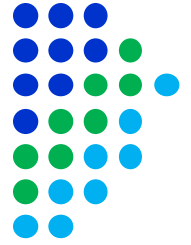
GM HID data files were run through MaSTR™ for a total of 864 analyses

3 quants, 6 combos, 2 pairs, 2 POIs, 4 ratios = 288

Run via burn-in of 8,000 iterations followed by eight chains of 10,000 or 40,000 iterations with a conditioning profile, and 10,000 without
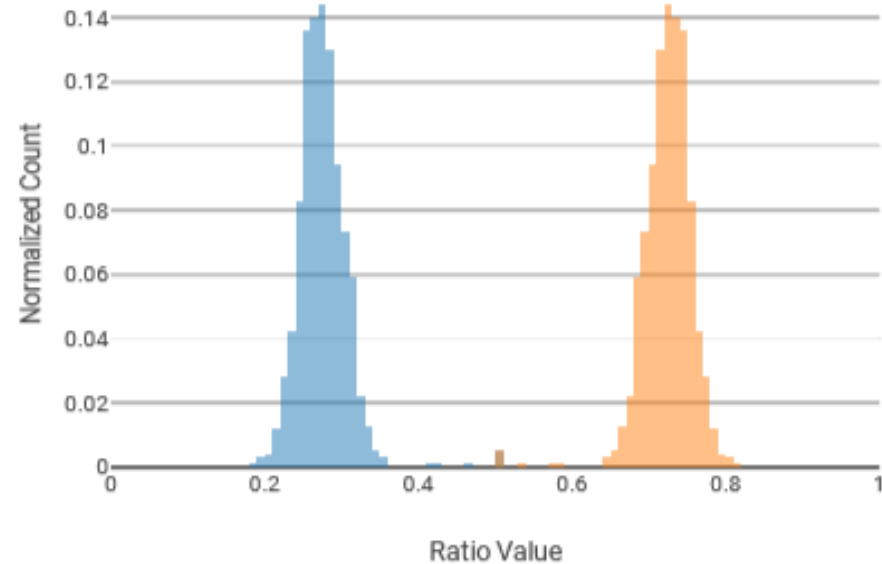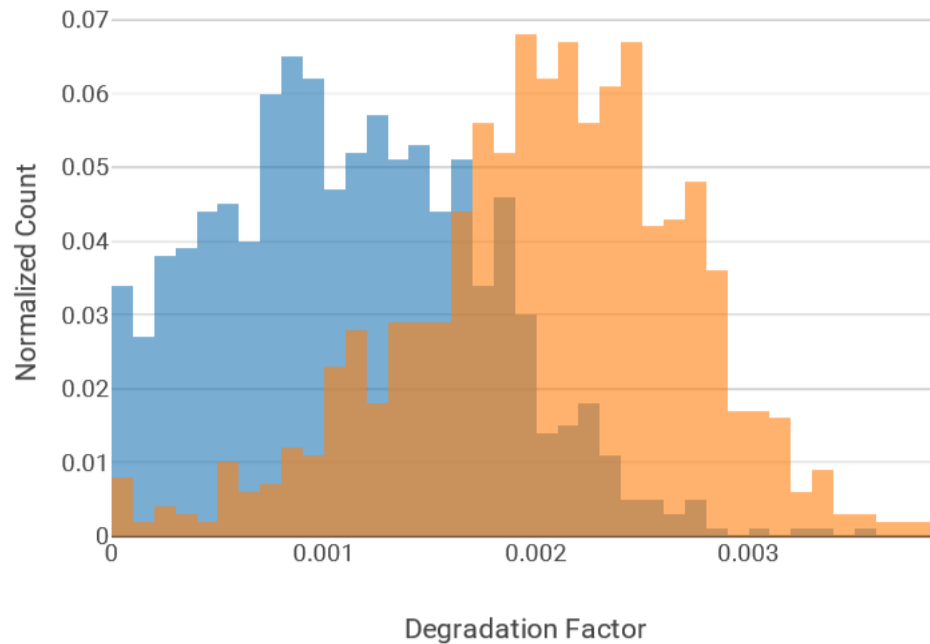
Software Assessment of Ratio

1:3 Mixture of M1:F1
0.5 ng of P:P Mixture
8 chains of 10,000 iterations

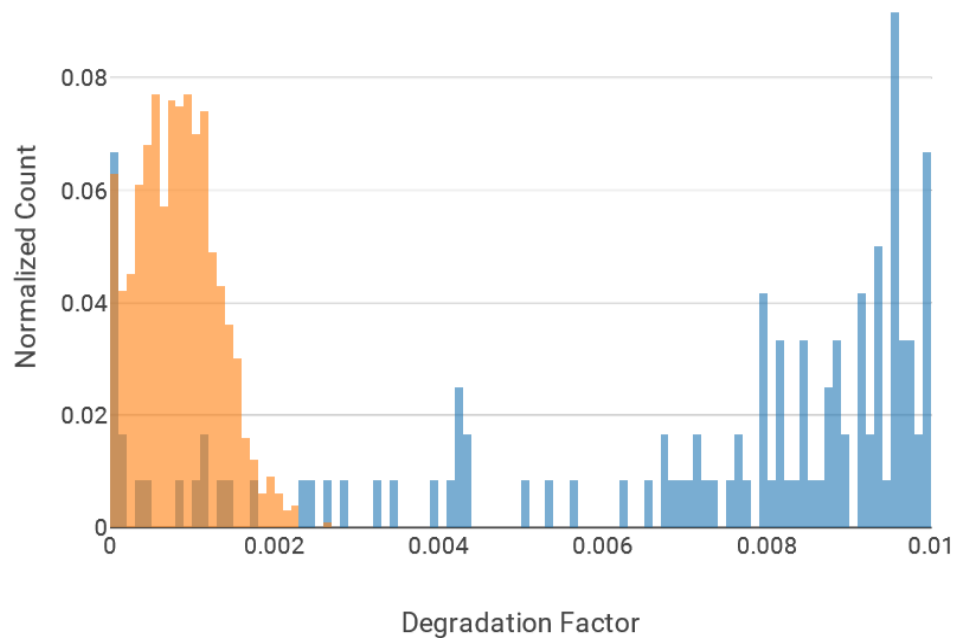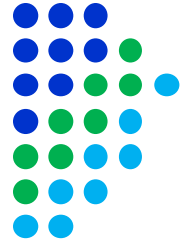Trace v. Histogram Plots

Software Assessment of Degradation

Degradation values of
<0.003 reflect low-levels
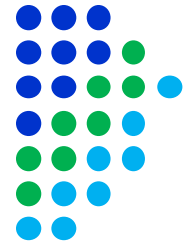of degradation

P:P Mixture
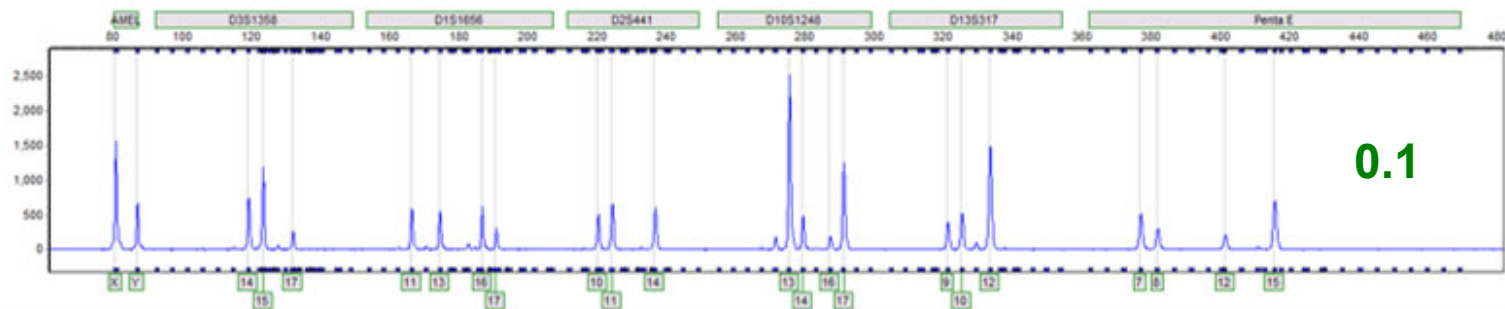(Same sample)

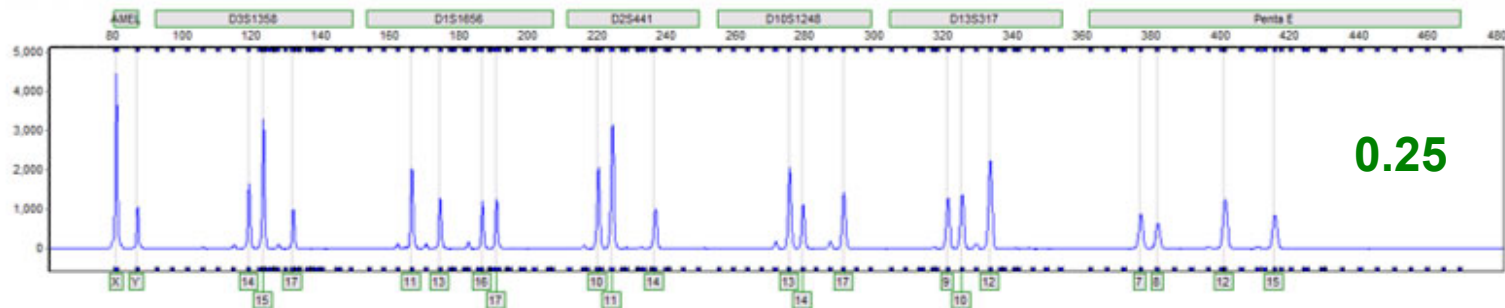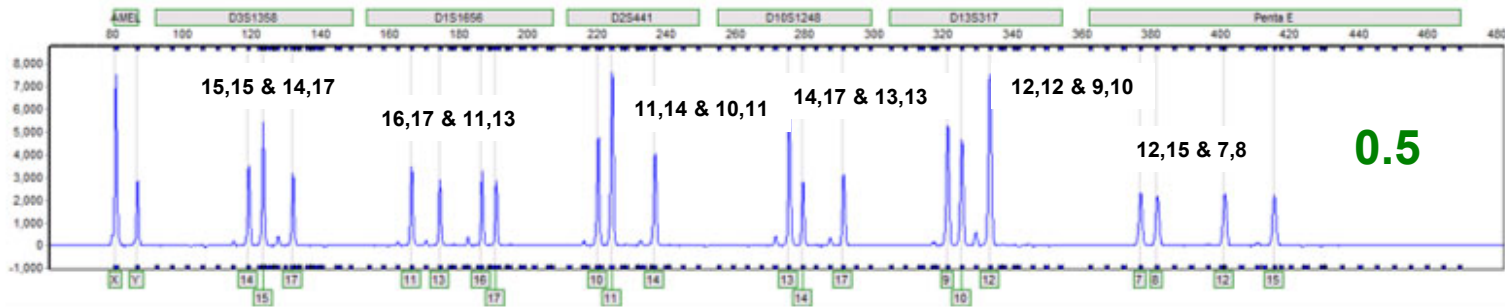# Software Assessment of Degradation

Degradation values of
>0.003 and up to 0.01
reflect higher-levels of
degradation

P:**150** Mixture
1:3 ratio, 0.5 ng, 8 x 10,000
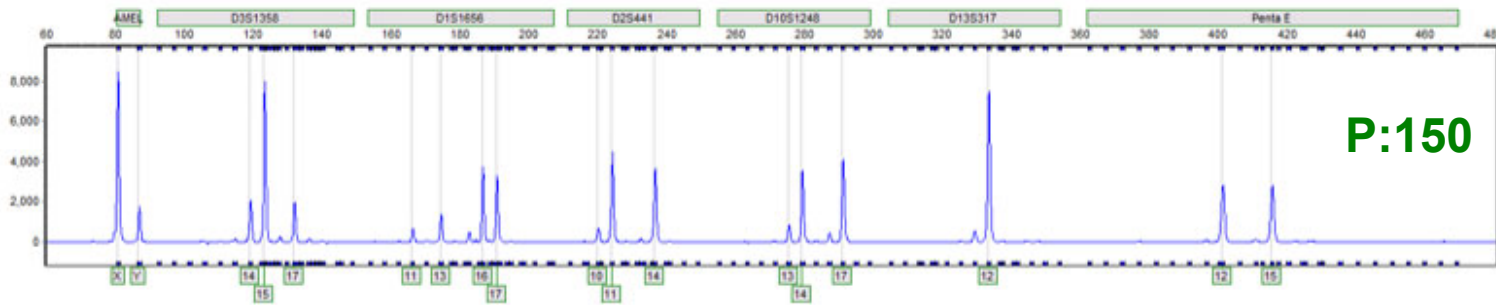(Different sample)

F2:M2

15,15 & 14,17

16,17 & 11,13

11,14 & 10,11

14,17 & 13,13

12,12 & 9,10

12,15 & 7,8

0.5

0.25

0.1

1:1, P:P, **0.5, 0.25 & 0.1 ngs**

Blue Channel of Fusion 6C

F2:M2

15,15 & 14,17

16,17 & 11,13

11,14 & 10,11

14,17 & 13,13

12,12 & 9,10

12,15 & 7,8

P:P
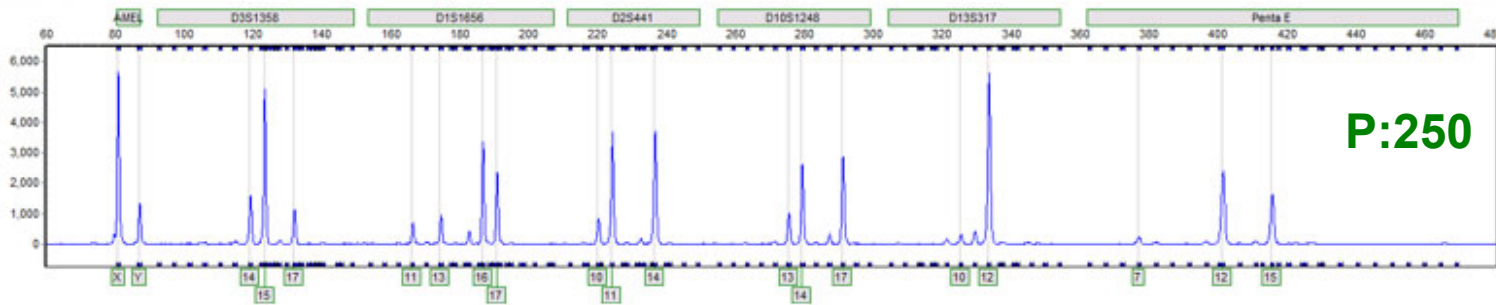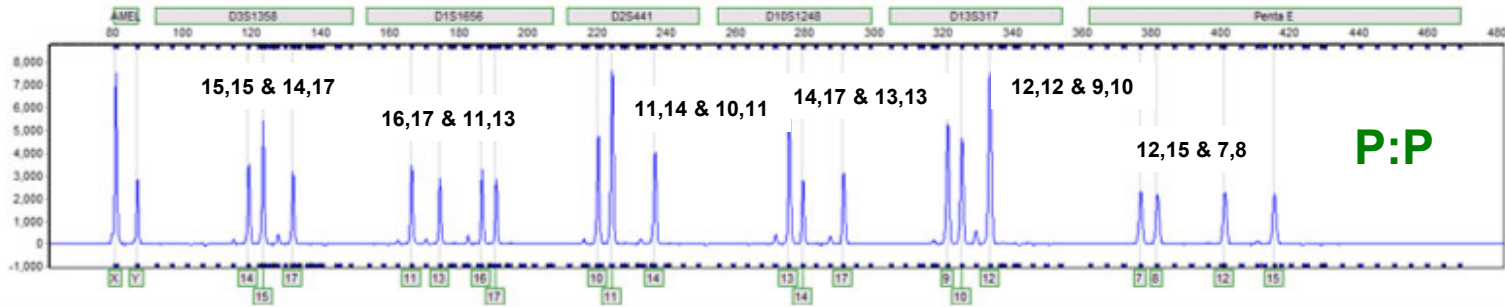
P:250

P:150

0.5 ng of 1:1, **P:P, P:250 & P:150**

F2:M2

15,15 & 14,17   16,17 & 11,13   11,14 & 10,11   14,17 & 13,13   12,12 & 9,10   12,15 & 7,8   P:P
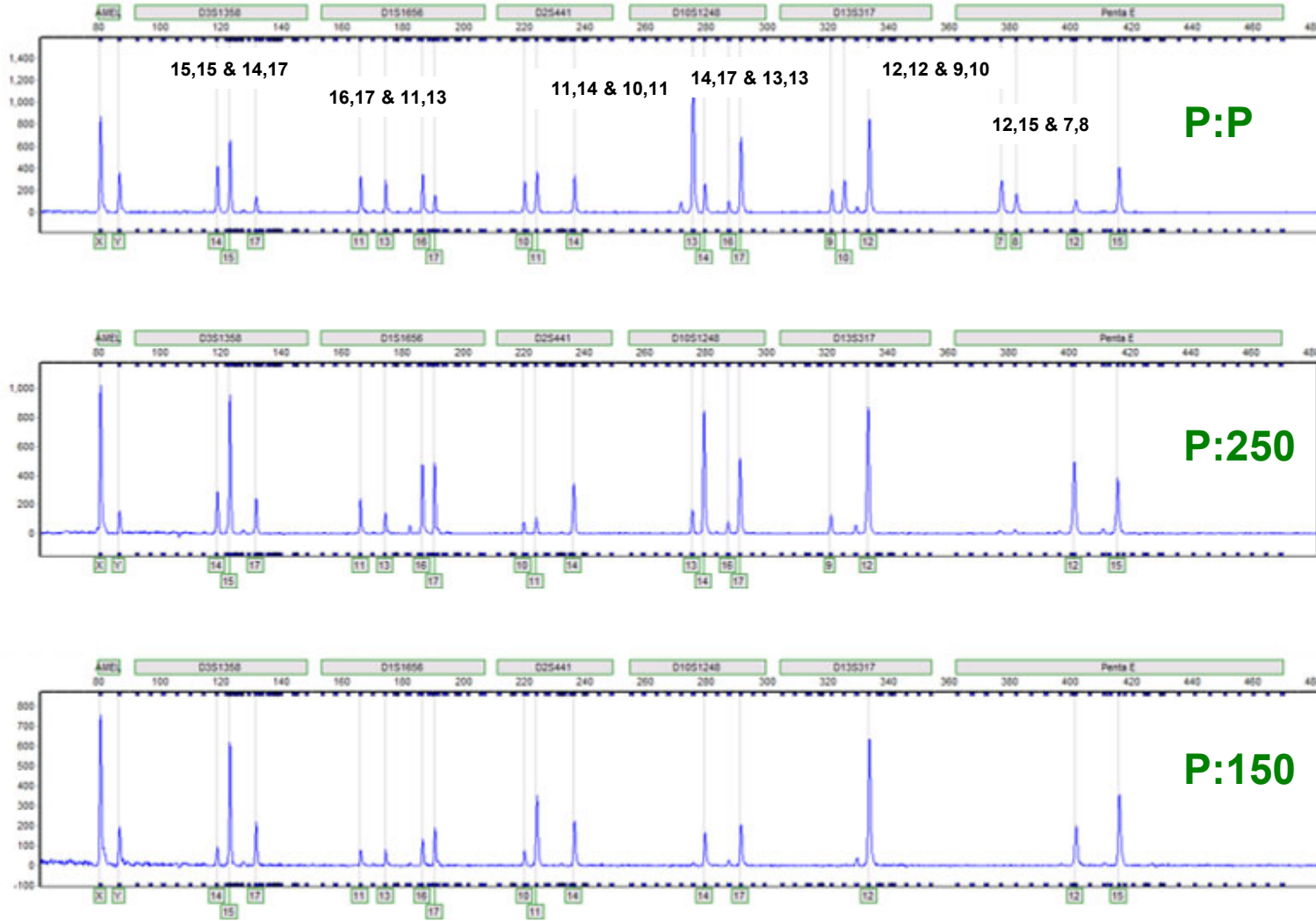
P:250

P:150

0.1 ng of 1:1, **P:P, P:250 & P:150**

0.25 ng, P:150
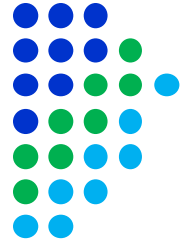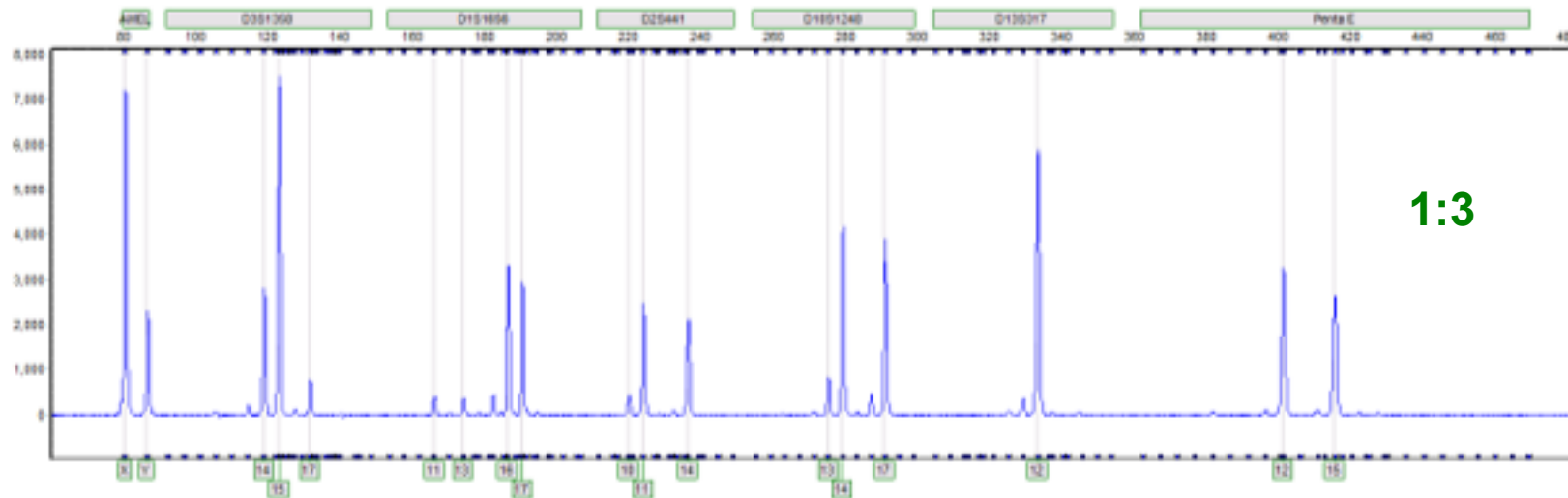Male is the minor

Decreasing Ratios

F2:M2

15,15 & 14,17

16,17 & 11,13

14,17 & 13,13

12,12 & 9,10

11,14 & 10,11

12,15 & 7,8

1:1

1:3

Decreasing Ratios

0.25 ng, P:150
Male is the minor

F2:M2

15,15 & 14,17

16,17 & 11,13

11,14 & 10,11

14,17 & 13,13

12,12 & 9,10

12,15 & 7,8

1:6

1:10
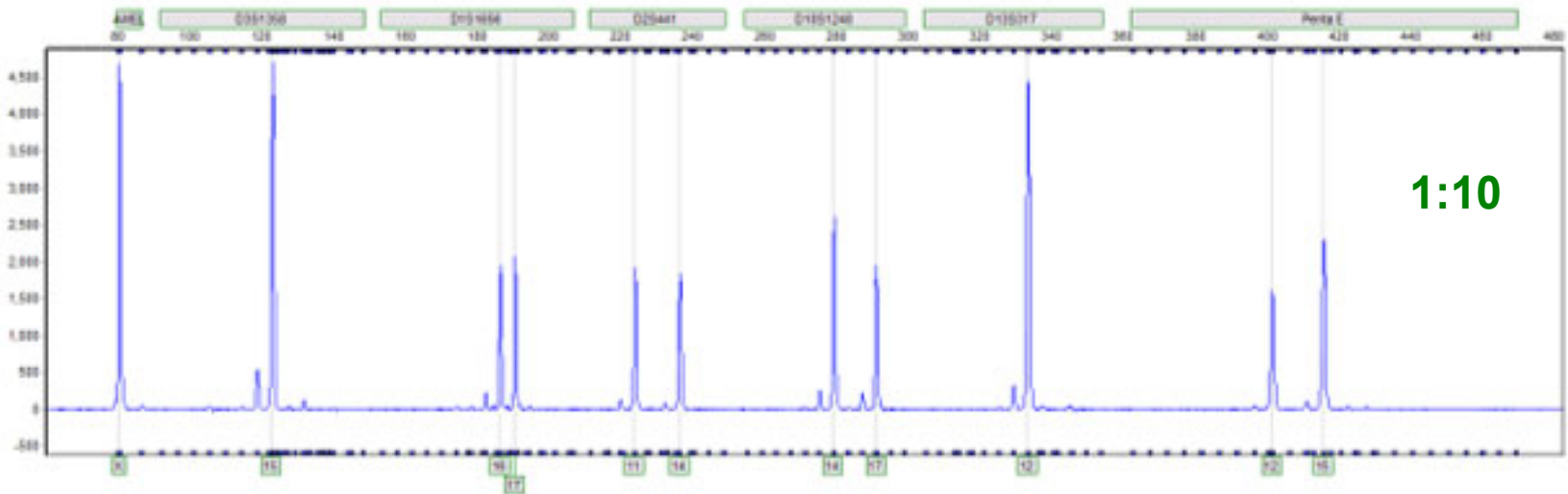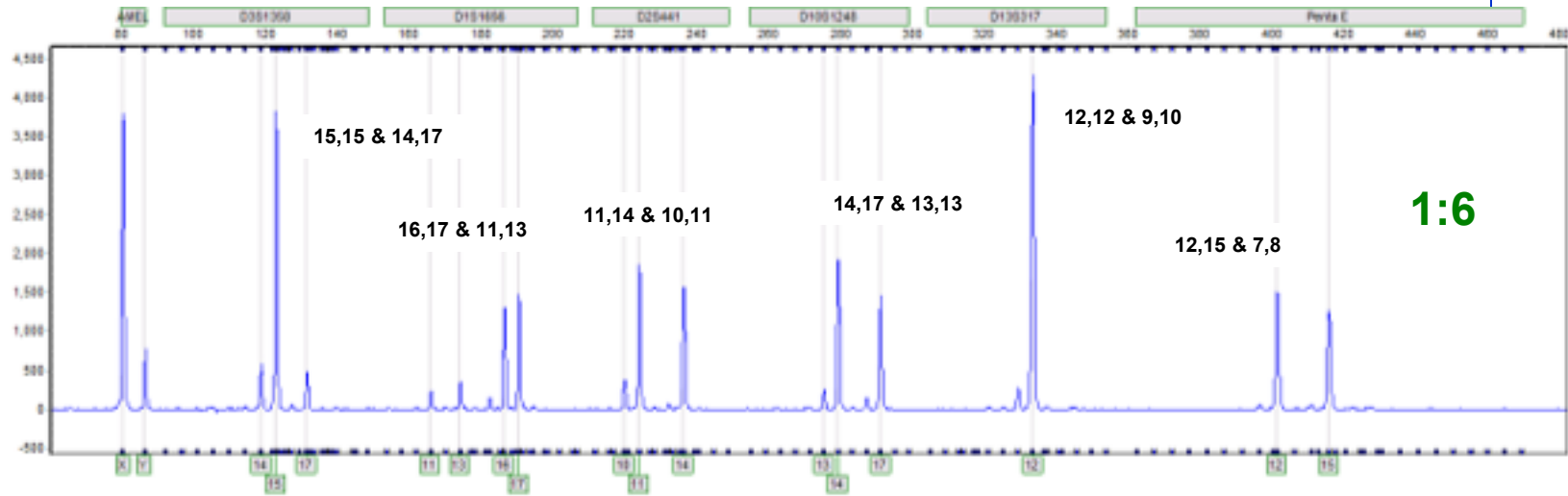
Decreasing Input (ng) & Degradation

0.1 ng, 1:1

F2:M2

250:250

15,15 & 14,17

16,17 & 11,13

11,14 & 10,11

14,17 & 13,13

12,12 & 9,10

12,15 & 7,8

150:250

150:150

1:1 Mix Ratio with M1 as the POI

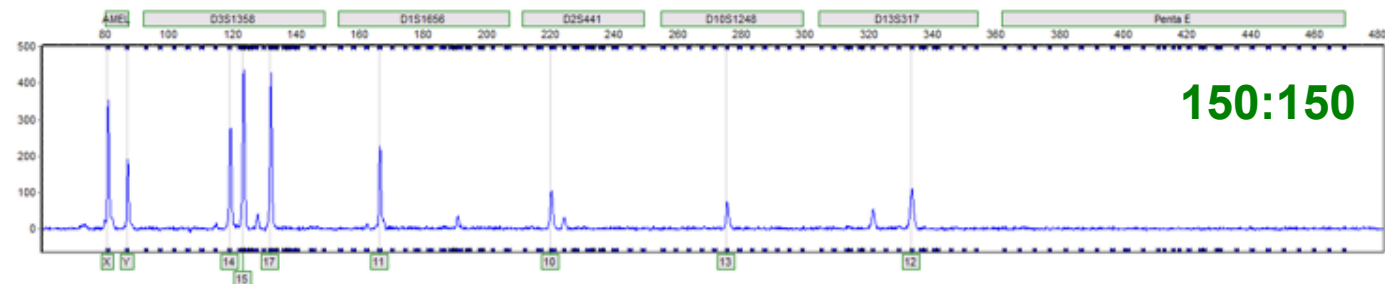|	P:P	P:250	P:150	250:250	150:250	150:150
M1:F1 (0.5 ngs)	3.01E+01	3.15E+01	3.08E+01	1.46E+01	8.05E+00	8.70E+00
M1:F1 (0.25 ngs)	3.02E+01	3.14E+01	3.14E+01	6.59E+00	-4.92E-01	1.07E+01
M1:F1 (0.1 ngs)	1.22E+01	1.30E+01	1.57E+01	1.81E+00	-5.14E-01	-2.01E+00
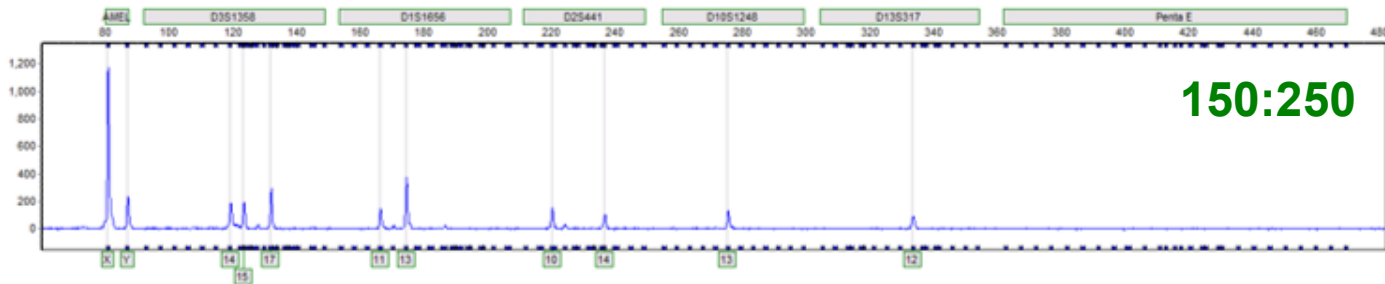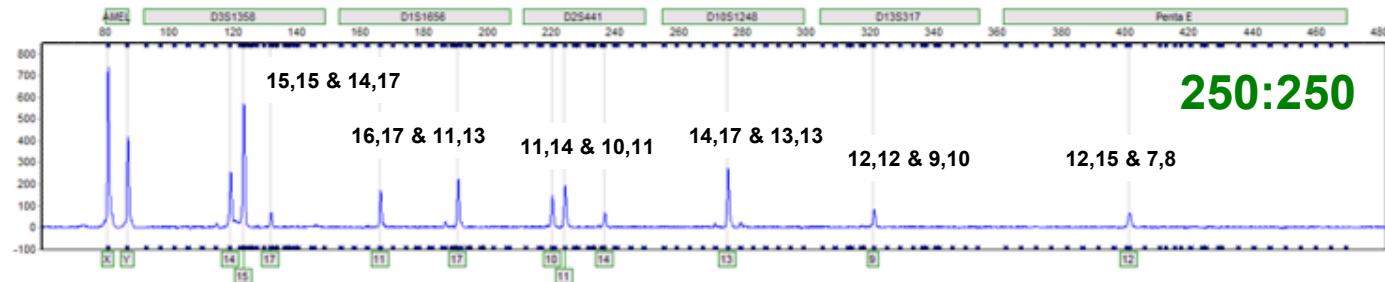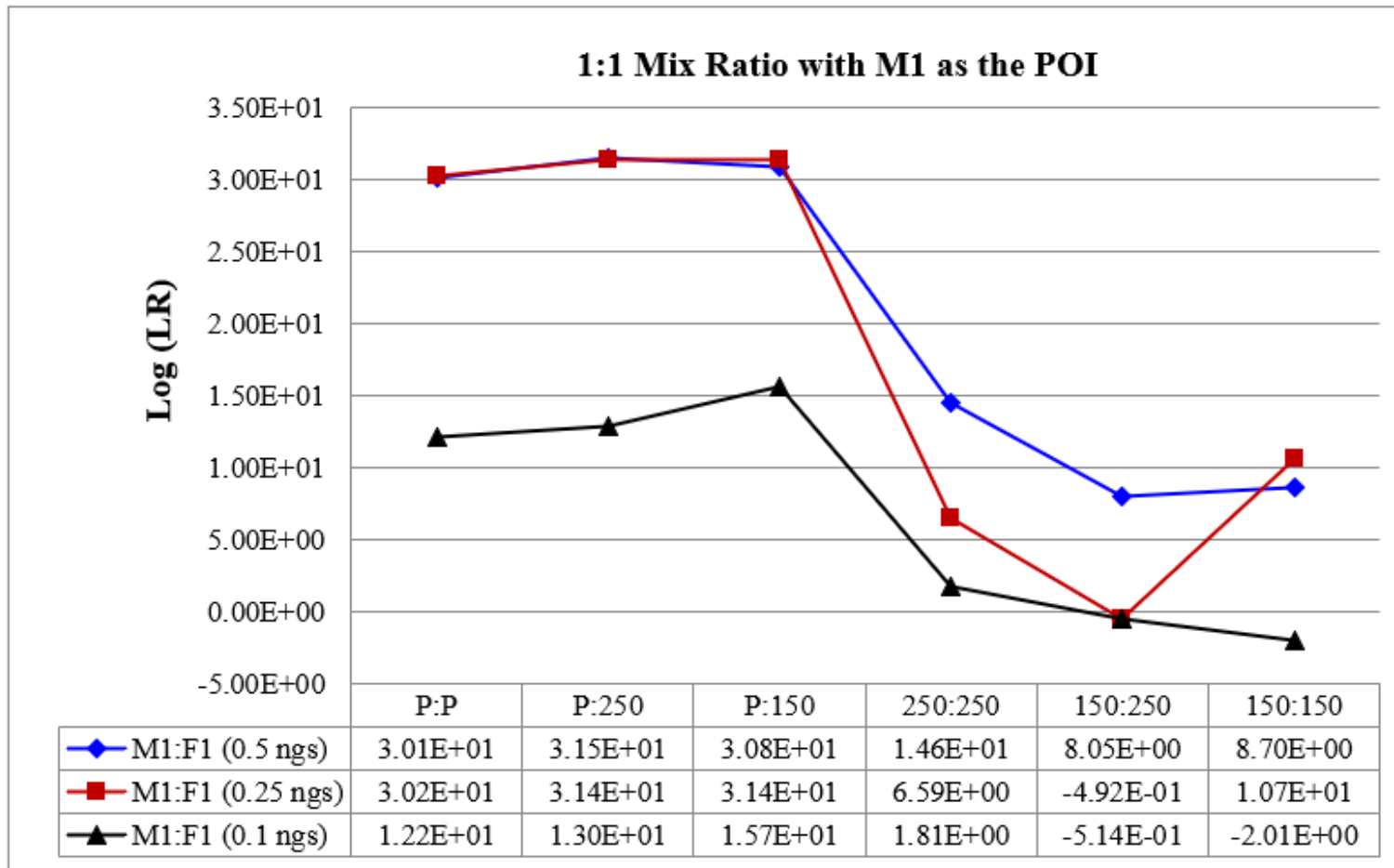
When **M1 is the POI** and F1 is the conditioning profile

1:1 Mix Ratio with F1 as the POI

	P:P	P:250	P:150	250:250	150:250	150:150
M1:F1 (0.5 ngs)	3.39E+01	1.41E+01	6.79E+00	2.47E+01	2.16E+01	2.31E+01
M1:F1 (0.25 ngs)	3.39E+01	1.61E+01	9.01E+00	1.69E+01	2.00E+01	1.11E+01
M1:F1 (0.1 ngs)	3.20E+01	3.57E+00	2.21E+00	1.61E+00	1.87E+00	8.75E+00
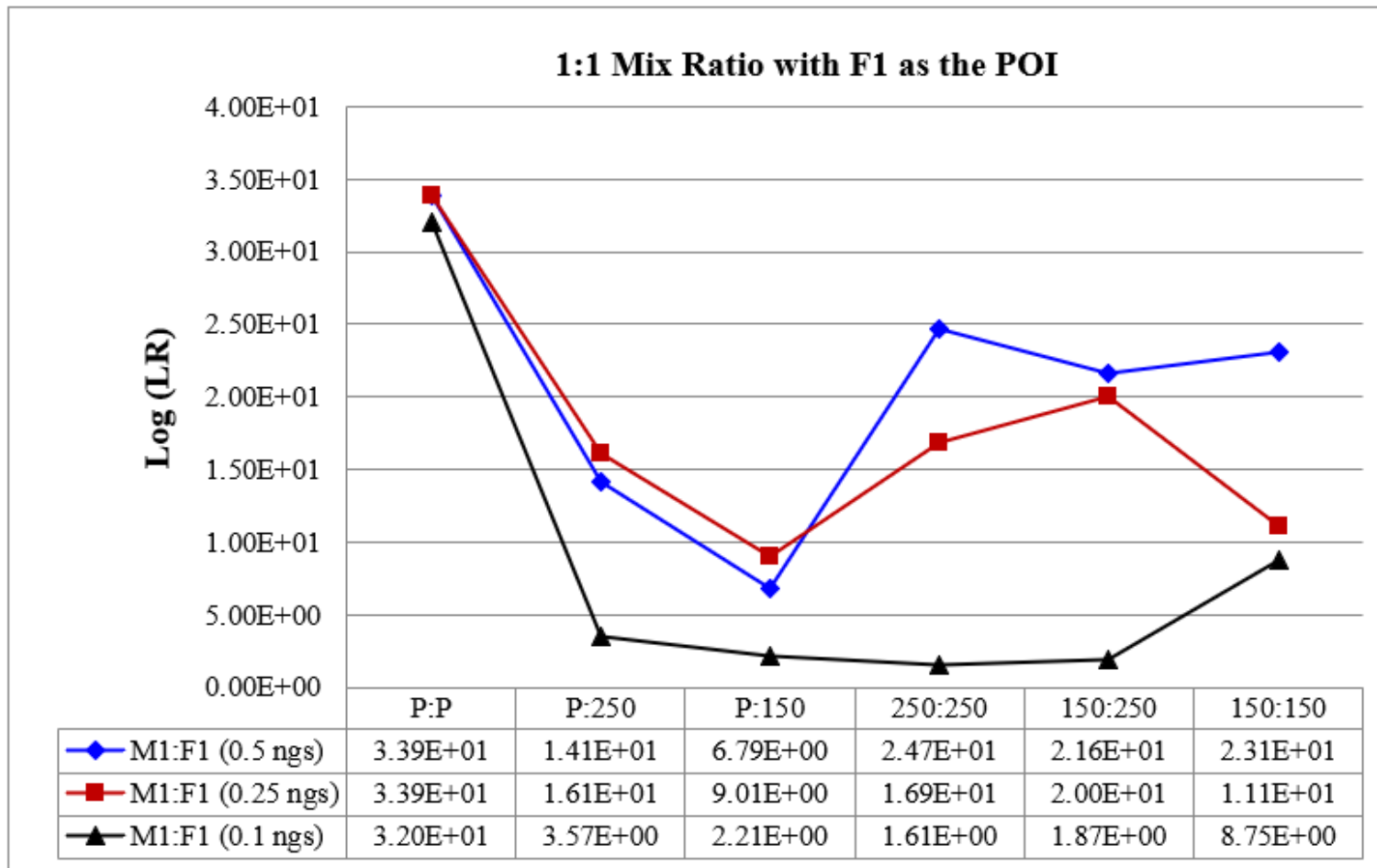
When **F1 is the POI** and M1 is the conditioning profile

1:1 Mix Ratio with F2 as the POI

	P:P	P:250	P:150	250:250	150:250	150:150
F2:M2 (0.5 ngs)	3.20E+01	3.15E+01	3.10E+01	2.21E+01	1.21E+01	1.35E+01
F2:M2 (0.25 ngs)	3.19E+01	3.19E+01	3.19E+01	1.49E+01	7.23E+00	1.21E+01
F2:M2 (0.1 ngs)	3.04E+01	3.11E+01	3.10E+01	5.81E+00	3.05E+00	-7.54E-01
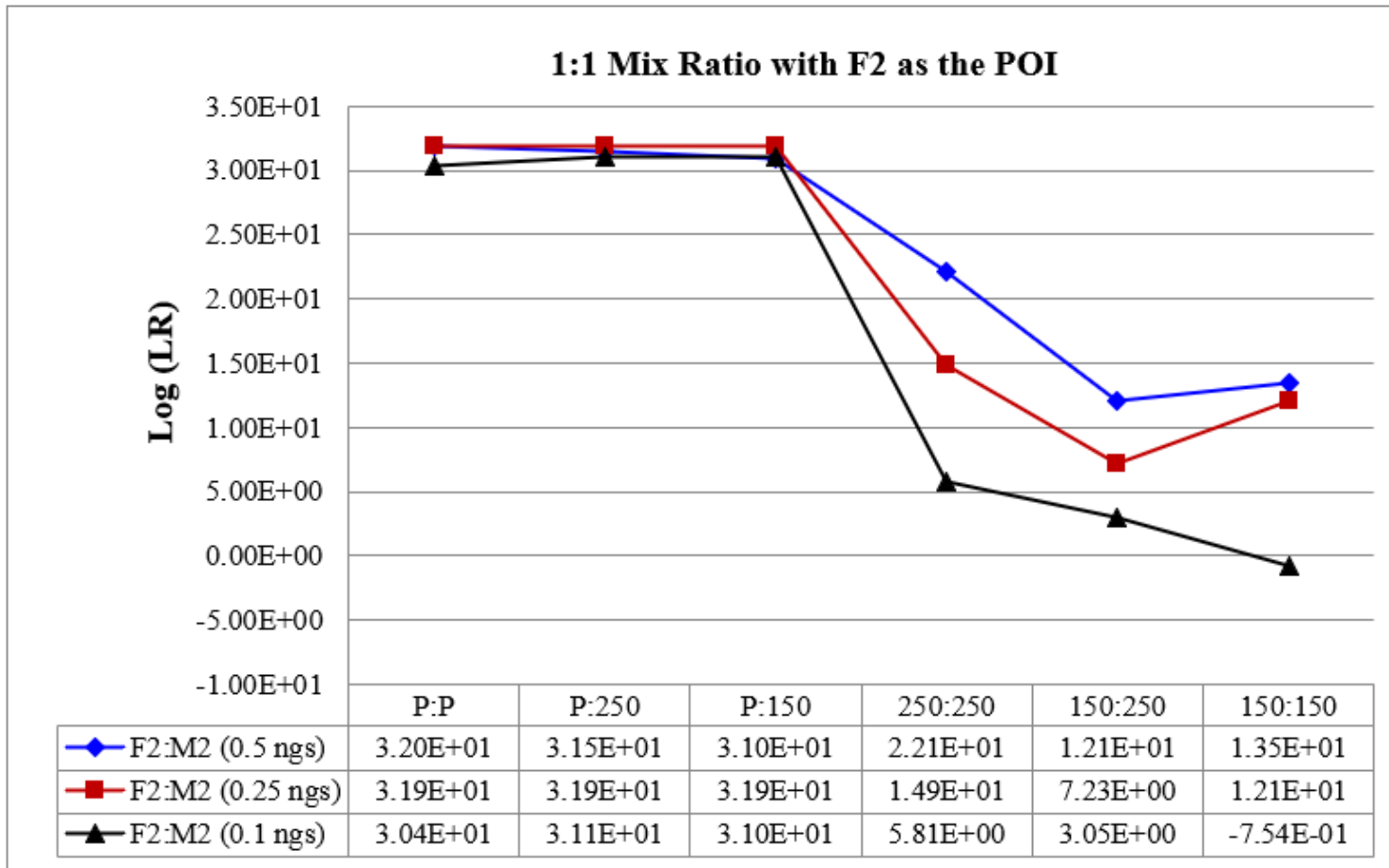
When **F2 is the POI** and M2 is the conditioning profile

1:1 Mix Ratio with M2 as the POI

	P:P	P:250	P:150	250:250	150:250	150:150
F2:M2 (0.5 ngs)	3.48E+01	1.18E+01	1.59E+00	1.93E+01	2.49E+01	2.26E+01
F2:M2 (0.25 ngs)	3.47E+01	9.63E+00	-5.63E+00	2.42E+01	1.31E+01	4.12E+00
F2:M2 (0.1 ngs)	3.12E+01	-1.83E+00	4.23E+00	3.89E+00	5.30E+00	4.94E-01
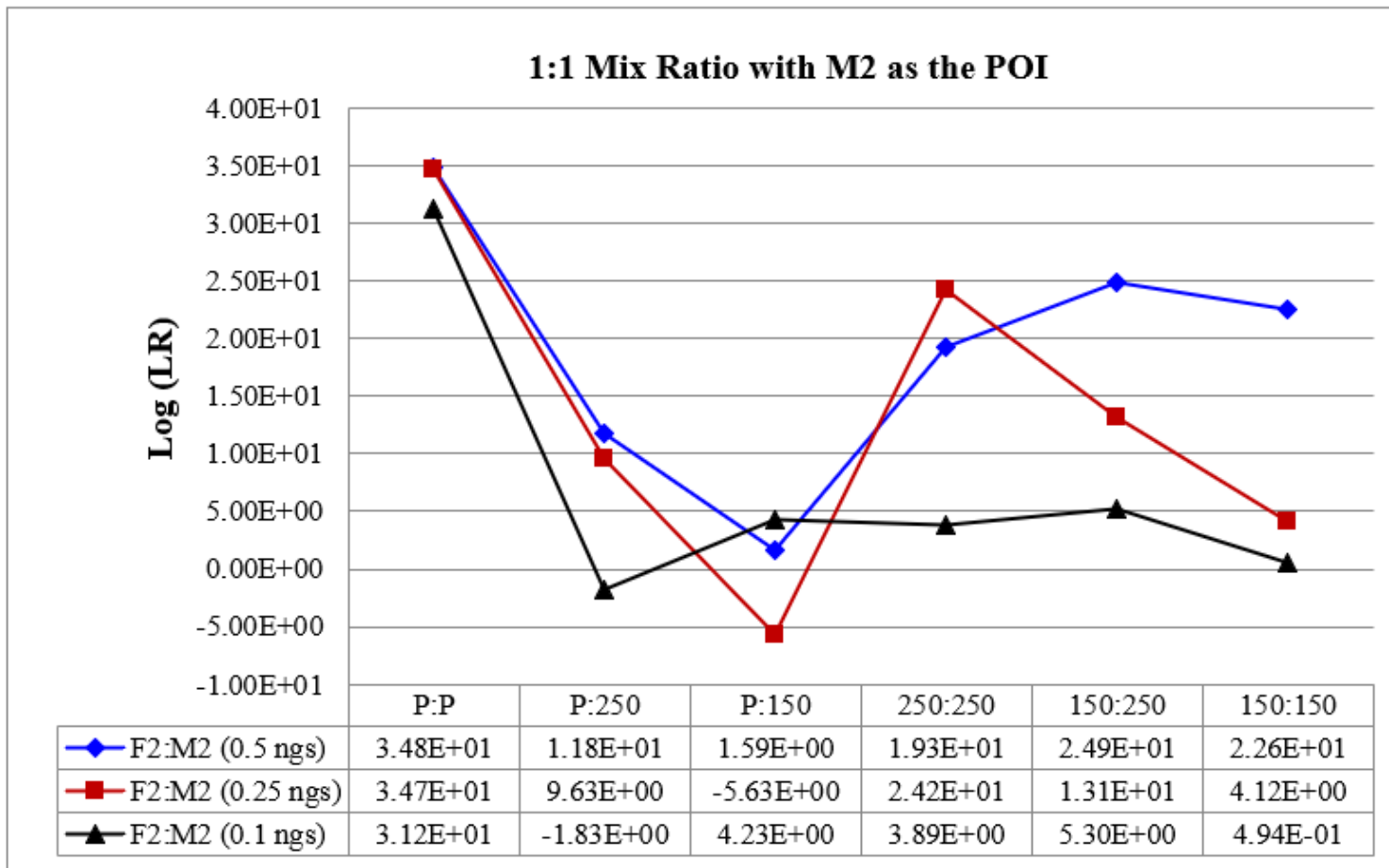
When **M2 is the POI** and F2 is the conditioning profile

Plot reflecting the 288 data points associated with eight chains of 10,000 iterations and with inclusion of a conditioning profile as the second contributor

The code associated with the key is as follows:

"sample" associated with **A** (when M1 or F2 are the POI and the major profile) or **B** (when F1 or M2 are the POI and the minor profile)
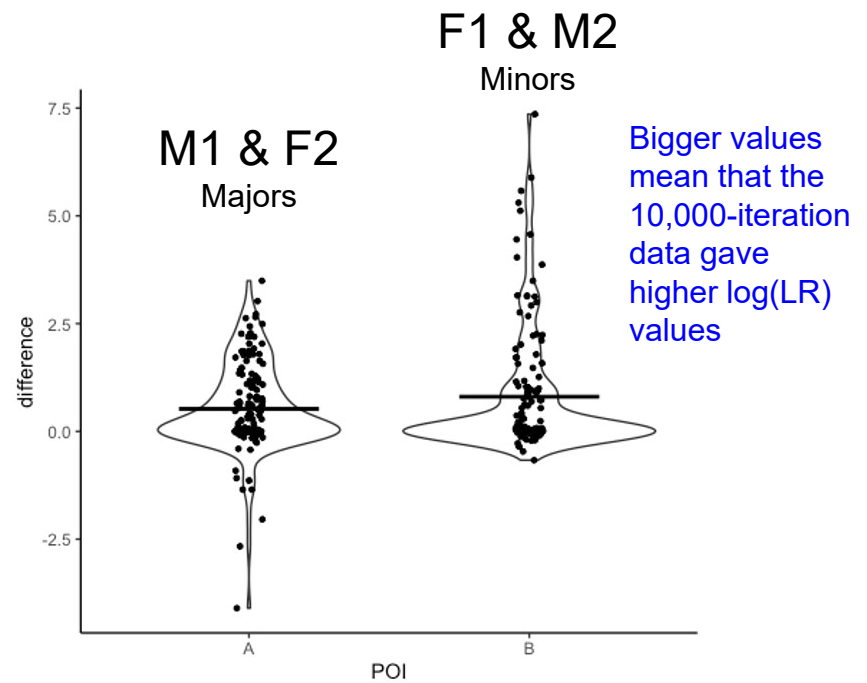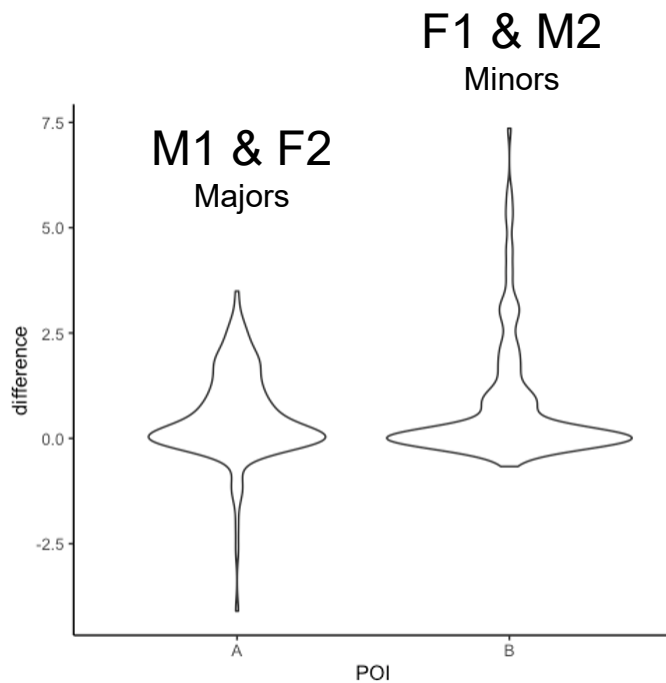
"amt" of input template associated with **X** (0.5 ngs, square data points), **Y** (0.25 ngs, triangle data points), or **Z** (0.1 ngs, circle data points) and in all cases, with the ratio of contributors as 1:1, 1:3, 1:6 or 1:10, and level of degradation associated with P:P, P:250, P:150, 250:250, 150:250, or 150:150
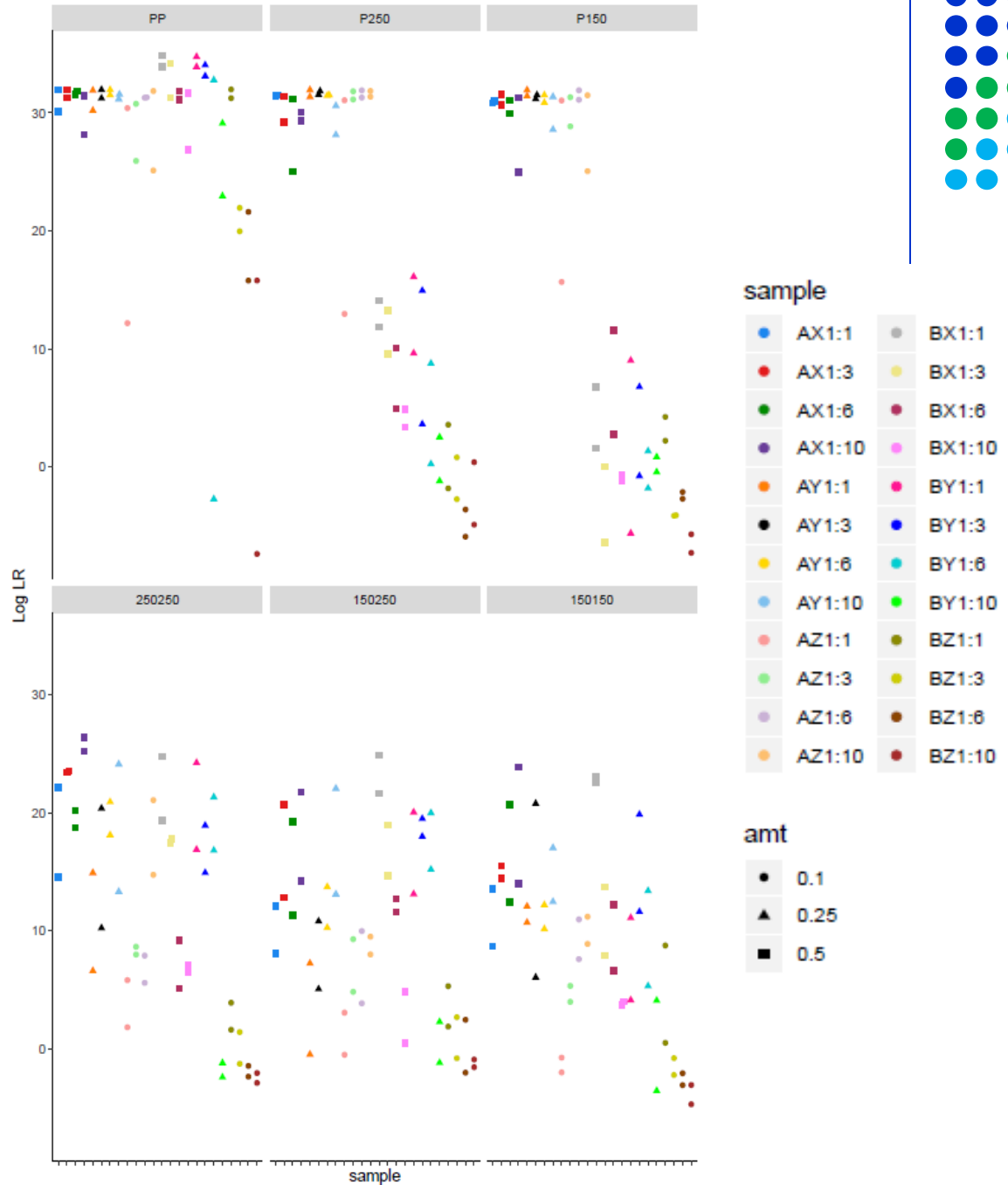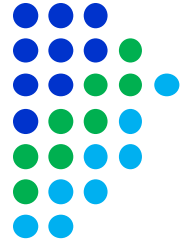
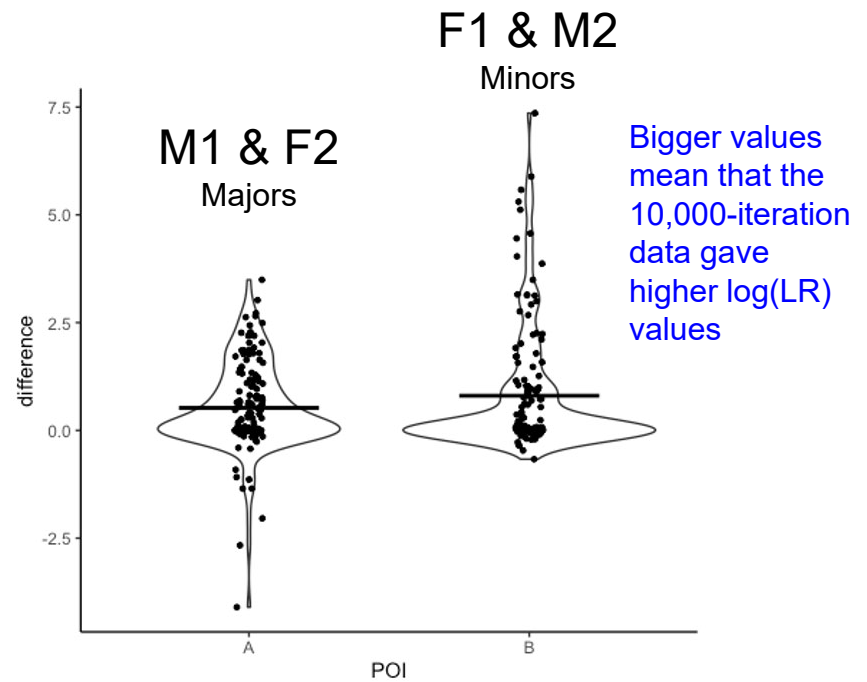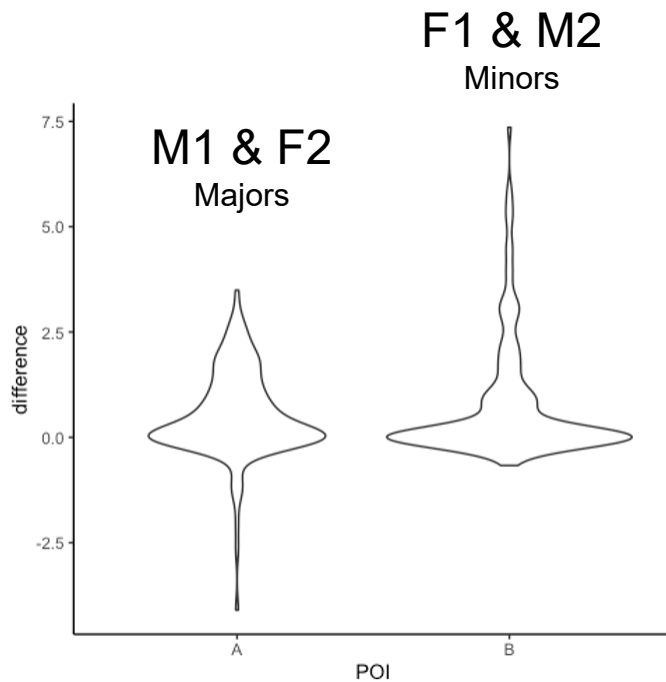# Violin Plots
10,000 v. 40,000 Iterations

The "difference" is the (number of log units) between each sample in the two datasets, calculated as log(LR) values for the 40,000-iteration MaSTR™ analysis subtracted from the log(LR) values for the analysis performed at 10,000 iterations
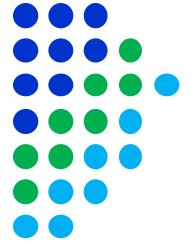
Violin Plots
10,000 v. 40,000 Iterations

F1 & M2
Minors

M1 & F2
Majors

F1 & M2
Minors

M1 & F2
Majors

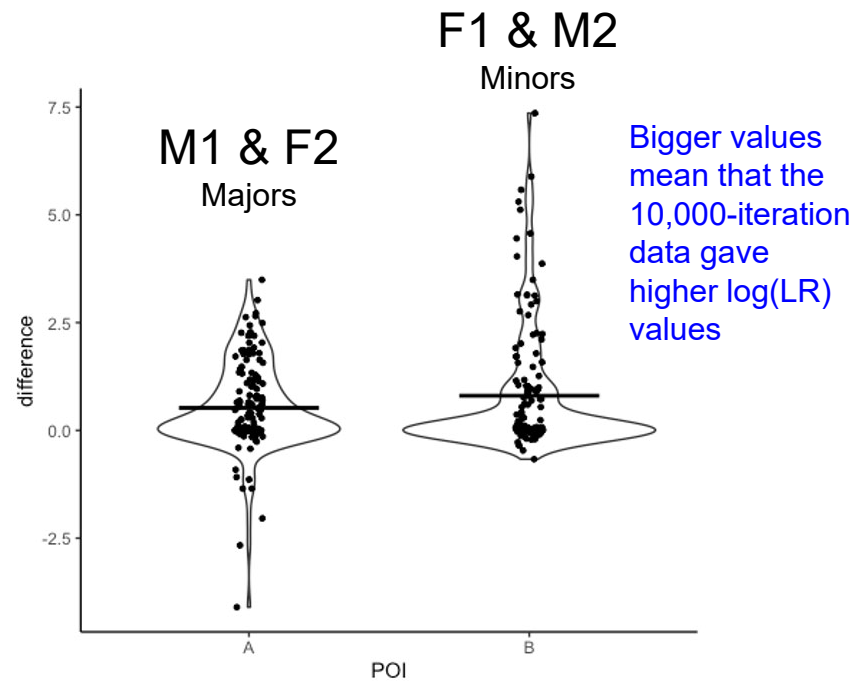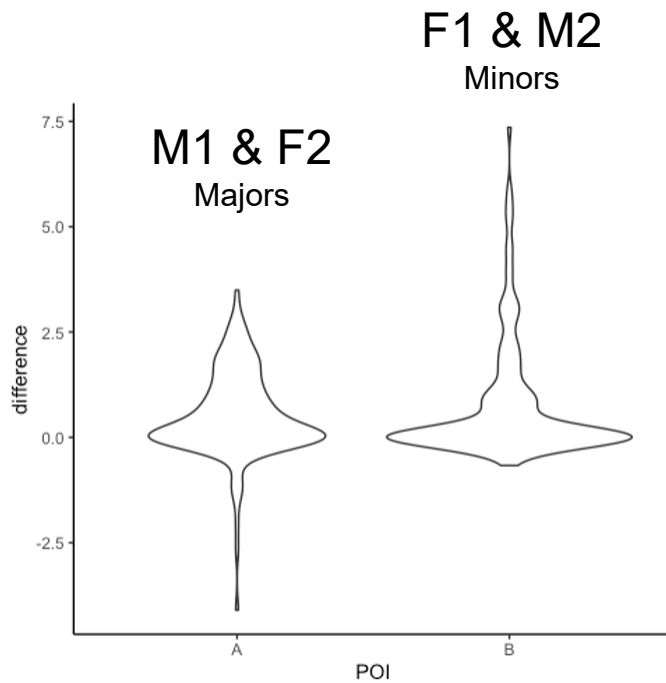Bigger values mean that the 10,000-iteration data gave higher log(LR) values

The overall mean was ~0.66 log units (a difference of ~4.5 in the LR), slightly favoring the 10,000-iteration approach when calculating LRs
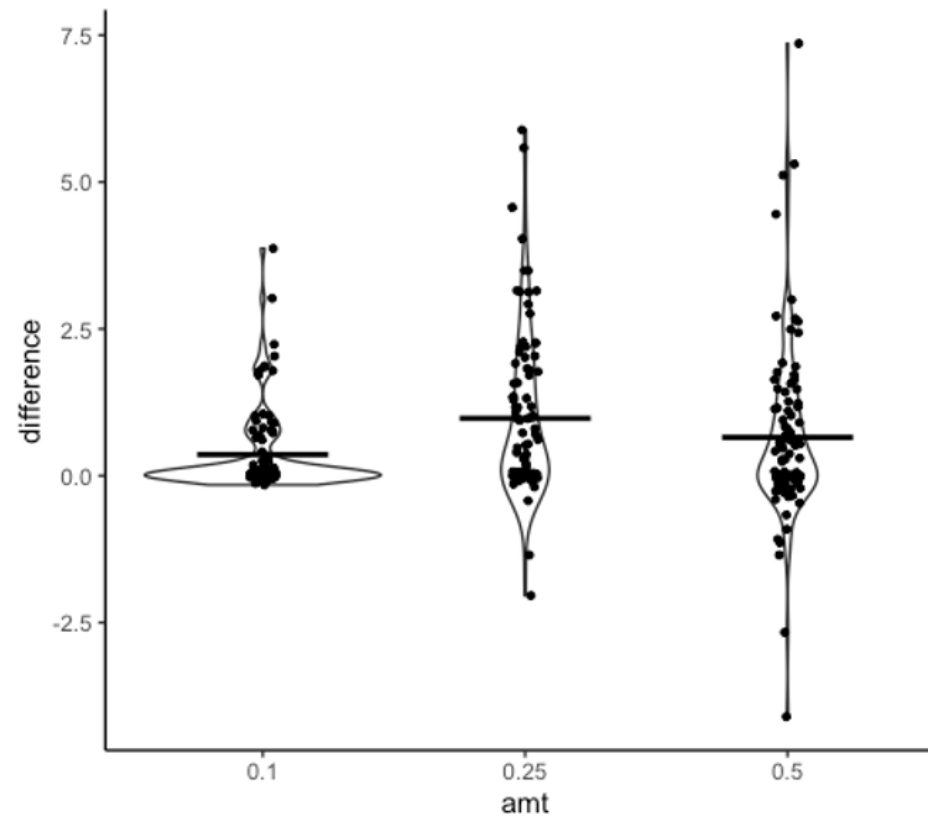
Violin Plots
10,000 v. 40,000 Iterations

~91.3% of the log values were within +/- 2.5 log units (LR difference of ~316), and ~70.8% of the values within +/- 1.0 log unit (LR difference of 10)
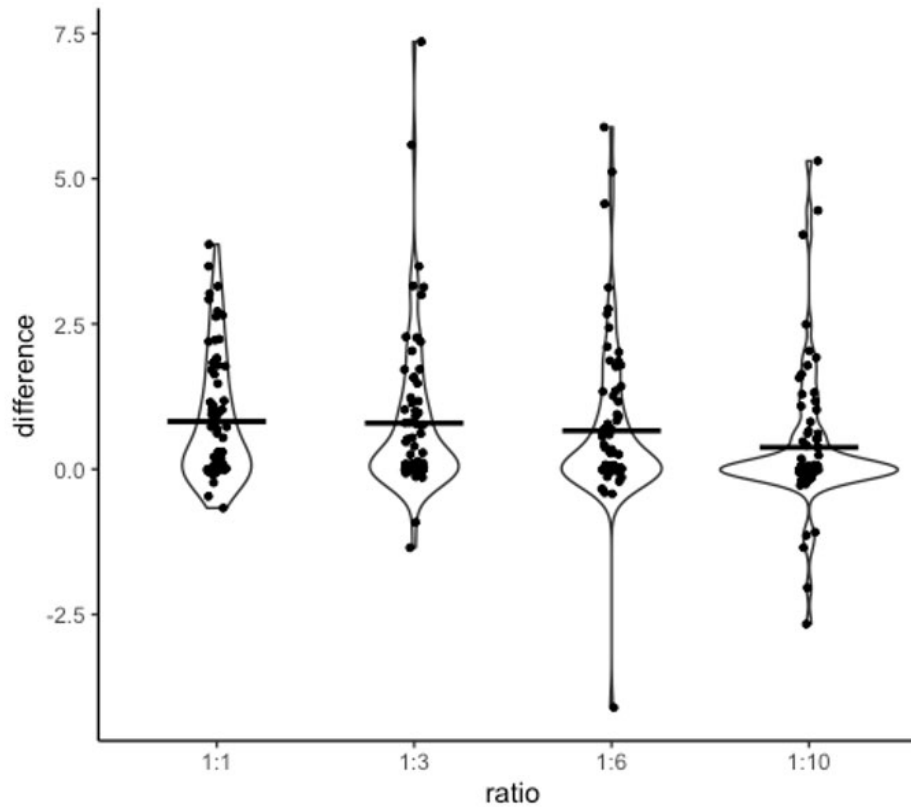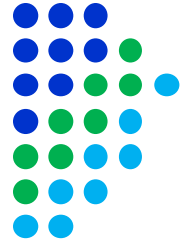
Template Amount

The 10,000-iteration approach is slightly favored when considering mixtures with 0.1 ng of template
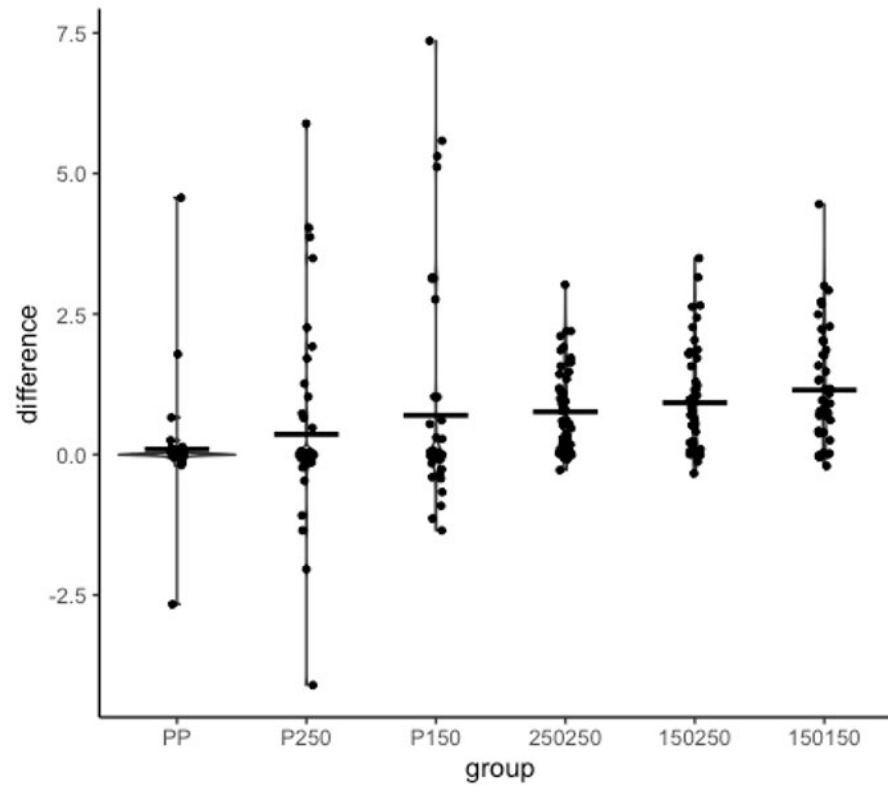
Ratios

The 10,000-iteration approach is slightly favored for a mixture ratio of 1:1, with decreasing impact as the ratio increases
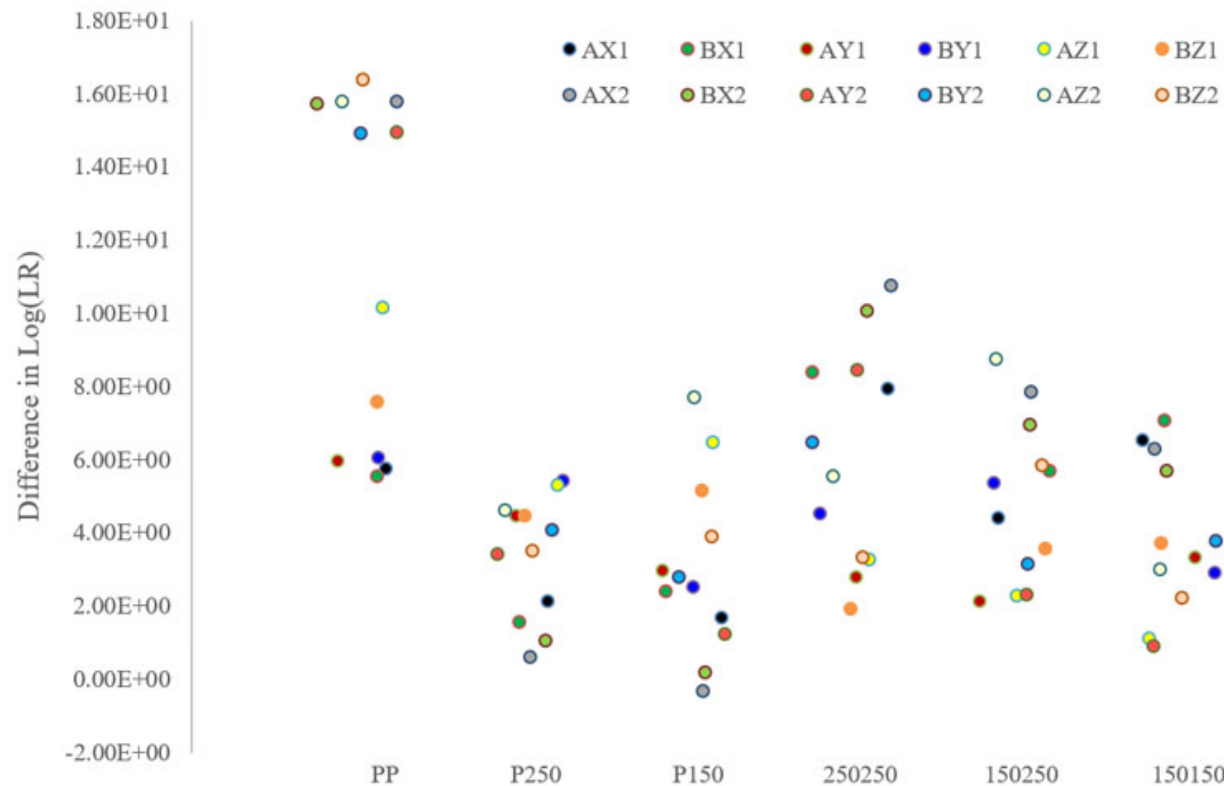
Degradation Status

The 10,000-iteration approach is slightly favored as degradation increases

Conditioning Profile

F2:M2 closer to a true 1:1, whereas M1:F1 between 1:1 to 1:2

Bigger values mean that the conditioning profile LRs are higher than without a conditioning profile
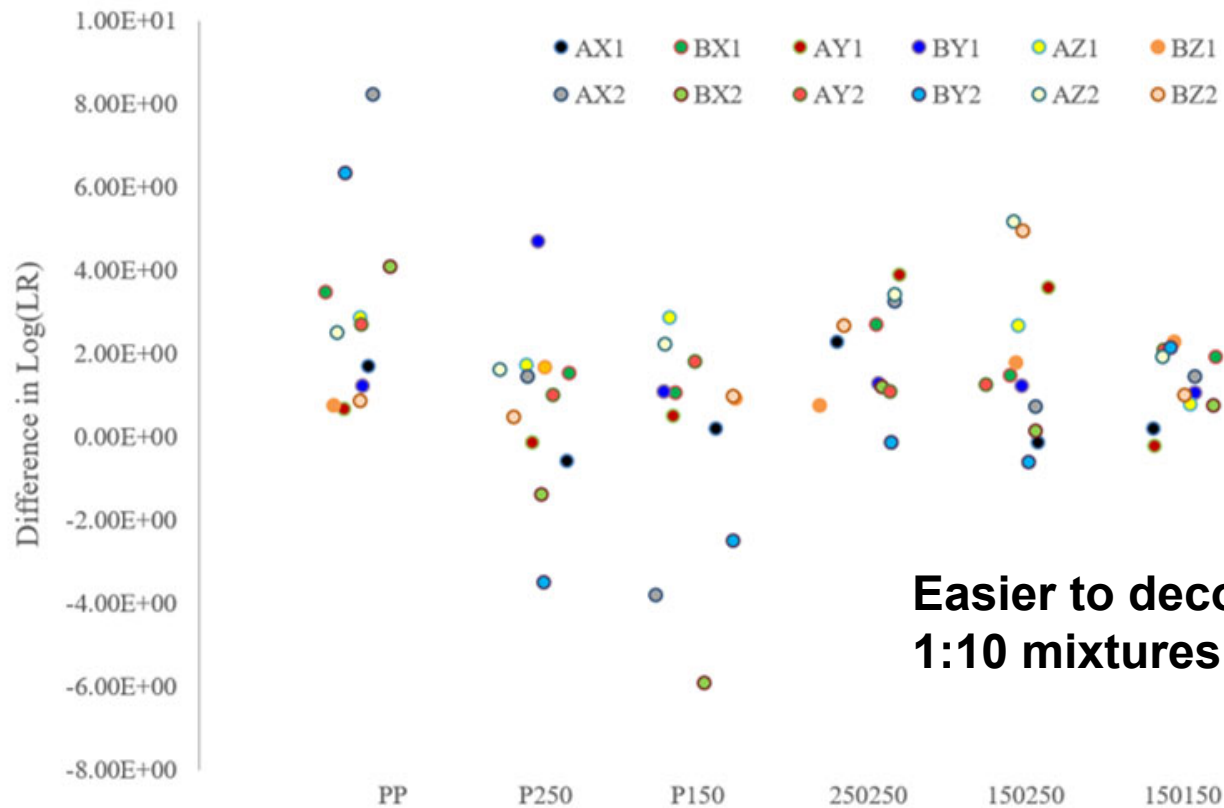
1:1 mixtures

Key:
A = major, **B** = minor
X = 0.5 ng, **Y** = 0.25 ng,
Z = 0.1 ng
1 = M1:F1, **2** = F2:M2

Average difference of 5.49

Conditioning Profile

Bigger values mean that the conditioning profile LRs are higher than without a conditioning profile

1:10 mixtures

Easier to deconvolute 1:10 mixtures

Average difference of 1.44

Take Home Messages

MaSTR™ calculated expected LRs for 2-person mixtures when assessing major & minor profiles (1:1, 1:3, 1:6, 1:10), different template amounts (0.1, 0.25, 0.5 ng), and differentially degraded DNA (P:P, P:250, P:150, 250:250, 150:250, 150:150).

Analysis with eight chains of 10,000 or 40,000-iterations gave comparable results

Analysis of 3 and 4-person mixtures is in progress (manuscript in preparation)

Thanks!!
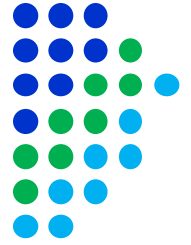
SoftGenetics – MaSTR™
John Fosnacht, Teresa Snyder-Leiby, Sarah Copeland, Dan Erb, etc

Teresa, Abby, Sidney – wonderful students!!

Eberly College of Science, Department of Biochemistry & Molecular Biology, Forensic Science Program at Penn State

Disclaimer: The authors of this presentation have no financial interests in SoftGenetics

Daisy says hello!

mmh20@psu.edu