STRmix™ V2.9.1

Idaho State Police Laboratory

(PowerPlex® Fusion 6C 3500)

STRmix™ Implementation

This document describes the determination of laboratory-specific parameters within STRmix™ V2.9 for the Idaho State Police crime laboratory (hereafter, ISP). Parameters were determined for PowerPlex® Fusion 6C data generated within ISP (29 PCR cycles, separated using 3500 CE instrumentation with a 1.2 kV/15 second injection protocol).

STRmix™ Parameters

There are a number of parameters that are not optimised by the MCMC in a STRmix™ analysis. These parameters must be set by the user and are either determined by analysis of empirical data or modelled within STRmix™ using the Model Maker function. The laboratory-specific parameters that are determined prior to use of STRmix™ are:

- Analytical/detection thresholds,
- Stutter ratios,
- Drop-in parameters,
- Saturation threshold,
- Allelic and stutter peak height variance parameters, and
- Locus Specific Amplification Efficiency (LSAE) variance parameter.

These parameters need to be defined for each STR kit, each PCR protocol (e.g. cycle number variation, reduced volume reaction), each CE platform (e.g. 3130 or 3500), each injection protocol, and potentially each time there is a significant update to the instrumentation (e.g. a camera or laser change). Laboratory-specific parameters were determined for the ISP laboratory using supplied data. The results of these analyses are described within this report.

Analytical Thresholds

The assignment of a fluorescent signal as DNA product as opposed to baseline or noise is important in DNA profile analysis. This differentiation is usually undertaken using a set threshold above which peaks are deemed to represent DNA product if they also meet certain morphological requirements, and below which they are ignored, regardless of morphology. The issue, then, is to assign a threshold, often termed the limit of detection (LOD) or analytical threshold (AT), to minimise the detection of instrument noise whilst maximising the detection of legitimate fluorescent signal.

Optimum AT values have previously been determined by the ISP laboratory for all Fusion 6C loci and set at 70 rfu. During the present study, reduced AT values were used to detect more of the DNA data. The values used have been provided within the relevant sections of this report. The optimum AT (70 rfu) determined by the ISP laboratory should be used during subsequent validation and casework use of STRmix™.

Stutter

Within STRmix™ V2.9.1 there is the ability to model any type of stutter a laboratory might observe within their casework profiles. This is referred to as *generalised stutter modelling*. The modelling of

these different stutter variants is informed by the analysis of stutter ratios for each stutter type from a laboratory's own empirical data.

Each type of stutter must be defined in relation to the stutter's parent peak; nomenclature has been developed and implemented within STRmix™ for this purpose. The relative position of a stutter product to its parent allele can be defined by the nomenclature (*i,j*), where *i* represents the number of whole STR units the stutter is located from the parent peak, and *j* represents any additional base pairs required to further define the location of the stutter. For example, (-1,0) describes a stutter product that is one whole repeat unit shorter than the parent allele (i.e. back stutter) whereas (-1,2) describes a stutter product that is two base pairs shorter than the parent allele (i.e. half back stutter).

Single-source profiles (124) were amplified for the purpose of determining suitable stutter models to implement within STRmix™. Six samples (Casework_1E.hid, Casework_2E.hid, Casework_4S.hid, Casework_8.hid, Casework_9.hid & Casework_12.hid) were excluded from this analysis as they did not appear to be single source.

The profiles were analysed using GeneMapper™ *ID-X* software v1.6 at 20rfu. Labels were retained for all allelic peaks. Labels were also retained for back stutter (-1,0), forward stutter (1,0), double back stutter (-2,0) and half back stutter (-1,2) at all loci.

There are three parameters used within STRmix™ to calculate expected stutter ratios. These parameters are optimised during the implementation phase of STRmix™ within a laboratory. The three parameters are the maximum allowable stutter ratio, an allele regression text (.txt) file, and, optionally, a stutter exceptions file (a .csv file). These parameters must be determined for each stutter variant being modelled.

The first parameter is the maximum allowable stutter ratio. The maximum allowable stutter ratio reduces interpretation run time by only permitting peaks in a stutter position below a certain stutter ratio to be considered as originating solely from stutter. It is important to reiterate that stutter is modelled in a fully continuous fashion within STRmix™; the maximum allowable stutter ratio threshold described above is only used prior to the MCMC process to eliminate unreasonable genotypes from being considered, thereby improving run time. If desired, no maximum stutter ratio thresholds can be set within STRmix™ however this will increase interpretation run time.

The second parameter used to model stutter within STRmix™ is a text file containing the regression parameters (slope and intercept) for each locus. These were determined by regressing *SR* against allelic designation using a linear least squares regression approach. The resulting model developed for each locus is $SR = m \times allele + c$, where 'allele' represents the allelic designation of the allele under consideration. This equation is used by STRmix™ to calculate per-allele expected stutter ratios based on allelic designation.

A better explanatory variable for stutter ratio for some loci with compound or complex repeat structures has been shown to be the longest uninterrupted stretch of common repeats (LUS) within the allele [1-3] rather than allelic designation. Values for LUS are determined by sequencing alleles. A number of common alleles for forensic loci have been sequenced. A summary of these appear on STRBase [4, 5]. To determine expected stutter ratios using the LUS model, observed *SR*s are regressed against the LUS designation of an allele rather than the allelic designation. The resulting model developed is $SR = m \times LUS + c$, where 'LUS' represents the LUS designation of the allele under

consideration. Expected stutter ratios are then calculated using this model and input into a file, referred to as a stutter exceptions file (a .csv file), which is the third parameter used to model stutter within STRmix™. When modelling stutter during deconvolution, STRmix™ will first refer to the stutter exceptions file to obtain the expected stutter ratio for a particular allele. If no exceptions file is saved or if a value for the observed allele is not present within the file (represented by a zero in the stutter exceptions file), STRmix™ will default to using the regression text file.

Some loci are not modelled well either using allelic designation or LUS. One approach for such loci is to calculate the average observed stutter ratio for each allele and input these values into the stutter exceptions file. In the present study, at least 3 observations were required to calculate the average observed *SR* for a given allele. An alternative approach is to model stutter ratio using a per-locus average. This can be implemented by setting the slope within the allele regression text file to zero and setting the intercept to equal the average observed *SR* across all alleles at the locus. This approach may be taken where SR appears to remain relatively constant across all alleles at the locus.

The resulting stutter models determined using the ISP dataset are described in turn for each of the stutter variants examined.

Back stutter (-1,0)

The largest back stutter ratio observed in the stutter data set was 0.2255 (22.55%) at locus D12S391. The maximum allowable back stutter ratio has been set to 0.30 (30%) for the ISP PowerPlex® Fusion 6C System 3500 STRmix™ kit.

Plots summarising the back stutter ratios observed are provided in Appendix 1. These plots were reviewed and used to determine the best explanatory variable to model expected *SR* for each locus. The decisions made with regards to the best explanatory variable selected are summarised in Table 1 below. A summary of the STRmix™ back stutter regression parameters for the ISP PowerPlex® Fusion 6C System 3500 data is provided in Table 2.

The filename for the back (-1,0) stutter regression file is *Idaho_Fusion6C_3500_Back Stutter Regression.txt.* A stutter exceptions file has also been prepared and will be used to model back stutter at those loci where LUS regression or allele average was chosen as the best explanatory variable. The stutter exceptions file is named, *Idaho_Fusion6C_3500_Back Stutter Exceptions.csv.*

Within STRmix™, back stutter peak height variability will be modelled as being *inversely proportional to the observed height of the parent allele*. Back stutter modelling has been enabled for all autosomal loci within the ISP PowerPlex® Fusion 6C System 3500 STRmix™ kit.

Table 1: Summary of best explanatory variable selected to model *SR* for back stutter within the ISP data for each of the Fusion 6C loci.

Locus	Best Explanatory Variable
D3S1358	Allele Average
D1S1656	LUS Regression
D2S441	Allele Average
D10S1248	Allele Regression
D13S317	Allele Regression
Penta E	Allele Regression
D16S539	Allele Regression
D18S51	Allele Regression
D2S1338	Allele Average
CSF1PO	Allele Regression
Penta D	Allele Average
TH01	LUS Regression
vWA	Allele Regression
D21S11	Allele Average
D7S820	Allele Regression
D5S818	Allele Regression
TPOX	Allele Regression
D8S1179	Allele Average
D12S391	Allele Regression
D19S433	Allele Average
SE33	Allele Average
D22S1045	Allele Regression
DYS391	N/A
FGA	Allele Regression
DYS576	N/A
DYS570	N/A

Table 2: Back stutter (-1,0) regression parameters used to model expected stutter ratio based on allelic designation. The model used to calculate expected stutter ratios within STRmix™ is $SR = m \times allele + c$, where 'allele' is the allelic designation of the allele of interest.

Locus	Slope (*m*)	Intercept (*c*)
D3S1358	0.00856	-0.04866
D1S1656	0.00402	0.02366
D2S441	0.00029	0.04384
D10S1248	0.00975	-0.05715
D13S317	0.01017	-0.05865
Penta E	0.00375	-0.01473
D16S539	0.01188	-0.06289
D18S51	0.0076	-0.03295
D2S1338	0.00425	-0.00471
CSF1PO	0.01533	-0.10245
Penta D	0.00278	-0.01208
TH01	0.00137	0.01081
vWA	0.01215	-0.1265
D21S11	0.00582	-0.09423
D7S820	0.0101	-0.05068
D5S818	0.01099	-0.05879
TPOX	0.00559	-0.02359
D8S1179	0.0044	0.01619
D12S391	0.00993	-0.09959
D19S433	0.01016	-0.06904
SE33	0.00297	0.03714
D22S1045	0.01513	-0.138
DYS391	0	0
FGA	0.00723	-0.08497
DYS576	0	0
DYS570	0	0

To note, should an allele be observed in data where the expected stutter ratio is less than 0.001 (0.1%), STRmix™ will use the minimum stutter ratio of 0.001 (0.1%) within its calculations.

It is also important for analysts to be aware of the limitations of data modelling. Given the number of samples profiled and the range of coverage of alleles profiled it is hoped that the stutter ratios calculated are reflective of the typical behaviour of profiles observed in casework, however, as with any data set there may be instances when stutter ratios observed in casework differ slightly to what is expected. The variance parameters discussed further in the Model Maker section do allow for a degree of variation in the peak heights of stutter so in most instances this is accounted for by the modelling in STRmix™. However, there may be occasions where analysts should consider the stutter ratios observed relative to the plots displayed in the appendices of this document as well as the following factors discussed below.

Analysts should also carefully consider alleles that have either not been observed in the stutter data set or have a limited number of observations. If variants such as these are observed in evidence profiles this could lead to an elevated posterior mean stutter variance value and proposed genotype combinations and their respective weights should be considered carefully in relation to any data that is present in the stutter data set. Any alleles not observed in the data set could also behave 'off trend' and genotype weights at a locus where a less common allele is observed should be reviewed carefully along with the other diagnostics included in the STRmix™ report.

Another factor to consider is the existence of iso-alleles, specifically with vWA alleles 14 and 15, although other variants at other loci may exist. It has been observed there are at least two different 'populations' of alleles that exhibit different stutter ratios. This is likely due to different sequence variants of these alleles that stutter in different amounts due to different underlying repeat structure[1]. The allele regression variable has been selected to determine the expected vWA stutter ratio for ISP data. Through the modelling of this data and the known behaviour of the vWA 14 and 15 alleles, it appears to be a better predictor than using the LUS regression or allele average explanatory variable. Despite this, on occasion this may lead to potential over- or under-estimation of the expected stutter ratio for vWA 14 and 15 alleles. Analysts need to be aware of this when reviewing the STRmix™ interpretation of profiles containing these alleles.

Forward stutter (1,0)

The largest forward stutter ratio observed was 0.0873 (8.73%) at locus D22S1045. The maximum allowable forward stutter ratio has been set to 0.15 (15%) for the ISP PowerPlex® Fusion 6C System 3500 STRmix™ kit.

Plots summarising the forward stutter ratios observed are provided in Appendix 2. These plots were reviewed and used to determine the best explanatory variable to model expected *SR* for each locus. Allelic designation was noted to be a good predictor of *SR* for forward stutter at D22S1045; regression against allelic designation was selected to model forward stutter at this locus. Allelic designation was not observed to be a good predictor of *SR* for forward stutter for the remaining loci. For each of the remaining loci, the average observed forward stutter ratio across all alleles at the locus was calculated and will be used to model forward stutter peaks. A summary of the STRmix™ forward stutter regression parameters for the ISP PowerPlex® Fusion 6C System data is provided in Table 3.

The filename for the forward (1,0) stutter regression file is *Idaho_Fusion6C_3500_Forward Stutter Regression.txt.*

Within STRmix™, forward stutter peak height variability will be modelled as being *inversely proportional to the expected height of the stutter peak*. Forward stutter modelling has been enabled for all autosomal loci within the ISP PowerPlex® Fusion 6C System 3500 STRmix™ kit settings.

Table 3: Forward stutter (1,0) regression parameters used to model expected stutter ratio based on allelic designation. The model used to calculate expected stutter ratios within STRmix™ is $SR = m \times allele + c$, where 'allele' is the allelic designation of the allele of interest. Note that for those loci where slope = 0, the

[1] This is a known issue and is further explained on the STRmix™ support website.

expected stutter ratio is equal to the average observed per-locus stutter ratio or the minimum STRmix™ stutter ratio of 0.1% (i.e. the intercept value).

Locus	Slope (*m*)	Intercept (*c*)
D3S1358	0	0.01005
D1S1656	0	0.01147
D2S441	0	0.00942
D10S1248	0	0.00633
D13S317	0	0.00991
Penta E	0	0.01035
D16S539	0	0.01239
D18S51	0	0.01291
D2S1338	0	0.01306
CSF1PO	0	0.01572
Penta D	0	0.00988
TH01	0	0.02945
vWA	0	0.00904
D21S11	0	0.01243
D7S820	0	0.00788
D5S818	0	0.01169
TPOX	0	0.00516
D8S1179	0	0.0089
D12S391	0	0.01114
D19S433	0	0.00892
SE33	0	0.01021
D22S1045	0.00715	-0.06123
DYS391	0	0
FGA	0	0.0105
DYS576	0	0
DYS570	0	0

Half back stutter (-1,2)

Half back stutter was investigated at all loci, however, was only observed with any frequency at the D1S1656 and SE33 loci only (158 and 198 observations respectively, the next highest observed locus was D21S11 with 8 observations). The largest half back stutter ratio observed was 0.0656 (6.56%) at locus SE33. The maximum allowable half back stutter ratio has been set to 0.1 (10%) for the ISP PowerPlex® Fusion 6C System 3500 STRmix™ kit.

Plots summarising the half back stutter ratios observed are provided in Appendix 3. These plots were reviewed and used to determine the best explanatory variable to model expected *SR* for each locus. Allelic designation was not observed to be a good predictor of *SR* for half back stutter for the D1S1656 or SE33 loci. Similar to forward stutter, the average observed half back *SR* was calculated at each locus across all alleles and will be used to model half back stutter. A summary of the STRmix™ half back

stutter regression parameters for ISP 3500 data is provided in Table 4. The filename for the half back (-1,2) stutter regression file *Idaho_Fusion6C_3500_Half Back Stutter Regression.txt*

Within STRmix™, half back stutter peak height variability will be modelled as being *inversely proportional to the expected height of the stutter peak*. Half back stutter modelling has only been enabled at the SE33 and D1S1656 loci within the ISP PowerPlex® Fusion 6C System 3500 STRmix™ kit.

Double back stutter (-2,0)

The largest double back stutter ratio observed was 0.0257 (2.57%) at locus D12S391. The maximum allowable double back stutter ratio has been set to 0.05 (5%) for the ISP PowerPlex® Fusion 6C System 3500 STRmix™ kit.

Plots summarising the double back stutter ratios observed are provided in Appendix 4 (there are no plots for loci where no data was observed). These plots were reviewed and used to determine the best explanatory variable to model expected SR for each locus. Allelic designation was noted to be a good predictor of *SR* for double back stutter at D12S391; regression against allelic designation was selected to model double back stutter at this locus. Allelic designation was not observed to be a good predictor of *SR* for double back stutter for the remaining loci. For each of the remaining loci, the average observed double back stutter ratio across all alleles at the locus was calculated and will be used to model double back stutter peaks. No double back stutter data was observed at the Penta D locus however, double back stutter modelling will still be enabled at this locus using the minimum SR in STRmix™ (0.1%). A summary of the STRmix™ double back stutter regression parameters for the ISP PowerPlex® Fusion 6C System data is provided in Table 5.

Within STRmix™, double back stutter peak height variability will be modelled as being *inversely proportional to the expected height of the stutter peak*. Double back stutter modelling has been enabled for all autosomal loci within the ISP PowerPlex® Fusion 6C System 3500 STRmix™ kit settings.

The filename for the double back (-2,0) stutter regression file is *Idaho_Fusion6C_3500_Double Back Stutter Regression.txt*

Table 4: Half back stutter (-1,2) regression parameters used to model expected stutter ratio based on allelic designation. Half back stutter modelling will only be enabled at the D1S1656 and SE33 loci.

Locus	Slope	Intercept
D3S1358	0	0
D1S1656	0	0.01718
D2S441	0	0
D10S1248	0	0
D13S317	0	0
Penta E	0	0
D16S539	0	0
D18S51	0	0
D2S1338	0	0
CSF1PO	0	0
Penta D	0	0
TH01	0	0
vWA	0	0
D21S11	0	0
D7S820	0	0
D5S818	0	0
TPOX	0	0
D8S1179	0	0
D12S391	0	0
D19S433	0	0
SE33	0	0.04408
D22S1045	0	0
DYS391	0	0
FGA	0	0
DYS576	0	0
DYS570	0	0

Table 5: Double back stutter (-2,0) regression parameters used to model expected stutter ratio based on allelic designation. The model used to calculate expected stutter ratios within STRmix™ is $SR = m \times allele + c$, where 'allele' is the allelic designation of the allele of interest. Note that where slope = 0, the expected stutter ratio will be equal to the average observed per-locus stutter ratio (i.e. the intercept value).

Locus	Slope (*m*)	Intercept (*c*)
D3S1358	0	0.00727
D1S1656	0	0.00883
D2S441	0	0.00399
D10S1248	0	0.00684
D13S317	0	0.00402
Penta E	0	0.00322
D16S539	0	0.00759
D18S51	0	0.01009
D2S1338	0	0.00973
CSF1PO	0	0.00785
Penta D	0	0
TH01	0	0.00556
vWA	0	0.00571
D21S11	0	0.00676
D7S820	0	0.00437
D5S818	0	0.00561
TPOX	0	0.00236
D8S1179	0	0.00587
D12S391	0.00153	-0.02042
D19S433	0	0.00552
SE33	0	0.01024
D22S1045	0	0.00907
DYS391	0	0
FGA	0	0.00913
DYS576	0	0
DYS570	0	0

Drop-in parameters

Drop-in is the observation of non-reproducible, unexplained peaks within a DNA profile. Drop-in rates for a laboratory platform (multiplex and instrument combination) should be monitored. This can be done by recording counts and corresponding heights of drop-in peaks observed in negative controls along with a count of the total number of negative controls examined.

There are four parameters used for the modelling of drop-in in STRmix™. These are:

1. Z: the analytical/detection threshold
2. A cap on the maximum allowable height per drop-in peak
3. The drop-in frequency
4. α,β: two parameters that define a gamma distribution

The ISP laboratory has not observed sufficient drop-in peaks within their Fusion 6C profiles to model observed drop-in rates. A uniform prior distribution has been chosen, applying a consistent probability of drop-in between AT and the drop-in cap. ISP's remaining drop-in parameters were provided by the STRmix scientific support team based on their experience of similar platforms (kit, cycle and CE). The settings displayed in Table 7 are proposed as the inaugural drop-in settings to be used within the laboratory for this kit, CE and cycle number combination. These will be reviewed as part of the sensitivity and specificity studies and may be updated (subject to performance check) when sufficient negative control data has been recorded.

Table 1: ISP laboratory drop-in parameters for 29 cycle Fusion 6C data separated using a 3500 genetic analyser with 15 second injection time.

Drop-in cap	150 rfu
Drop-in frequency	0.0001
Drop-in parameters α, β	0,0 (Uniform)

Saturation

The peaks in a DNA profile are measured using fluorescence. The amount of fluorescence is proportional to the amount of DNA present. This fluorescence is captured by a camera. It is expected that as more DNA is added into a PCR the resulting peak height (measured in relative fluorescent units) in an electropherogram will increase. The camera can become saturated when there is too much fluorescence detected. This means it is no longer possible to accurately measure the height of the peaks observed or estimate how much DNA is really represented by this result. Following this it is no longer possible to accurately model saturated peak heights using STRmix™. Saturation is mostly instrument related and not kit or method dependent.

Based on work undertaken as part of previous validations, a saturation threshold of 30,000 rfu has been selected for use within the ISP Laboratory. See "Estimation of STRmix™ V2.8 parameters for the Idaho State Police Laboratory (Fusion 5C 3500)" documentation for more information.

Peak height variance and LSAE using Model Maker

Empirical observations and experience suggest that profiles differ in variance (hereafter, "quality"). Within STRmix™ the variability of peaks within profiles is described using a model containing a variance constant. Allele and stutter peaks have separate variances; c^2 and k^2, respectively. Furthermore, each stutter variant being modelled has its own k^2 variance constant. The c^2 and k^2 terms are variables which are determined after sampling from a gamma distribution within the MCMC.

The prior gamma distributions that are sampled from during an interpretation are optimised using the Model Maker functionality of STRmix™. Model Maker works by using a component-wise MCMC. In component 1 each DNA profile has its mass parameters optimised and uses a stable gamma distribution for allele, stutter, and LSAE variance constants. In component 2 the mass parameters for

each profile are held constant and the hyper parameters for each gamma distribution are varied. Components are 1000 accepts long and they cycle through a number of times depending on the user input value.

Single-source PowerPlex® Fusion 6C profiles (100) of varying quality (i.e. template amount) were prepared by ISP. Input template amount ranged from 0.0078 ng to 4.0 ng. The dataset used is expected to be indicative of the peak height variability likely to be encountered in casework DNA profiles. Following amplification and CE, all profiles were analysed using GeneMapper® *ID-X* V1.6. A reduced AT of 20 rfu (all dye channels) was used to maximise the stutter information detected. Labels were retained for all allelic peaks. Labels were retained for back stutter (-1,0), forward stutter (1,0), and double back stutter (-2,0) at all loci, and half back stutter (0,-2) at the SE33 and D1S1656 loci. Following analysis, the Model Maker functionality of STRmix™ was used to assess peak height variability within the dataset. Two hundred component-wise cycles were used during the analysis (200,000 accepts total). A summary of the Model Maker results is given in Table 9. These results include parameters (α, β) that describe the prior gamma distributions for allele and stutter peak height variance and the mean that of the prior exponential distribution for LSAE variance. Plots of the prior gamma distributions and LSAE variance distribution developed are provided in Figure 3 to Figure 6 below.

Table 2: Summary of Model Maker results for the ISP dataset (PowerPlex® Fusion 6C 29 cycle 3500 CE data).

Number of profiles analysed	Allele variance parameters: α, β (mode)	Back stutter variance parameters: α, β (mode)	Forward stutter variance parameters: α, β (mode)	Half back stutter variance parameters: α, β (mode)	Double back stutter variance parameters: α, β (mode)	Mean LSAE variance
95[2]	5.631, 1.203 (5.571)	1.511, 13.111 (6.700)	1.624, 18.489 (11.537)	1.520, 3.814 (1.983)	2.218, 5.548 (6.757)	0.004

[2] 5 out of the 100 profiles were not utilised in the Model Maker run, due to them containing at least one peak with a height above the saturation threshold of 30,000 rfu.

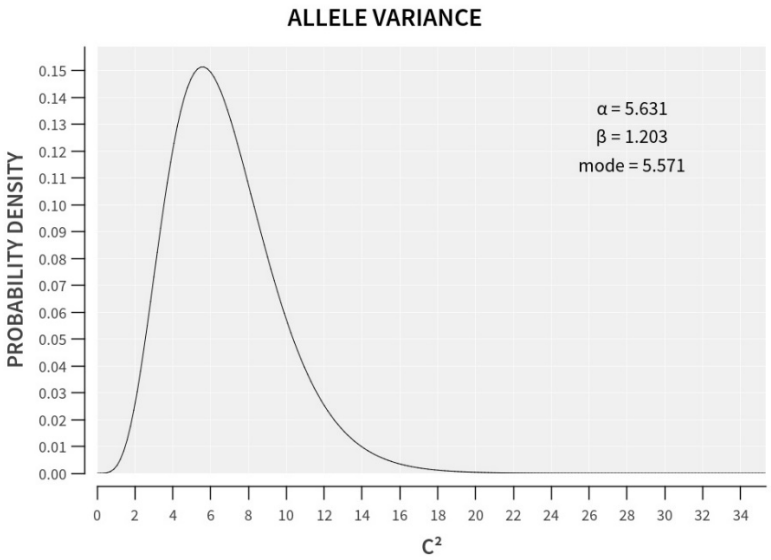


Figure 1: Allelic peak height variance prior gamma distribution determined using the Model Maker functionality of STRmix™ for the ISP dataset (PowerPlex® Fusion 6C 29 cycle 3500 CE data).

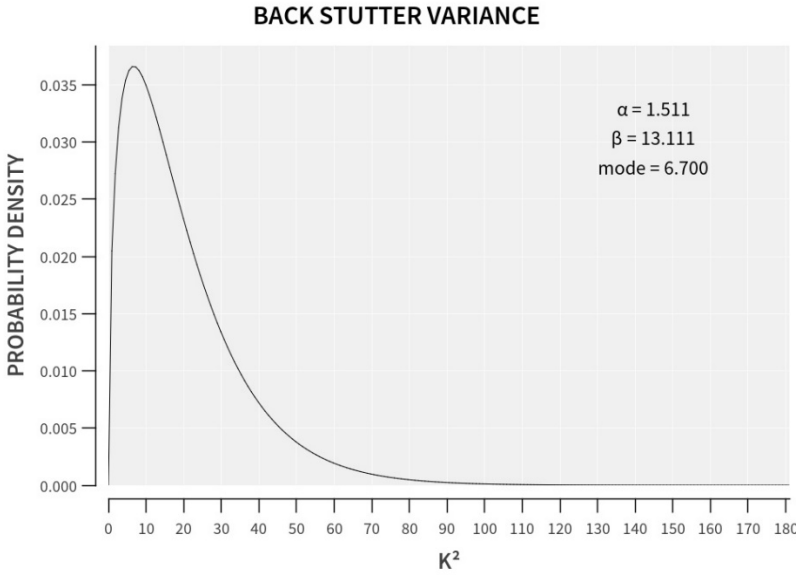


Figure 2: Back stutter (-1,0) peak height variance prior gamma distribution determined using the Model Maker functionality of STRmix™ for the ISP dataset (PowerPlex® Fusion 6C 29 cycle 3500 CE data).

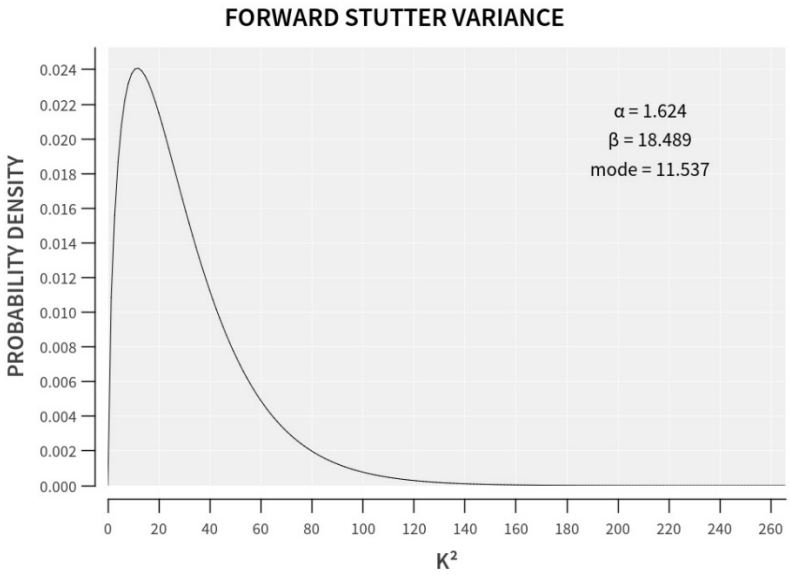


Figure 3: Forward stutter (1,0) peak height variance prior gamma distribution determined using the Model Maker functionality of STRmix™ for the ISP dataset (PowerPlex® Fusion 6C 29 cycle 3500 CE data).

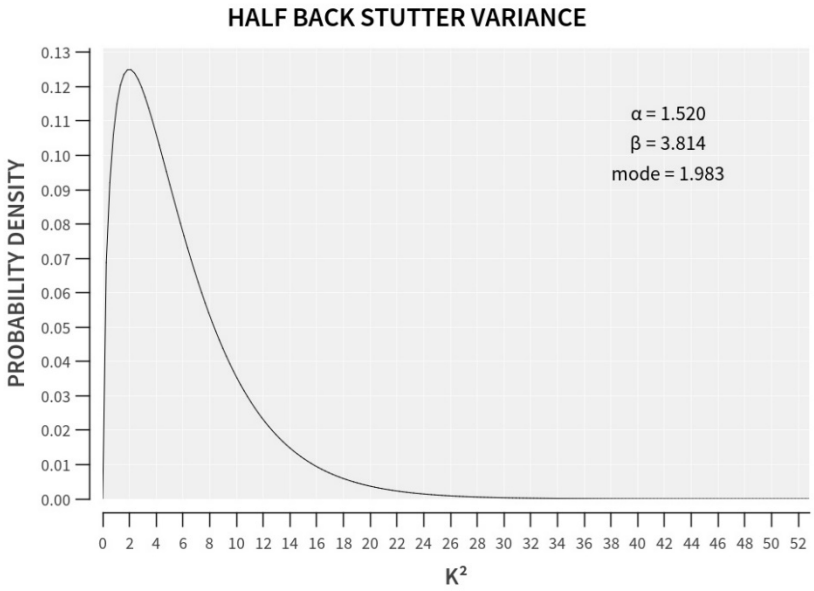


Figure 4: Half back stutter (0,-2) peak height variance prior gamma distribution determined using the Model Maker functionality of STRmix™ for the ISP dataset (PowerPlex® Fusion 6C 29 cycle 3500 CE data).

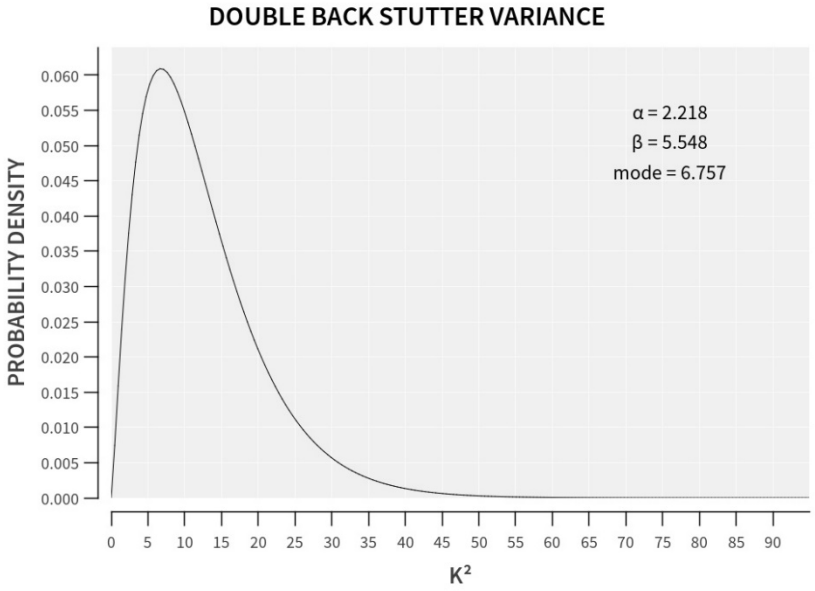


Figure 5: Double back stutter (-2,0) peak height variance prior gamma distribution determined using the Model Maker functionality of STRmix™ for the ISP dataset (PowerPlex® Fusion 6C 29 cycle 3500 CE data).

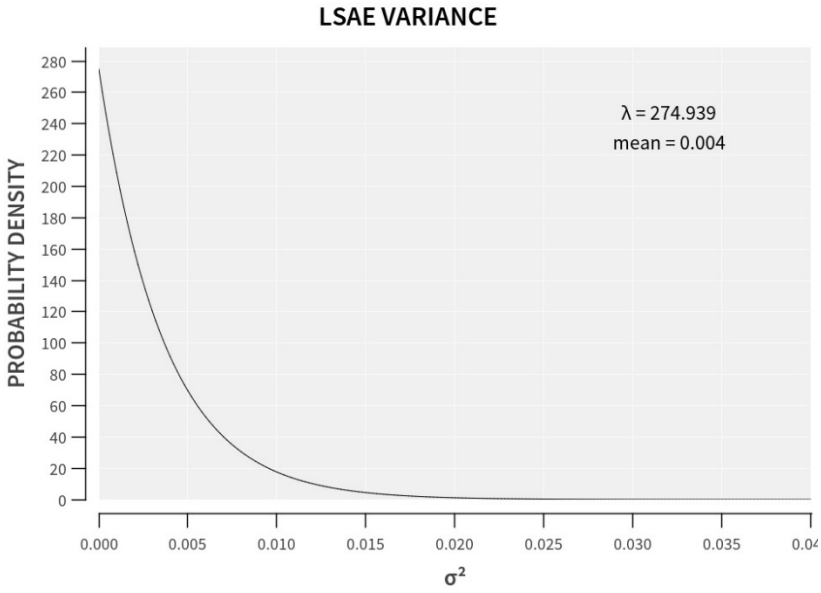


Figure 6: Locus Specific Amplification Efficiency variance prior exponential distribution determined using the Model Maker functionality of STRmix™ for the ISP dataset (PowerPlex® Fusion 6C 29 cycle 3500 CE data).

The diagnostics output by Model Maker were also reviewed. In particular, the correlation plots were examined and assessed. These plots are reproduced in Figure 9 to Figure 13 below. No obvious correlation was observed (the desired result). However, three outliers (present in the top right

quadrant of the back stutter correlation plot) were observed and investigated further. They were all found to be due to a higher-than-expected stutter of a Penta E 5 allele in samples DNA9_500pg, DNA9_1ng, and DNA9_2ng. Given the 5 allele peak heights (respectively 2351 rfu, 2858 rfu, and 4450 rfu) and an expected stutter ratio for a 5 allele of 0.00402 – obtained from the ISP stutter regression file – STRmix™ would expect a stutter peak with height 9 rfu, 11 rfu, and 17 rfu, respectively. However, the observed heights were 33 rfu, 110 rfu, and 199rfu. In general, the 5 allele is a rather rare allele – with only 3 observations in the stutter dataset – and the instance of the higher stutter ratio was only observed in samples with higher DNA input amount. STRmix™ is able to account for some degree of difference in the expected and observed peak heights however, scenarios such as described above with the Penta E 5 allele may lead to potential under estimation of peak heights and influence genotype weights. Similar to, the known behaviour of vWA 14 and 15 alleles, analysts should be aware of this when reviewing the STRmix™ interpretation of profiles containing Penta E 5 alleles.

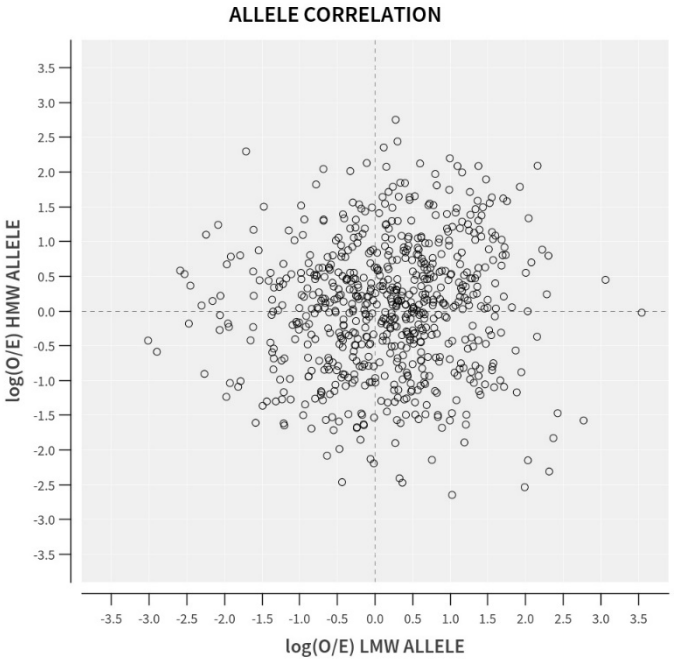


Figure 7: STRmix™ Model Maker correlation plot for allelic peaks.

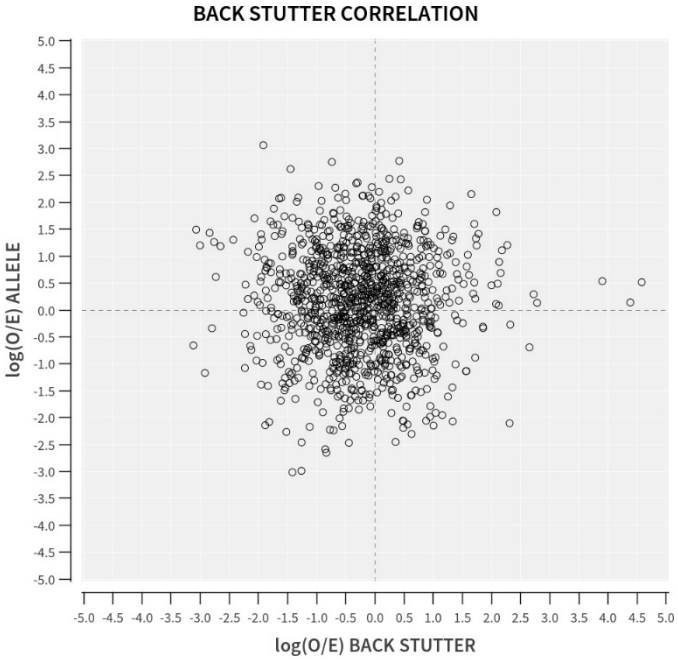


Figure 8: STRmix™ Model Maker correlation plot for back stutter (-1,0) peaks.

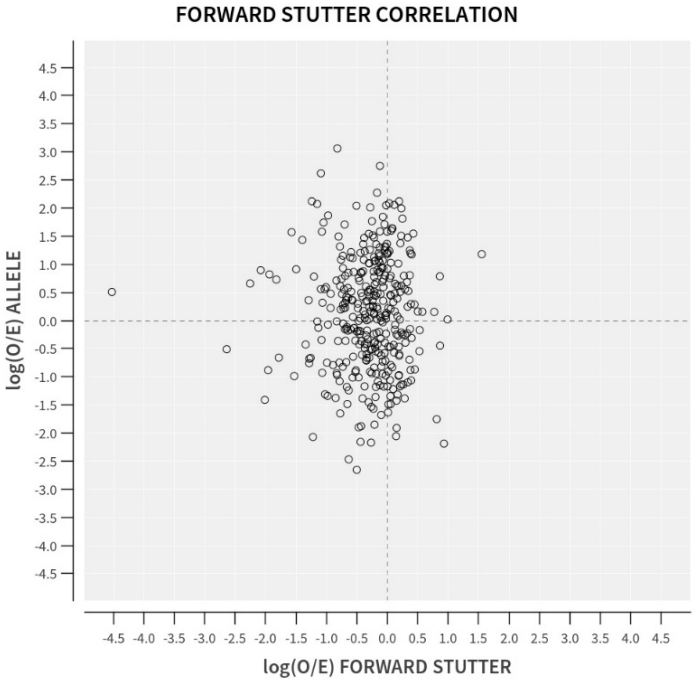


Figure 9: STRmix™ Model Maker correlation plot for forward stutter (1,0) peaks.

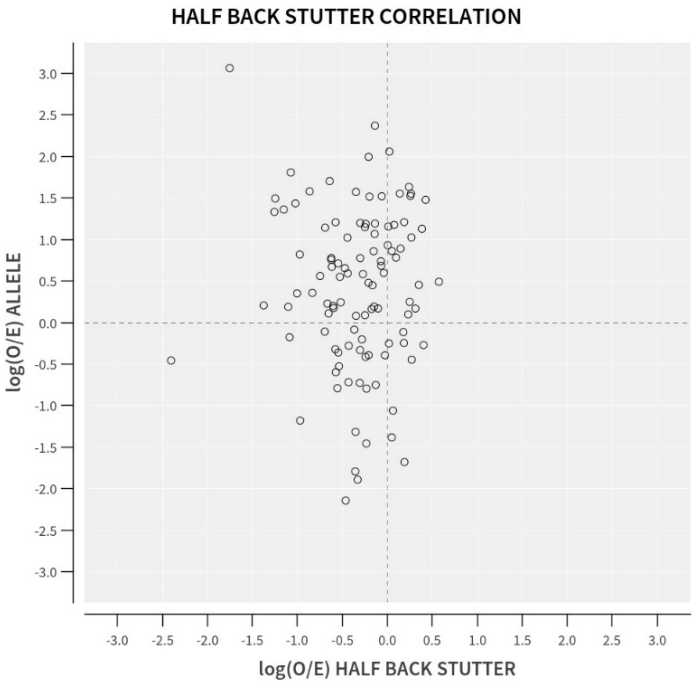


Figure 10: STRmix™ Model Maker correlation plot for half back stutter (0,-2) peaks.

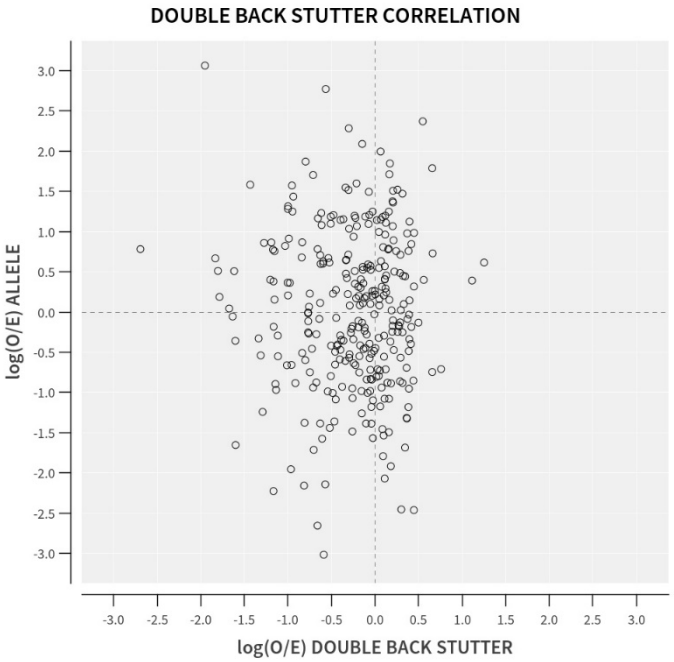


Figure 11: STRmix™ Model Maker correlation plot for double back stutter (-2,0) peaks.

As a final check of the variance parameters determined, heterozygote balance was calculated for all heterozygous loci within the Model Maker dataset. Heterozygote balance (*Hb*) was calculated as:

$$Hb = \frac{O_{HMW}}{O_{LMW}}$$

Where O_{HMW} refers to the observed height of the high molecular weight allele and O_{LMW} refers to the observed height of the low molecular weight allele. Previous work has suggested that there is a relationship between the variation in peak height and the variation in *Hb* [6, 7]. In single-source profiles, variability in *Hb* reduces as the average peak height (APH) at a locus increases. The variance of *Hb* can be used as a proxy for the variance of individual peaks. This allows an approximate comparison between the variance from the STRmix™ MCMC approach and a readily determined variable from empirical data (*Hb*).

A plot of log(*Hb*) versus APH (the black circles) for the Model Maker dataset is provided in Figure 14 below. The expected 95% bounds are indicated within the plot using red dashed lines. The bounds were calculated as $\pm\sqrt{2}\times 1.96\times\sqrt{\frac{c^2}{APH}}$, where c^2 = 8.42 is the 75th percentile from the allele peak height variance prior gamma distribution. Under the assumption of a normal distribution, it is expected that ~95% of data points will fall within +/- 2 standard deviations (95% bounds) of the mean. For this dataset, the 95% bounds encapsulate sufficient data (coverage = 96.5%) demonstrating that the values for variance appear sufficiently optimised.

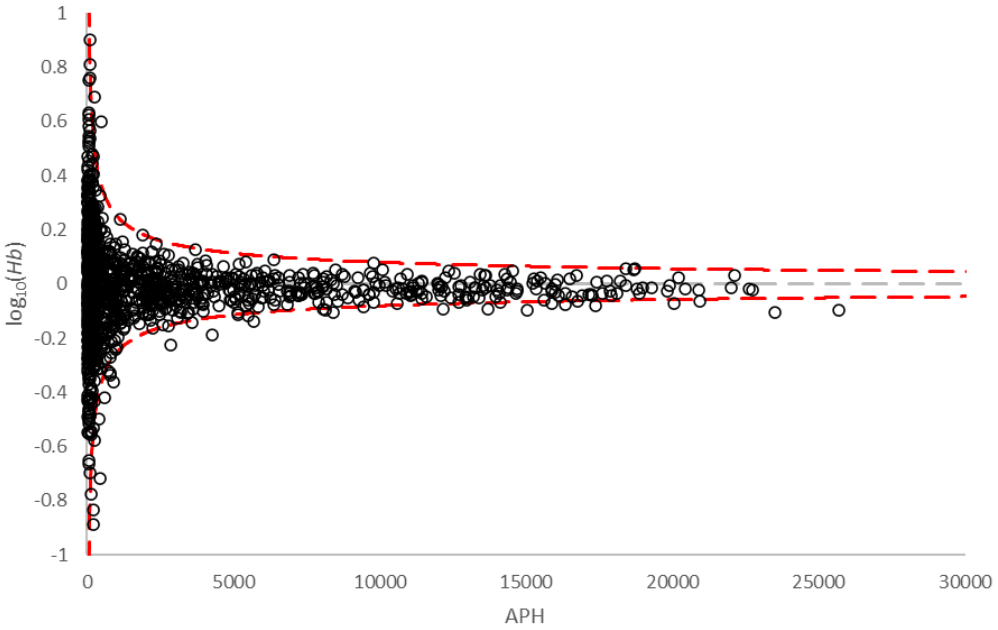


Figure 12: Plot of log(*Hb*) versus APH for single-source profiles from the ISP Model Maker dataset. The red dashed lines indicate the 95% bounds.

Kit settings

The recommended STRmix™ V2.9.1 default parameters for the interpretation of 29 cycle PowerPlex® Fusion 6C profiles analysed on a 3500 CE instrument with a 1.2 kV/15 s injection protocol within ISP are given in Figure 13 to Figure 18.

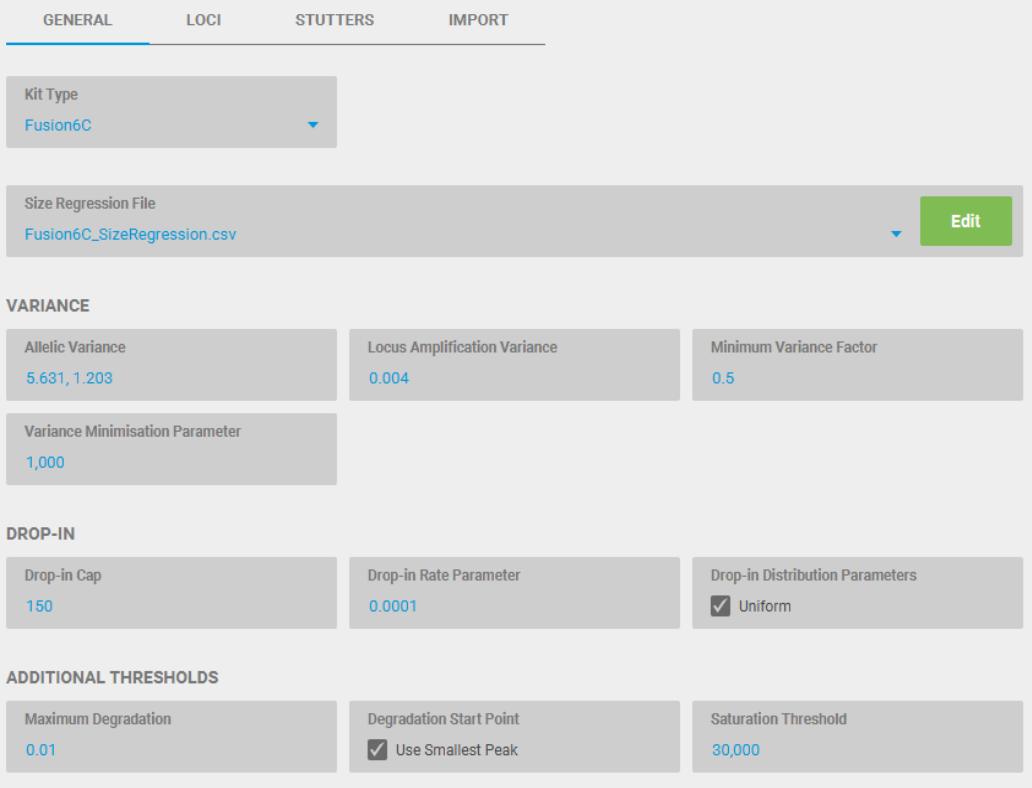


Figure 13: STRmix™ kit settings for 29 cycle PowerPlex® Fusion 6C profiles separated on a 3500 CE instrument within ISP. General kit settings shown.

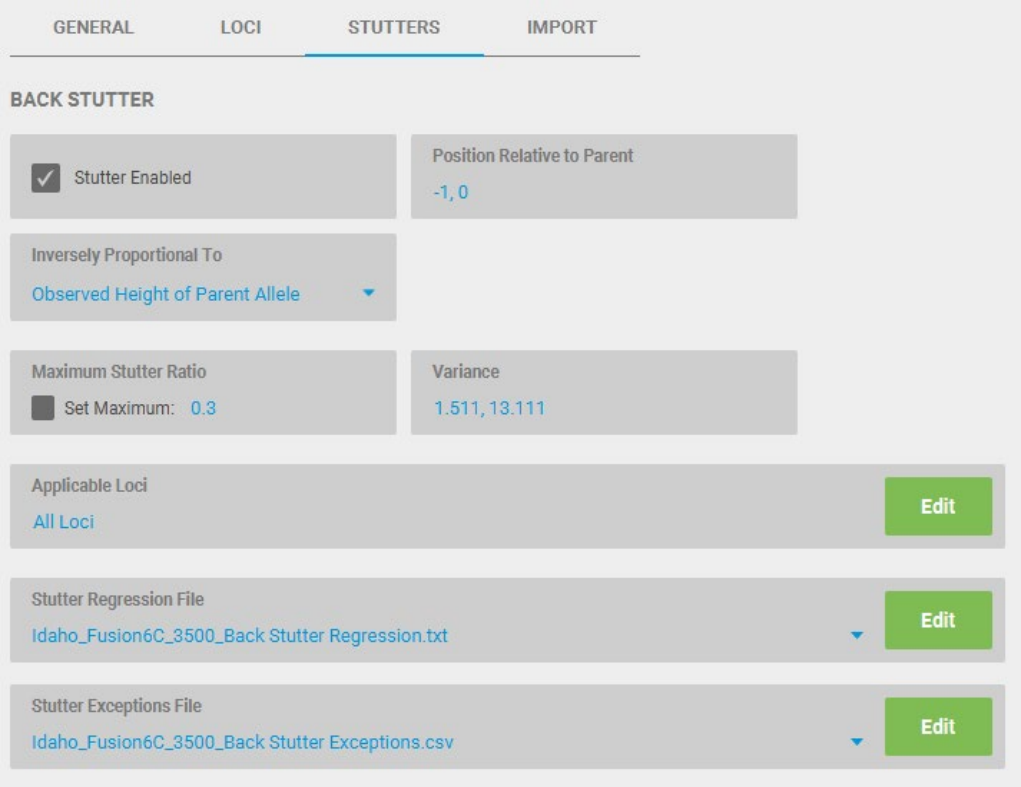


Figure 14: STRmix™ kit settings for 29 cycle PowerPlex® Fusion 6C profiles separated on a 3500 CE instrument within ISP. Back stutter settings shown.

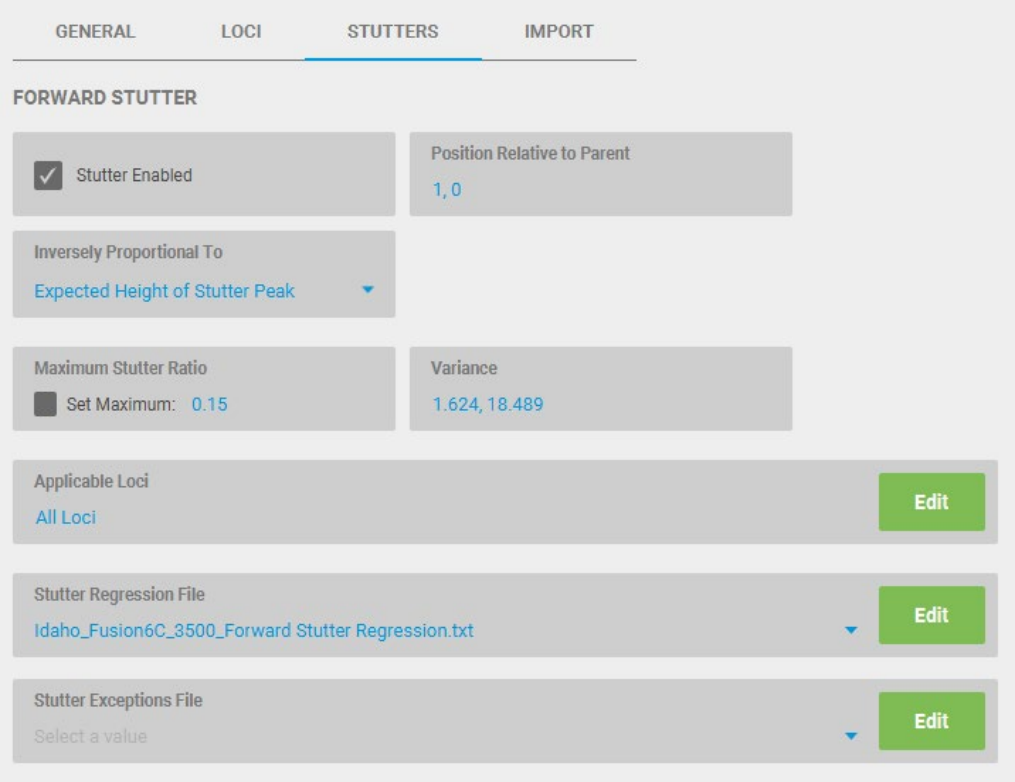


Figure 15: STRmix™ kit settings for 29 cycle PowerPlex® Fusion 6C profiles separated on a 3500 CE instrument within ISP. Forward stutter settings shown.

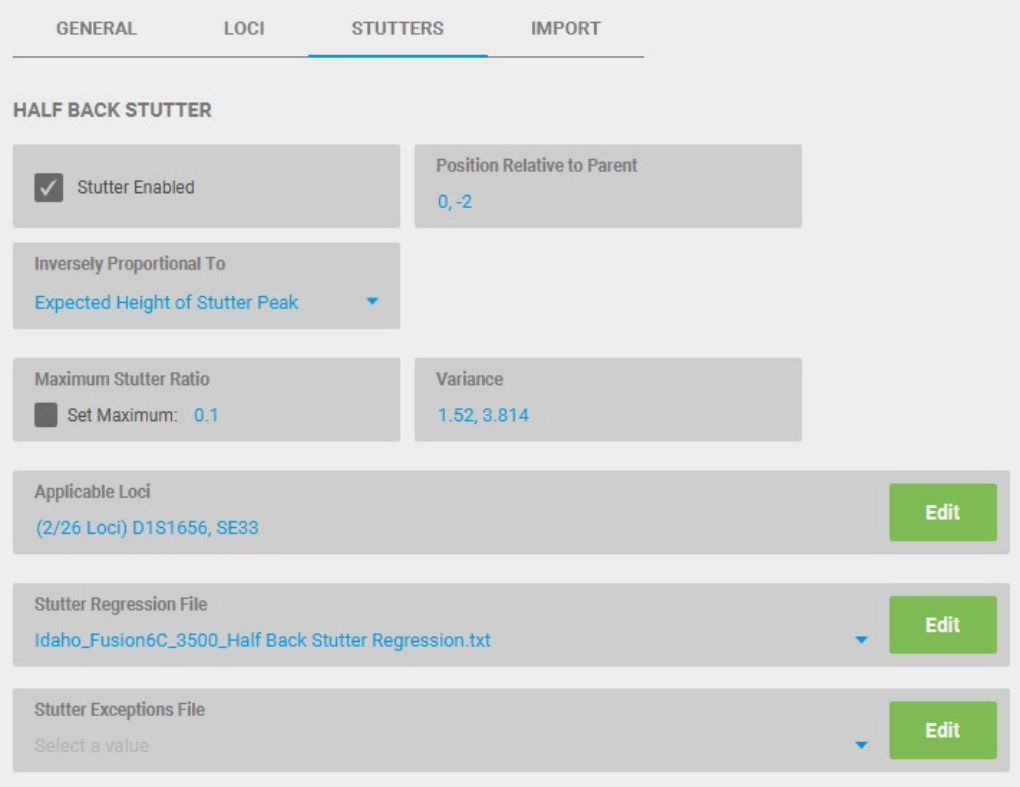


Figure 16: STRmix™ kit settings for 29 cycle PowerPlex® Fusion 6C profiles separated on a 3500 CE instrument within ISP. Half back stutter settings shown.

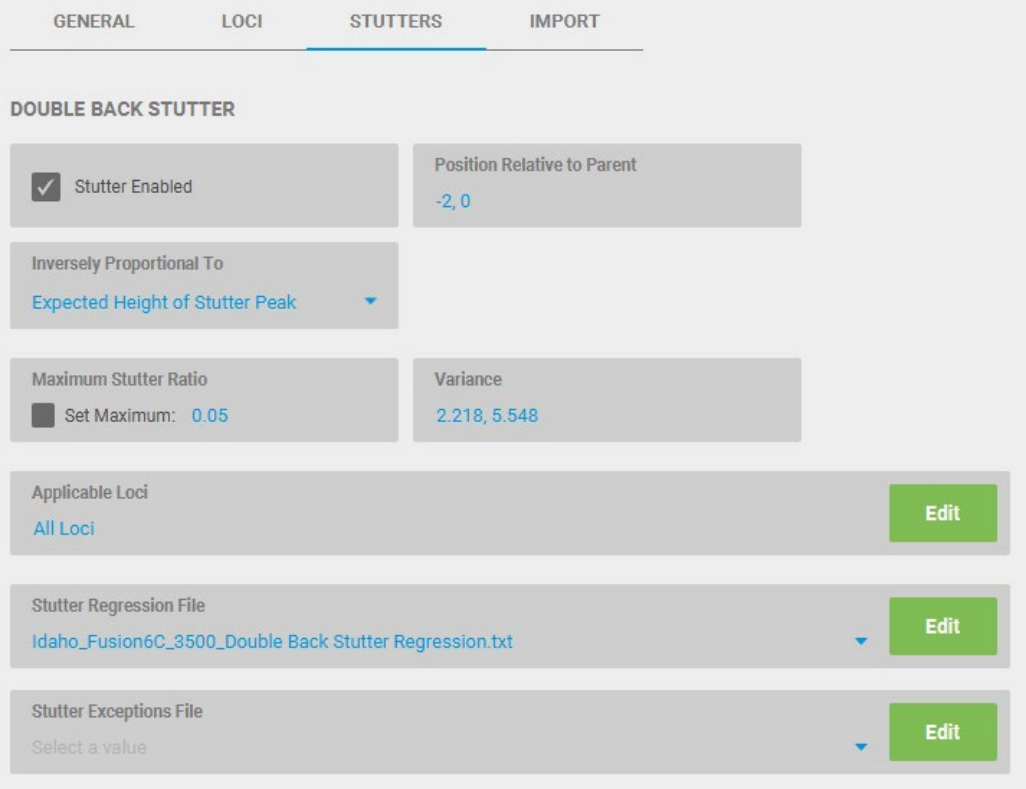


Figure 17: STRmix™ kit settings for 29 cycle PowerPlex® Fusion 6C profiles separated on a 3500 CE instrument within ISP. Double back stutter settings shown.

GENERAL | LOCI | STUTTERS | IMPORT

LOCUS NAME	GENDER?	REPEAT LENGTH	IGNORE?	DETECTION THRESHOLD
AMEL	☑			
D3S1358	☐	4	☐	70
D1S1656	☐	4	☐	70
D2S441	☐	4	☐	70
D10S1248	☐	4	☐	70
D13S317	☐	4	☐	70
Penta E	☐	5	☐	70
D16S539	☐	4	☐	70
D18S51	☐	4	☐	70
D2S1338	☐	4	☐	70
CSF1PO	☐	4	☐	70
Penta D	☐	5	☐	70
TH01	☐	4	☐	70
vWA	☐	4	☐	70
D21S11	☐	4	☐	70
D7S820	☐	4	☐	70
D5S818	☐	4	☐	70
TPOX	☐	4	☐	70
D8S1179	☐	4	☐	70
D12S391	☐	4	☐	70
D19S433	☐	4	☐	70
SE33	☐	4	☐	70
D22S1045	☐	3	☐	70
DYS391	☐	4	☑	70
FGA	☐	4	☐	70
DYS576	☐	4	☑	70
DYS570	☐	4	☑	70

Figure 18: STRmix™ kit settings for 29 cycle PowerPlex® Fusion 6C profiles separated on a 3500 CE instrument within ISP. Locus settings shown.

Performance check

To demonstrate the suitability of STRmix™ V2.9.1 and the kit parameters for the interpretation of Fusion 6C profiles generated within ISP laboratory performance checks were undertaken investigating the behaviour of the *LR* using a range of mixed DNA profiles. This is referred to as Section D, sensitivity and specificity, in the original ISP validation of STRmix™.

Section D: Sensitivity and specificity of mixed DNA profiles

This section covers the following recommendations:

4.1.2. Hypothesis testing with contributors and non-contributors

4.1.6. Mixed specimens

4.1.6.1. Various contributor ratios (e.g., 1:1 through 1:20, 2:2:1, 4:2:1, 3:1:1, etc.)

4.1.6.2. Various total DNA template quantities

4.1.6.3. Various numbers of contributors. The number of contributors evaluated should be based on the laboratory's intended use of the software. A range of contributor numbers should be evaluated in order to define the limitations of the software

4.1.6.5. Sharing of alleles among contributors

4.1.7. Partial profiles, to include the following:

4.1.7.1. Allele and locus drop-out

4.1.13. Sensitivity, specificity and precision, as described for Developmental Validation

A demonstration of sensitivity and specificity for a range of mixtures prepared by the Idaho Laboratory was undertaken as per [8].

With respect to interpretation methods, sensitivity is defined as the ability of the software to reliably resolve the DNA profile of the known contributor(s) to a DNA profile for a range of starting DNA templates. The log(*LR*) for known contributors (i.e. H_p true) should be high and should trend to 0 as less information is present within the profile. In this context, 'information' includes the amount of DNA from the contributor of interest, the use of conditioning profiles during interpretation (for example, the complainant's DNA on intimate samples), the use of PCR replicates, and decreased profile complexity. Specificity is defined as the ability of the software to reliably exclude non-contributors (i.e. H_d true) within a DNA profile for a range of starting DNA templates. The log(*LR*) should trend upwards to 0 as less information is present within the profile.

A series of mixed DNA profiles ranging from two to four contributors was prepared by the Idaho State Police Laboratory. These mixtures cover a broad range of template amounts and mixture proportions and are likely to be representative of DNA profiles recovered during casework analysis. The

contributors include homozygous and heterozygous alleles and there are varying amounts of allele sharing across the different loci (recommendation 4.1.6.5). Given the template amounts, allele and/or locus dropout was expected to occur within the profiles containing lower DNA amounts (recommendation 4.1.7.1). In total, fifty-eight mixtures were prepared and run in duplicate[3]. Following amplification and CE, the profiles were analysed within GeneMapper® *ID-X* V1.6 using the ISP Laboratory's Fusion 6C™ casework analysis method for 3500 CE data.

Following analysis, each mixture was interpreted within STRmix™. The experimental number of contributors (NOC) was used when setting up the interpretations. Likelihood ratios were assigned to true and non-contributors by searching each deconvolution against a database that contained the DNA profiles of the known donors as well as 500 non-contributor profiles. The non-contributor profiles were simulated from the NIST Caucasian allele frequencies. An *LR* was assigned for each database individual considering the following propositions:

H_p: The DNA originates from the database individual and *N*-1 unknown, unrelated individuals

H_d: The DNA originates from *N* unknown, unrelated individuals

Where *N* is the experimental NOC. *LR*s were assigned using the NIST Caucasian allele frequencies with θ = 0.01 and the sub-source *LR* used as the point of comparison.

Plots of log(*LR*) versus STRmix posterior mean DNA template amount (rfu) for the two-, three-, and four-person mixtures are given Figure 19. Each plot has been reproduced with the scale of the x-axis adjusted to better display data points for low template (template ≤ 500 rfu). These plots follow the approach used in [8] but STRmix™ reported template rather than PCR input template was used. For non-contributors, the log(*LR*) was plotted against the lowest template across all known donors to a mixture. Exclusions (*LR* = 0) are plotted as log(*LR*) = -40.

Inspection of the plots in Figure 19 shows that as template increases, the *LR*s assigned for known donors and non-contributors diverge. As template decreases, *LR*s for known donors and non-contributors trend to log(*LR*) = 0. A log(*LR*) of zero may be considered to be 'uninformative', or 'neutral'. The plots in Figure 19 demonstrate that STRmix™ was able to reliably distinguish between true donors and non-contributors, even where per contributor template was relatively low.

The plots in Figure 19 can help inform the limits of STRmix™, particularly the lower limit of DNA where an H_p true hypothesis still results in a log(*LR*) greater than 0 and the limit where false positives may arise (a log(*LR*) greater than 0 where H_d is true).

[3] Profile 10.1_C1 failed to run due to an apparent pull-up peak at the SE33 locus. In this instance this profile could not be run under the assumption of two contributors.

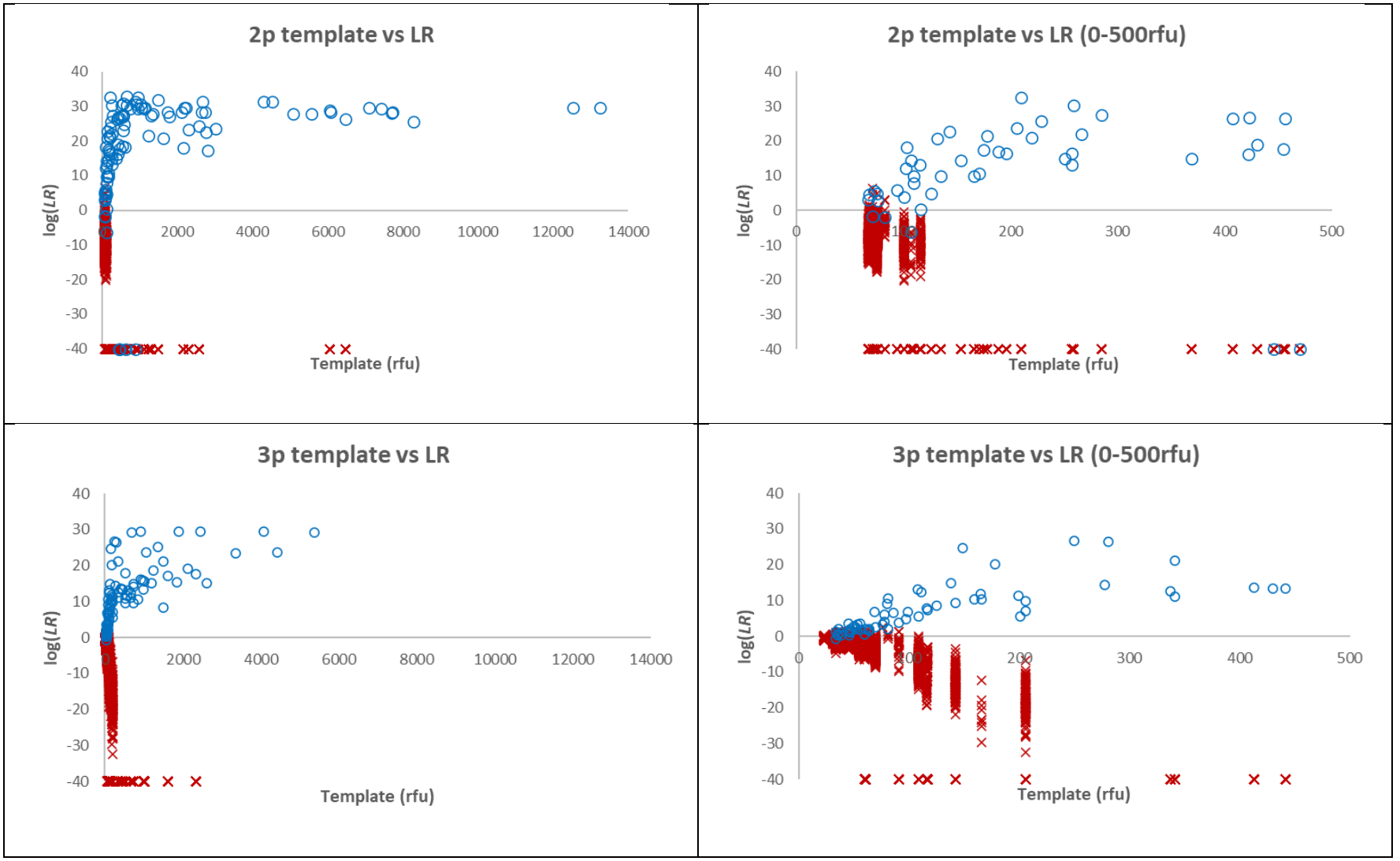


Figure 19: Log(*LR*) versus STRmix™ posterior mean template (rfu) for known donors (plotted using circles) and non-contributors (plotted using crosses). Separate plots are provided for the two-, three-, and four-person mixtures examined. Each plot has been reproduced with the scale of the *x*-axis adjusted to better display data points for low-template contributors (Template ≤ 500 rfu).

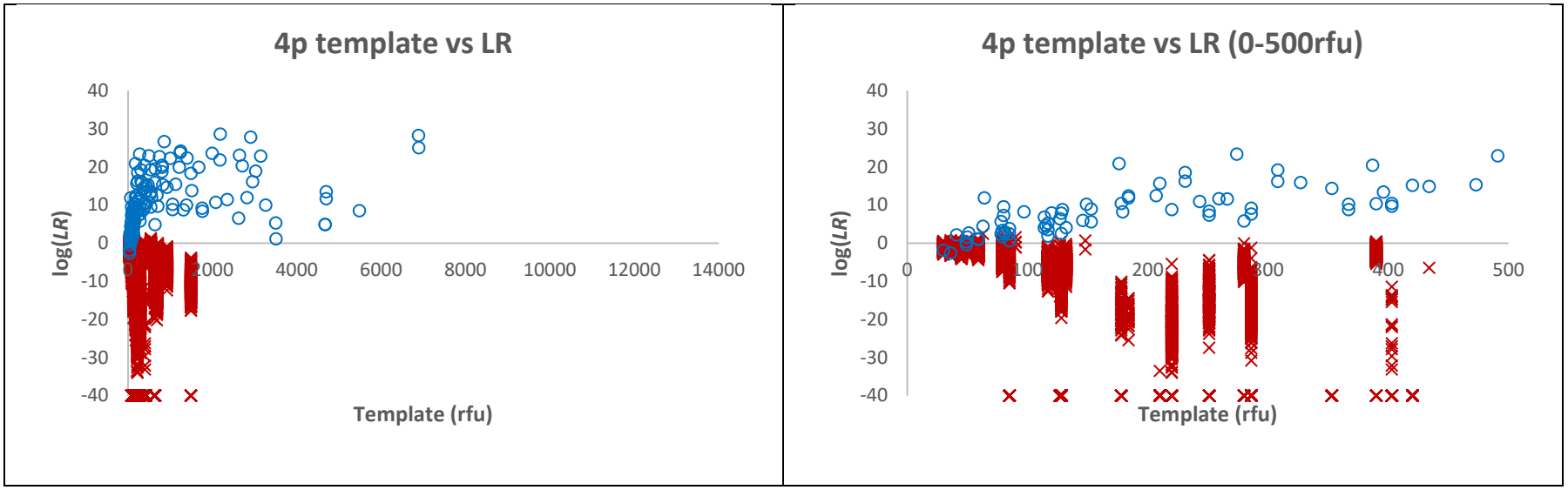


Figure 19 (continued).

Review of specific results

Four false exclusions were observed with the 2-person data. These were all due to retention of artefacts during GeneMapper™*ID-X* analysis.

These false exclusions were investigated by undertaking '*LR* from Previous' calculations that assigned a LR to the known contributor that had been excluded. Likelihood ratios of zero were observed at one or two loci when the majority of the other loci in each profile supported the inclusionary hypothesis. Typically, an LR of zero at just one locus can act as a diagnostic flag and indicate that the input file information requires further scrutiny [9]. Two of the four exclusions were single locus exclusion whilst the other two displayed *LR*s of zero at two loci, each of these is discussed below. Careful review of input data is recommended (either pre-deconvolution or when evaluating STRmix™ reports) as well as consideration of the 'Per Locus Likelihood Ratios' when comparisons and LR assignments undertaken.

A known contributor (100) to sample 10.1_C1(2) was excluded due to the retention of a pullup peak (38) at SE33 (Figure 20). This led to exclusion of Known 100 whose genotype 26.2, 29.2 could not be accepted under the assumption of two contributors. The deconvolution of this sample resulted in a high GR (2.78) and an elevated allele variance value likely due to the peak height imbalance between the 26.2 and the 38 peaks. An *LR* greater than zero (8.7676E33) was assigned when this pull-up peak was removed and the interpretation repeated.

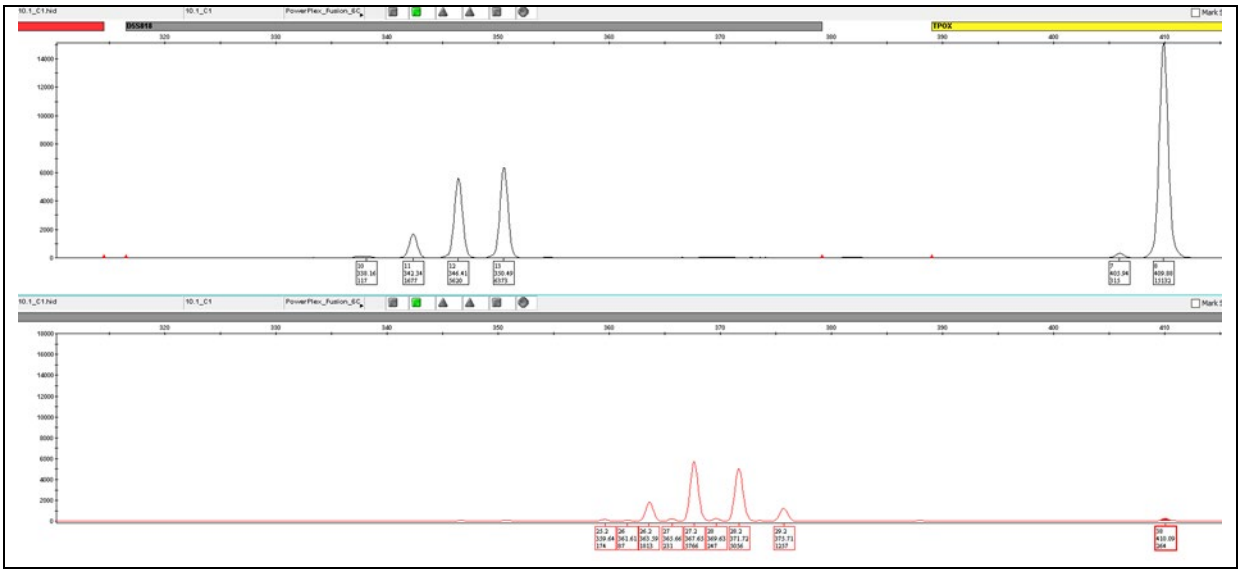


Figure 20: Two images showing the 38 peak at SE33, caused by pullup of the tall 8 peak at TPOX, left on at analysis in error.

The three other false exclusions within the 2p dataset were observed with samples 19.1_C1, 19.1_C1(2) and 19.1_C2. Known contributor (5) was excluded when considered in relation to profiles 19.1_C1 and 19.1_C1(2). Investigation revealed this was due to the retention of a pullup peak (5) at Penta E and an artefact at D19S433 (9.2). Sample 19.1_C2 produced a single locus exclusion at Penta E only, again due to the retention of a '5' pullup peak.

The largest false inclusion (log(*LR*) 6.31 (*LR* ≈ 2,042,796) was observed when non-contributor 'Known 63' was considered in relation to the deconvolution results of profile '3.1_C5(2)' a two person mixture. Review of the mixture and the 'Known 63' reference profile revealed a high degree of similarity with approximately 65% of the non-contributor's autosomal alleles represented within the mixture. There is also a high degree of similarity between the non-contributor and one of the true contributor profiles (Known 65) with a common allele at each locus. This high degree of similarity coupled with the low level nature of the mixture (drop-out and double drop-out combinations accepted across the profile) resulted in an inclusionary *LR* for the non-contributor. Similar observations were observed with the two-person mixture '19.1_C5', where a log(LR) of 4.97 was assigned when non-contributor 'Known 63' was considered in relation to this mixture. Approximately 60% of 'Known 63's alleles are present in this mixture and a high degree of similarity was noted between this non-contributor and the true contributor (Known 5). Other instances of high non-contributor *LR*s (log(*LR*) > 2) involving these database entries (Known 5 and 63) and the 3:1 series of mixtures were also observed and are again likely due to the high degree of similarity and accepted drop-out combinations in one or more of the contributor positions.

These observations are not unexpected given the high degree of similarity between the profiles. Rather than a software issue, these results are better classified as a low-grade adventitious match arising from the fact that the non-contributor has many alleles in common with a known donor used to construct the mixture.

A limited number (7) of falsely exclusionary log(LR)s were also observed across the two, three and four contributor data sets. These appear to be due to stochastic effects in profiles where the true contributors have low posterior mean template amount (less than 200 rfu). This can mean that observed profile does not represent the true experimental design. STRmix™ accepts an array of different genotype combinations and due to the fluctuating peak heights of each contributor across the profile the weights assigned to the genotypes of the true contributors may in some instances be low. Again, this is not an issue with the software more a function of the PCR process and low template samples. The lowest log(*LR*) assigned was -628998 when 'Known 100' was considered in relation to a low level mixture '10.1_C5'. A review of this profile did indeed reveal quite strong stochastic sampling effects with variable presence of the genotypes of 'Known 100' across the molecular weight range.

A lower log(*LR*) than perhaps may be expected given the posterior mean template amount (3505 rfu) was observed in the 4p dataset. The log(*LR*) assigned when true contributor 'Known 90' was compared to mixture '1.1.1.1_C1' was 1.107 (*LR* ~ 12.79). This mixture was reviewed further, and it was found that there are two stutter peaks that were expected (according to the ISP Fusion 6C stutter models) missing from the input file. These were highlighted within the 'Evidence Peak Issues' section of the STRmix™ report (see Figure 21).

EVIDENCE PEAK ISSUES

1.1.1.1_C1_A04.HID.CSV

LOCUS	PEAK	ISSUE	DECISION
Missing Stutter Peaks			
CSF1PO	10	Allele 10 (3635 RFU) is missing Back Stutter at position 9 (expected height of 185 RFU)	Continue
Penta D	12	Allele 12 (10771 RFU) is missing Back Stutter at position 11 (expected height of 209 RFU)	Continue

Figure 21: Excerpt from the STRmix™ report of mixture '1.1.1.1_C1' displaying the missing peak information within the 'Evidence Peak Issues' section

In addition to the low *LR* assigned to a true contributor, elevated diagnostics (LSAE variance, allele variance and to some extent back stutter variance) were also observed. These peaks were not apparent in the electropherogram when reviewed in GeneMapper™*ID-X,* however, it was noted that they were each in pull up positions under relatively strong allelic peaks. Review in an alternate analysis software, FaSTR™DNA, was able to assist the troubleshooting process and it appears that the signal from each of them may have been lost due to the effects of spectral overlap. Whilst this usually results in the 'pull-up' of peaks in other dyes, it has been observed that spectral overlap can also cause 'pull-down' of signal in other dyes and in this instance the loss of the stutter analyte signal [10]. The left pane of Figure 22 displays the data viewed in GeneMapper™*ID-X* and the right pane the same data in FaSTR™DNA. If this is observed within a profile, rework options such as reamplification at a lower input template amount or deconvolution ignoring the affected locus/loci should be considered. In this type of situation, ignoring the locus within an '*LR* from Previous' calculation is not advised as missing peaks can significantly affect the progression of the deconvolution, the resulting weights and subsequent *LR*s.

When these peaks were added to the input file at the expected peak height (for demonstration purposes only, NOT advised for casework), the log(*LR*)s assigned for all true contributors to this profile were greater than 7 and specifically the log(*LR*) assigned considering 'Known 90' was ~7.389. Elevated diagnostic values (Gelman Rubin and posterior mean LSAE variance) were still observed; however, these are likely due to the overall complexity of the four person profile and if encountered in casework it would be recommended that this profile is re-deconvoluted with increased accepts.

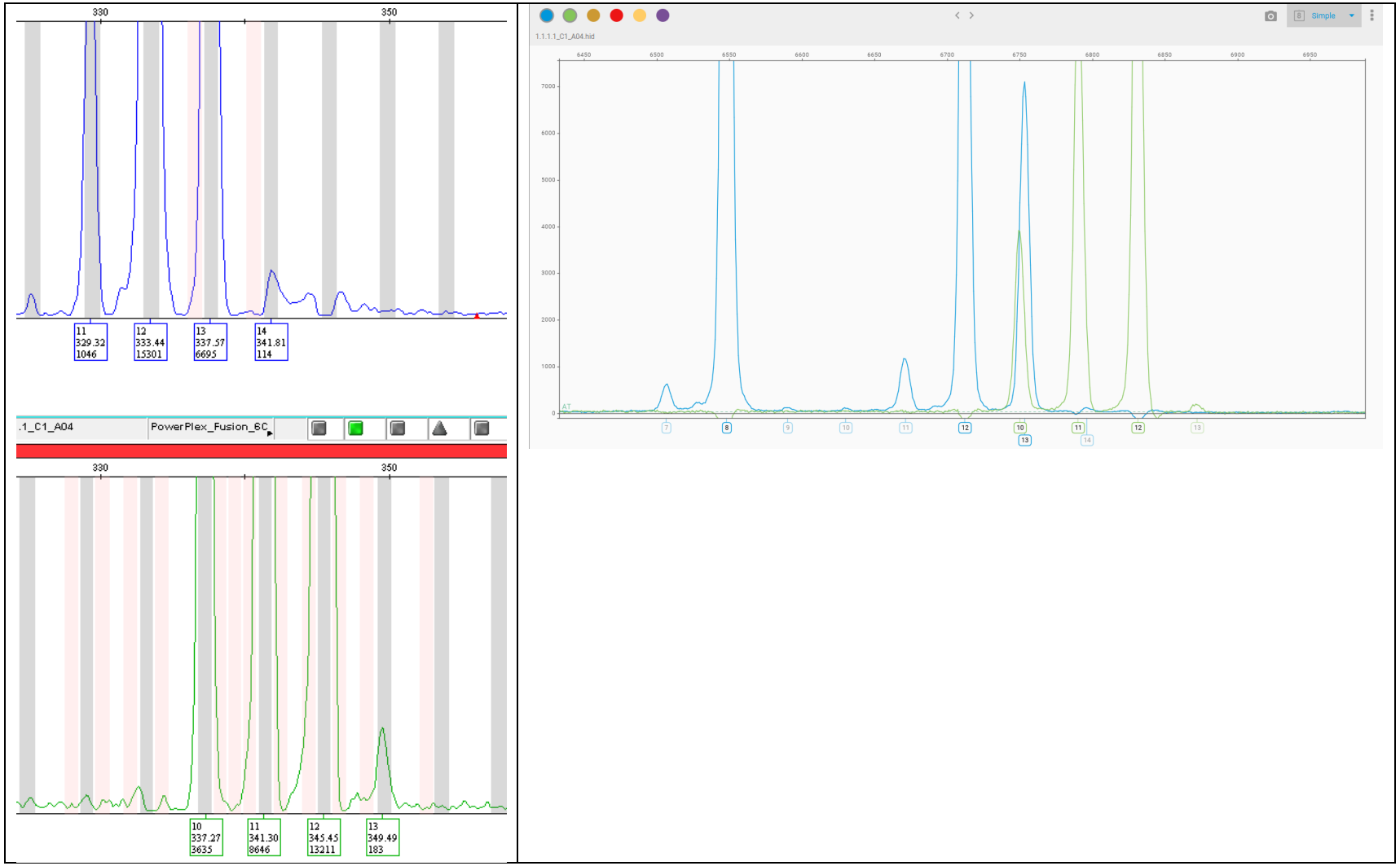


Figure 22: Excerpts from the electropherogram of mixture '1.1.1.1_C1' displaying the information observed at the D13S317 (blue dye) and CSF1PO (green dye) loci in two different software. The left pane displays the data viewed in GeneMapper™*ID-X* and the right pane the same data in FaSTR™DNA

Review of Run Diagnostics

STRmix™ includes a number of diagnostics within its reports. These have been deliberately included to assist the user when evaluating the reliability of an interpretation. These may be conveniently categorised into 'primary' and 'secondary' diagnostics. Primary diagnostics include the mixture proportions, genotype weights, and locus *LR*s. Secondary diagnostics include the average log(likelihood), the Gelman-Rubin convergence diagnostic, and the posterior mean variance parameters. In instances where non-intuitive primary diagnostics are observed, the STRmix™ results should be closely scrutinised however elevated secondary diagnostics do not necessarily invalidate an interpretation. Provided that the primary diagnostics are intuitive, the results are likely still reliable. The diagnostics for the ISP Fusion 6C dataset are displayed in Appendix 5.

In summary, the plots in Figure 19 demonstrate that at high template STRmix™ correctly and reliably gave high *LR*s for known contributors and a low or exclusionary *LR* for non-contributors. At low template and higher contributor number profiles STRmix™ correctly and reliably reported that the analysis of the sample tends towards uninformative or inconclusive. The plots also help to inform the limits of STRmix™, particularly the lower limit of DNA where an H_p true hypothesis still results in an *LR* greater than 1 and the limit where false positives may arise (an *LR* greater than 1 where H_d is true). As detailed in Appendix 5, the diagnostic values were generally as expected given the observed profile but analysts are advised to apply caution when elevated or extreme values are encountered or if unusual profile morphology is observed.

Conclusion

This document describes the upgrade and performance check of STRmix™ V2.9.1 and the Fusion 6C kit with data generated within the ISP laboratory. It has been shown that it is suited for its intended use for the interpretation of profiles generated from crime scene samples.

Signatures:

Taylor Maichak

DNA Technical Lead

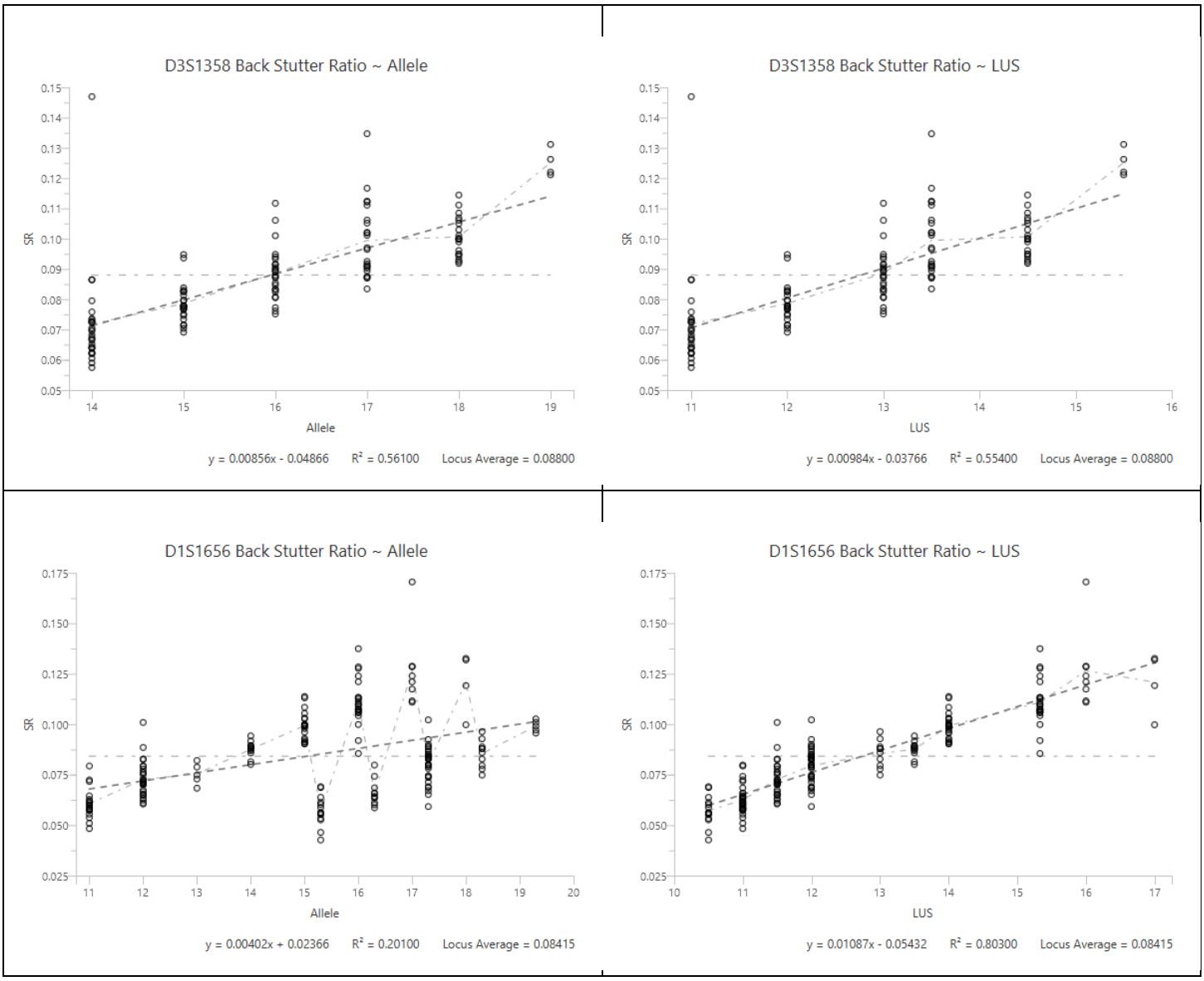
This work has been reviewed and it has been determined that STRmix™ V2.9.1 is suitable for its intended use for interpretation of crime profiles within ISP. The project work has met the validation requirements as required by A2LA and FBI QAS.

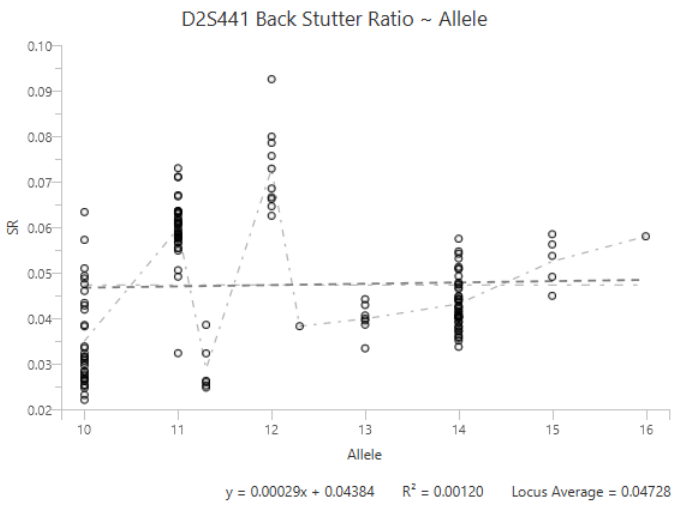
Quality Manager

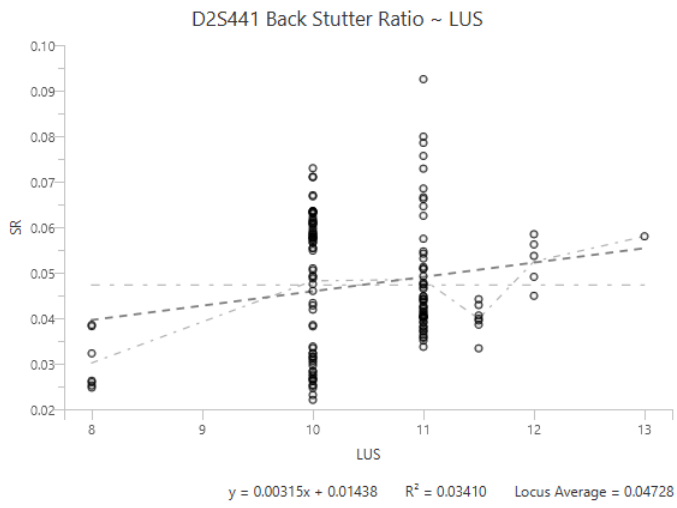
References

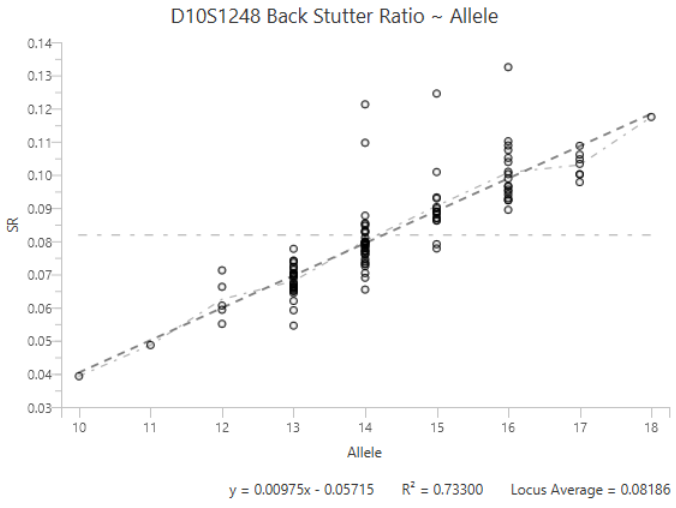
1. Bright, J.-A., et al., *Developing allelic and stutter peak height models for a continuous method of DNA interpretation.* Forensic Science International: Genetics, 2013. **7**(2): p. 296-304.
2. Brookes, C., et al., *Characterising stutter in forensic STR multiplexes.* Forensic Science International: Genetics, 2012. **6**(1): p. 58-63.
3. Walsh, P.S., N.J. Fildes, and R. Reynolds, *Sequence analysis and characterization of stutter products at the tetranucleotide repeat locus vWA.* Nucleic Acids Research, 1996. **24**: p. 2807-2812.
4. Butler, J.M. and D.J. Reeder. *Short Tandem Repeat DNA Internet DataBase*. [cited 2014 27 October 2014]; Available from: www.cstl.nist.gov/biotech/strbase.
5. Ruitberg, C.M., D.J. Reeder, and J.M. Butler, *STRBase: a short tandem repeat DNA database for the human identity testing community.* Nucleic Acids Research, 2001. **29**(1): p. 320 - 322.
6. Bright, J.-A., et al., *Determination of the variables affecting mixed MiniFiler™ DNA profiles.* Forensic Science International: Genetics, 2011. **5**(5): p. 381-385.
7. Bright, J.-A., J. Turkington, and J. Buckleton, *Examination of the variability in mixed DNA profile parameters for the Identifiler multiplex.* Forensic Science International: Genetics, 2009. **4**(2): p. 111-114.
8. Taylor, D., *Using continuous DNA interpretation methods to revisit likelihood ratio behaviour.* Forensic Science International: Genetics, 2014. **11**: p. 144-153.
9. Russell, L., et al., *A guide to results and diagnostics within a STRmix™ report.* WIREs Forensic Science, 2019. **1**(6): p. e1354.
10. Lin, M.-H., et al., *Developmental validation of FaSTR™ DNA: Software for the analysis of forensic DNA profiles.* Forensic Science International: Reports, 2021. **3**: p. 100217.

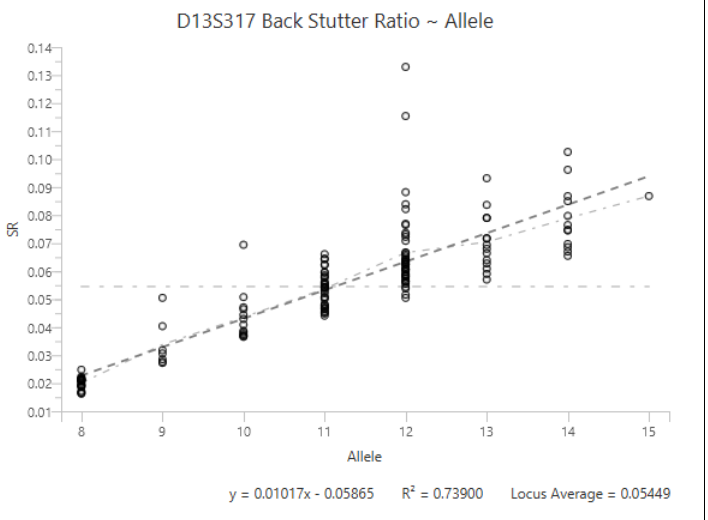
Appendix 1: Back stutter (-1,0) regression plots for the autosomal Fusion 6C loci determined using the ISP stutter dataset. Plots for *SR* versus allelic designation are provided on the left, plots for *SR* versus LUS designation are provided on the right (for loci with compound or complex repeat structures only). The faint, horizontal dash-dot line indicates the average observed per-locus *SR*. The other dash-dot line connects the average observed per-allele *SR*s. The darker dashed line displays the linear regression of SR against allele or LUS

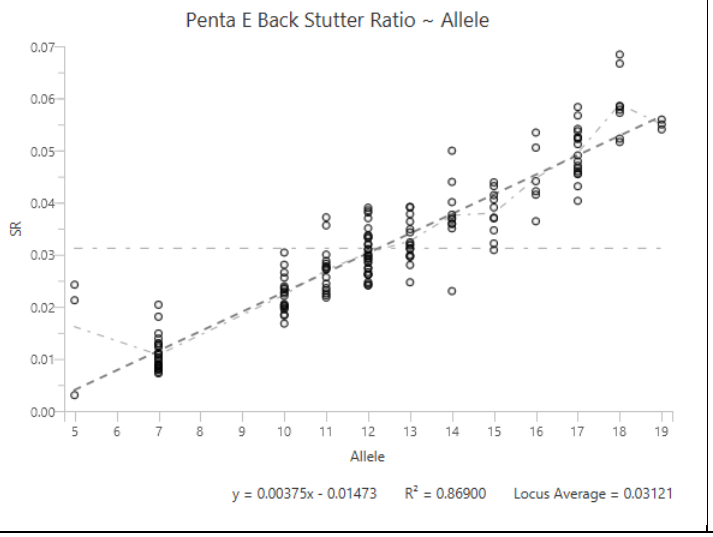


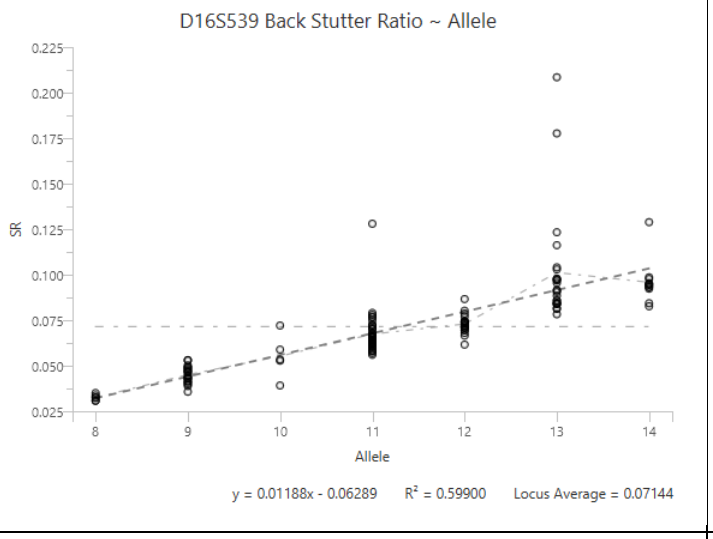

D2S441 Back Stutter Ratio ~ Allele
SR
Allele
y = 0.00029x + 0.04384
R² = 0.00120
Locus Average = 0.04728

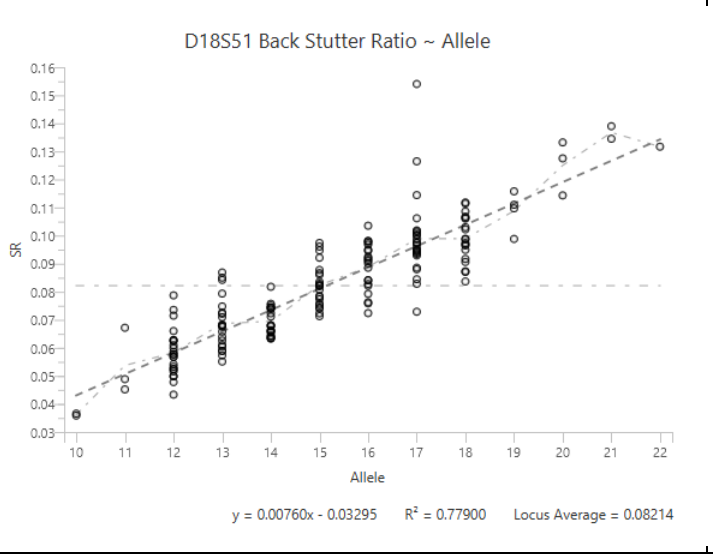

D2S441 Back Stutter Ratio ~ LUS
SR
LUS
y = 0.00315x + 0.01438
R² = 0.03410
Locus Average = 0.04728

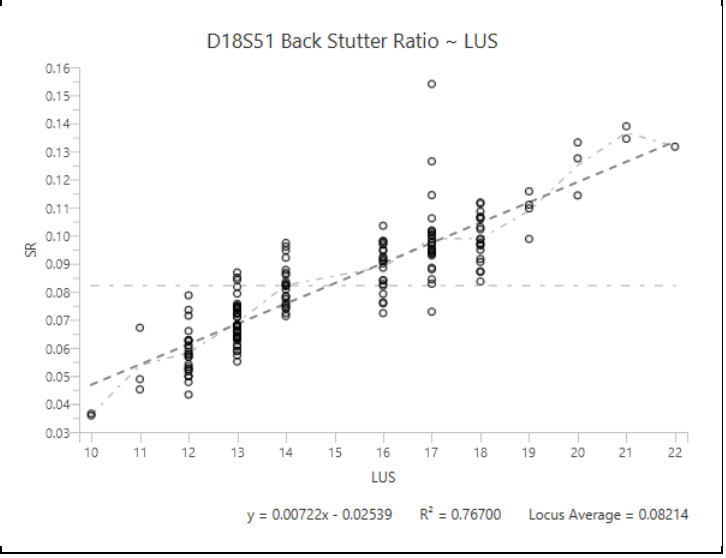

D10S1248 Back Stutter Ratio ~ Allele
SR
Allele
y = 0.00975x - 0.05715
R² = 0.73300
Locus Average = 0.08186

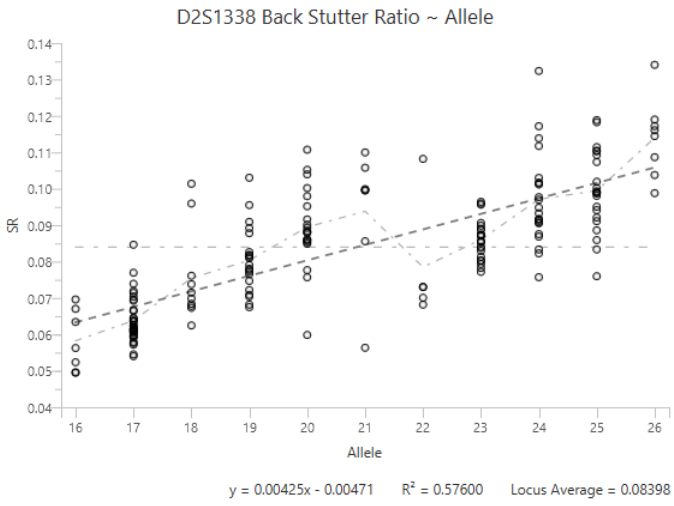

D13S317 Back Stutter Ratio ~ Allele
SR
Allele
y = 0.01017x - 0.05865
R² = 0.73900
Locus Average = 0.05449

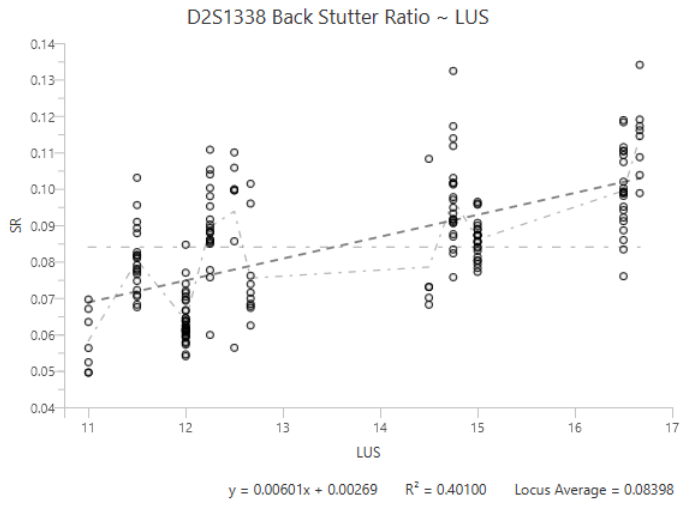

Penta E Back Stutter Ratio ~ Allele
SR
Allele
y = 0.00375x - 0.01473
R² = 0.86900
Locus Average = 0.03121

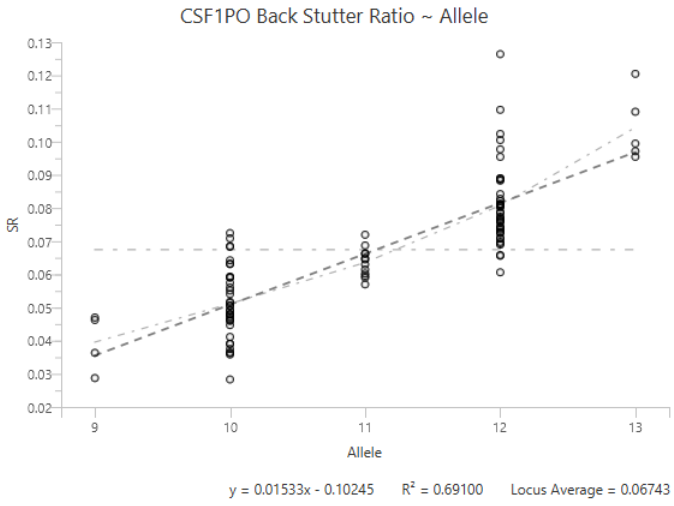

D16S539 Back Stutter Ratio ~ Allele
SR
Allele
y = 0.01188x - 0.06289
R² = 0.59900
Locus Average = 0.07144

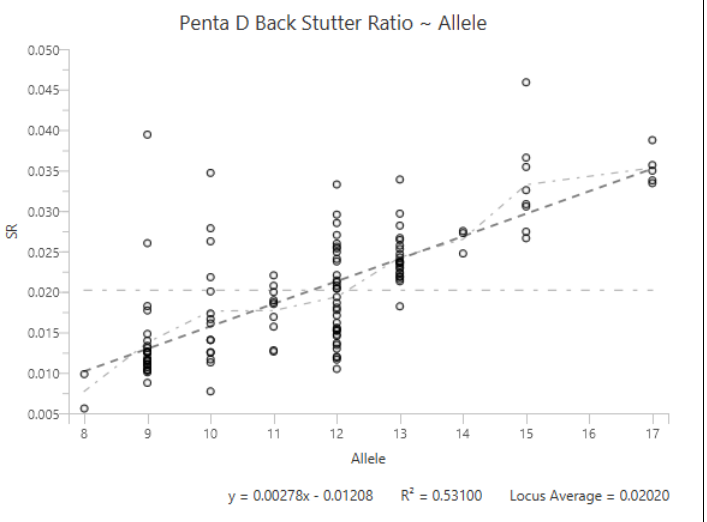

D18S51 Back Stutter Ratio ~ Allele
SR
Allele
y = 0.00760x - 0.03295
R² = 0.77900
Locus Average = 0.08214

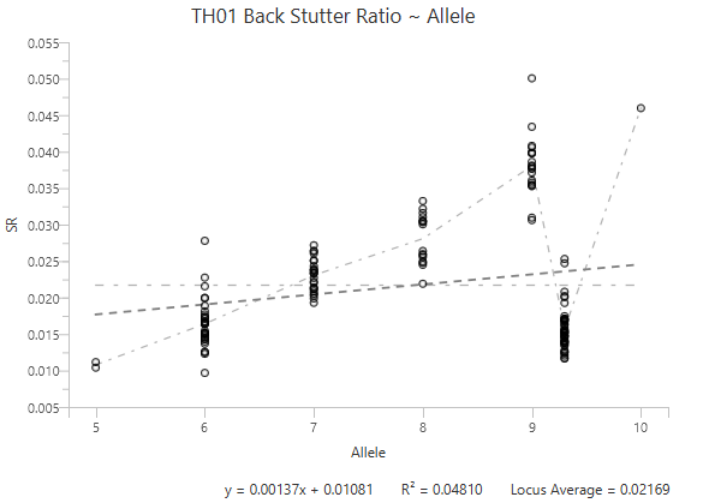

D18S51 Back Stutter Ratio ~ LUS
SR
LUS
y = 0.00722x - 0.02539
R² = 0.76700
Locus Average = 0.08214

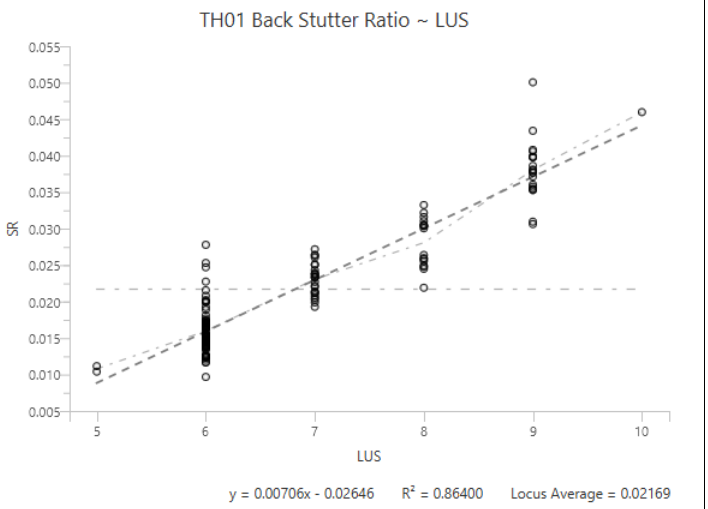

D2S1338 Back Stutter Ratio ~ Allele
SR
Allele
y = 0.00425x - 0.00471
R² = 0.57600
Locus Average = 0.08398

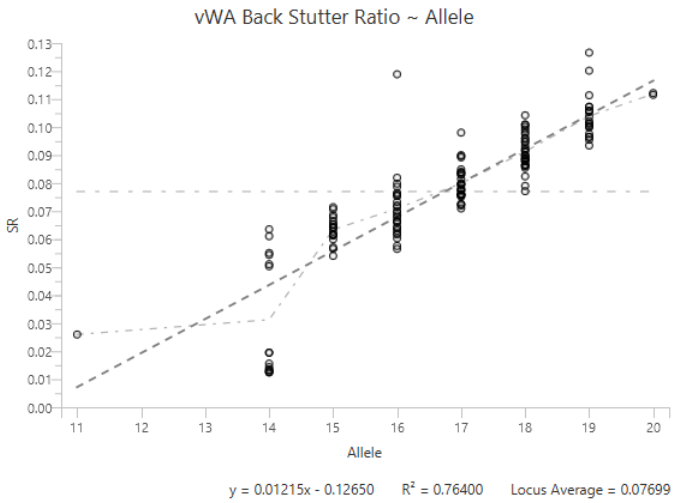

D2S1338 Back Stutter Ratio ~ LUS
SR
LUS
y = 0.00601x + 0.00269
R² = 0.40100
Locus Average = 0.08398

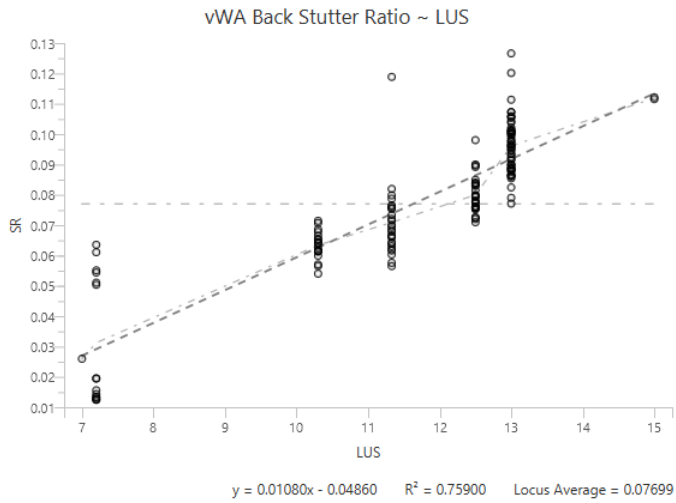

CSF1PO Back Stutter Ratio ~ Allele
SR
Allele
y = 0.01533x - 0.10245
R² = 0.69100
Locus Average = 0.06743

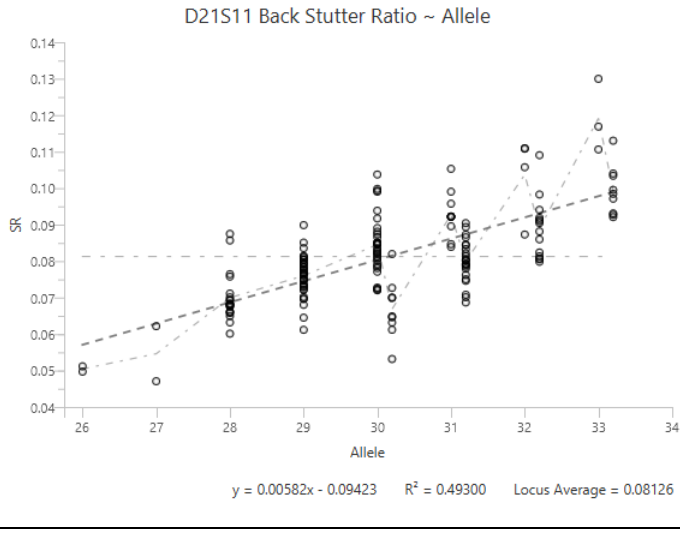

Penta D Back Stutter Ratio ~ Allele
SR
Allele
y = 0.00278x - 0.01208
R² = 0.53100
Locus Average = 0.02020

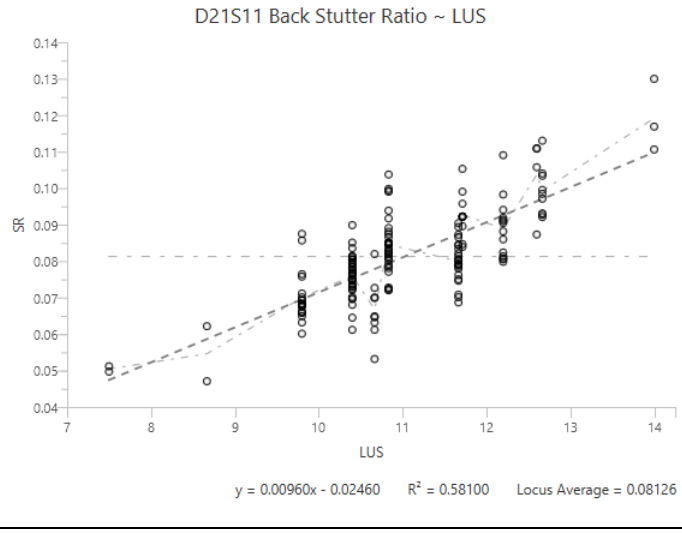

TH01 Back Stutter Ratio ~ Allele
SR
Allele
y = 0.00137x + 0.01081
R² = 0.04810
Locus Average = 0.02169

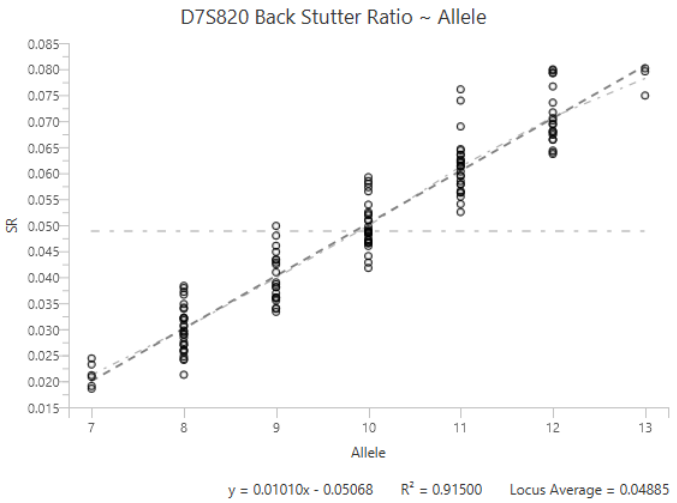

TH01 Back Stutter Ratio ~ LUS
SR
LUS
y = 0.00706x - 0.02646
R² = 0.86400
Locus Average = 0.02169

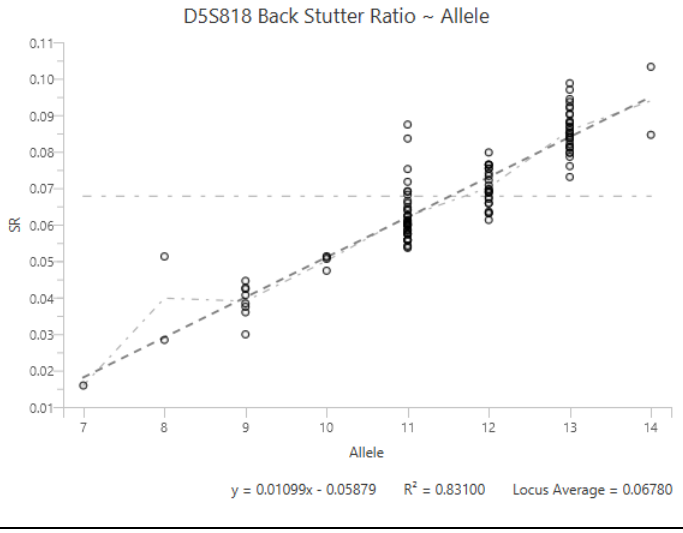

vWA Back Stutter Ratio ~ Allele
SR
Allele
y = 0.01215x - 0.12650
R² = 0.76400
Locus Average = 0.07699

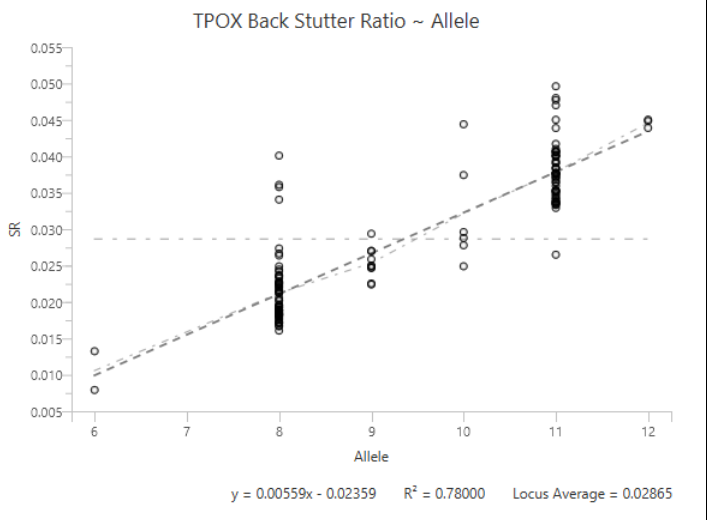

vWA Back Stutter Ratio ~ LUS
SR
LUS
y = 0.01080x - 0.04860
R² = 0.75900
Locus Average = 0.07699

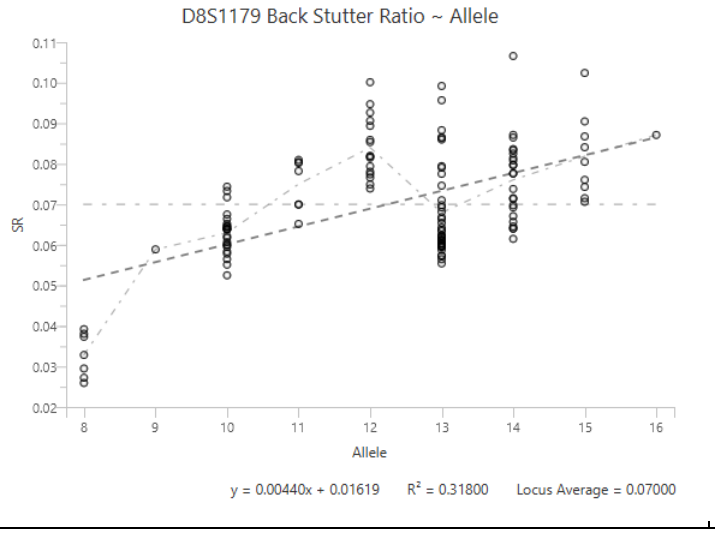

D21S11 Back Stutter Ratio ~ Allele
SR
Allele
y = 0.00582x - 0.09423
R² = 0.49300
Locus Average = 0.08126

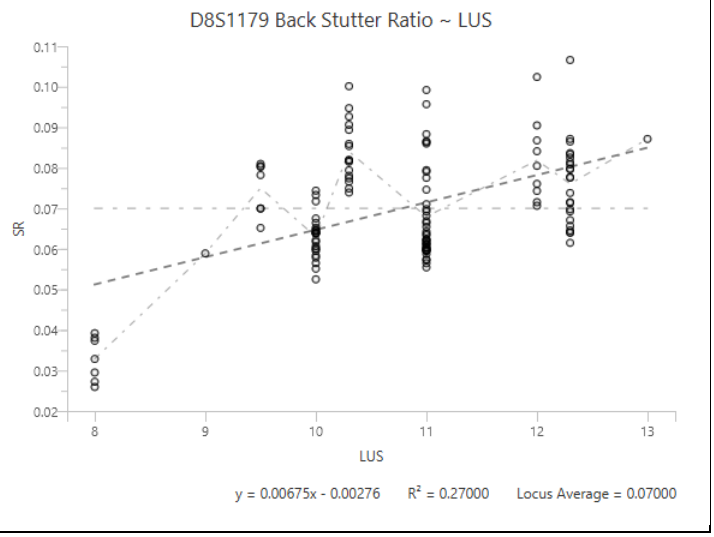

D21S11 Back Stutter Ratio ~ LUS
SR
LUS
y = 0.00960x - 0.02460
R² = 0.58100
Locus Average = 0.08126

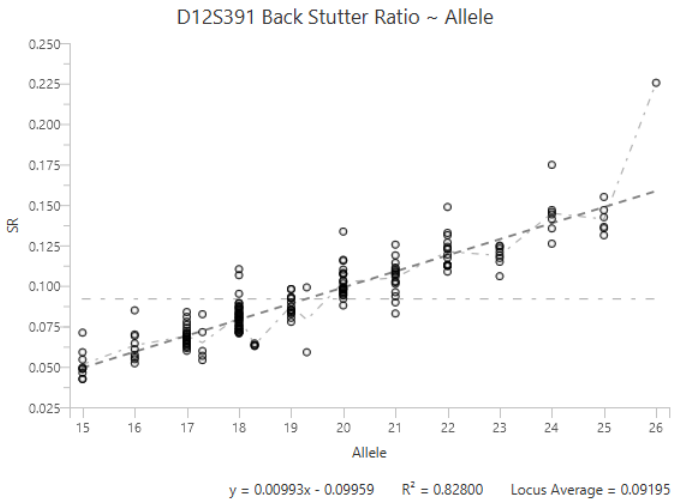

D7S820 Back Stutter Ratio ~ Allele
SR
Allele
y = 0.01010x - 0.05068
R² = 0.91500
Locus Average = 0.04885

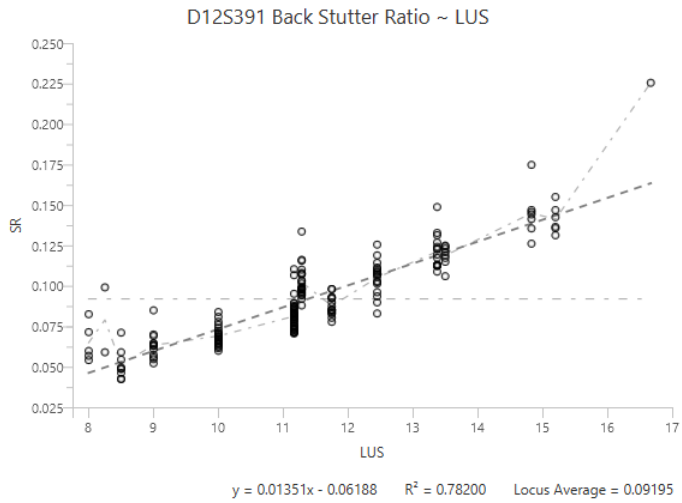

D5S818 Back Stutter Ratio ~ Allele
SR
Allele
y = 0.01099x - 0.05879
R² = 0.83100
Locus Average = 0.06780

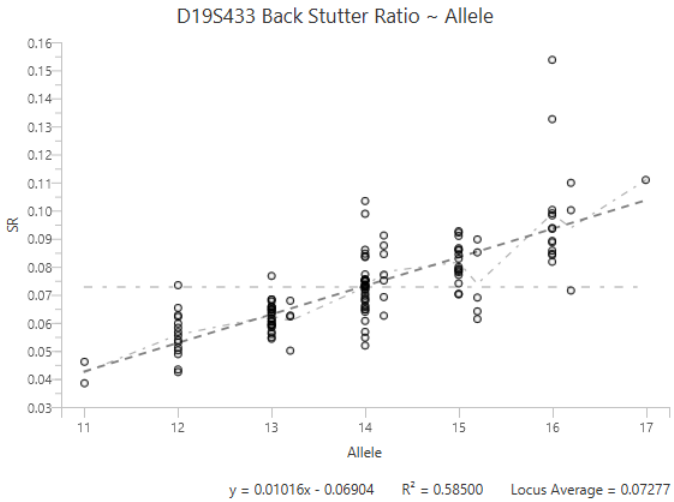

TPOX Back Stutter Ratio ~ Allele
SR
Allele
y = 0.00559x - 0.02359
R² = 0.78000
Locus Average = 0.02865

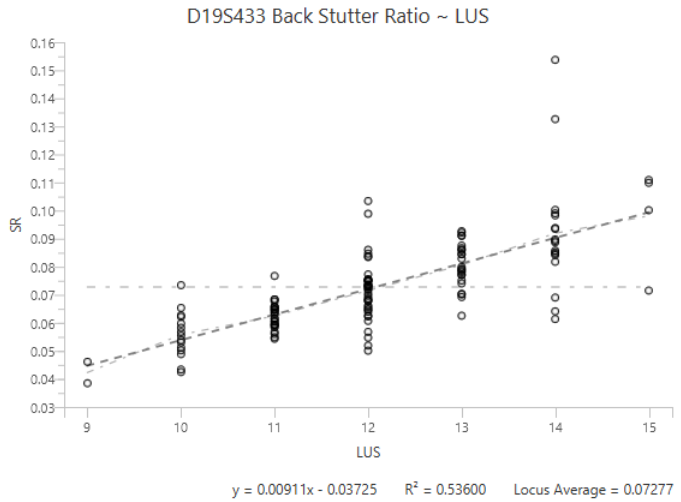

D8S1179 Back Stutter Ratio ~ Allele
SR
Allele
y = 0.00440x + 0.01619
R² = 0.31800
Locus Average = 0.07000

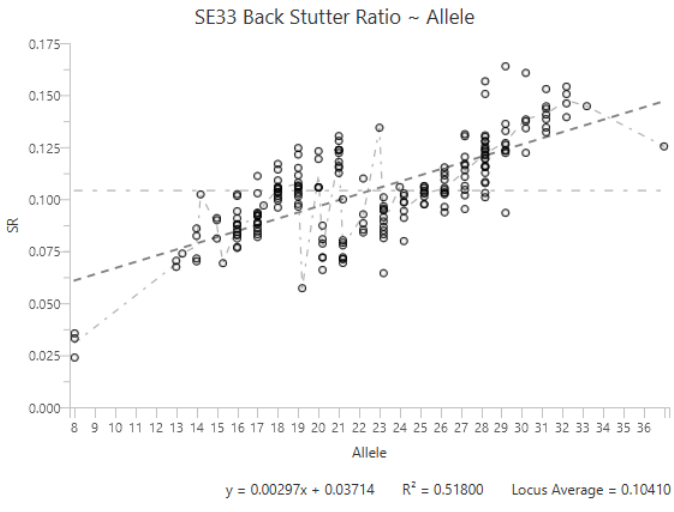

D8S1179 Back Stutter Ratio ~ LUS
SR
LUS
y = 0.00675x - 0.00276
R² = 0.27000
Locus Average = 0.07000

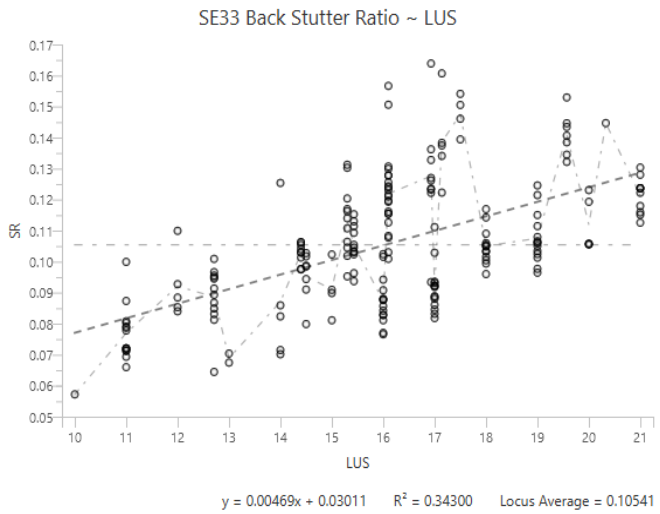

D12S391 Back Stutter Ratio ~ Allele
SR
Allele
y = 0.00993x - 0.09959
R² = 0.82800
Locus Average = 0.09195

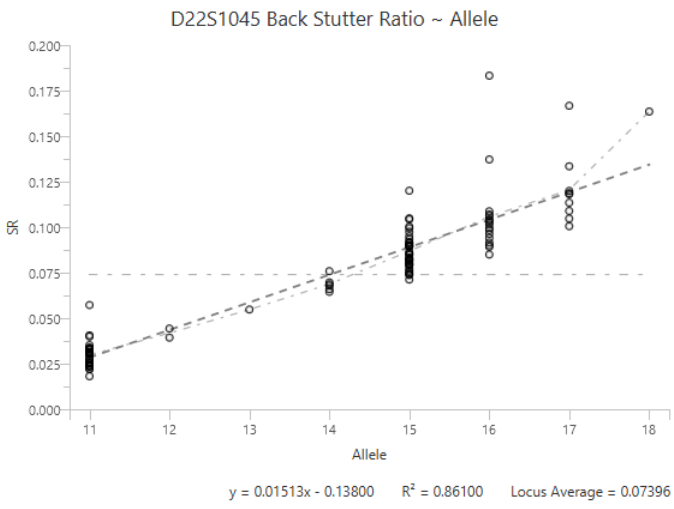

D12S391 Back Stutter Ratio ~ LUS
SR
LUS
y = 0.01351x - 0.06188
R² = 0.78200
Locus Average = 0.09195

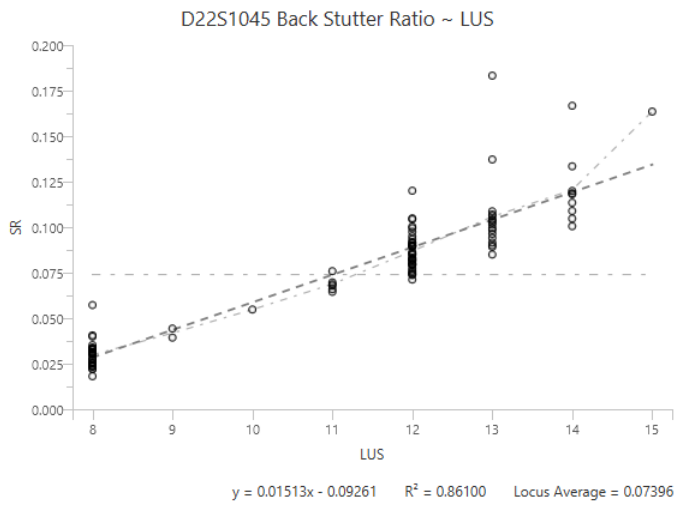

D19S433 Back Stutter Ratio ~ Allele
SR
Allele
y = 0.01016x - 0.06904
R² = 0.58500
Locus Average = 0.07277

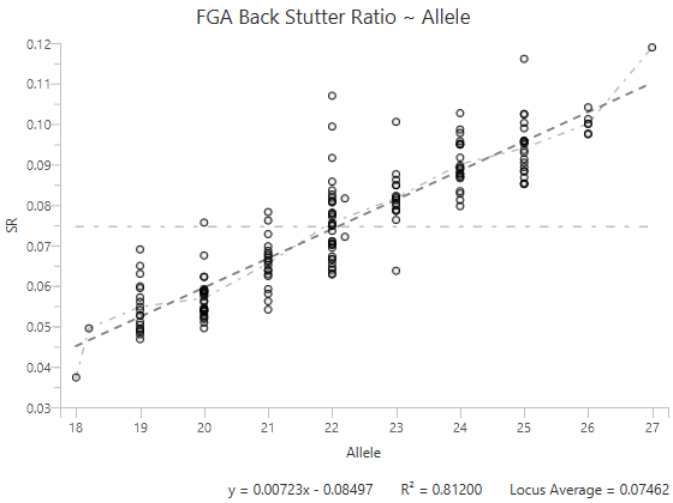

D19S433 Back Stutter Ratio ~ LUS
SR
LUS
y = 0.00911x - 0.03725
R² = 0.53600
Locus Average = 0.07277

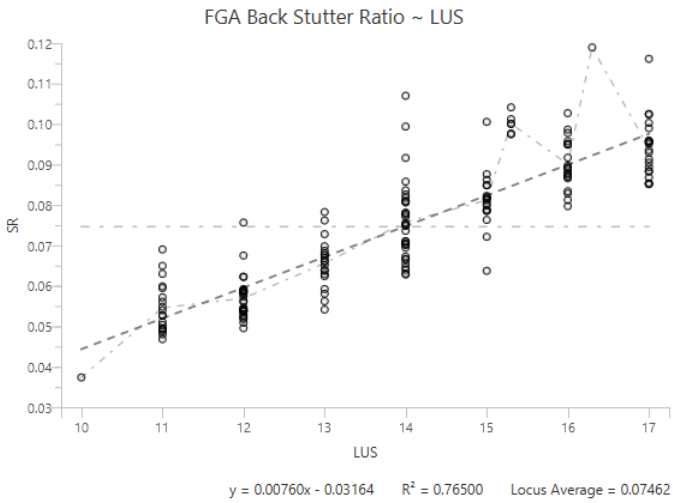

SE33 Back Stutter Ratio ~ Allele
SR
Allele
y = 0.00297x + 0.03714
R² = 0.51800
Locus Average = 0.10410


SE33 Back Stutter Ratio ~ LUS
SR
LUS
y = 0.00469x + 0.03011
R² = 0.34300
Locus Average = 0.10541

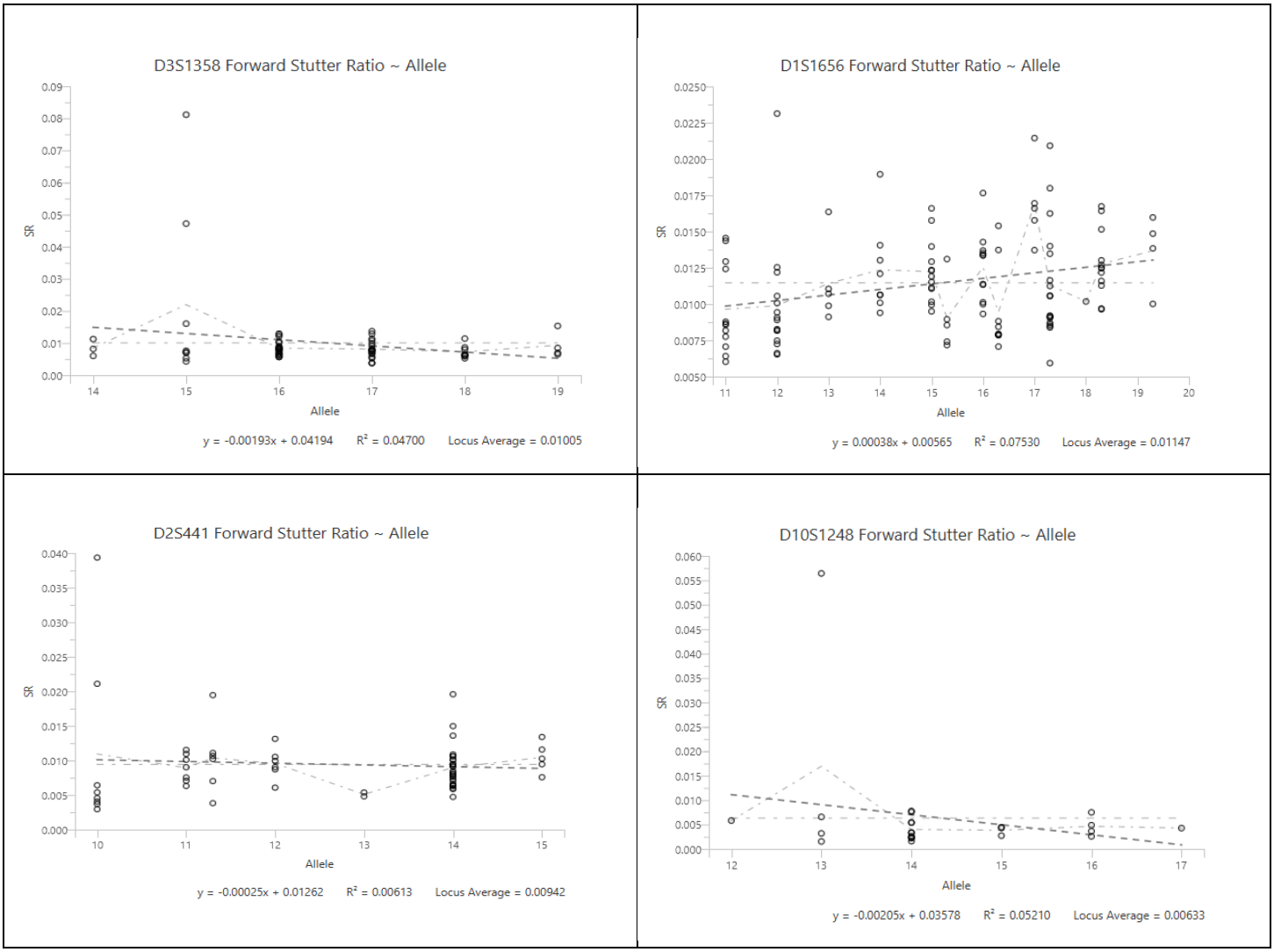

D22S1045 Back Stutter Ratio ~ Allele
SR
Allele
y = 0.01513x - 0.13800
R² = 0.86100
Locus Average = 0.07396

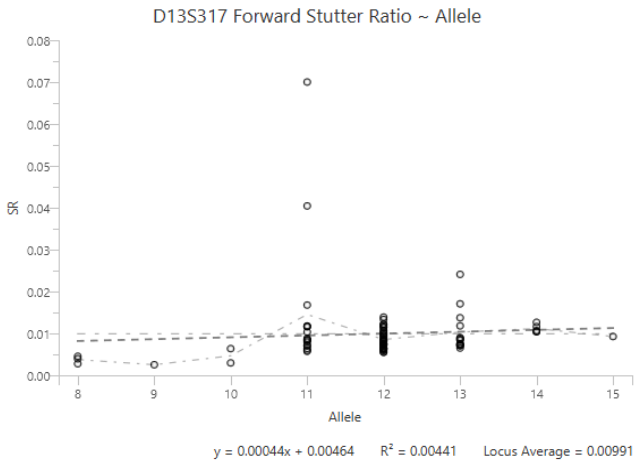

D22S1045 Back Stutter Ratio ~ LUS
SR
LUS
y = 0.01513x - 0.09261
R² = 0.86100
Locus Average = 0.07396

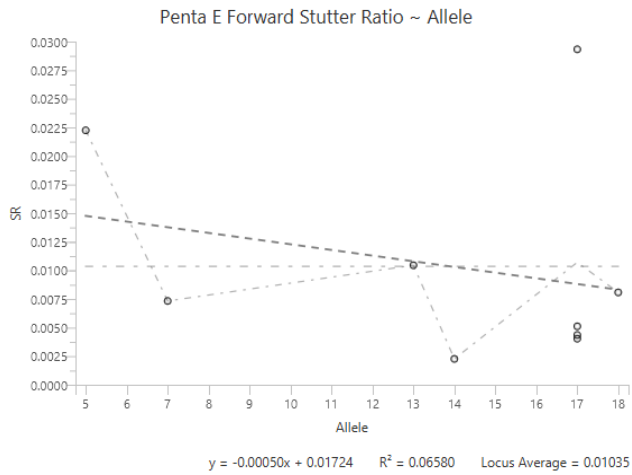

FGA Back Stutter Ratio ~ Allele
SR
Allele
y = 0.00723x - 0.08497
R² = 0.81200
Locus Average = 0.07462

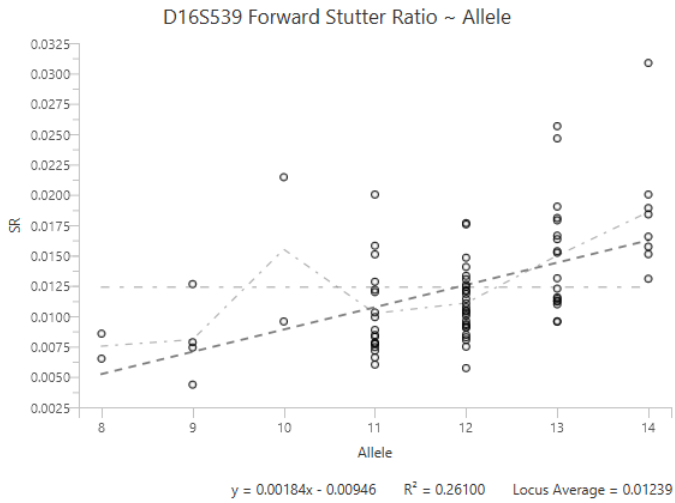

FGA Back Stutter Ratio ~ LUS
SR
LUS
y = 0.00760x - 0.03164
R² = 0.76500
Locus Average = 0.07462

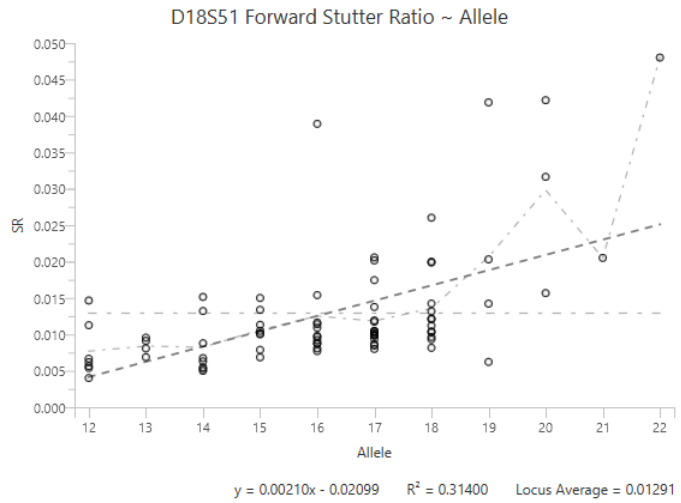
Appendix 2: Forward stutter (1,0) regression plots for the autosomal Fusion 6C loci determined using the ISP stutter dataset. The faint, horizontal dash-dot line indicates the average observed per-locus *SR*. The other dash-dot line connects the average observed per-allele *SR*s. The darker dashed line displays the linear regression of SR against allele.

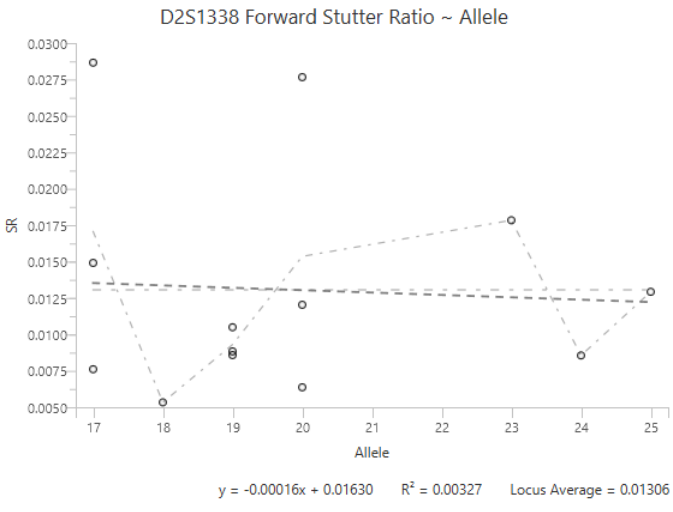


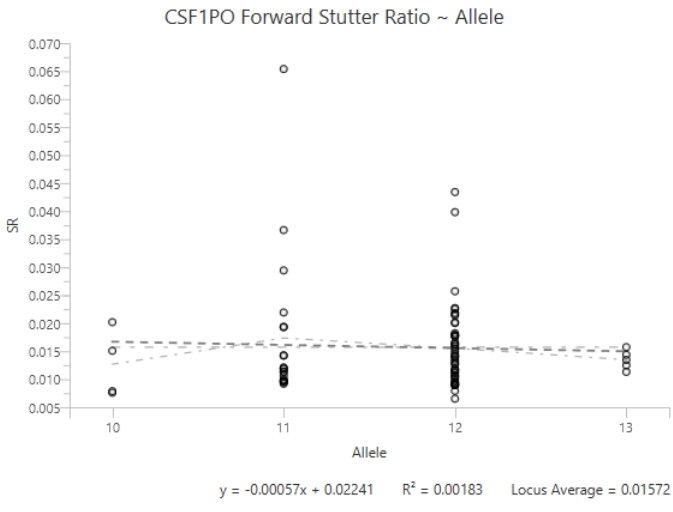

D13S317 Forward Stutter Ratio ~ Allele
SR
Allele
y = 0.00044x + 0.00464
R² = 0.00441
Locus Average = 0.00991

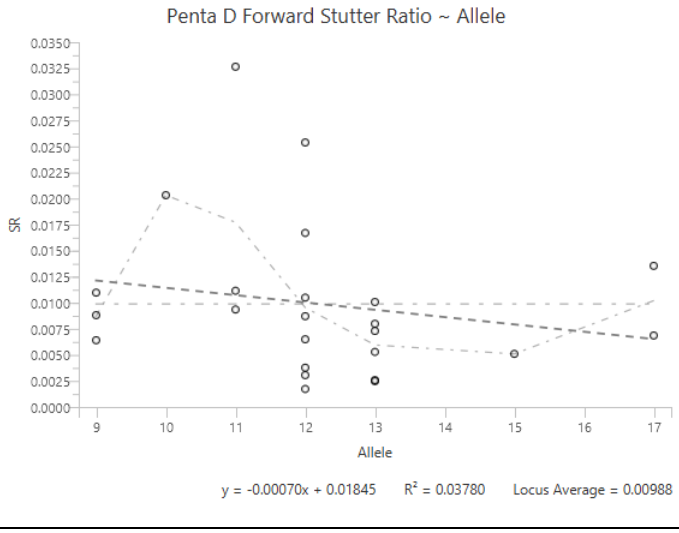

Penta E Forward Stutter Ratio ~ Allele
SR
Allele
y = -0.00050x + 0.01724
R² = 0.06580
Locus Average = 0.01035

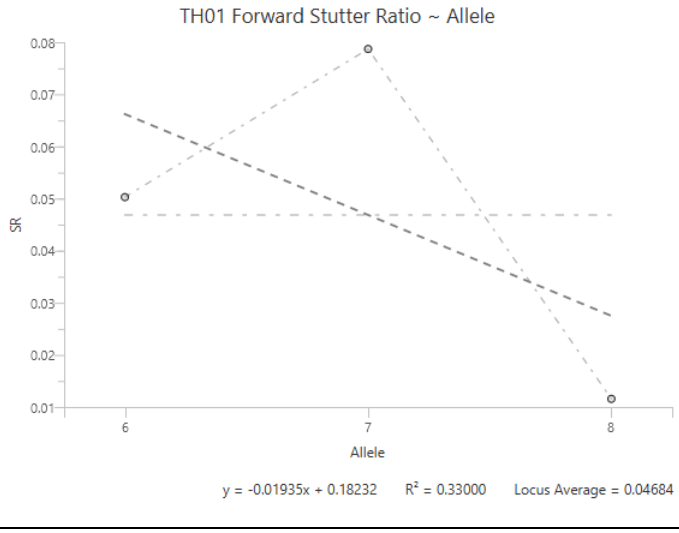

D16S539 Forward Stutter Ratio ~ Allele
SR
Allele
y = 0.00184x - 0.00946
R² = 0.26100
Locus Average = 0.01239

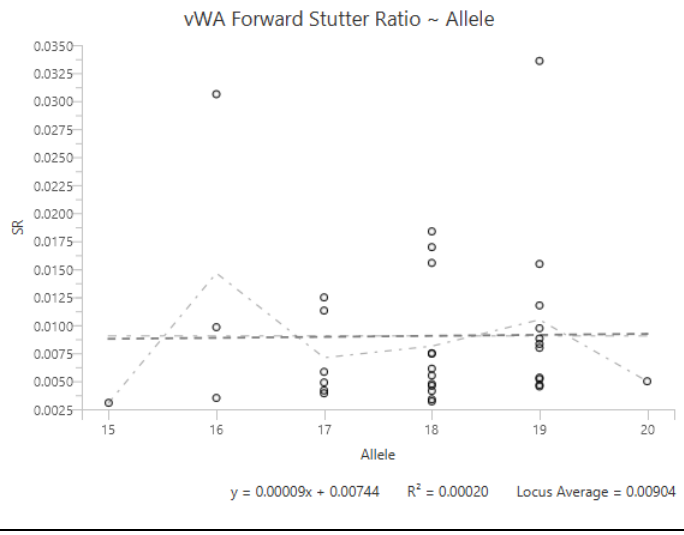

D18S51 Forward Stutter Ratio ~ Allele
SR
Allele
y = 0.00210x - 0.02099
R² = 0.31400
Locus Average = 0.01291

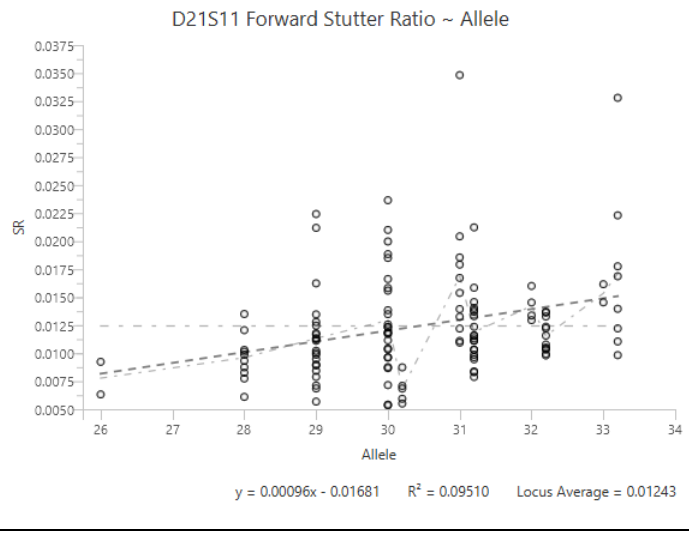

D2S1338 Forward Stutter Ratio ~ Allele
SR
Allele
y = -0.00016x + 0.01630
R² = 0.00327
Locus Average = 0.01306

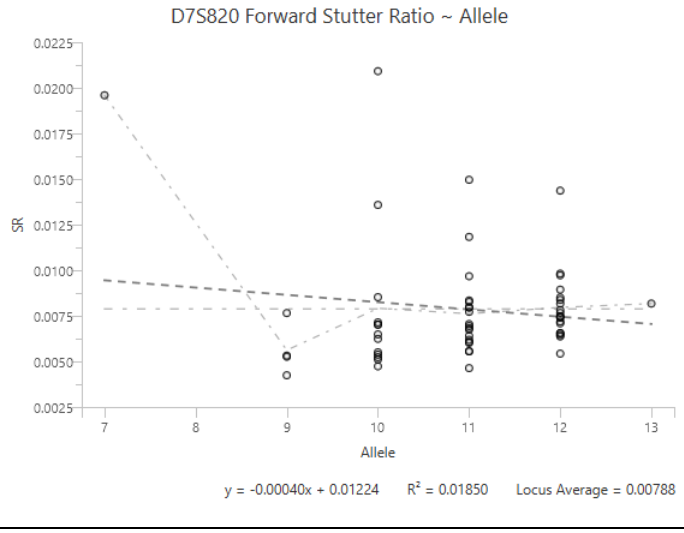

CSF1PO Forward Stutter Ratio ~ Allele
SR
Allele
y = -0.00057x + 0.02241
R² = 0.00183
Locus Average = 0.01572

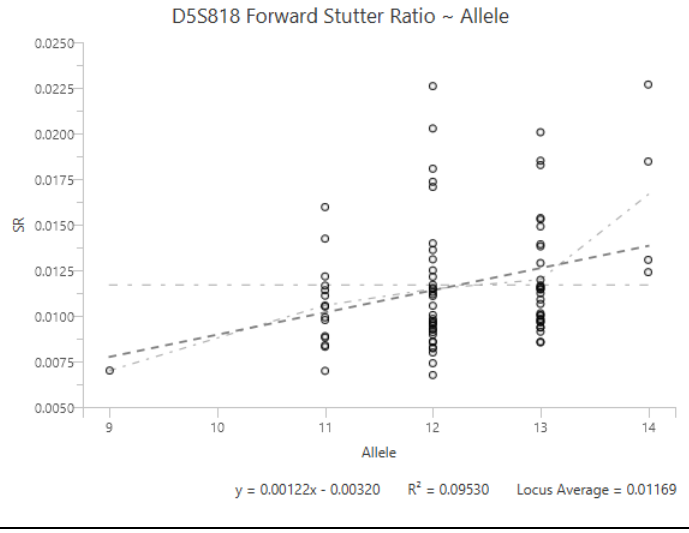

Penta D Forward Stutter Ratio ~ Allele
SR
Allele
y = -0.00070x + 0.01845
R² = 0.03780
Locus Average = 0.00988

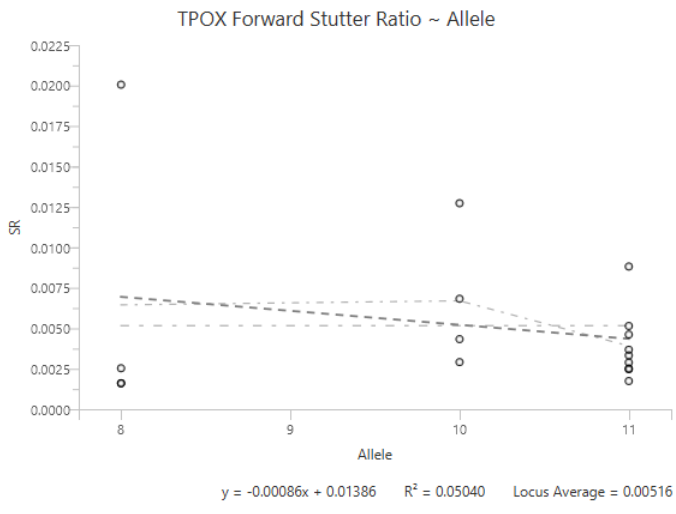

TH01 Forward Stutter Ratio ~ Allele
SR
Allele
y = -0.01935x + 0.18232
R² = 0.33000
Locus Average = 0.04684

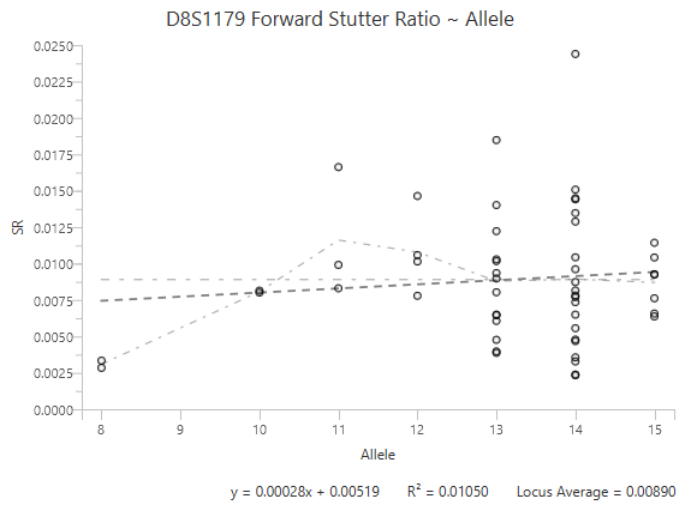

vWA Forward Stutter Ratio ~ Allele
SR
Allele
y = 0.00009x + 0.00744
R² = 0.00020
Locus Average = 0.00904

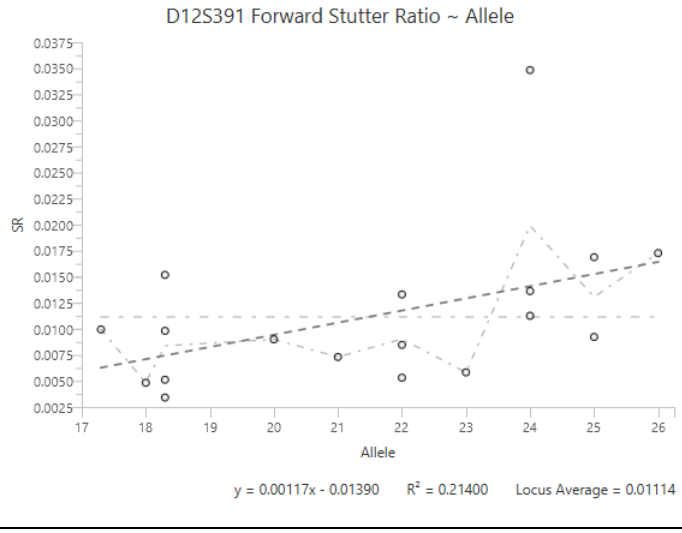

D21S11 Forward Stutter Ratio ~ Allele
SR
Allele
y = 0.00096x - 0.01681
R² = 0.09510
Locus Average = 0.01243

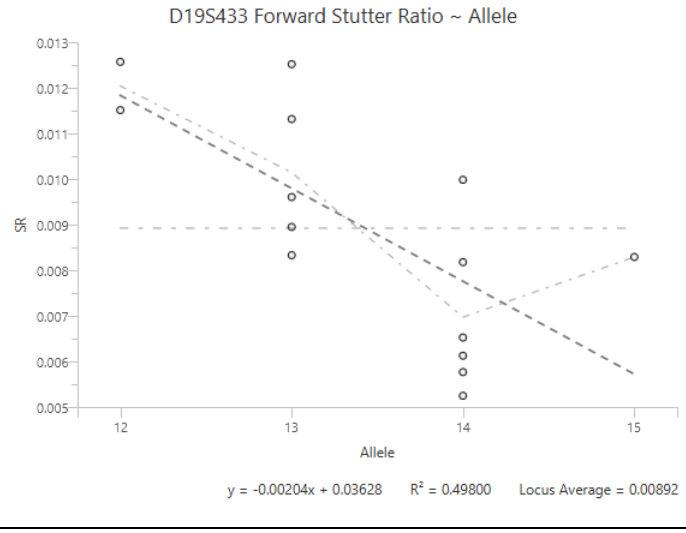

D7S820 Forward Stutter Ratio ~ Allele
SR
Allele
y = -0.00040x + 0.01224
R² = 0.01850
Locus Average = 0.00788

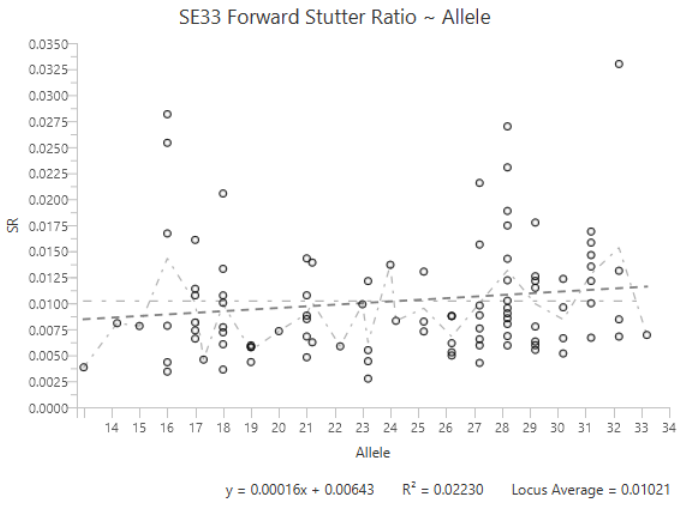

D5S818 Forward Stutter Ratio ~ Allele
SR
Allele
y = 0.00122x - 0.00320
R² = 0.09530
Locus Average = 0.01169

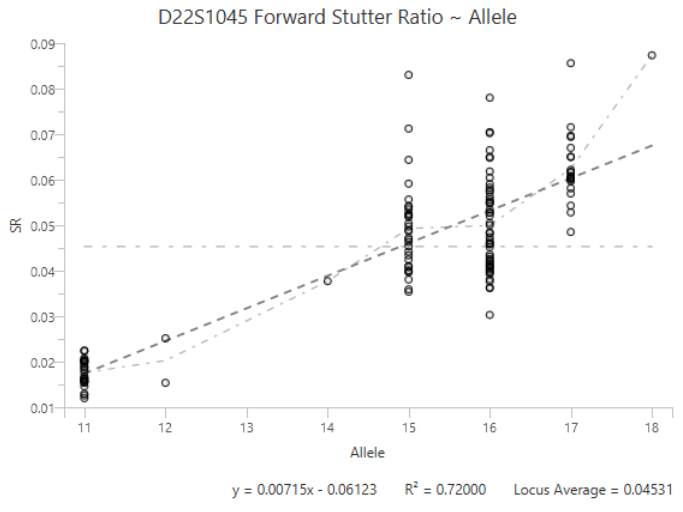

TPOX Forward Stutter Ratio ~ Allele
SR
Allele
y = -0.00086x + 0.01386
R² = 0.05040
Locus Average = 0.00516

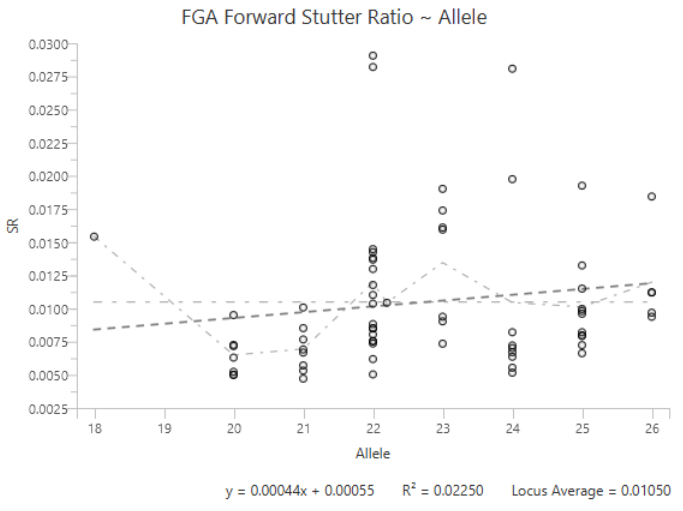

D8S1179 Forward Stutter Ratio ~ Allele
SR
Allele
y = 0.00028x + 0.00519
R² = 0.01050
Locus Average = 0.00890


D12S391 Forward Stutter Ratio ~ Allele
SR
Allele
y = 0.00117x - 0.01390
R² = 0.21400
Locus Average = 0.01114

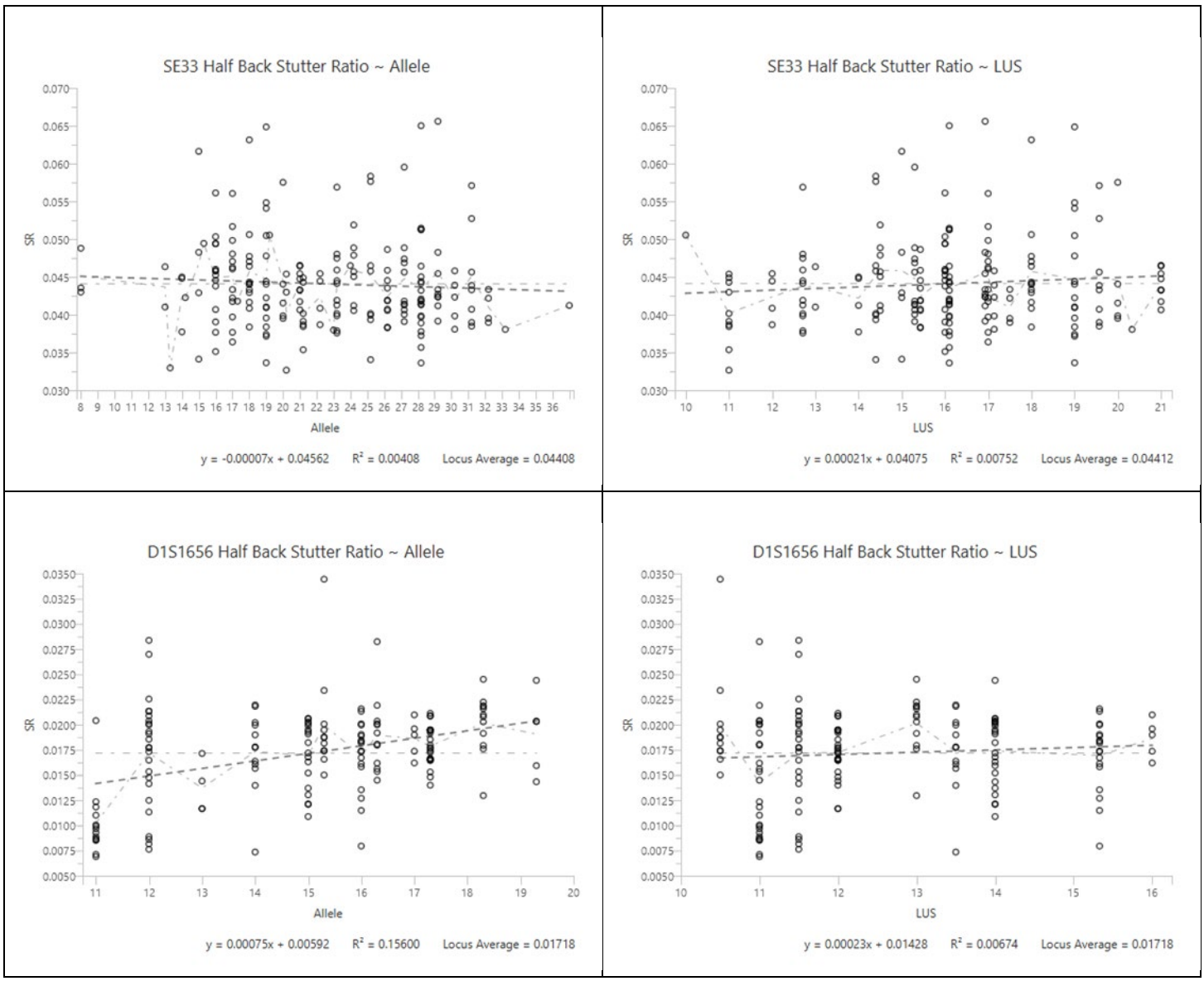

D19S433 Forward Stutter Ratio ~ Allele
SR
Allele
y = -0.00204x + 0.03628
R² = 0.49800
Locus Average = 0.00892


SE33 Forward Stutter Ratio ~ Allele
SR
Allele
y = 0.00016x + 0.00643
R² = 0.02230
Locus Average = 0.01021

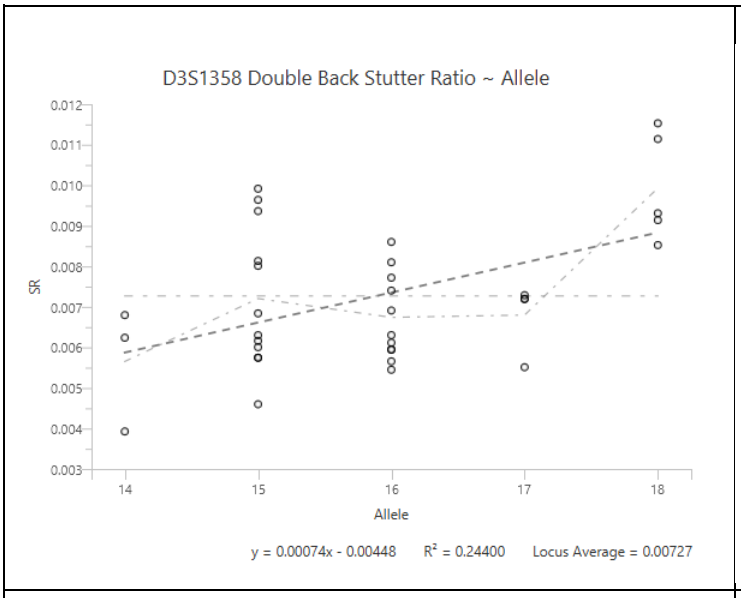

D22S1045 Forward Stutter Ratio ~ Allele
SR
Allele
y = 0.00715x - 0.06123
R² = 0.72000
Locus Average = 0.04531

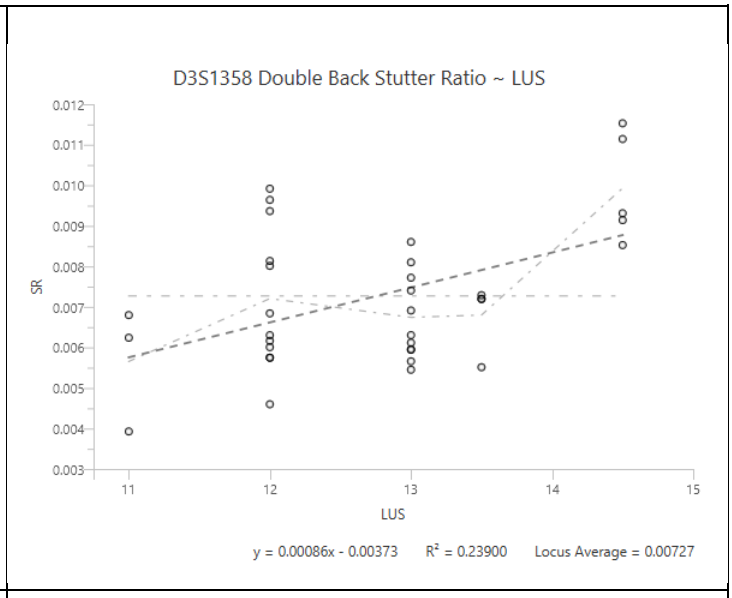

FGA Forward Stutter Ratio ~ Allele
SR
Allele
y = 0.00044x + 0.00055
R² = 0.02250
Locus Average = 0.01050

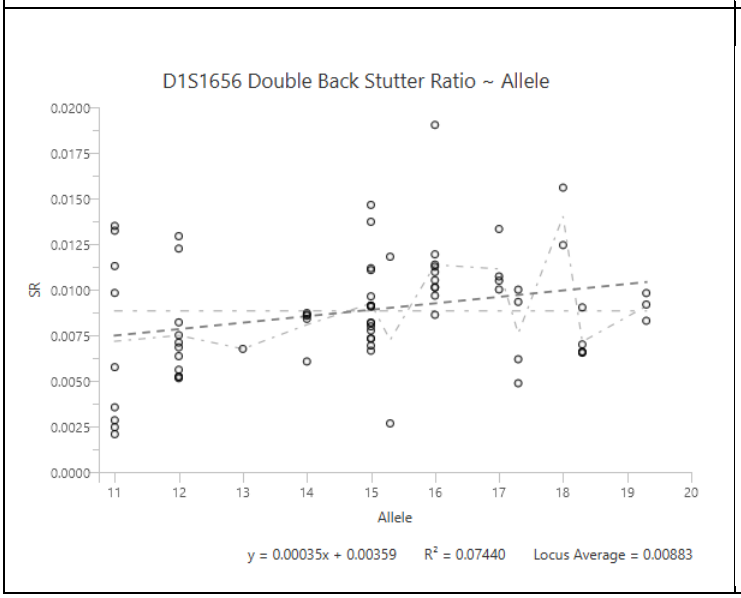
Appendix 3: Half back stutter (-1,2) regression plots for the SE33 and D1S1656 loci determined using the ISP stutter dataset . Plots for *SR* versus allelic designation are provided on the left, plots for *SR* versus LUS designation are provided on the right. The faint, horizontal dash-dot line indicates the average observed per-locus *SR*. The other dash-dot line connects the average observed per-allele *SR*s. The darker dashed line displays the linear regression of SR against allele.

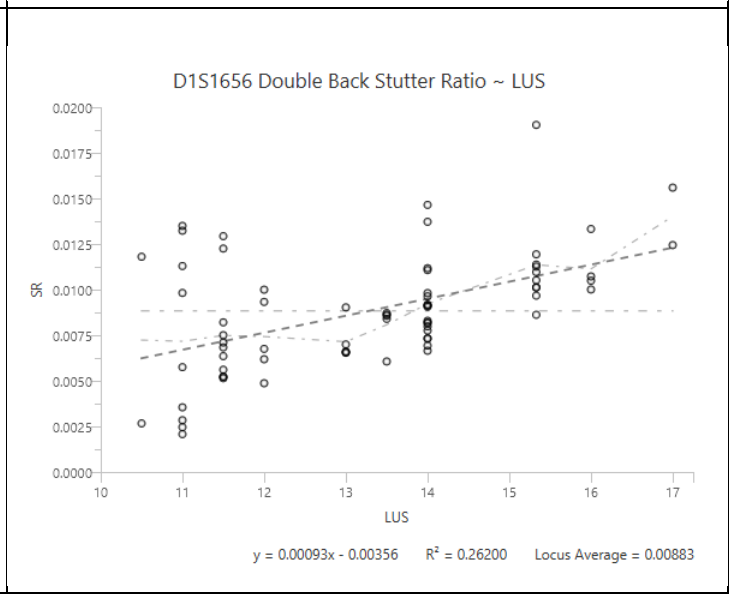


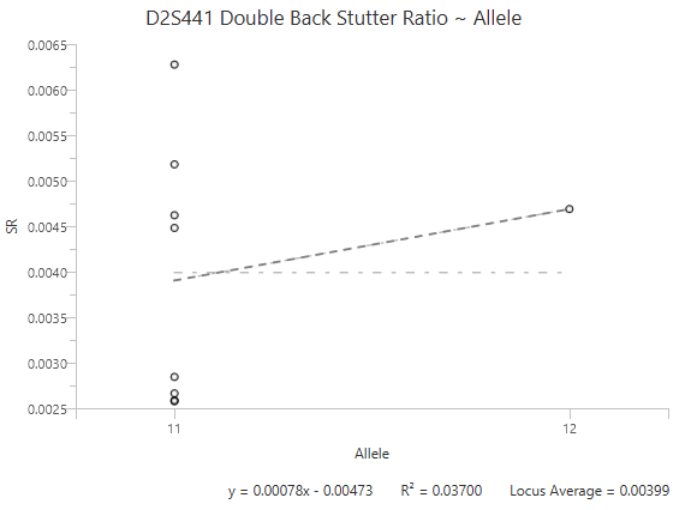
Appendix 4: Double back stutter (-2,0) regression plots for the ISP stutter dataset . Plots for *SR* versus allelic designation are provided on the left, plots for *SR* versus LUS designation are provided on the right. The faint, horizontal dash-dot line indicates the average observed per-locus *SR*. The other dash-dot line connects the average observed per-allele *SR*s. The other dash-dot line connects the average observed per-allele *SR*s. The darker dashed line displays the linear regression of SR against allele.

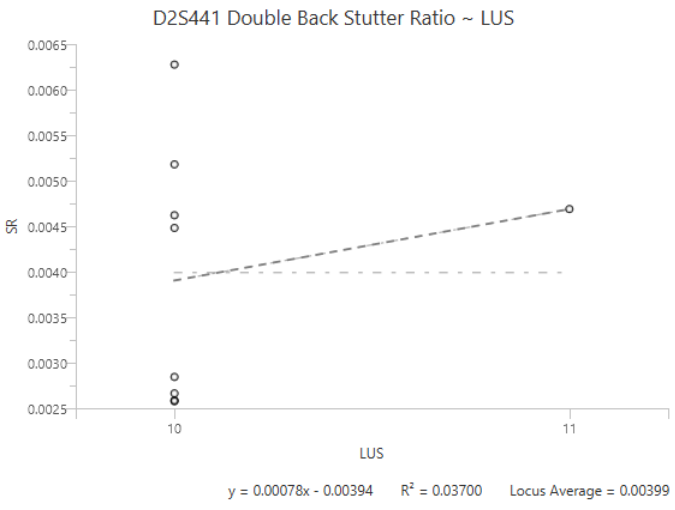


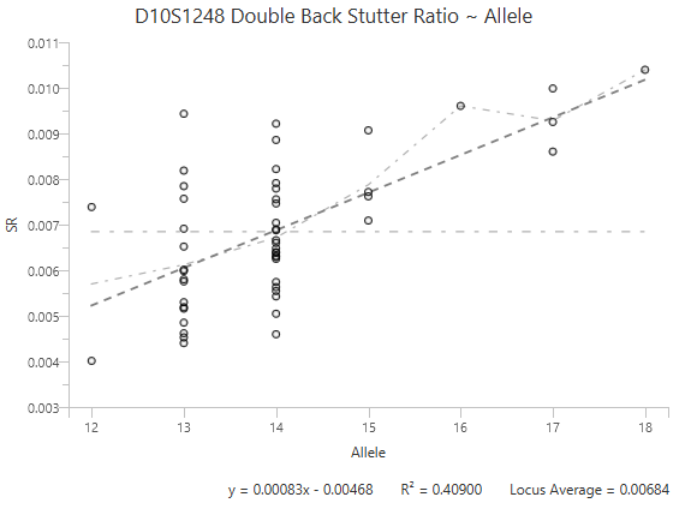


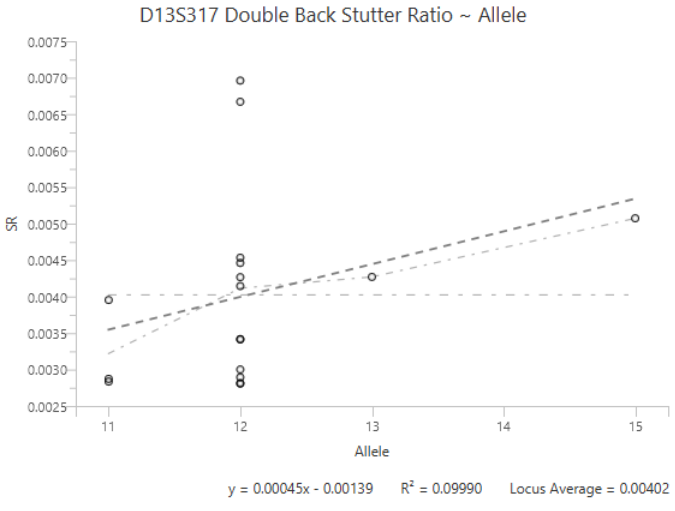


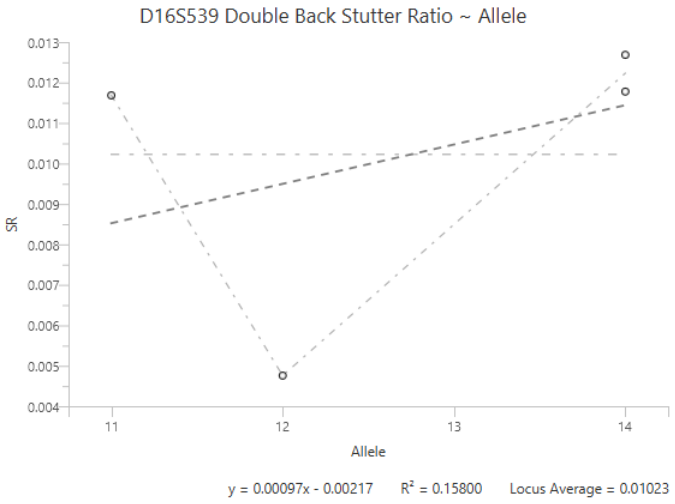


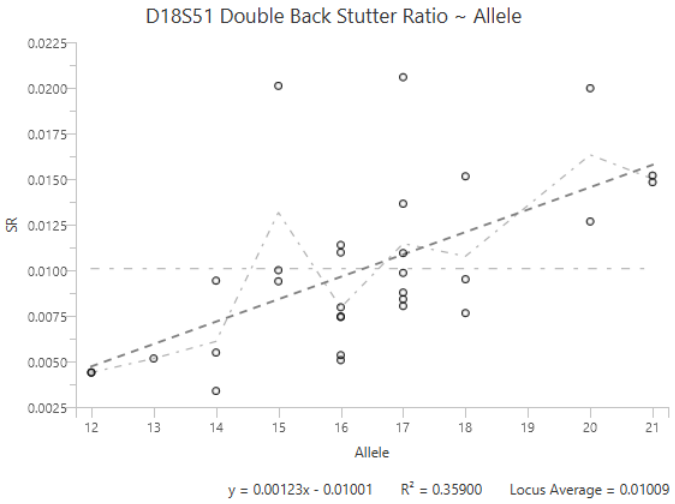

D2S441 Double Back Stutter Ratio ~ Allele
SR
Allele
y = 0.00078x - 0.00473
R² = 0.03700
Locus Average = 0.00399

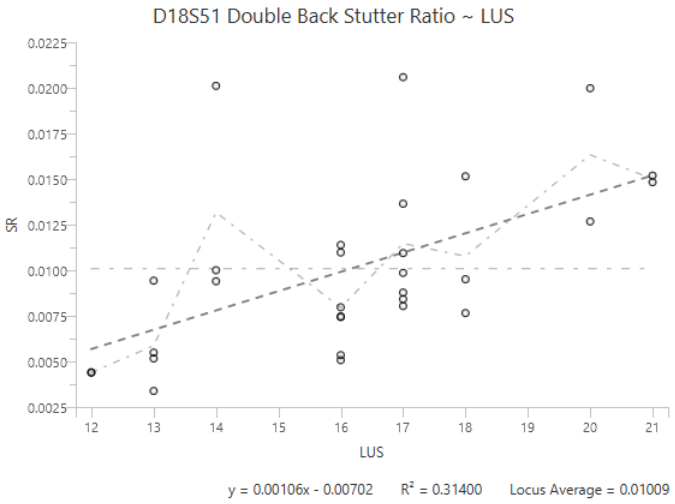

D2S441 Double Back Stutter Ratio ~ LUS
SR
LUS
y = 0.00078x - 0.00394
R² = 0.03700
Locus Average = 0.00399

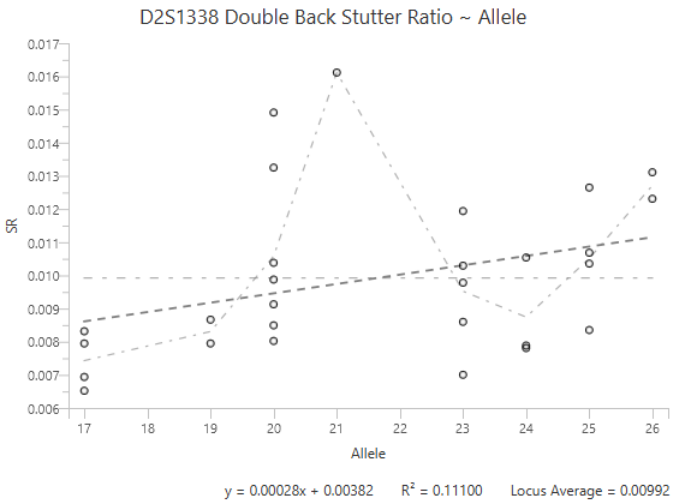

D10S1248 Double Back Stutter Ratio ~ Allele
SR
Allele
y = 0.00083x - 0.00468
R² = 0.40900
Locus Average = 0.00684

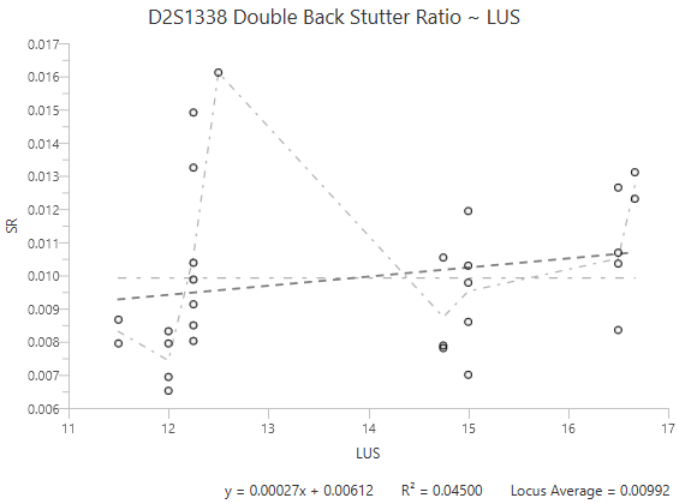

D13S317 Double Back Stutter Ratio ~ Allele
SR
Allele
y = 0.00045x - 0.00139
R² = 0.09990
Locus Average = 0.00402

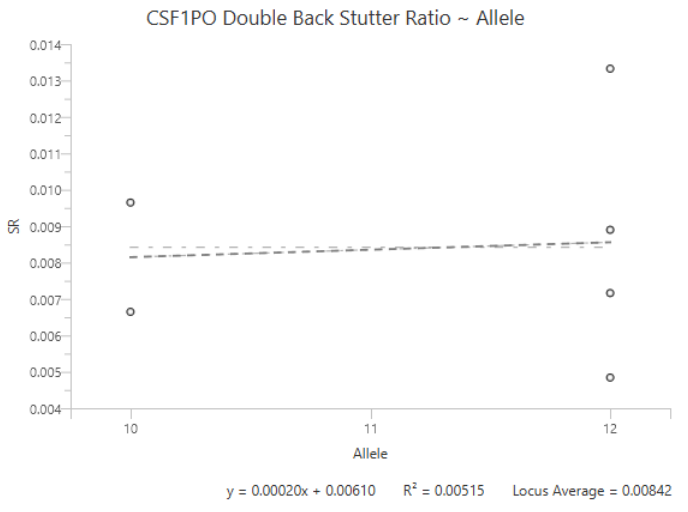

D16S539 Double Back Stutter Ratio ~ Allele
SR
Allele
y = 0.00097x - 0.00217
R² = 0.15800
Locus Average = 0.01023

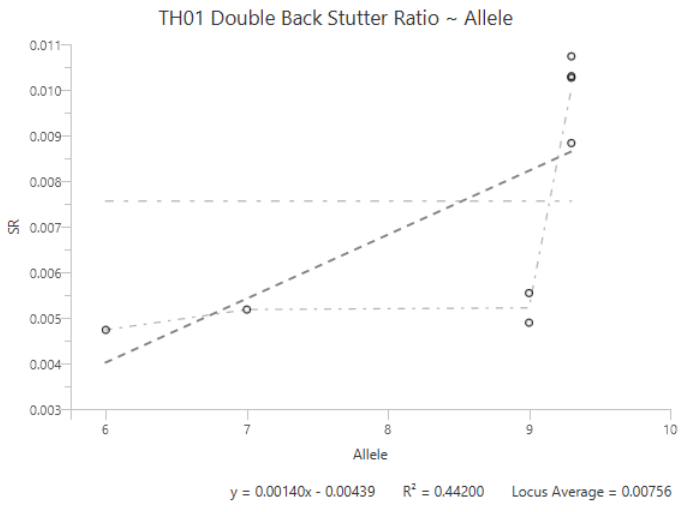

D18S51 Double Back Stutter Ratio ~ Allele
SR
Allele
y = 0.00123x - 0.01001
R² = 0.35900
Locus Average = 0.01009

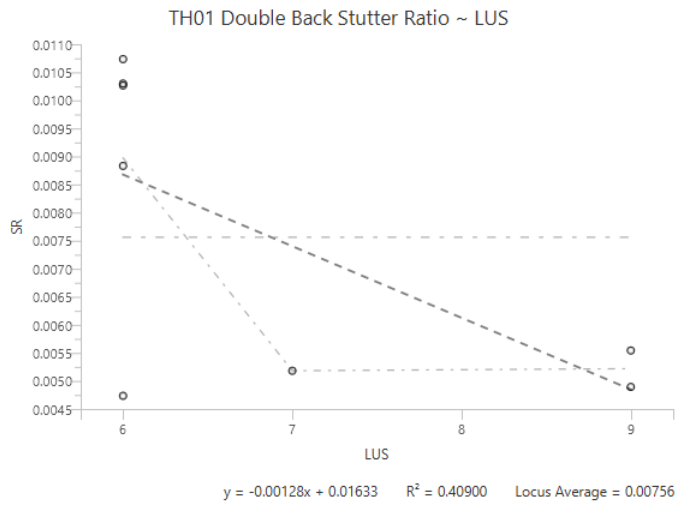

D18S51 Double Back Stutter Ratio ~ LUS
SR
LUS
y = 0.00106x - 0.00702
R² = 0.31400
Locus Average = 0.01009

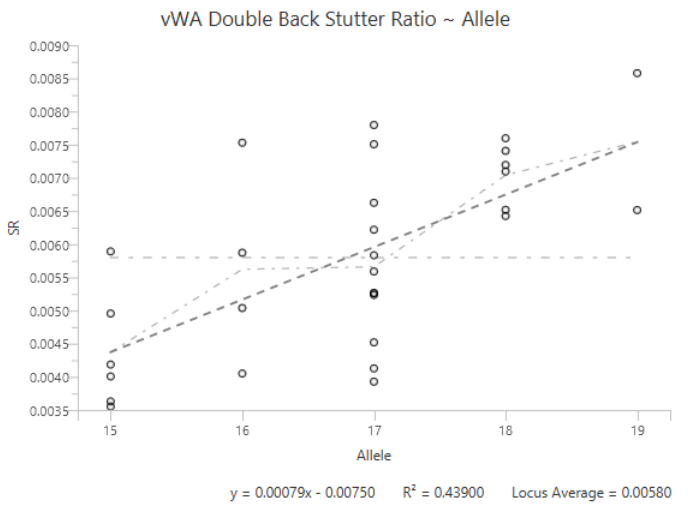

D2S1338 Double Back Stutter Ratio ~ Allele
SR
Allele
y = 0.00028x + 0.00382
R² = 0.11100
Locus Average = 0.00992

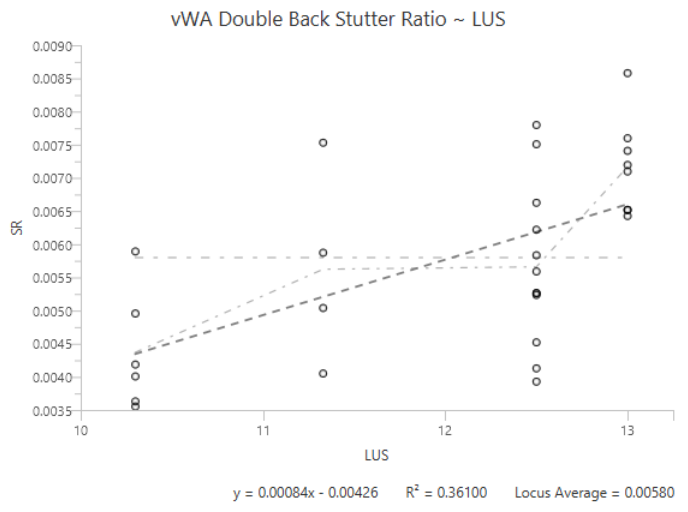

D2S1338 Double Back Stutter Ratio ~ LUS
SR
LUS
y = 0.00027x + 0.00612
R² = 0.04500
Locus Average = 0.00992

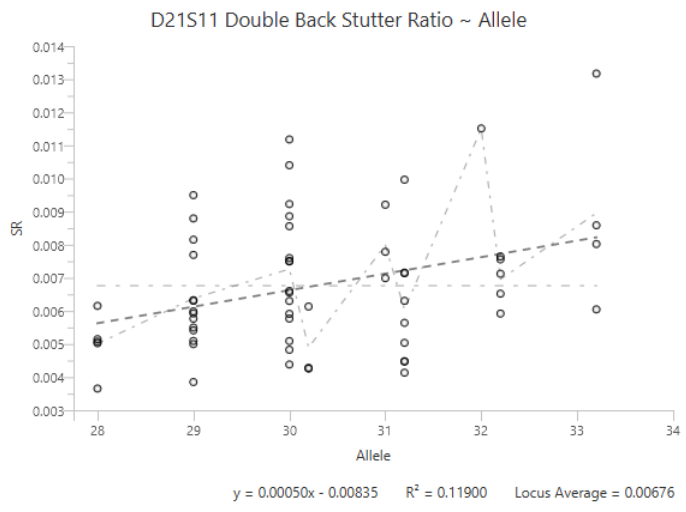

CSF1PO Double Back Stutter Ratio ~ Allele
SR
Allele
y = 0.00020x + 0.00610
R² = 0.00515
Locus Average = 0.00842

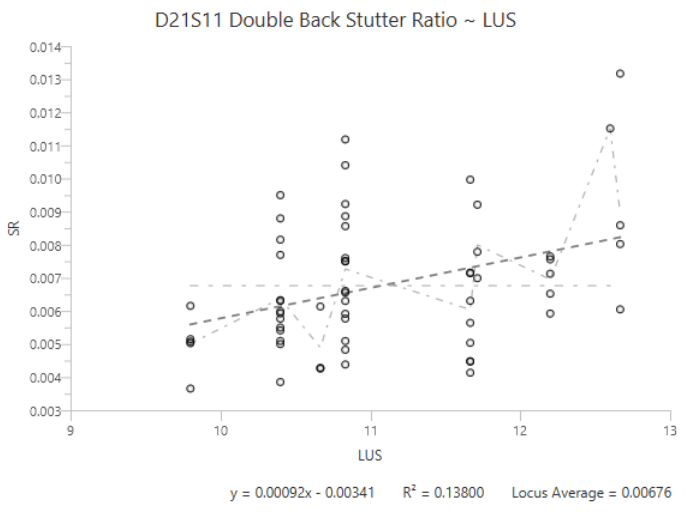

TH01 Double Back Stutter Ratio ~ Allele
SR
Allele
y = 0.00140x - 0.00439
R² = 0.44200
Locus Average = 0.00756

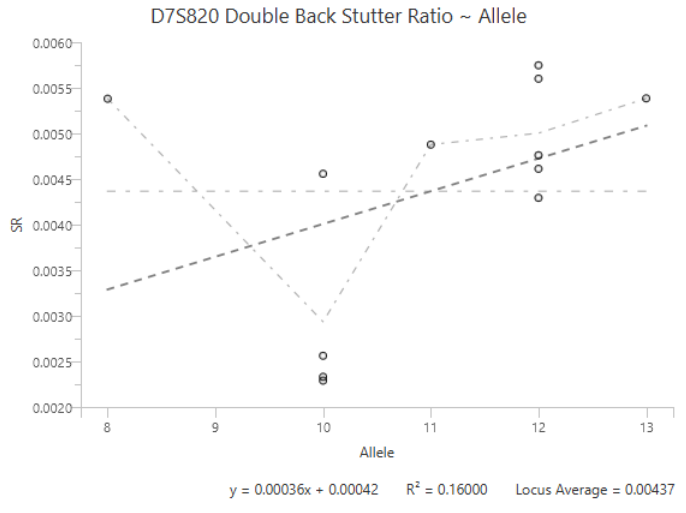

TH01 Double Back Stutter Ratio ~ LUS
SR
LUS
y = -0.00128x + 0.01633
R² = 0.40900
Locus Average = 0.00756

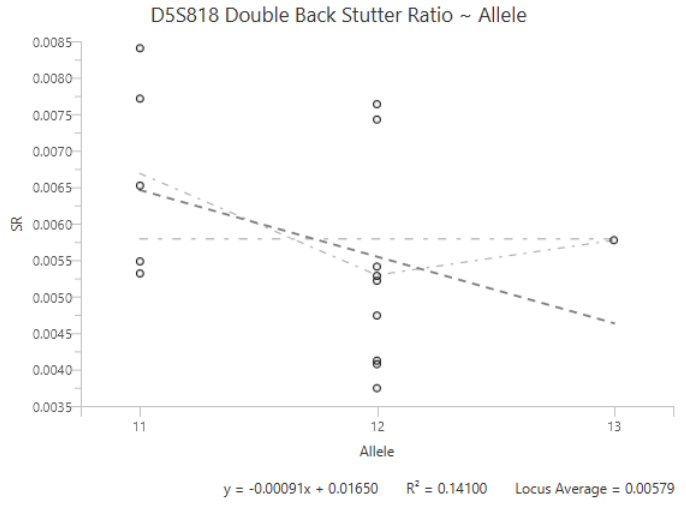

vWA Double Back Stutter Ratio ~ Allele
SR
Allele
y = 0.00079x - 0.00750
R² = 0.43900
Locus Average = 0.00580

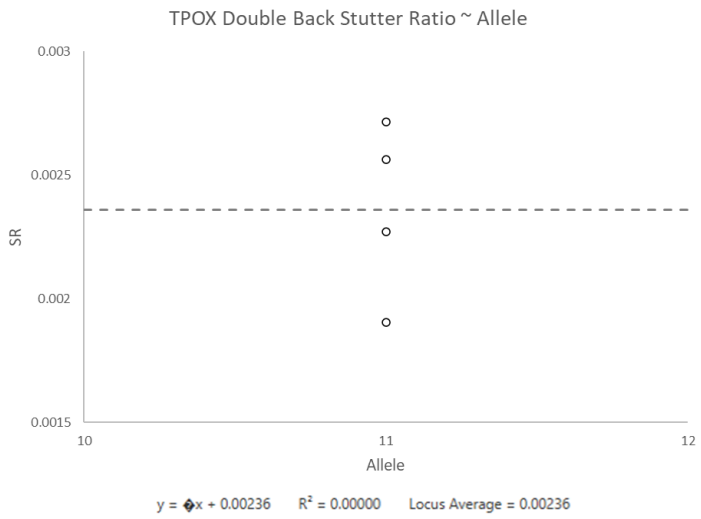

vWA Double Back Stutter Ratio ~ LUS
SR
LUS
y = 0.00084x - 0.00426
R² = 0.36100
Locus Average = 0.00580

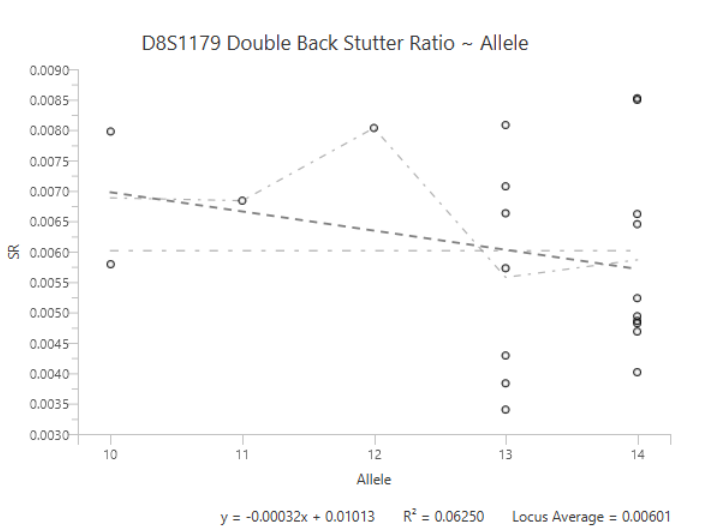

D21S11 Double Back Stutter Ratio ~ Allele
SR
Allele
y = 0.00050x - 0.00835
R² = 0.11900
Locus Average = 0.00676

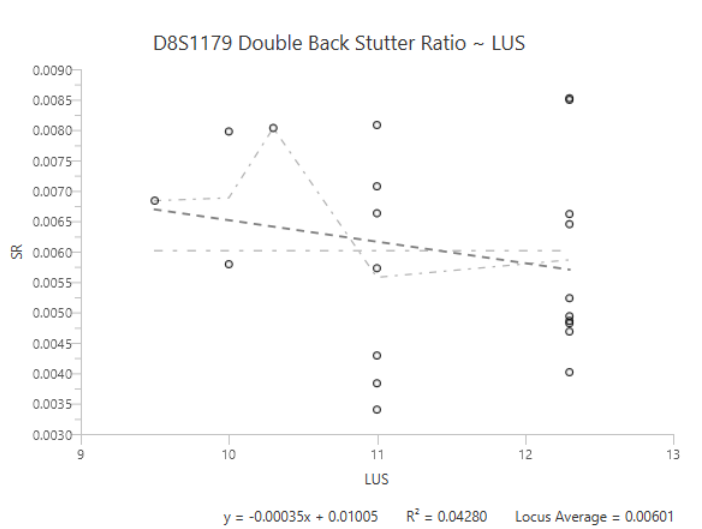

D21S11 Double Back Stutter Ratio ~ LUS
SR
LUS
y = 0.00092x - 0.00341
R² = 0.13800
Locus Average = 0.00676

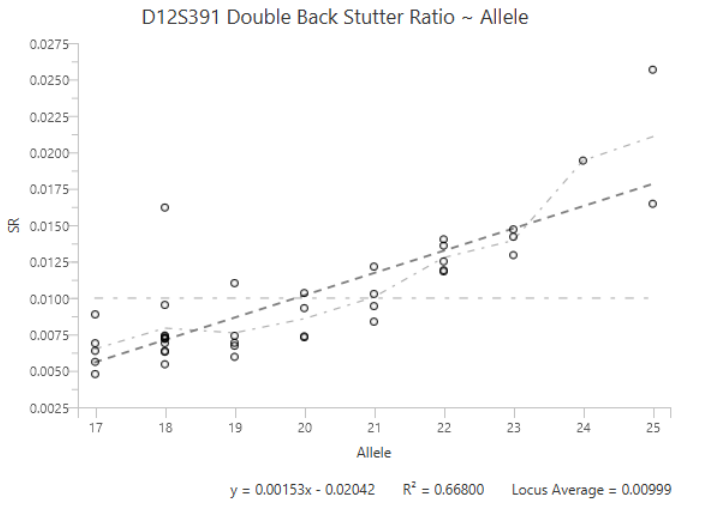

D7S820 Double Back Stutter Ratio ~ Allele
SR
Allele
y = 0.00036x + 0.00042
R² = 0.16000
Locus Average = 0.00437

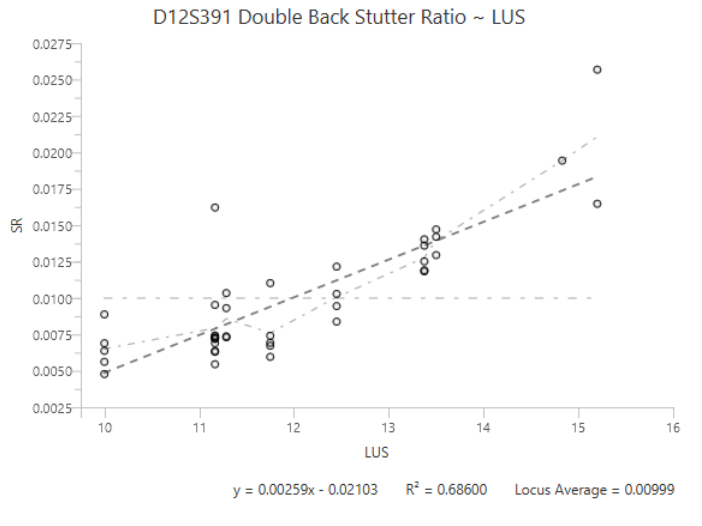

D5S818 Double Back Stutter Ratio ~ Allele
SR
Allele
y = -0.00091x + 0.01650
R² = 0.14100
Locus Average = 0.00579

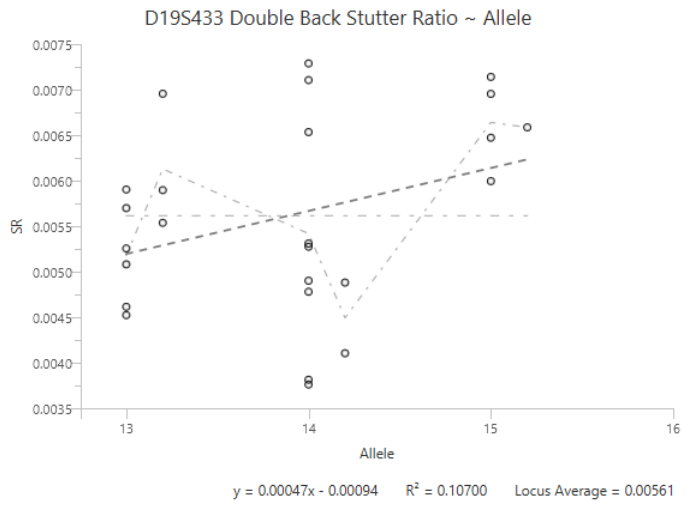

TPOX Double Back Stutter Ratio ~ Allele
SR
Allele
y = x + 0.00236 R² = 0.00000 Locus Average = 0.00236

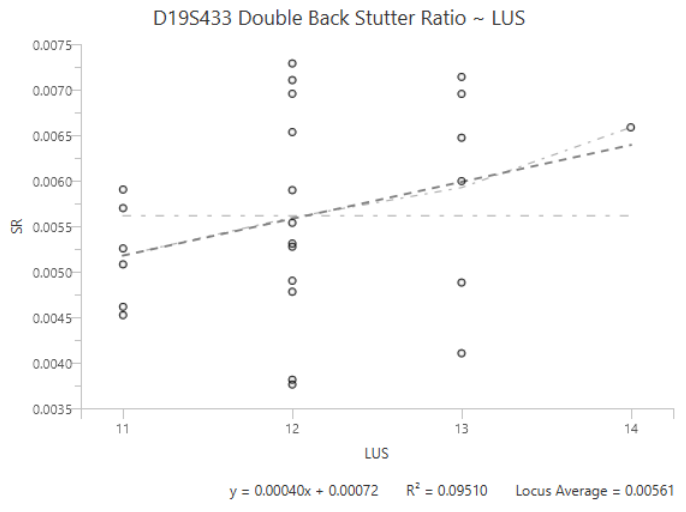

D8S1179 Double Back Stutter Ratio ~ Allele
SR
Allele
y = -0.00032x + 0.01013 R² = 0.06250 Locus Average = 0.00601

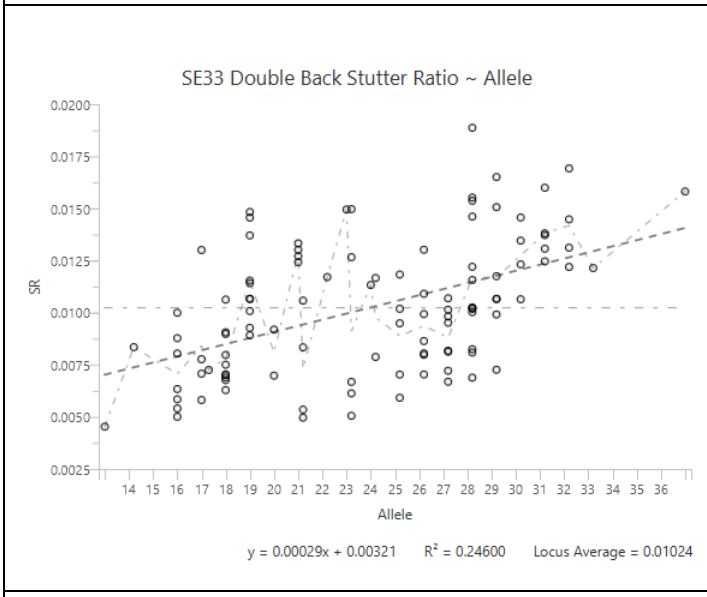

D8S1179 Double Back Stutter Ratio ~ LUS
SR
LUS
y = -0.00035x + 0.01005 R² = 0.04280 Locus Average = 0.00601

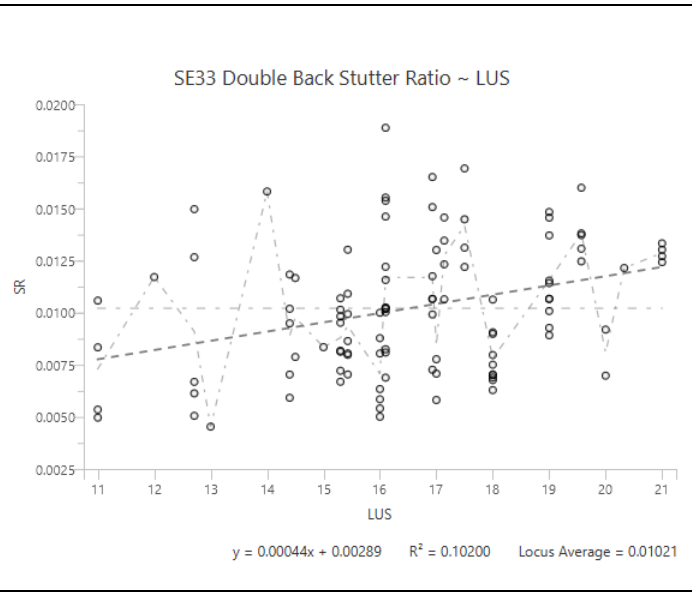

D12S391 Double Back Stutter Ratio ~ Allele
SR
Allele
y = 0.00153x - 0.02042 R² = 0.66800 Locus Average = 0.00999

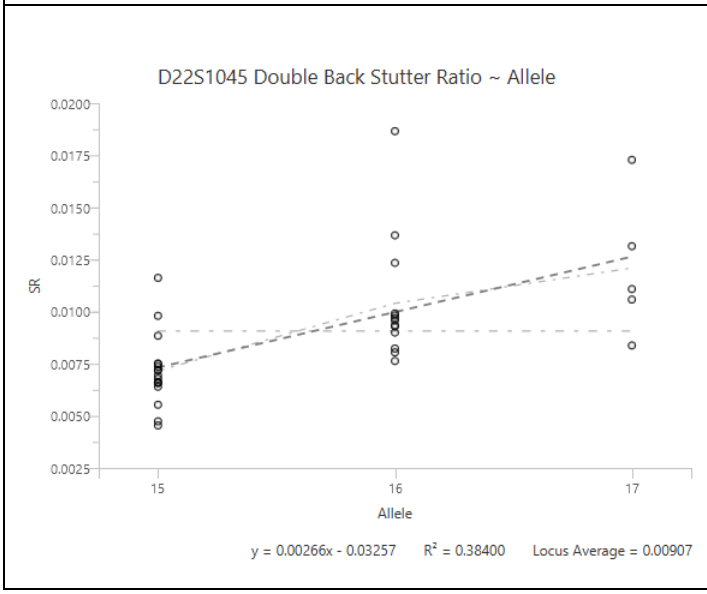

D12S391 Double Back Stutter Ratio ~ LUS
SR
LUS
y = 0.00259x - 0.02103 R² = 0.68600 Locus Average = 0.00999

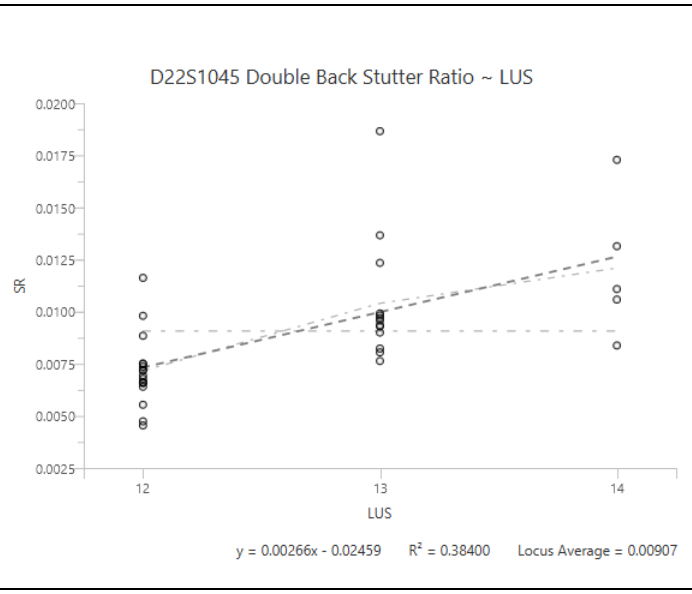

D19S433 Double Back Stutter Ratio ~ Allele
SR
Allele
y = 0.00047x - 0.00094
R² = 0.10700
Locus Average = 0.00561

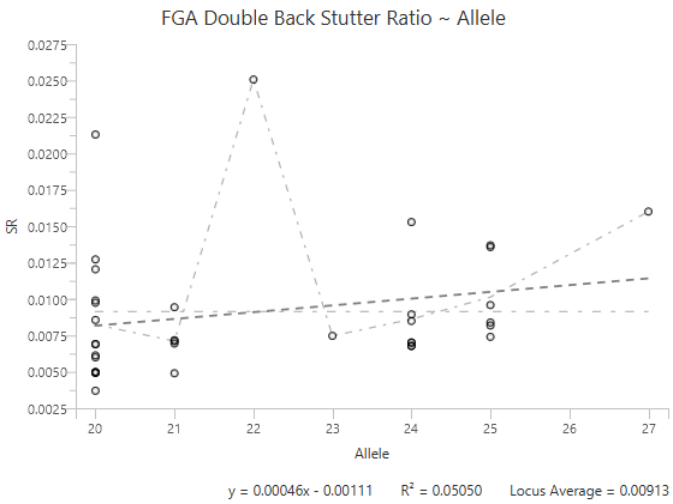

D19S433 Double Back Stutter Ratio ~ LUS
SR
LUS
y = 0.00040x + 0.00072
R² = 0.09510
Locus Average = 0.00561

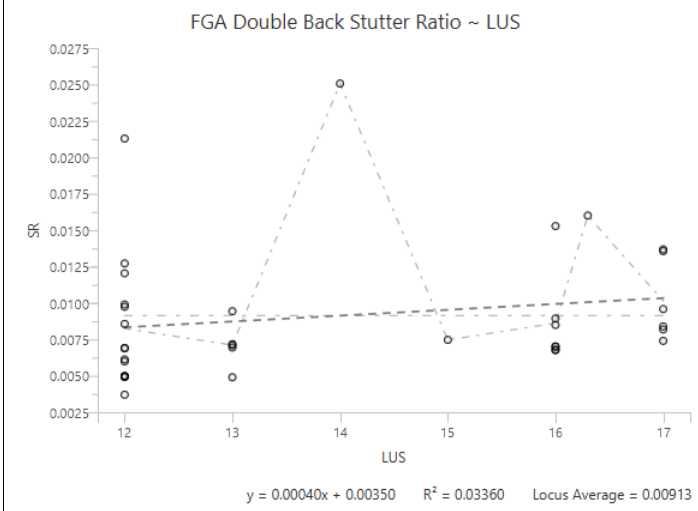

SE33 Double Back Stutter Ratio ~ Allele
SR
Allele
y = 0.00029x + 0.00321
R² = 0.24600
Locus Average = 0.01024


SE33 Double Back Stutter Ratio ~ LUS
SR
LUS
y = 0.00044x + 0.00289
R² = 0.10200
Locus Average = 0.01021


D22S1045 Double Back Stutter Ratio ~ Allele
SR
Allele
y = 0.00266x - 0.03257
R² = 0.38400
Locus Average = 0.00907


D22S1045 Double Back Stutter Ratio ~ LUS
SR
LUS
y = 0.00266x - 0.02459
R² = 0.38400
Locus Average = 0.00907


FGA Double Back Stutter Ratio ~ Allele
SR
0.0275
0.0250
0.0225
0.0200
0.0175
0.0150
0.0125
0.0100
0.0075
0.0050
0.0025
20
21
22
23
24
25
26
27
Allele
y = 0.00046x - 0.00111
R² = 0.05050
Locus Average = 0.00913


FGA Double Back Stutter Ratio ~ LUS
SR
0.0275
0.0250
0.0225
0.0200
0.0175
0.0150
0.0125
0.0100
0.0075
0.0050
0.0025
12
13
14
15
16
17
LUS
y = 0.00040x + 0.00350
R² = 0.03360
Locus Average = 0.00913

Appendix 5: Review of secondary diagnostics

Secondary diagnostics are a useful guide to provide confidence the STRmix™ interpretation has progressed as expected. Individual secondary diagnostics may indicate whether a more comprehensive review is warranted, however analysts should not rely on these diagnostics alone. Elevated values for one of these diagnostics may not necessarily mean the results are unfit for purpose. To put in context the range of diagnostic values that can be expected from ISP data, a discussion of the secondary run diagnostics obtained from the Section D interpretations is provided below.

Effective sample size (ESS)

This is a measure of the degree of correlation within the accepts of a STRmix™ deconvolution. It is used within the Highest Posterior Density (HPD) method to help take into account uncertainty in the weights. ESS is used to convert the full sample set of iterations that includes many with correlation into a set of independent samples that may be resampled from during the HPD process. If a sample set for a chain is fully correlated then an ESS value of 1 would be observed and this indicates a problem with the deconvolution. As there are 8 chains, full correlation across each of these chains would display as 8. No ESS values of 8 were observed within this limited data set but analysts are advised to review this as part of their STRmix™ output and interpretation process. It has been demonstrated that correlation can be seed (starting point) related, so if a value of 8 is observed simply rerunning the deconvolution with a different seed is recommended.

Average log(likelihood):

STRmix™ uses a biological model to generate an expected DNA profile which is then compared with the observed profile. When assessing the fit of the expected profile with the observed, STRmix™ calculates a 'grade', referred to as a log(likelihood). The average log(likelihood) diagnostic reported in the STRmix™ output is the average of the log(likelihood) values across all post-burn-in iterations. The larger this value is, the better STRmix™ has been able to describe the observed data. A low or negative value suggests that STRmix™ has not been able to describe the data very well given the information it has been provided with. Reasons why this value may be low or negative include:

1. The profile is simply low level and there is very little data making up the likelihood,
2. There are large stochastic events in the STRmix™ run (e.g. large heterozygote peak imbalances or variation in mixture proportions across the profile). These may be forced by mis-assignment of the number of contributors.
3. Data has been removed that was real, in particular stutter peaks, and must now be described within STRmix™ by dropout, and where data is absent (rather than removed) eg due CE resolution limitations or pull up effects
4. Artefactual peaks have been left labelled and must now be accounted for within STRmix™ by e.g. drop-in.

As per point 1 above, it is important to note that low or negative average log(likelihood) values may legitimately be produced when interpreting low level DNA profiles. As such, low or negative average log(likelihood) values do not necessarily indicate that the STRmix™ results are unreliable.

The average log(likelihood) diagnostic for each Section D interpretation is plotted against experimentally designed NOC in Figure 23 below. The smallest value observed was approximately 3.63 and was recorded for a low level three-person mixture (3.2.1_C5) where only twelve autosomal peaks are present in the input file with heights below 200 rfu. The largest value observed was approximately 116 and was recorded for a high template four-person mixture (10.5.2.1 C1).

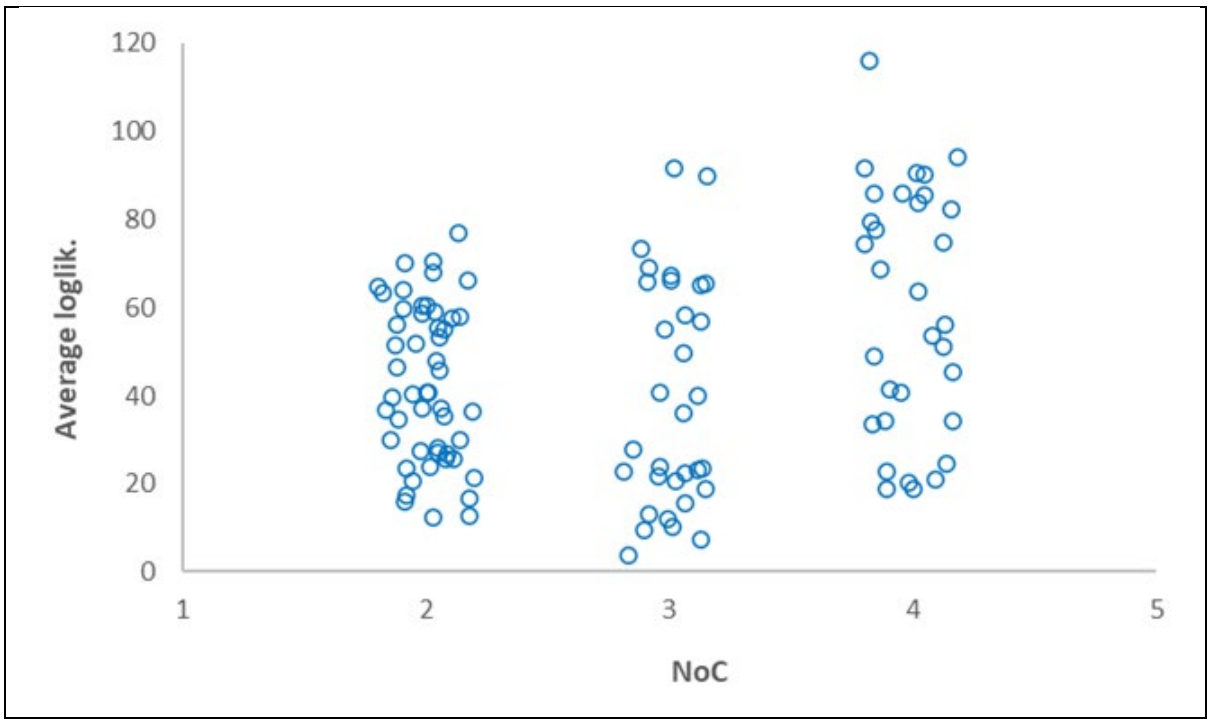


Figure 23: Plot of average log(likelihood) diagnostic versus apparent number of contributors.

Gelman-Rubin convergence diagnostic:

Ideally, each MCMC chain will reach the area of high probability within the sample space during burn-in and will continue to sample from this space during the post-burn-in MCMC. This is referred to as 'convergence'. If the chains spend their time in different spaces during the post-burn-in MCMC then it is likely that the analysis has not been run for long enough. The Gelman-Rubin (GR) convergence diagnostic included in the STRmix™ report can indicate to the user if the Markov chains have not sufficiently converged. If the chains have been sampling from the same space, then the GR diagnostic should be close to 1.0. Notionally, values above 1.2 indicate that the chains may not be nearing convergence. It is important to note that the GR diagnostic output by STRmix™ is a summary statistic: values less than 1.2 do not guarantee that all parameters have converged whilst values greater than 1.2 do not necessarily indicate that the results are unreliable.

In rare instances, one (or more) chain(s) may fail to find the area of high probability space altogether. This is referred to as a wandering chain and typically leads to substantially elevated GR diagnostics. Often, the genotypes accepted at one or more loci will not be intuitive in instances where there has been a wandering chain. Simply re-running the interpretation will typically recover the GR and produce sensible results. However, not all causes of an elevated GR can be addressed in this way, therefore as with all run diagnostics it is recommended that both the input and primary and secondary outputs of runs with excessive values are closely scrutinized.

The GR convergence diagnostic for each Section D interpretation is plotted against experimentally designed NOC in Figure 24 below. The largest GR observed was approximately 3.59 and was produced following interpretation of a high template four-person mixture (10.5.2.1 C1_F02). A review of the interpretation report revealed that there is a missing stutter peak (10.3) at the D2S441 locus. It is likely this peak failed to be resolved from the neighbouring 11 peak during the CE process. Whilst strong inclusionary *LR*s were assigned for all known donors to this mixture, the consideration of rework action (reinjection of PCR product onto the CE machine or ignoring the affected locus within a repeat deconvolution) is recommended as missing stutter can impact the MCMC process and weights assigned.

Slightly elevated GRs for higher order mixtures (i.e. 4p) are not unexpected. These GRs can indicate that the chains have made it into the same probability space but have possibly not had quite enough time to explore the space. Reliable results may still be produced in circumstances where an elevated GR diagnostic has been produced, however, careful review of the weights for samples where GRs > 1.2 are observed is recommended. It is suggested that analysts can re-run interpretations with GRs > 1.2 with extended burn-in and post-burn-in accepts, as described below.

Eleven other instances of moderately elevated GRs were observed, within this dataset; ranging from ~1.45 to ~3.29. Five of these profiles were also identified as having missing stutter peaks whilst the remaining six profiles were complex either four person mixtures and/or profiles where contributors were designed to contribute relatively similar amounts (close proportions). Re-running these six profiles with 10x burn-in and 10x post burn-in accepts resulted in improvements to the GR with most falling below the desired 1.2. No significant changes to the LRs assigned to the known donors to these samples were observed.

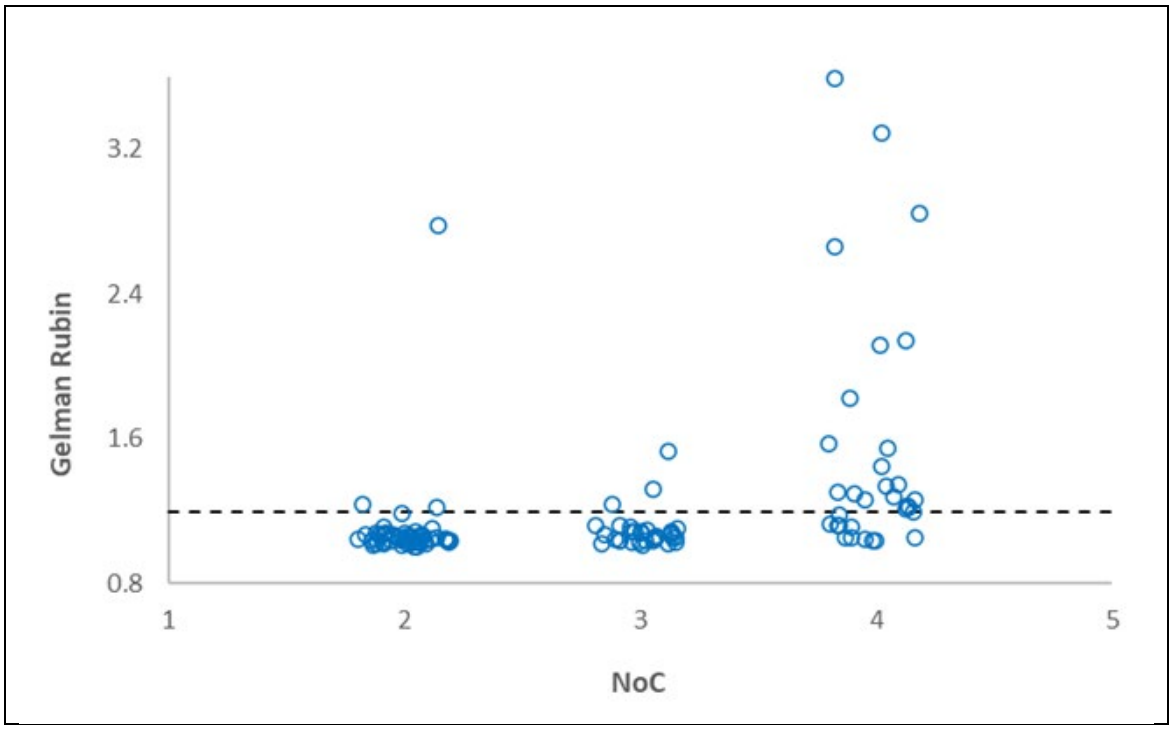


Figure 24: Plot of Gelman-Rubin (GR) convergence diagnostic versus experimentally designed number of contributors. The dashed line indicates a GR value of 1.2.

All other mildly elevated GRs were likely to be due to profile complexity either higher order mixtures or mixtures where the contributors were designed to be in even proportions.

Posterior variance parameters:

Within the STRmix™ report, the posterior mean variance parameters are overlaid on the relevant prior distributions. Ideally, each of the posterior variance parameters should sit within the body of the relevant prior distribution. Values that fall in the right hand tail of the prior distribution may warrant further investigation. A large allele variance parameter in conjunction with a low or negative average log(likelihood) diagnostic may indicate that the number of contributors to the profile has been mis-assigned. Excessive stutter variance parameters may be due to the inadvertent application of a stutter filter during CE profile analysis. As with the other secondary diagnostics described above, elevated variance parameters do not necessarily invalidate the results. Provided that the primary diagnostics are intuitive, the STRmix™ results are likely reliable.

The posterior variance parameters for each Section D interpretation along with their prior distributions are provided in Figure 25 below. Several elevated values were observed, these are discussed below.

The posterior LSAE variance values for the deconvolutions spanned a range of values with some resulting in values that sit in the right-hand tail of the distribution. The input files of deconvolutions resulting in LSAE variance values greater than 0.013 were reviewed further. The majority of these profiles displayed either differences in the peak heights between the dyes or across a dye often in low level or complex profiles where stochastic sampling effects may be occurring. For example, the highest posterior mean LSAE variance (0.022) value was observed following the deconvolution of profile '4.3.2.1 C4_A02'. This profile was relatively low level with the heights of the FGA peaks (purple dye) being somewhat lower than the other dye channels. There also appeared to be an increase in peak heights across the red dye, from low molecular weight to high. This is counter to the expected behaviour of DNA profiles and the STRmix™ model which generally assumes a decrease in peak height across the molecular weight range. In other profiles clear differences in the peak heights in the blue (often higher), and green and purple (often lower) dye sets were noted. As only one template value is proposed in each iteration, if there are inter- or intra- locus balance issues STRmix™ may propose higher LSAE values to account for deviations from the proposed template/degradation value. This can in turn lead to higher LSAE variance values being required. Whilst the *LR*s assigned to true contributors and non-contributors were as expected for these profiles, analysts are advised to use caution if inter- or intra- dye balance issues are noted; the weights and other diagnostics should be reviewed carefully and where appropriate rework to address issues should be undertaken.

The highest, and slightly elevated, allele variance value was observed following the interpretation of '1.1.1.1_C2_D04'. Similar to profile '1.1.1.1_C1' already discussed above within 'review of specific results', this profile was found to have a stutter peak (9 at CSF1PO) missing from the input file. As already discussed missing peaks can have a significant effect on the MCMC process and may lead to other elevated diagnostics or unintuitive weights.

The six back stutter variance values that were greater than 60 were investigated further. In each instance missing stutter peaks were detected in the input files of each sample. As discussed previously, STRmix™ will record peaks that it suspects are missing from the input file in the Interpretation Report. STRmix™ analysts will also be alerted prior to the run commencing if missing peaks are detected giving them the opportunity to investigate futher. Five out of the six of these missing peaks appeared to be due to the non-resolution (during the CE process) of two peaks separated by only one base pair. The remaining peak identified was the 9 peak discussed above (possibly missing due to 'pull-down' of the fluorescent signal).

A single elevated posterior forward stutter variance value (~ k^2 = 77) was observed following the deconvolution of 10.5.2.1 C1_F02. A missing back stutter peak (a 10.3 at the D2S441 locus) possibly impacted the deconvolution (as discussed previously) leading to an increased GR value of 3.59 indicating non-convergence of the chains and also led to elevated back and forward stutter variance values. Reinserting the missing peak and rerunning the deconvolution led to lower back and forward stutter variance values. The GR was still elevated but this is likely due to the overall complexity of the profile.

No strongly elevated posterior mean variance values were observed for half back stutter or double back stutter.

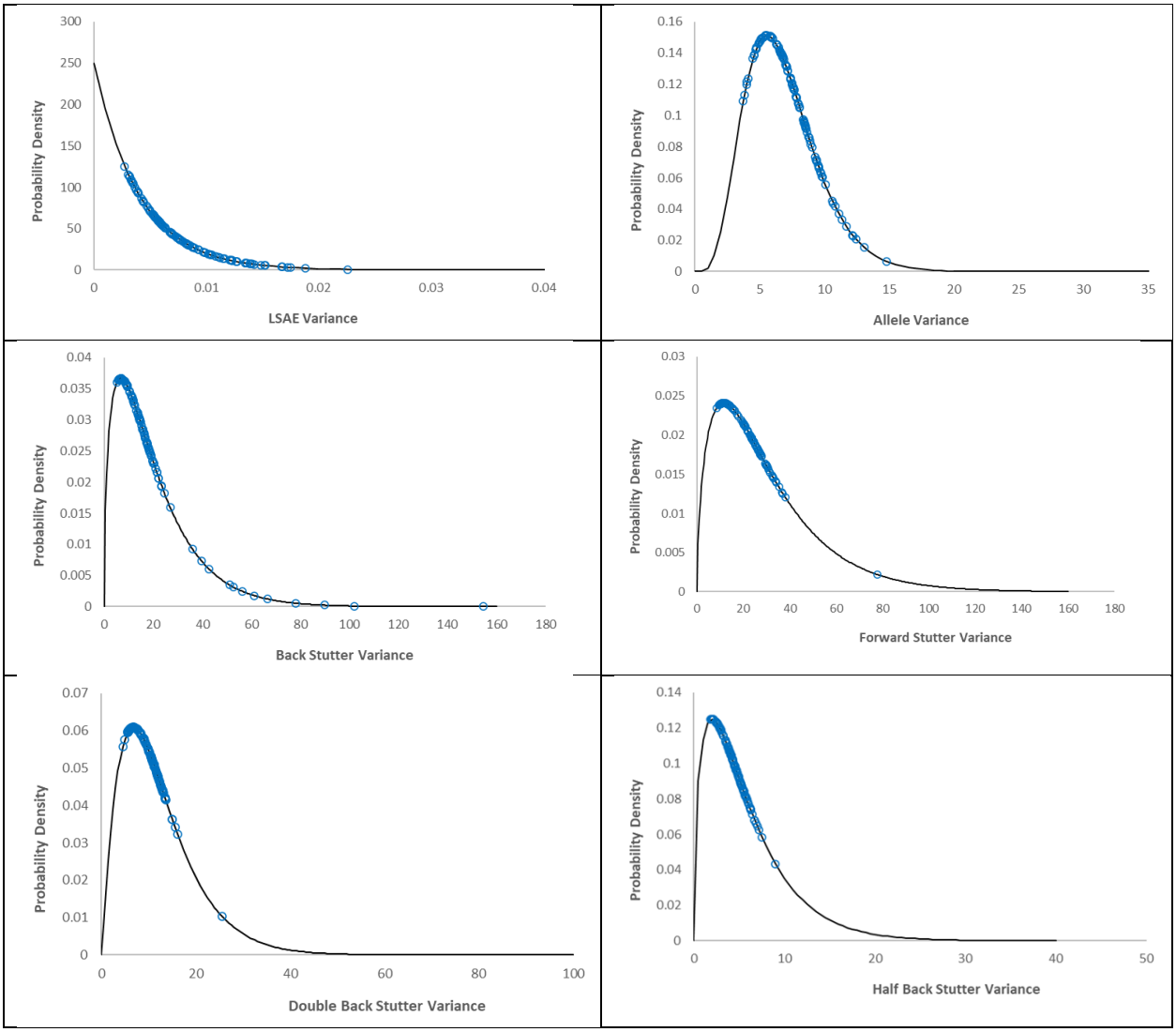


Figure 25: Plots of the posterior mean LSAE and peak height variance values (blue circles) from each of the ISP STRmix™ V2.9.1 Fusion 6C deconvolutions overlaid on their respective prior distributions.