



Department of Forensic Biology

Charles S. Hirsch Center for Forensic Sciences

421 East 26th Street, New York, NY 10016

Telephone: 212-323-1200 Fax: 212-323-1590

Email: DNALab@ocme.nyc.gov

Official Website: www.nyc.gov/ocme

NYC OCME Internal Validation of STRmix™ v2.7 for Fusion 5C/3500xL Data - STRmix™ Parameters

STRmix™ is a probabilistic genotyping software that utilizes a fully continuous approach to DNA sample interpretation. It was developed by the Institute of Environmental Science and Research (ESR) in New Zealand, the New Zealand Crown Research Institute, and Forensic Science South Australia (FSSA). By using a variety of biological parameters, STRmix™ can deconvolute mixtures, provide a statistical weight to each possible genotype, and calculate likelihood ratio (LR) comparisons between reference and evidence profiles (1-2).

Prior to using STRmix™, laboratory-specific parameters must be optimized. These parameters are determined through analysis of empirical data or modeled within STRmix™ using Model Maker (1-3). Each parameter depends on both STR amplification kits and CE platforms/protocols used and includes:

- a. analytical threshold (AT),
- b. CE saturation limit,
- c. expected stutter ratios,
- d. drop-in parameters,
- e. allelic and stutter peak height variance prior distributions,
- f. the hyper-parameter for the variance of locus specific amplification effects (LSAE), and
- g. population settings including allele frequencies and Theta (θ) values.

This document describes the methods and samples used to estimate the parameters described above for use of STRmix™ v2.7. Dye-specific analytical thresholds, stutter ratios, drop-in parameters, and saturation were determined using Promega PowerPlex® Fusion 5C, 29 cycle, half-reaction amplification, following standard OCME protocol, with data analyzed on two Applied Biosystems® 3500xLs using GeneMarker® version 3.0.0 (4-5). Variances for allelic and stutter peaks as well as LSAE were calculated using Model Maker within STRmix™ v2.7 from the analysis of empirical data. (NOTE: The New York City Office of Chief Medical Examiner will be referred to as NYC OCME.)

Analytical Threshold

In the analysis of electropherograms, the analytical threshold (AT) is set to distinguish baseline noise peaks from true allelic peaks. This threshold is important in sample analysis and is assigned to minimize the detection of artifacts caused mostly by instrument noise and, therefore, increasing confidence in the peaks that fall above this threshold as true allelic peaks.

For the NYC OCME, ATs were set according to the internal validation of Fusion 5C data run on 3500xLs (4). The AT for each dye channel is as follows:

Table 1. NYC OCME analytical thresholds for Fusion 5C data run on 3500xL genetic analyzers per dye. RFU = relative fluorescent units.

Dye	AT (RFU)
Fluorescein (Blue)	85
JOE (Green)	120
TMR (Yellow)	130
CXR (Red)	160

Unless otherwise stated, these ATs were used for the analysis of the data within this report.

Saturation

DNA profiles are read from amplified DNA using fluorescent labels conjugated to the DNA primers. The fluorescence is measured using the CE camera in relative fluorescence units (RFU). As the amount of fluorescently labeled DNA increases, the amount of fluorescence emitted increases. When there is too much fluorescence, the camera can become saturated and, beyond this point, the level of fluorescence cannot be accurately measured. STRmix™ uses this value to determine the upper limit to peak heights it will model.

High template samples which included saturated loci, determined by peak morphology, were used to establish the saturation point. Allelic and back stutter peaks at loci with simple repeat structures were used to calculate the expected peak height. The expected peak height was calculated using –

$$E_a = \frac{O_{a-1,0}}{SR_{a-1,0}}$$

where E_a is the expected allele peak height, $O_{a-1,0}$ is the observed back stutter peak height, and $SR_{a-1,0}$ is the expected back stutter ratio for allele a as determined through the stutter ratio analysis (3). The expected allele peak heights were plotted against the observed allele peak heights, along with a line for $y = x$.

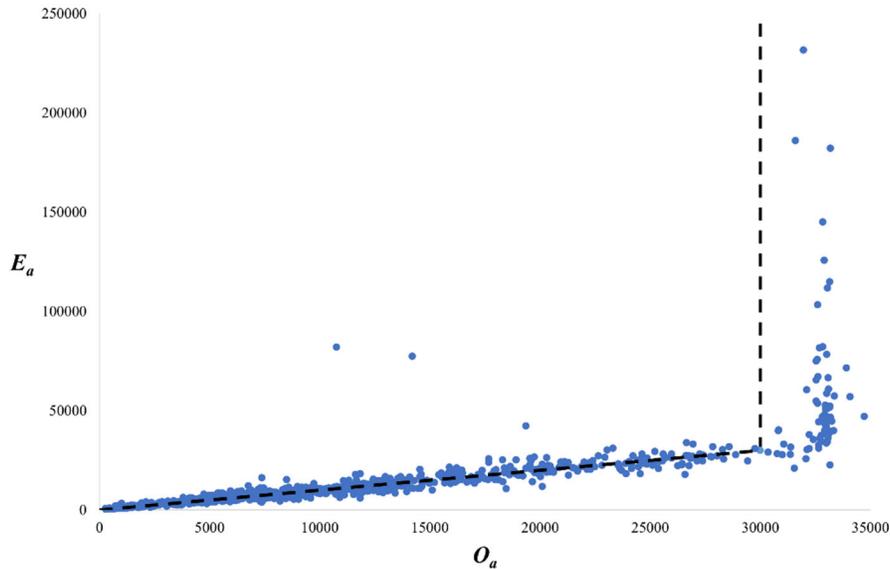


Figure 1. Observed (O_a) versus expected (E_a) peak heights with dashed lines for $y = x$ and $x = 30,000$.

The point at which the data deviates from the $y = x$ line represents the approximate saturation level. Based on the data, a point (30,000 RFU) just below the approximate saturation level was selected as a conservative value.

Stutter

Stutter is a common artifact observed in STR electropherograms caused during replication of DNA during the amplification process. It is important to delineate this artifact from true allelic peaks to interpret DNA samples with more accuracy. This is notably important in the interpretation of mixture samples where there may be a minor or trace contributor at a similar height as potential stutter peaks.

Previously, stutter has been well characterized as locus specific; however, it has been shown that using allele specific stutter ratios is an improved method to describe this artifact (6-8). For earlier versions of STRmix™, NYC OCME used allele specific stutter ratios for back and forward stutter only. STRmix™ v2.7 analysis has generalized stutter modeling to include stutter ratios for any type of stutter. The following stutter types using allele specific stutter ratios will be modeled at the NYC OCME:

- Back Stutter – one repeat unit *shorter* than the parent allele,
- Forward Stutter – one repeat unit *larger* than the parent allele,

- Half Back Stutter – two base pairs *shorter* than the parent allele in some tetranucleotide loci, and
- Double Back Stutter – two repeat units *shorter* than the parent allele.

For each type of stutter modeled, three different parameters must be determined: the maximum allowable stutter ratio, a stutter regression line, and any stutter exceptions from the regression line. The NYC OCME has determined each parameter for the four types of stutter mentioned above.

Sample Choice

Two independent sets of samples were needed for the Stutter Study and Model Maker experiments. 246 female and male DNA donor samples were genotyped using PowerPlex® Fusion 5C to use in validation experiments. Stutter data can only be collected for alleles present in the dataset; therefore, a Python program was used to maximize the coverage of unique alleles from this population of donors. A set of 100 donors was used for the Stutter Study (9).

Stutter Sample Analysis

For the set of samples chosen for stutter analysis, each donor was amplified at target amounts of 4 ng, 2 ng, 1 ng, 750 pg, 525 pg, 250 pg, 125 pg, 75 pg and 50 pg. All samples were analyzed using GeneMarker® HID v3.0.0 (5) with stutter filters off and at a lowered AT to maximize the amount of stutter data observed to better inform the model (3). The lowered ATs used were based on the average noise peak height observed for the 3500xLs plus three times the standard deviation, rounded to the nearest multiple of 5 (4, 9). These ATs were chosen to prevent masking of true stutter peaks while having an AT high enough to prevent analyzing around excessive noise peaks. These values were calculated per channel and are as follows:

Table 2. NYC OCME lowered analytical thresholds for Fusion 5C data run on 3500xL genetic analyzers per dye for stutter analysis. All numbers are in RFU.

Dye	AT	Average Noise Peak Height for 3500xLs	Standard Deviation	Calculated Lowered AT	Final Lowered AT
Fluorescein (Blue)	85	7.0	6.9	27.7	30
JOE (Green)	120	11.7	11.3	45.6	45
TMR (Yellow)	130	15.8	14.2	58.4	60
CXR (Red)	160	19.7	18.3	74.6	75

For each sample, labels were only retained for allelic peaks and any of the four previously mentioned stutter variants. All labels for other artifacts (i.e. pull-up, spikes, drop-in, etc.) were deleted. Any stutter affected allele was not used. Stutter affected alleles include any allele at a locus that is in a stutter position of another allele where the peak height may be impacted. Additionally, any locations where a peak fell at or above 30,000 RFU were not used. This is the saturation level of the instrument where the observed parent peak heights no longer correlate with

the expected parent peak heights (see Figure 1). At each locus, allelic and/or stutter peaks were labeled as either a parent allele or a specific type of stutter artifact. Data was collected for all samples where associated allelic ladders and size standards passed.

Maximum Allowable Stutter Ratio

The maximum allowable stutter ratio aids in reducing long run times. This setting only allows peaks in a stutter position to be considered stutter if the peak height ratio of that potential stutter peak to the parent peak falls below a specified threshold (1-2). To determine the maximum allowable stutter ratio for each stutter type, maximum stutter ratio values for each type of stutter, the coverage that a percent threshold would provide of the stutter data, and the highest stutter peaks in relation to the determined drop-in cap were evaluated. Table 3 shows the maximum allowable stutter ratio for each type of stutter modeled.

Table 3. Maximum allowable stutter ratio per stutter type.

Stutter Type	Maximum Allowable Stutter Ratio
Back Stutter	0.3
Forward Stutter	0.2
Half Back Stutter	0.1
Double Back Stutter	0.1

Regressions and Exceptions Files

STRmix™ v2.7 uses two types of files for each of the modeled stutter types, the stutter regressions file and the stutter exceptions file. Stutter was modeled for any location with a total of 100+ observed stutters and/or any location where the average stutter variant peak height was above the AT for that dye. Any location where stutter data did not conform to the above measures were not modeled for that type of stutter. By visually analyzing regressions plots for each locus and stutter type, a stutter model was chosen - i.e. allele regression, longest uninterrupted sequence (LUS) regression, or average stutter ratio. Regressions based on the allele designation were created using the relationship between the allele and stutter ratio at each locus. The linear regression equation using allele designation is $Stutter\ Ratio = m * Allele + c$ where m is the slope, c is the y-intercept, and $Allele$ is the allele designation of the parent peak (not the allele designation of the stutter). Regressions based on the LUS are created using the relationship of the longest uninterrupted sequence of the alleles and the stutter ratio for each locus. The linear regression using the longest uninterrupted sequence equation is $Stutter\ Ratio = m * LUS + c$ where m is the slope, c is the y-intercept, and LUS is the longest uninterrupted sequence of a particular parent allele. For locations where average stutter was used, a minimum of 5 observed stutters was required

to use that value for modeling stutter at a particular allele (3). For further details on stutter analysis refer to the NYC OCME Stutter Study (9). Table 4 provides a summary of the different models chosen for each locus and type of stutter.

The regressions in the stutter regressions file describe the linear relationship between the allele designations and the stutter ratio by providing the slope and the y-intercept for each locus. These values are used to determine expected stutter heights per allele (3). Table 5 shows the data for the back stutter and forward stutter regressions files and Table 6 shows the data for the half back stutter and double back stutter regressions files. For loci where stutter was not modeled, a '0' was entered into the stutter regressions file.

Table 4. NYC OCME stutter model used at each locus for each type of stutter modeled. Allele regression = Allele; longest uninterrupted sequence regression = LUS; average observed = Average; a blank space indicates that the stutter type was not modeled at the location.

Locus	Back Stutter	Forward Stutter	Half Back Stutter	Double Back Stutter
D3S1358	Average	Average	Average	
D1S1656	LUS	Average	Allele	Average
D2S441	Average	Average	Average	Average
D10S1248	Allele	Average	Average	
D13S317	Allele	Average	Average	
Penta E	Average	Average		
D16S539	Allele	Average		
D18S51	Allele	Average	Average	Average
D2S1338	Average	Average	Allele	Allele
CSF1PO	Allele			
Penta D	Average			
TH01	LUS		Average	
vWA	Average	Average		
D21S11	Average	Average		Average
D7S820	Allele	Average	Average	
D5S818	Allele	Average		
TPOX	Average			
DYS391				
D8S1179	Average	Average		Average
D12S391	Allele	Average		Allele
D19S433	Average	Average		
FGA	Average	Average		Average
D22S1045	Allele	Average		

Table 5. NYC OCME per allele back and forward stutter values for STRmix™ v2.7 regressions files.

<i>Locus</i>	<i>Back Stutter</i>		<i>Forward Stutter</i>	
	Intercept	Slope	Intercept	Slope
<i>D3S1358</i>	-0.05479	0.007968	-0.01456	0.001409
<i>D1S1656</i>	0.016231	0.004786	0.018215	-0.0003
<i>D2S441</i>	0.047376	-0.00053	0.005033	0.000733
<i>D10S1248</i>	-0.06923	0.008943	-0.01029	0.001623
<i>D13S317</i>	-0.04284	0.007386	-0.01228	0.002308
<i>Penta E</i>	-0.01446	0.002983	0.009222	0.00017
<i>D16S539</i>	-0.02692	0.007207	-0.00784	0.001494
<i>D18S51</i>	-0.03244	0.007117	-0.00299	0.00075
<i>D2S1338</i>	-0.02299	0.004761	0.015805	0.000104
<i>CSF1PO</i>	-0.03809	0.00867	0	0
<i>Penta D</i>	-0.01059	0.002378	0	0
<i>TH01</i>	0.018192	0.000459	0	0
<i>vWA</i>	-0.09321	0.00965	0.032973	-0.00116
<i>D21S11</i>	-0.01958	0.003242	0.004489	0.000283
<i>D7S820</i>	-0.04017	0.008574	0.014813	-0.00028
<i>D5S818</i>	-0.05354	0.009808	-0.00528	0.001422
<i>TPOX</i>	-0.0297	0.006002	0	0
<i>DYS391</i>	0	0	0	0
<i>D8S1179</i>	0.006233	0.005008	0.012172	0.000178
<i>D12S391</i>	-0.12991	0.011527	-0.00641	0.000893
<i>D19S433</i>	-0.03849	0.007461	0.144265	-0.00834
<i>FGA</i>	-0.0367	0.004586	-0.0449	0.003078
<i>D22S1045</i>	-0.11898	0.014742	-0.03599	0.007296

Table 6. NYC OCME per allele half back and double back stutter values for STRmix™ v2.7 regressions files.

<i>Locus</i>	<i>Half Back Stutter</i>		<i>Double Back Stutter</i>	
	Intercept	Slope	Intercept	Slope
<i>D3S1358</i>	0.001794	0.000323	0	0
<i>D1S1656</i>	0.004607	0.000573	0.003072	0.000261
<i>D2S441</i>	-0.0125	0.001748	0.001215	0.000239
<i>D10S1248</i>	8.23E-03	4.80E-05	0	0
<i>D13S317</i>	0.017333	0.000558	0	0
<i>Penta E</i>	0	0	0	0
<i>D16S539</i>	0	0	0	0
<i>D18S51</i>	0.01031	-0.00034	0.010069	-9.16E-06
<i>D2S1338</i>	-0.01376	0.000969	-0.00596	0.00068
<i>CSF1PO</i>	0	0	0	0
<i>Penta D</i>	0	0	0	0
<i>TH01</i>	7.05E-03	-4.48E-05	0	0
<i>vWA</i>	0	0	0	0
<i>D21S11</i>	0	0	-0.00703	0.000487
<i>D7S820</i>	0.015658	-0.0005	0	0
<i>D5S818</i>	0	0	0	0
<i>TPOX</i>	0	0	0	0
<i>DYS391</i>	0	0	0	0
<i>D8S1179</i>	0	0	0.014875	-0.00047
<i>D12S391</i>	0	0	-0.01535	0.001249
<i>D19S433</i>	0	0	0	0
<i>FGA</i>	0	0	-0.02932	0.001795
<i>D22S1045</i>	0	0	0	0

The stutter exceptions file stores LUS and average stutter ratio values for alleles in the loci of the profiling kit and is created for each modeled stutter type. These are specifically used for loci where the LUS regression or the allele specific stutter averages best describe the data, as opposed to the allele regression in the stutter regressions files. Where available, the average value or the value calculated from the LUS regression are input into the appropriate allele for a locus. Where data is not available in the stutter exceptions file, indicated by a '0', the stutter regressions file is used. Regression graphs are available in Appendix A and the stutter exceptions files data are available in Appendix B.

Drop-in

Drop-in peaks are non-reproducible, unexplained peaks observed in a profile. STRmix™ models drop-in using the following parameters:

1. The analytical threshold,
2. A cap on the maximum allowed height for a proposed drop-in peak,
3. The drop-in frequency, and
4. The alpha and beta values that describe the gamma distribution.

The analytical threshold was defined as mentioned above. The remaining parameters were defined by the analysis of 124 amplification negative controls. To better inform the drop-in model, the analytical threshold was lowered to 50 RFU for all dye channels to capture more drop-in peaks. There were 20 observed drop-in events in 2,976 scored loci. The drop-in cap was defined as the largest drop-in seen during this study plus three times the standard deviation rounded to the nearest hundred. By using the Drop-in Calculator from the STRmix™ developers, the drop-in frequency and the α and β values for the gamma distribution were determined (10). The gamma distribution parameters define the gamma distribution STRmix™ uses to apply a penalty (i.e. probability) to peaks it is considering as possible drop-in. Table 7 describes the parameters calculated for drop-in.

Table 7. NYC OCME STRmix™ v2.7 drop-in parameters.

Drop-in cap	300 RFU
Drop-in frequency	0.0087
Distribution parameters α, β	22.31, 2.65

Peak Height Variances and Locus Specific Amplification Effects (LSAE) using Model Maker

The quality of profiles is inherently variable, as seen through extensive empirical data. Within STRmix™, allele peak height variances (c^2), stutter peak height variances (k^2), and locus specific amplification effects are defined using the Model Maker function and a set of samples ranging in quality that are likely to be observed in casework. For each type of parameter, a gamma distribution of the variance is determined, and the mode of that distribution will serve as the starting point within the MCMC for subsequent sample analysis using STRmix™.

Model Maker Sample Analysis

All sample analysis was performed according to the STRmix™ v2.7 Implementation and Validation Guide (3). A set of 10 different donor samples, chosen from the donors not used for the Stutter Study, were amplified at various target amounts ranging from 750 pg – 15 pg. Profiles were analyzed in GeneMarker® 3.0.0 with no stutter filter applied and at lowered ATs – the same ATs used for stutter analysis (Table 2). Labels for allelic peaks and their associated stutter, along with apparent drop-in peaks, were retained while all labels for other artifacts were removed.

Additionally, labels were removed for peaks that fell in a stutter position for an allele at a locus where that stutter type will not be modeled in STRmix™ (see Table 4). Input files for both the set of single source profiles and their reference profiles were created and imported into Model Maker for analysis.

Model Maker Results

Table 8 gives a summary of the Model Maker results. Figure 2 shows the plots of the gamma distributions for the LSAE variance, allele variance, and each type of stutter variance. Figure 3 displays the correlation plots for low molecular weight (LMW) alleles versus high molecular weight (HMW) alleles and allele versus stutter peaks for each type of stutter modeled. The distribution within these graphs is as expected, with no observed correlation. Additionally, Figure 4 shows that the analysis during the MCMC cycles during the Model Maker run converged, as observed by the plateauing of the log(LR) values.

Table 8. NYC OCME STRmix™ v2.7 summary of Model Maker results.

Number of profiles analyzed	164
Mean LSAE variance	0.030
Allele Variance (c²)	α : 7.427, β : 3.479 Mode: 22.360
Back Stutter Variance (k²)	α : 1.799, β : 19.052 Mode: 15.223
Forward Stutter Variance (k²)	α : 1.999, β : 11.703 Mode: 11.691
Half Back Stutter Variance (k²)	α : 2.597, β : 6.632 Mode: 10.591
Double Back Stutter Variance (k²)	α : 2.816, β : 6.792 Mode: 12.334

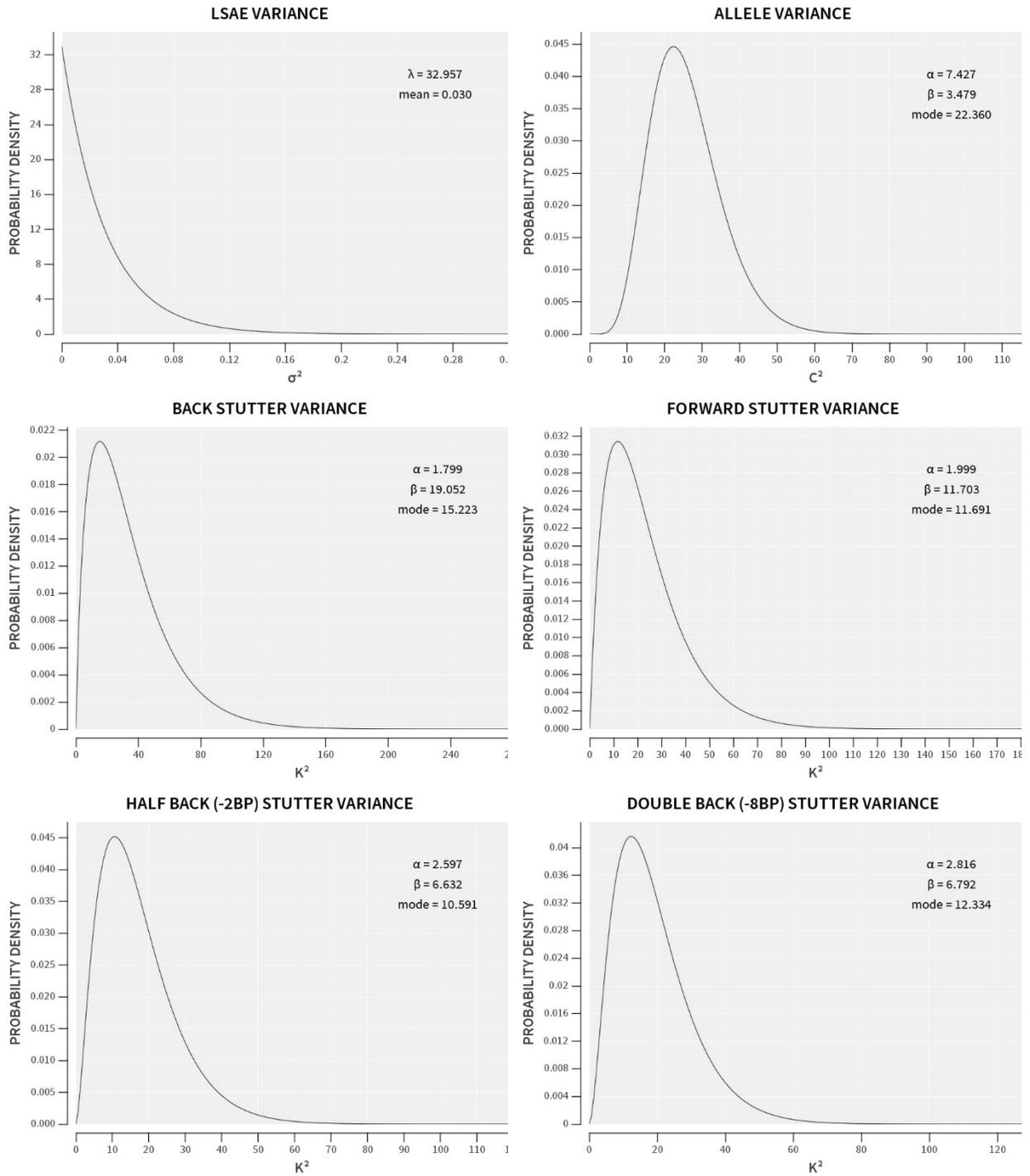


Figure 2. NYC OCME STRmix™ v2.7 Model Maker variance plots.

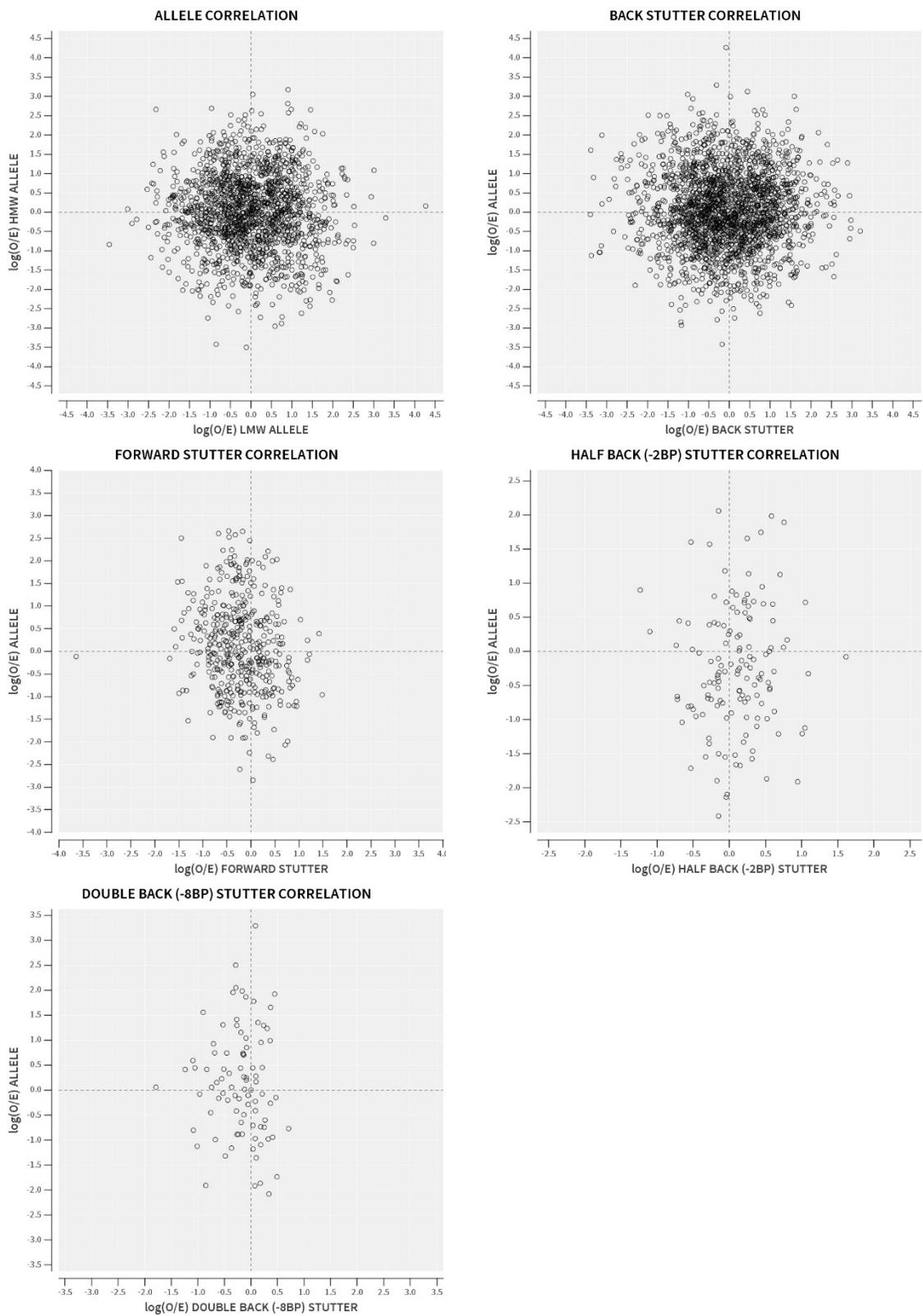


Figure 3. NYC OCME STRmix™ v2.7 Model Maker correlation plots.

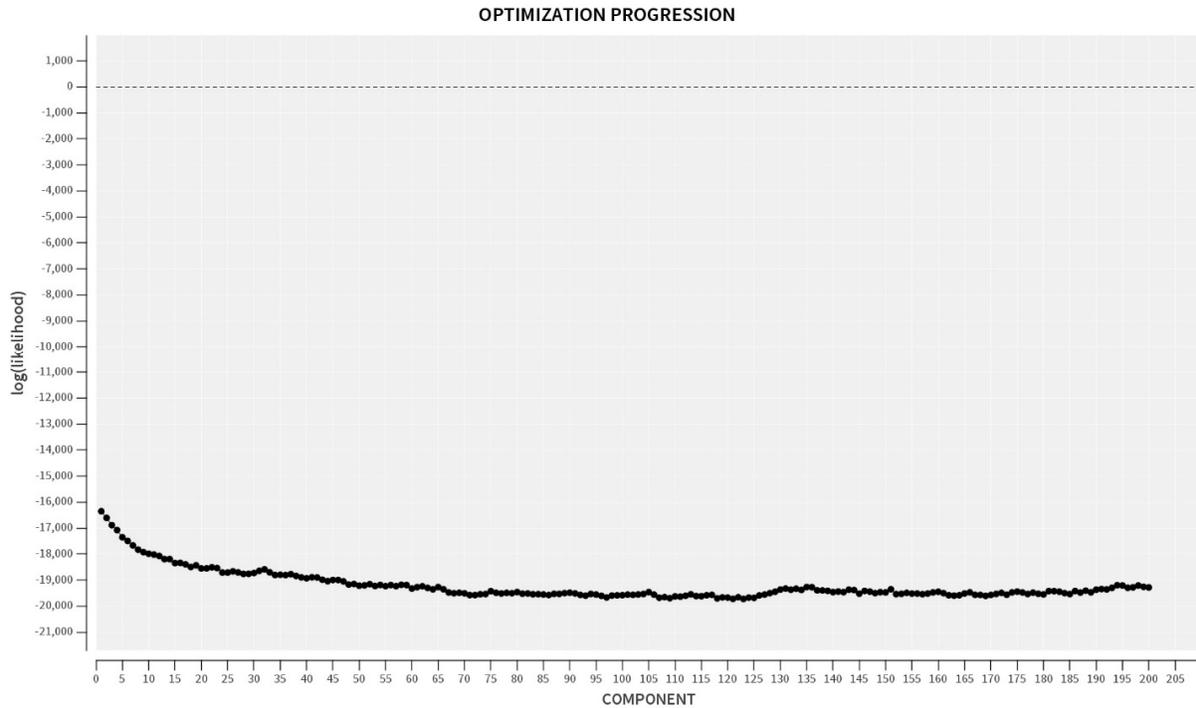


Figure 4. NYC OCME STRmix™ v2.7 optimization progression of the MCMC process.

Additionally, a check of the Model Maker output was performed to ensure that the allele variance was optimized during the MCMC process. The $\log(Hb)$, where Hb is the heterozygote balance, and the average peak heights (APH) for the set of data used for Model Maker were compared. The expected 95% bounds using the allelic variance from a chosen percentile of the prior gamma distribution, c^2 , was calculated and plotted in order to demonstrate that the value for allele variance produced by Model Maker is sufficiently optimized across all data. The $\log(Hb)$, APH , and the 95% bounds were calculated using –

$$\log(Hb) = \log\left(\frac{O_{HMW}}{O_{LMW}}\right)$$

$$APH = \frac{O_{HMW} + O_{LMW}}{2}$$

$$\log(Hb) = \pm\sqrt{2} * 1.96 * \sqrt{\frac{c^2}{APH}}$$

Figure 5 demonstrates that the allele variance was optimized during the MCMC process, with the 95% bounds capturing 95.0% of the data when c^2 is set to the 80th percentile of the prior gamma distribution.

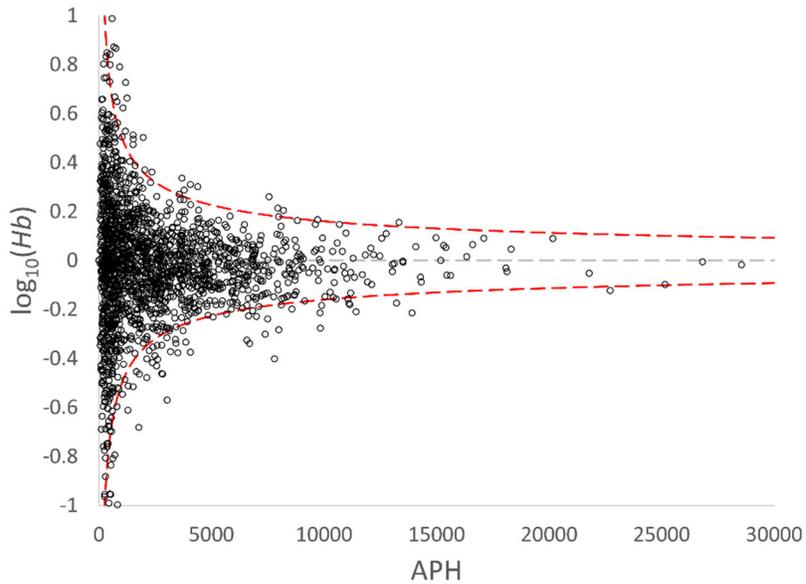


Figure 5. NYC OCME STRmix™ v2.7 log(Hb) versus APH for single source profiles.

Population, Allele Frequencies, and Theta Settings

For STRmix™ v2.7 analysis at the NYC OCME, NIST amended allele frequencies for Caucasian, Hispanic, Asian, and African American sub-groups will be used (11). Theta will be set to a value of 0.03 (12; 13). Prior probabilities for the unified likelihood ratio will be a population size of 8 million, based on census data (14), and an average number of children per family set to 3, based on the world average (15). Additionally, the minimum allele frequency for alleles not seen within the population frequency database is derived from the population size e.g. *allele frequency* = 1/8,000,000.

APPROVED

By Craig O'Connor at 10:28 pm, Aug 23, 2021

References

1. **Institute of Environmental Science and Research Limited.** *STRmix™ V2.7 User's Manual.* 6 September 2019.
2. —. *STRmix™ V2.7 Operation Manual.* 11 October 2019.
3. —. *STRmix™ V2.7 Implementation and Validation Guide.* 2 September 2019.
4. **NYC OCME.** *PowerPlex® Fusion System Amplification Kit on the Applied Biosystems® 3500xL Genetic Analyzer with GeneMarker® HID 2.9.5.* New York City : s.n., 2019.
5. **SoftGenetics®, LLC.** *GeneMarker® HID v3.0.0 User Manual.* State College, Pennsylvania : s.n., July 2019.
6. **Jo-Anne Bright, James M Curran, John S Buckelton.** Investigation into the performance of different models for predicting stutter. *Forensic Science International: Genetics.* 2013, pp. 422-427.
7. **Jo-Anne Bright, Duncan Taylor, James M Curran, John S Buckelton.** Developing allelic and stutter peak height models for a continuous method of DNA interpretation. *Forensic Science International: Genetics.* 2013, Vol. 7, pp. 296-304.
8. **Clare Brookes, Jo-Anne Bright, SallyAnn Harbison, John Buckleton.** Characterizing stutter in forensic STR multiplexes. *Forensic Science International: Genetics.* 2012, Vol. 6, pp. 58-63.
9. **NYC OCME.** *Stutter Study for GeneMarker® HID 3.0.0 and STRmix™ Version 2.7-PowerPlex® Fusion Data run on 3500xL Genetic Analyzers.* NYC : NYC OCME, 2021.
10. **ESR.** STRmix™ Technical and Scientific Support. *STRmix™.* [Online] ESR. <https://support.strmix.com/support/home>.
11. **National Institute of Standards and Technology.** NIST 1036 Revised U.S. Population Dataset (July 2017). *STRBase.* [Online] [Cited: 10 27, 2020.] <https://strbase.nist.gov/1036-Revised-Allele-Freqs-PopStats-July-19-2017.xlsx>.
12. **John Buckleton, James Curran, Jerome Goudet, Duncan Taylor, Alexandre Thiery, B.S. Weir.** Population-specific FST values for forensic STR markers: A worldwide survey. *Forensic Science International: Genetics.* Jul 2016, Vol. 23, pp. 91-100.
13. **Weir, Bruce S.** *Population Genetic Issues for Forensic DNA.* National Criminal Justice Reference Services. April 2018.

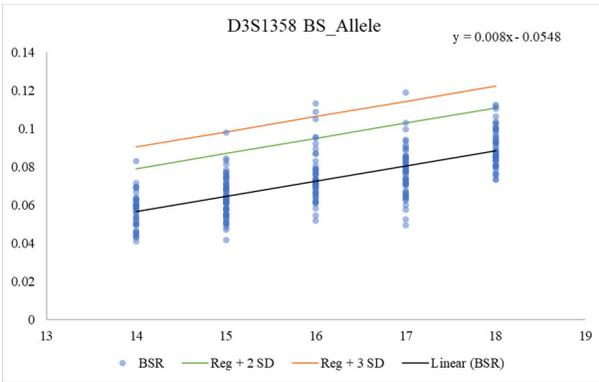
14. Quick Facts New York City, New York. *United States Census Bureau* . [Online]
<https://www.census.gov/quickfacts/newyorkcitynewyork>.

15. The World Factbook. *Central Intelligence Agency*. [Online] [Cited: December 8, 2020.]
<https://www.cia.gov/library/publications/the-world-factbook/geos/xx.html>.

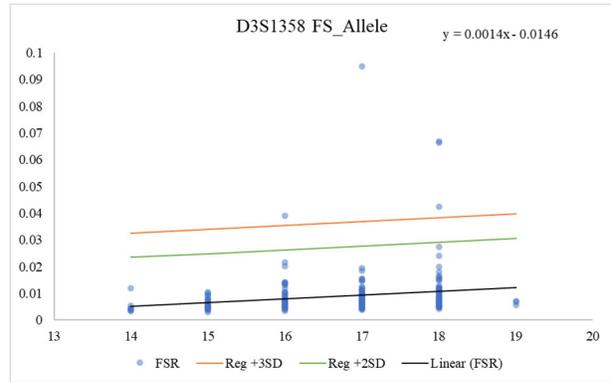
Appendix A: Graphs of regressions used for stutter analysis for each type of stutter modeled per locus. Allele designation or LUS is on x-axis and stutter ratio is on y-axis.

D3S1358

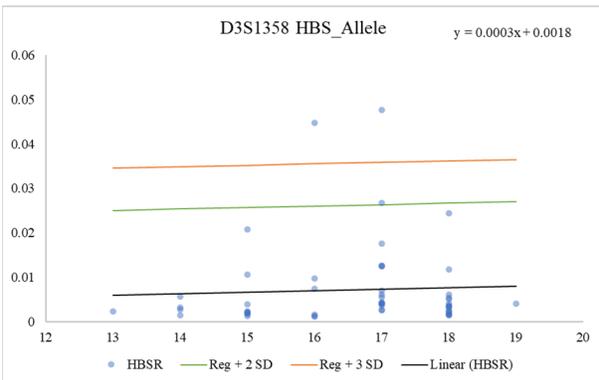
Back Stutter



Forward Stutter



Half Back Stutter

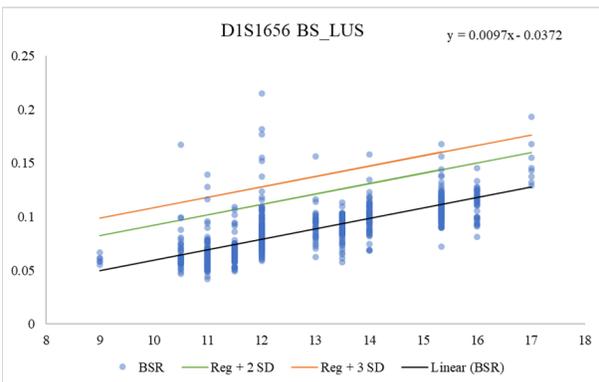


Double Back Stutter

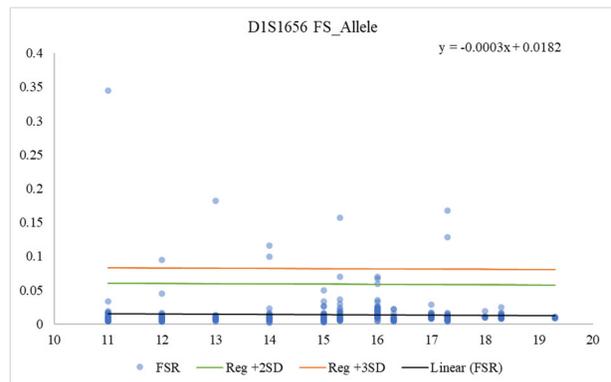
Not Modeled

D1S1656

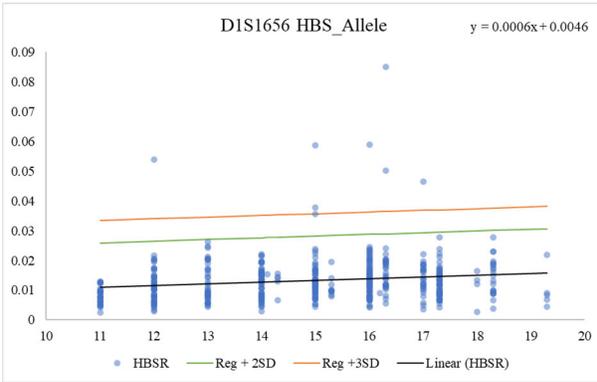
Back Stutter



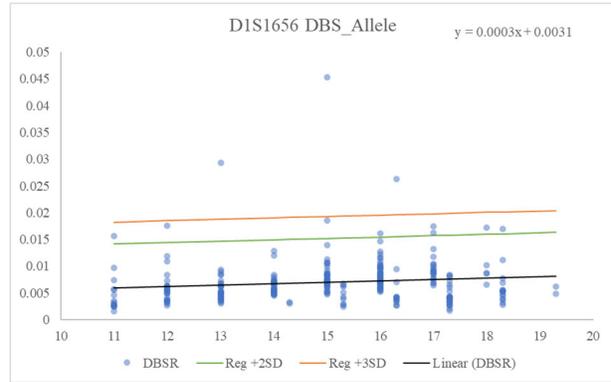
Forward Stutter



Half Back Stutter

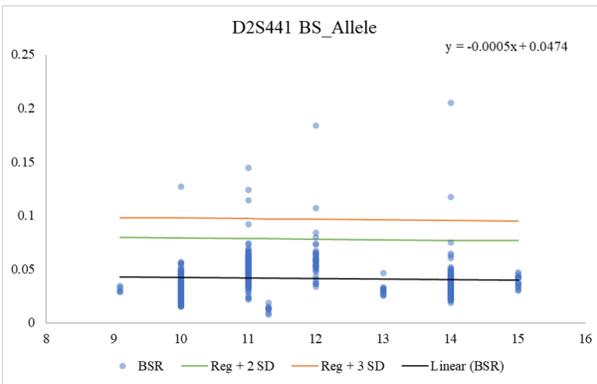


Double Back Stutter

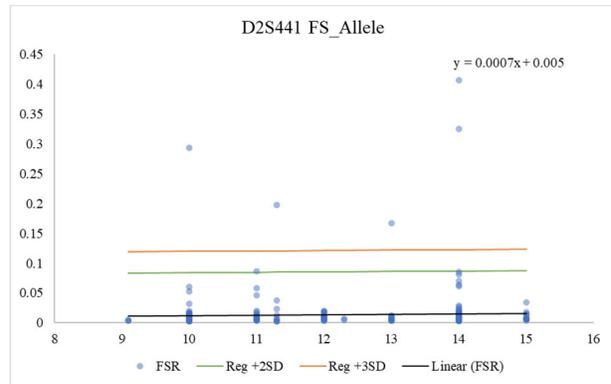


D2S441

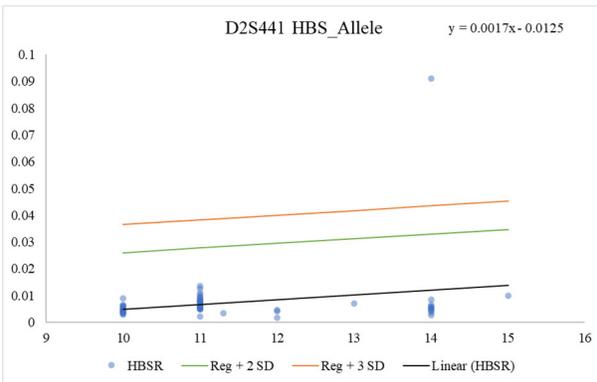
Back Stutter



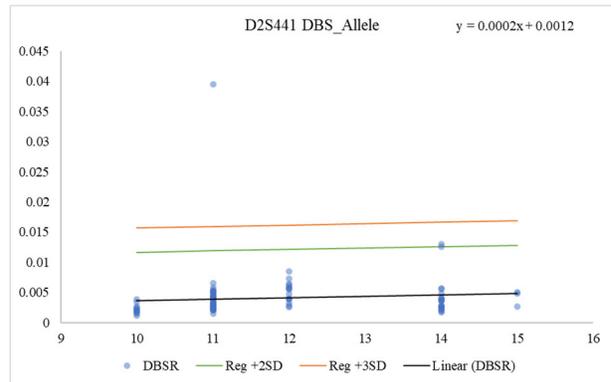
Forward Stutter



Half Back Stutter

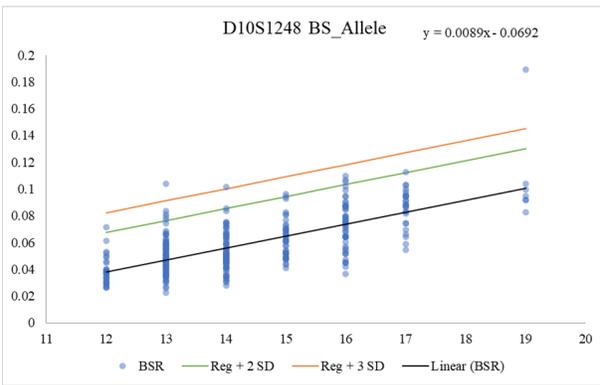


Double Back Stutter

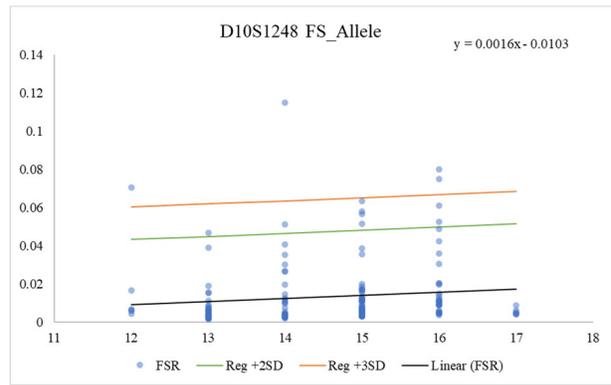


D10S1248

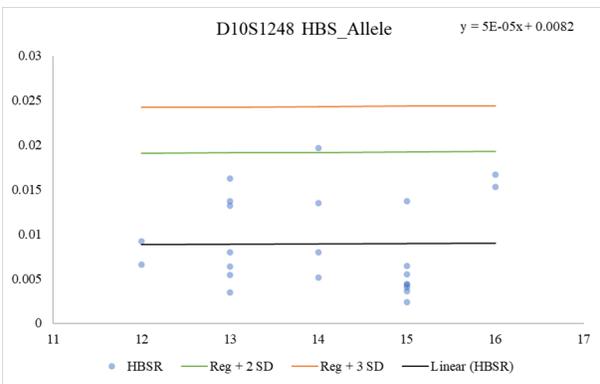
Back Stutter



Forward Stutter



Half Back Stutter

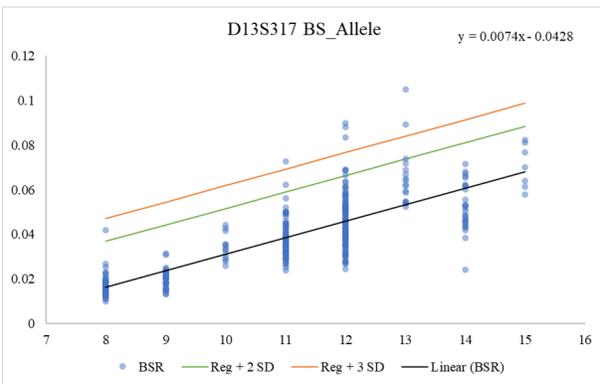


Double Back Stutter

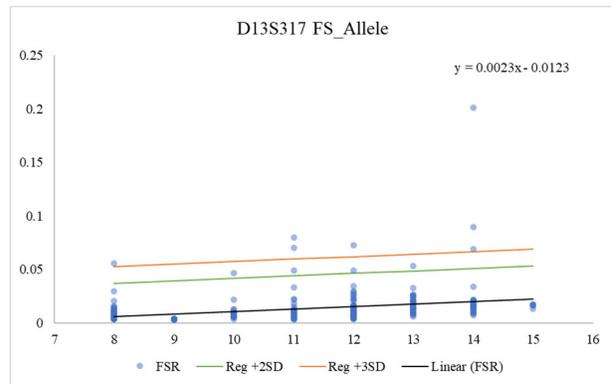
Not Modeled

D13S317

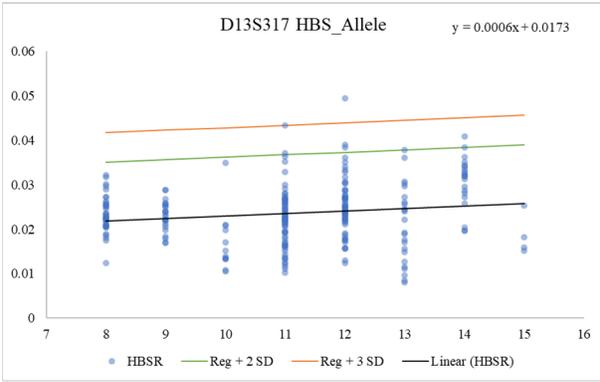
Back Stutter



Forward Stutter



Half Back Stutter

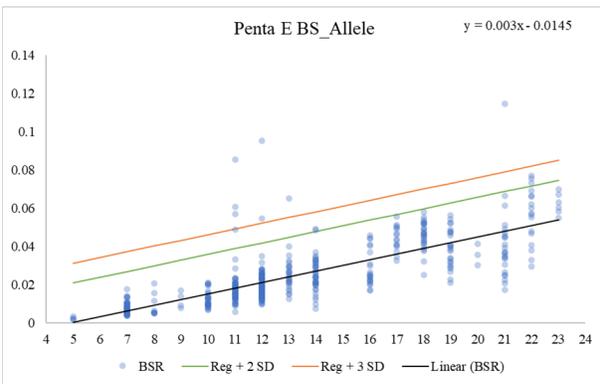


Double Back Stutter

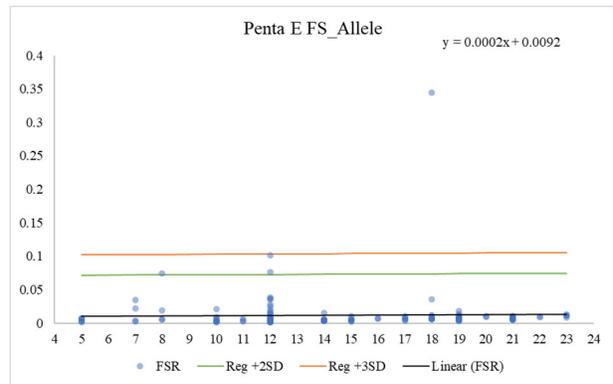
Not Modeled

Penta E

Back Stutter



Forward Stutter



Half Back Stutter

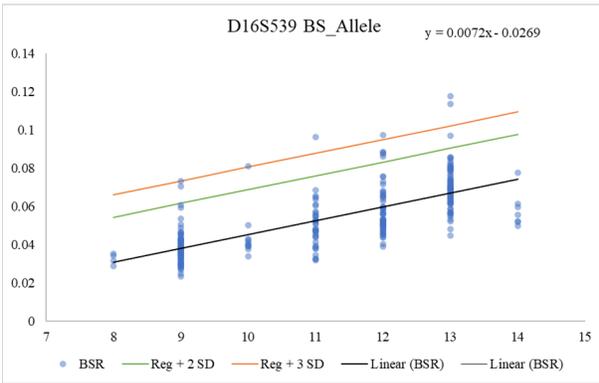
Not Modeled

Double Back Stutter

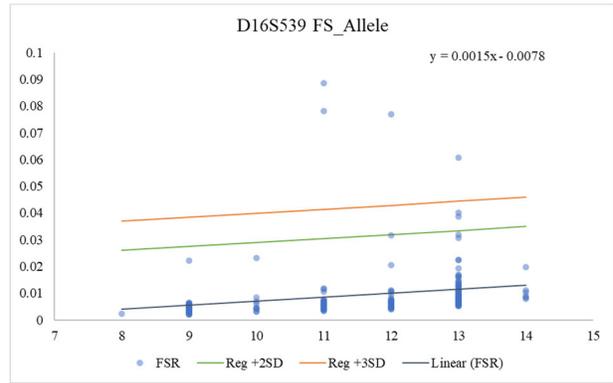
Not Modeled

D16S539

Back Stutter



Forward Stutter



Half Back Stutter

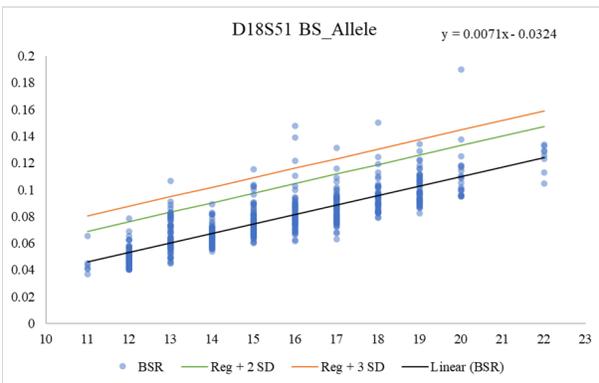
Not Modeled

Double Back Stutter

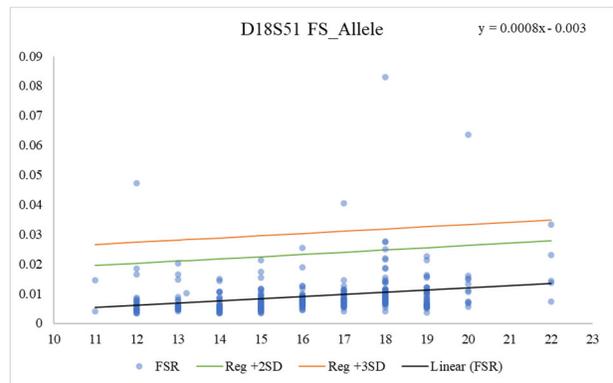
Not Modeled

D18S51

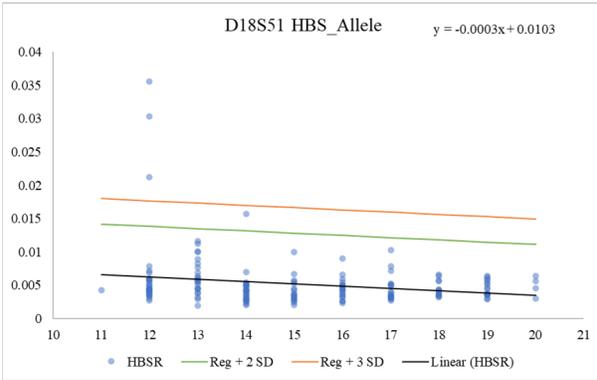
Back Stutter



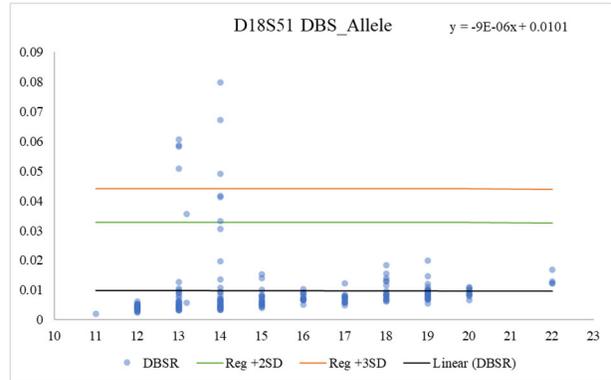
Forward Stutter



Half Back Stutter

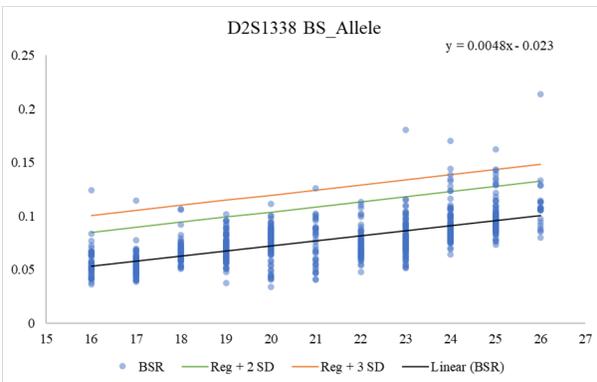


Double Back Stutter

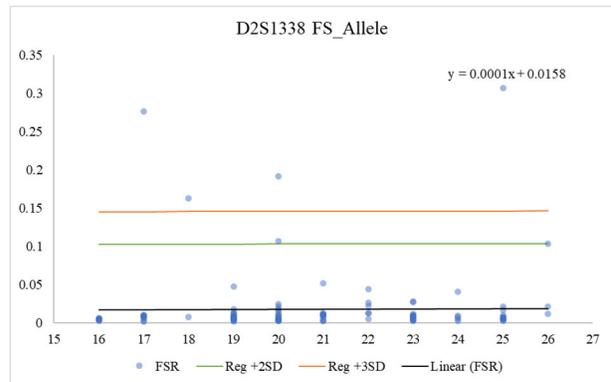


D2S1338

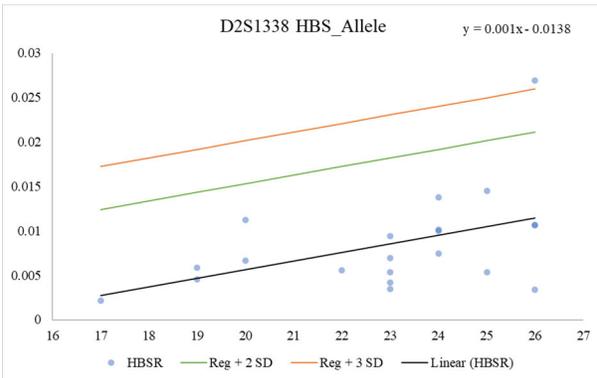
Back Stutter



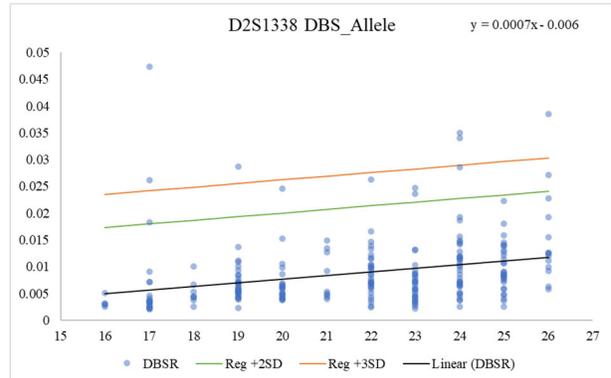
Forward Stutter



Half Back Stutter

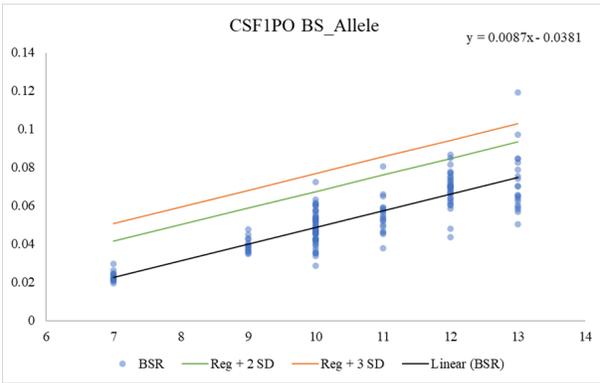


Double Back Stutter



CSF1PO

Back Stutter



Forward Stutter

Not Modeled

Half Back Stutter

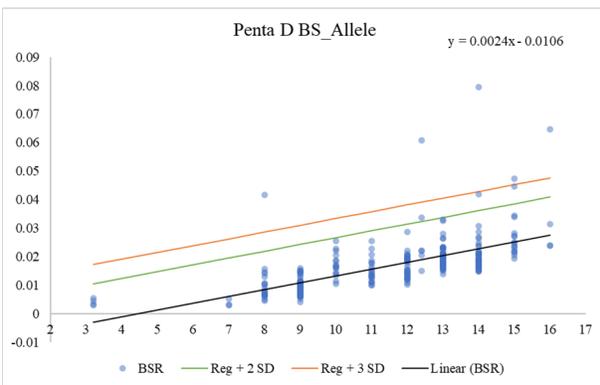
Double Back Stutter

Not Modeled

Not Modeled

Penta D

Back Stutter



Forward Stutter

Not Modeled

Half Back Stutter

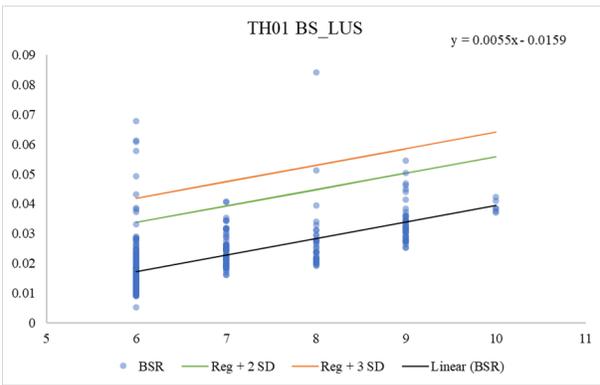
Double Back Stutter

Not Modeled

Not Modeled

TH01

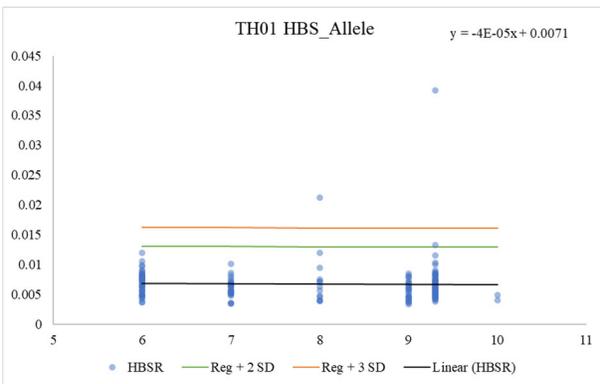
Back Stutter



Forward Stutter

Not Modeled

Half Back Stutter

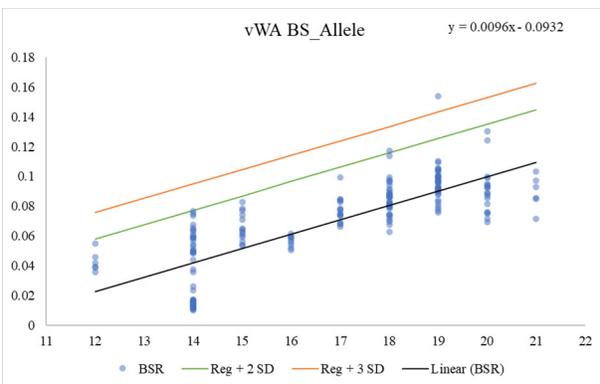


Double Back Stutter

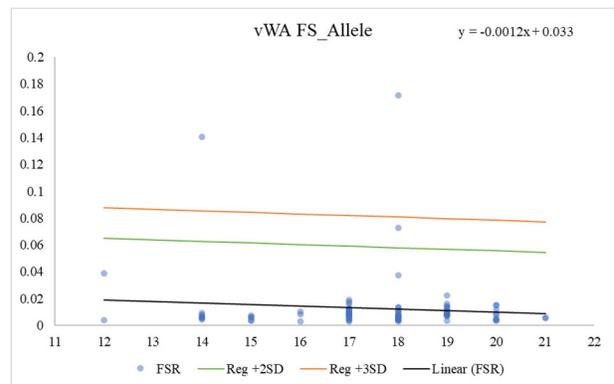
Not Modeled

vWA

Back Stutter



Forward Stutter



Half Back Stutter

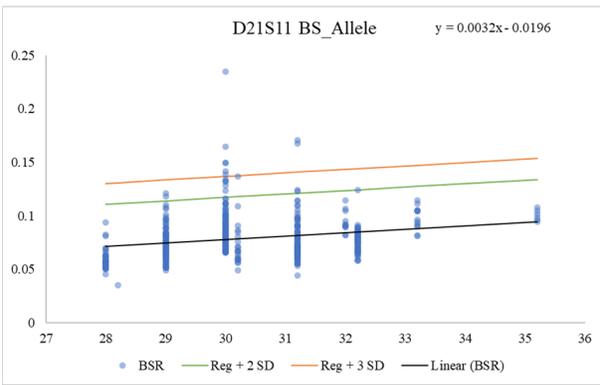
Not Modeled

Double Back Stutter

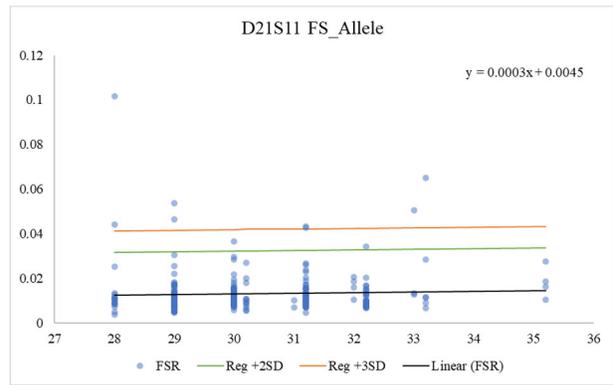
Not Modeled

D21S11

Back Stutter



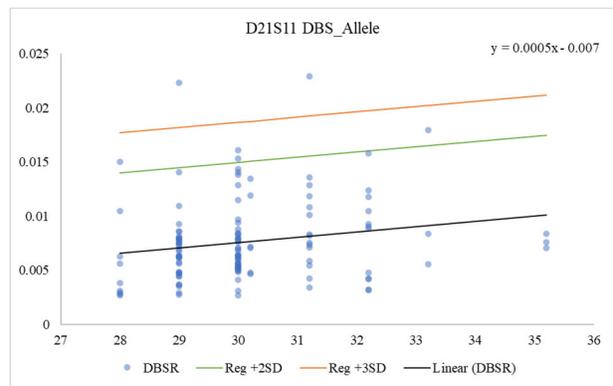
Forward Stutter



Half Back Stutter

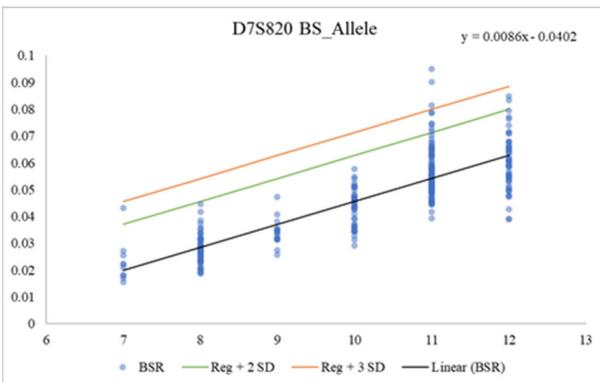
Not Modeled

Double Back Stutter

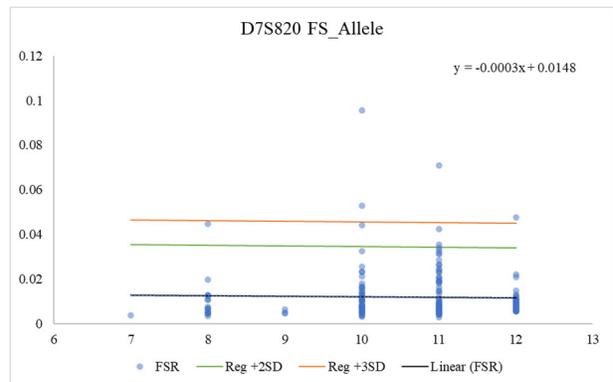


D7S820

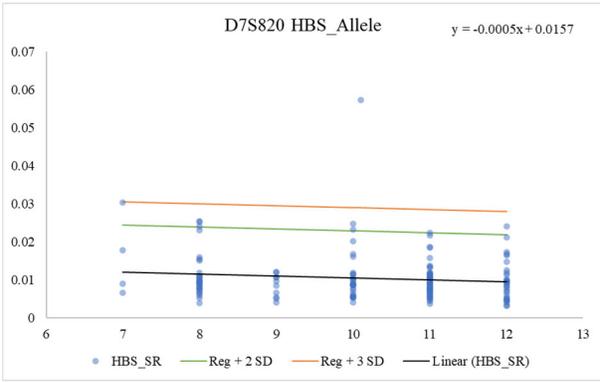
Back Stutter



Forward Stutter



Half Back Stutter

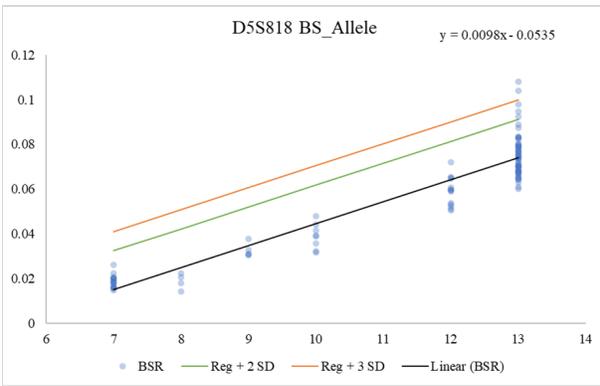


Double Back Stutter

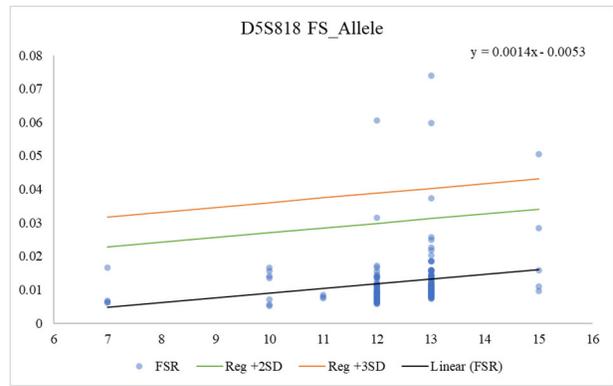
Not Modeled

D5S818

Back Stutter



Forward Stutter



Half Back Stutter

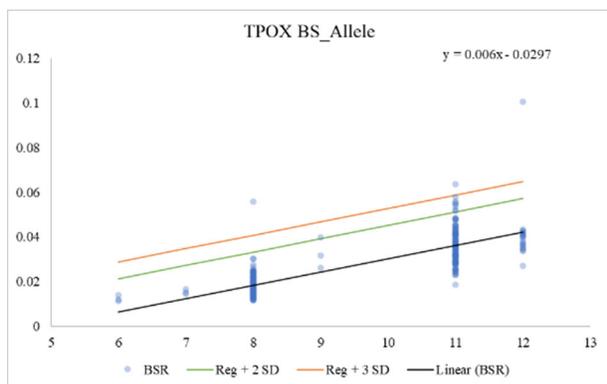
Not Modeled

Double Back Stutter

Not Modeled

TPOX

Back Stutter



Forward Stutter

Not Modeled

Half Back Stutter

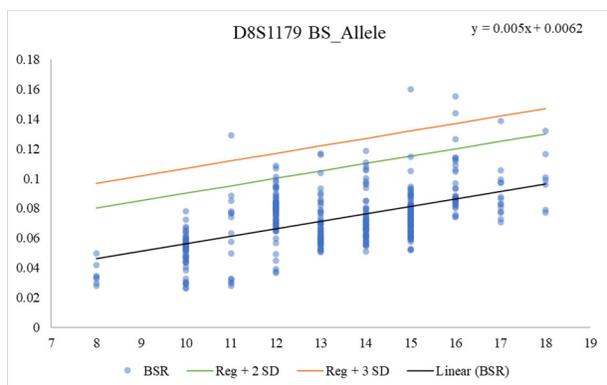
Not Modeled

Double Back Stutter

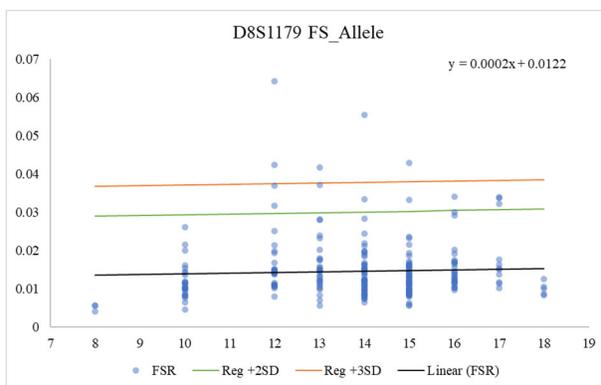
Not Modeled

D8S1179

Back Stutter



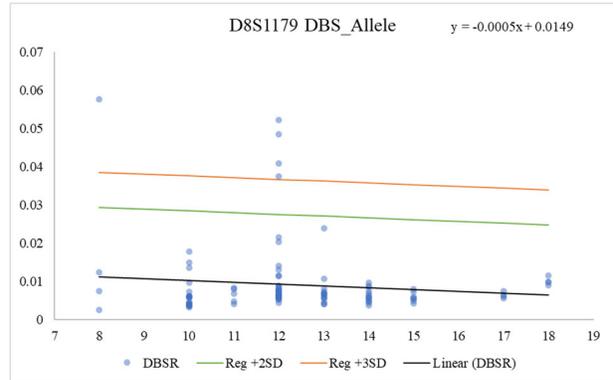
Forward Stutter



Half Back Stutter

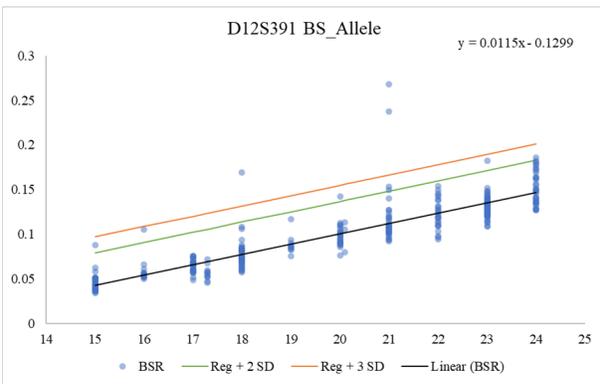
Not Modeled

Double Back Stutter

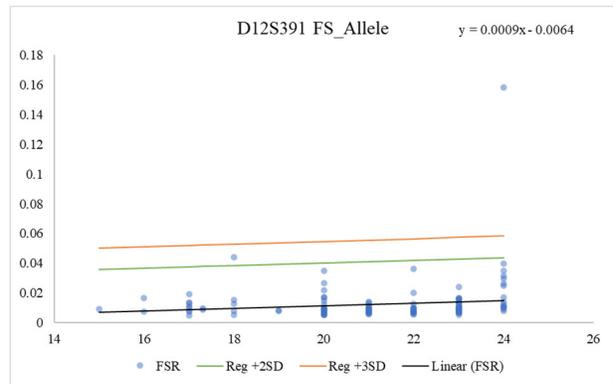


D12S391

Back Stutter



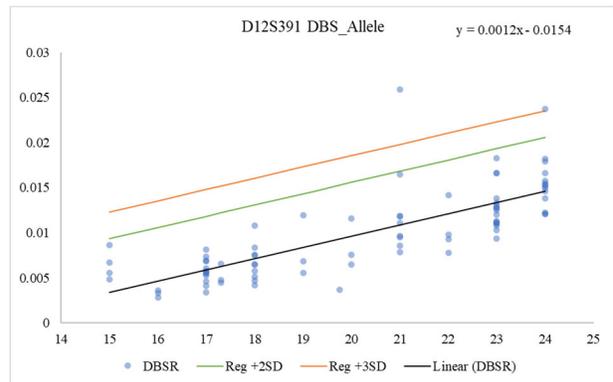
Forward Stutter



Half Back Stutter

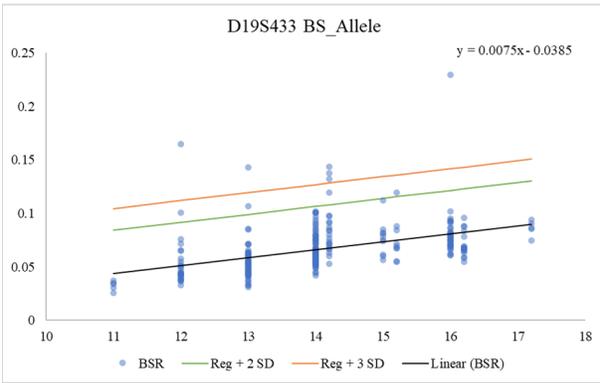
Not Modeled

Double Back Stutter

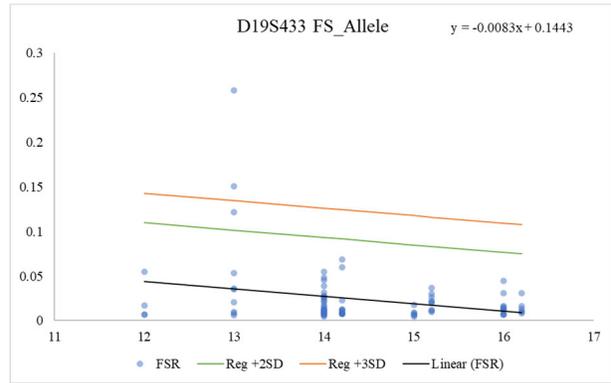


D19S433

Back Stutter



Forward Stutter



Half Back Stutter

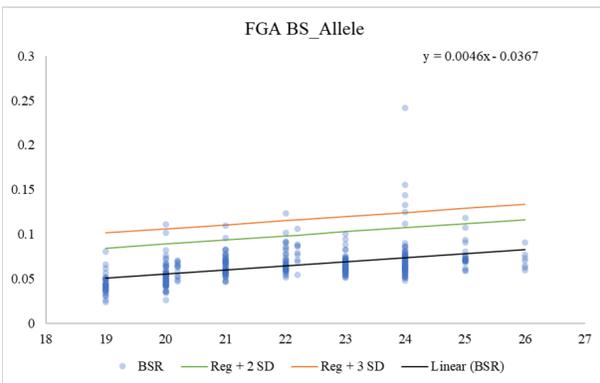
Not Modeled

Double Back Stutter

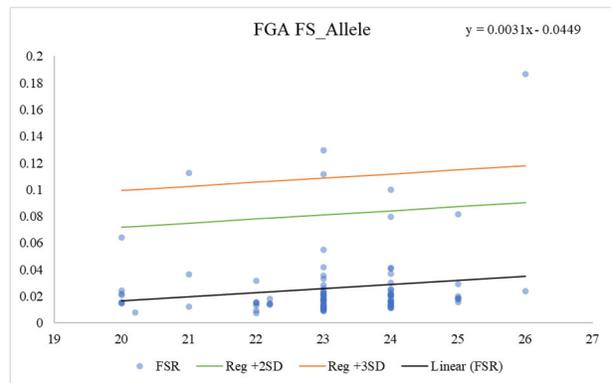
Not Modeled

FGA

Back Stutter



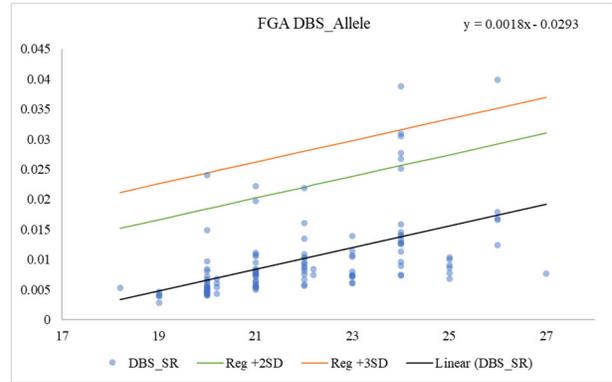
Forward Stutter



Half Back Stutter

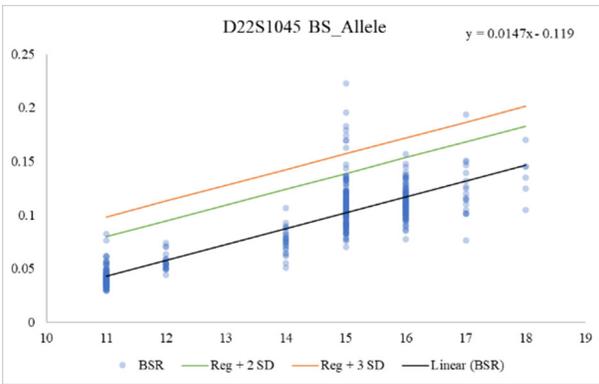
Not Modeled

Double Back Stutter

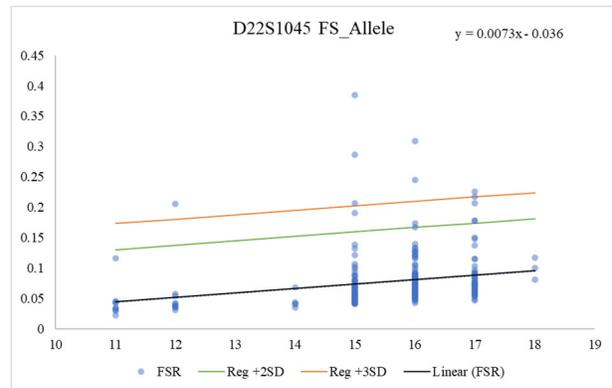


D22S1045

Back Stutter



Forward Stutter



Half Back Stutter

Not Modeled

Double Back Stutter

Not Modeled

Appendix B. NYC OCME STRmix™ v2.7 (a) back stutter, (b) forward stutter, (c) half back stutter, and (d) double back stutter exceptions files.

(a) back stutter

Allele	D3S1358	D151656	D25441	D10S1248	D13S317	Penta E	D16S539	D18S51	D251338	CSF1PO	Penta D	TH01	vWA	D21S11	D7S820	D5S818	TPOX	DYS391	D8S1179	D12S391	D19S433	FGA	D22S1045
3	0	0	0	0	0	0	0	0	0	0	0	0.000679	0	0	0	0	0	0	0	0	0	0	0
3.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0.006206	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0.011733	0	0	0	0	0	0	0	0	0	0	0
5.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0.01726	0	0	0	0	0	0	0	0	0	0	0
6.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6.3	0	0	0	0	0	0	0	0	0	0	0	0.000679	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0.007494	0	0	0	0	0	0.022787	0	0	0	0	0	0	0	0	0	0	0
7.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0.040342	0	0	0	0.009072	0	0	0	0	0.01051	0.028314	0	0	0	0	0.01788	0	0.035798	0	0	0	0
8.3	0	0	0	0	0	0	0	0	0	0	0	0.011733	0	0	0	0	0	0	0	0	0	0	0
9	0	0.050041	0	0	0	0	0	0	0	0	0.009183	0.033841	0	0	0	0	0	0	0	0	0	0	0
9.1	0	0.030964	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9.3	0	0	0	0	0	0	0	0	0	0	0	0.01726	0	0	0	0	0	0	0	0	0	0	0
10	0	0.05489	0.032083	0	0	0.012482	0	0	0	0	0.017096	0.039368	0	0	0	0	0	0	0	0.051612	0	0	0
10.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10.3	0	0	0	0	0	0	0	0	0	0	0	0.01726	0	0	0	0	0	0	0	0	0	0	0
11	0	0.069438	0.050787	0	0	0.019801	0	0	0	0	0.014967	0.044895	0	0	0	0	0.036614	0	0.062171	0	0.032464	0	0
11.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11.3	0	0	0.012691	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0.074287	0.060977	0	0	0.020152	0	0	0	0	0.016356	0.050422	0.042719	0	0	0	0.04144	0	0.0775	0	0.053714	0	0
12.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12.4	0	0	0	0	0	0	0	0	0	0	0.029057	0	0	0	0	0	0	0	0	0	0	0	0
13	0	0.079136	0.0308	0	0	0.025984	0	0	0	0	0.019737	0	0	0	0	0	0	0	0.068699	0	0.053815	0	0
13.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13.3	0	0.069438	0	0	0	0	0	0	0	0	0	0.028314	0	0	0	0	0	0	0	0	0	0	0
14	0.056921	0.093684	0.036289	0	0	0.025746	0	0	0	0	0.021677	0	0.033369	0	0	0	0	0	0.07379	0	0.066152	0	0.08715
14.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14.3	0	0.050041	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15	0.064306	0.098533	0.038352	0	0	0	0	0	0	0	0.027501	0	0.065771	0	0	0	0	0	0.07533	0	0.076671	0	0
15.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.075191	0
15.3	0	0.064588	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16	0.074894	0.111432	0	0	0	0.028467	0	0	0.05676	0	0	0.056506	0	0	0	0	0	0.096057	0	0.081463	0	0	0
16.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.070888	0	0
16.3	0	0.069438	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17	0.077591	0.11793	0	0	0	0.044203	0	0	0.054699	0	0	0	0.077282	0	0	0	0	0	0.089703	0	0	0	0
17.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.085986	0	0
17.3	0	0.079136	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18	0.090084	0.127629	0	0	0	0.045862	0	0	0.066828	0	0	0	0.084902	0	0	0	0	0	0.100083	0	0	0	0
18.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18.3	0	0.088835	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18.4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0.039248	0	0	0.06989	0	0	0	0.095987	0	0	0	0	0	0	0	0	0.043773	0
19.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19.3	0	0.098533	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19.4	0	0	0	0	0	0	0	0	0	0	0	0	0.090524	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0.075501	0	0	0	0.090524	0	0	0	0	0	0	0	0	0.052758	0
20.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.060441	0
20.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
21	0	0	0	0	0	0.041131	0	0	0.073853	0	0	0	0.08928	0	0	0	0	0	0	0	0	0.065012	0
21.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
21.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
21.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
22	0	0	0	0	0	0.056256	0	0	0.072827	0	0	0	0	0	0	0	0	0	0	0	0	0.069781	0
22.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.079812	0
22.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
23	0	0	0	0	0	0.062392	0	0	0.080149	0	0	0	0	0	0	0	0	0	0	0	0	0.065348	0
23.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0	0.093354	0	0	0	0	0	0	0	0	0	0	0	0	0.073649	0
24.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
24.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0	0.099745	0	0	0	0										

