Forensic and Scientific Services

Minor Process Change

Stage 2

		Project #:	199
Proposed by :	Angela Adamson, Emma Caunt, Cassandra James, Rhys Parry	**Date:**	25/03/2022
Title:	Summary – Model Maker results for Project #199		
Comment to be added to SOP:	☐ Yes QIS#_____ ☐ No	**Completed date:**	
Email communication sent:	☐ Yes Team/s_____ ☐ No	**Completed date:**	
Add to minor change register	☐ Yes	**Completed date:**	
Outline of Minor Change:			

Introduction

The ProFlex™ 96-well PCR System (ProFlex) thermal cyclers were implemented in Forensic DNA Analysis on the 10th January 2022, replacing the end of life GeneAmp® PCR System (9700) thermal cyclers.
Advice from the STRmix™ support group recommended re-running Model Maker to see whether the new thermal cyclers have affected the peak height [1]. If there were no substantial changes to the variances determined by Model Maker then it would be acceptable to keep using the existing STRmix™ parameters.

Summary of work undertaken

Results from single source samples that were analysed during the validation of the Proflex thermal cyclers as part of Project #199 were used.

A batch of 42 single source samples run once at a template of 0.5ng, and 6 samples run as a serial dilution at templates of 0.001ng, 0.005ng, 0.025ng, 0.125ng,0.25ng, 0.5ng and 0.7ng was created. This batch of 78 samples was amplified on each of the 6 Proflex instruments and once on a 9700 instrument. Samples were read at 80 rfu with -1 rpt Stutter and +1 rpt Stutter left labelled as per standard operating procedures.

Data obtained from each of the 6 Proflex thermal cyclers were combined into one single source (casework) input file and reference profile information was collated into a separate input file. The data obtained from the 9700 was kept in a separate single source (casework) input file. These files were analysed using the Model Maker function of STRmix™ v2.8.0.

The variances obtained from the Proflex instruments and the 9700 were compared with those used currently in casework assessment using STRmix™.

Study findings

A summary of each variance value calculated by Model Maker is included in Table 1 below, along with the values currently in place for routine analysis (sourced from Project#219 - Verification STRmix™ 2.7 for 3500xL).

Table 1 Summary of Model Maker output

	Current Settings			Proflex Model Maker			9700 Model Maker		
	α	**β**	**MODE**	**α**	**β**	**MODE**	**α**	**β**	**MODE**
Allele Variance C^2	10.197	1.801	16.564	14.095	1.366	17.888	10.327	1.663	15.511
Back (-1rpt) Stutter Variance k^2	1.703	14.134	9.936	2.082	8.192	8.864	3.399	4.194	10.061
+1rpt stutter Variance k^2	5.519	28.11	127.029	2.908	31.797	60.669	4.626	17.636	63.948
	λ	**MEAN**		**λ**	**MEAN**		**λ**	**MEAN**	
LSAE Variance	103.756*	0.01		69.312	0.014		57.382	0.017	

***Note:** Current setting used in STRmix™ v2.8 is 100.00 due to rounding by STRmix

Comparisons of the current values with those obtained from the Proflexes and 9700 showed that there were differences between them.

In order to visualise the above data, graphical representations comparing the STRmix™ settings with those generated from two different Model Maker runs (Proflexes and 9700), are shown in Figures 1-4.

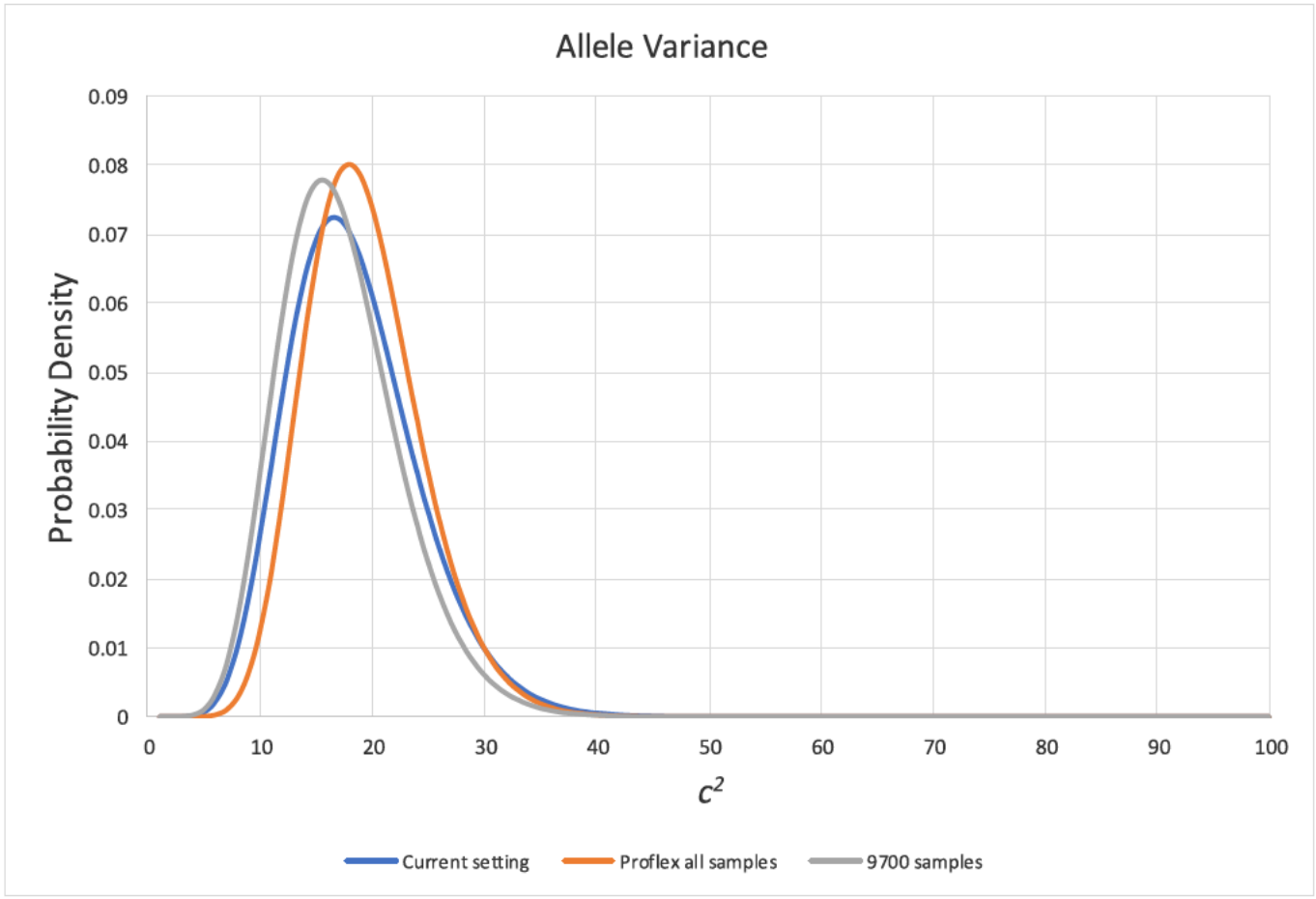

Figure 1 – Allele Variance

Figure 1 shows that the allele variances between the current settings, Proflexes and 9700 were all similar.

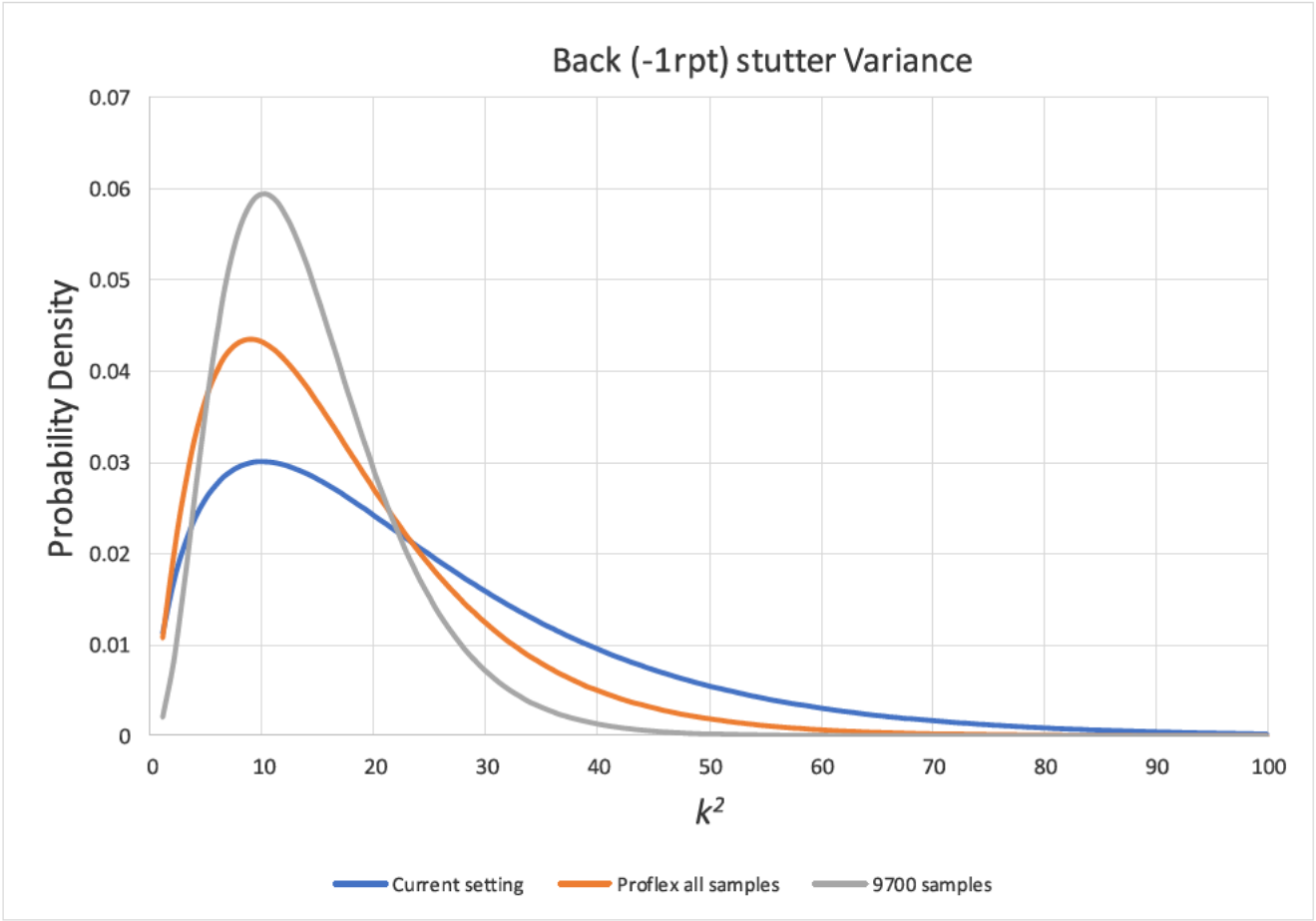

Figure 2 – Back (-1 rpt) stutter variance

The -1 rpt stutter (back stutter) variance values (Figure 2) have a similar mode however the distribution for Proflex and 9700 variance is considerably narrower than the existing distribution. This could result in more stutters being designated as allelic more so than the current settings being used. It therefore could be considered that the current settings would be more lenient than Proflex model maker settings.

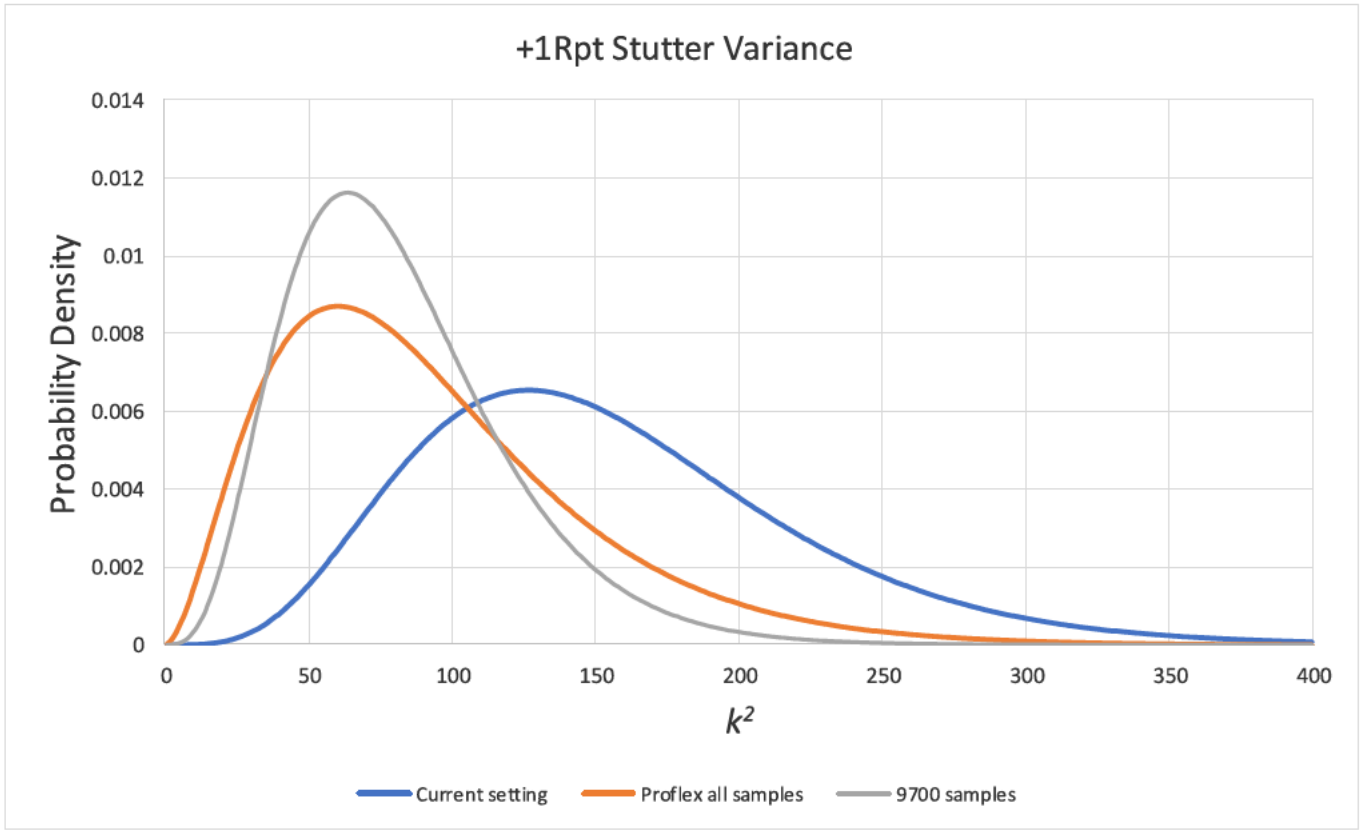

Figure 3 - +1 rpt stutter variance

The +1rpt stutter variance values (Figure 3) are very different with respect to mode and the shape of the distribution. This could result in more +1pt stutters being designated as allelic under the Proflex settings than under the current settings being used. It therefore could be considered that the current settings for +1 rpt stutters are more lenient than Proflex model maker settings.

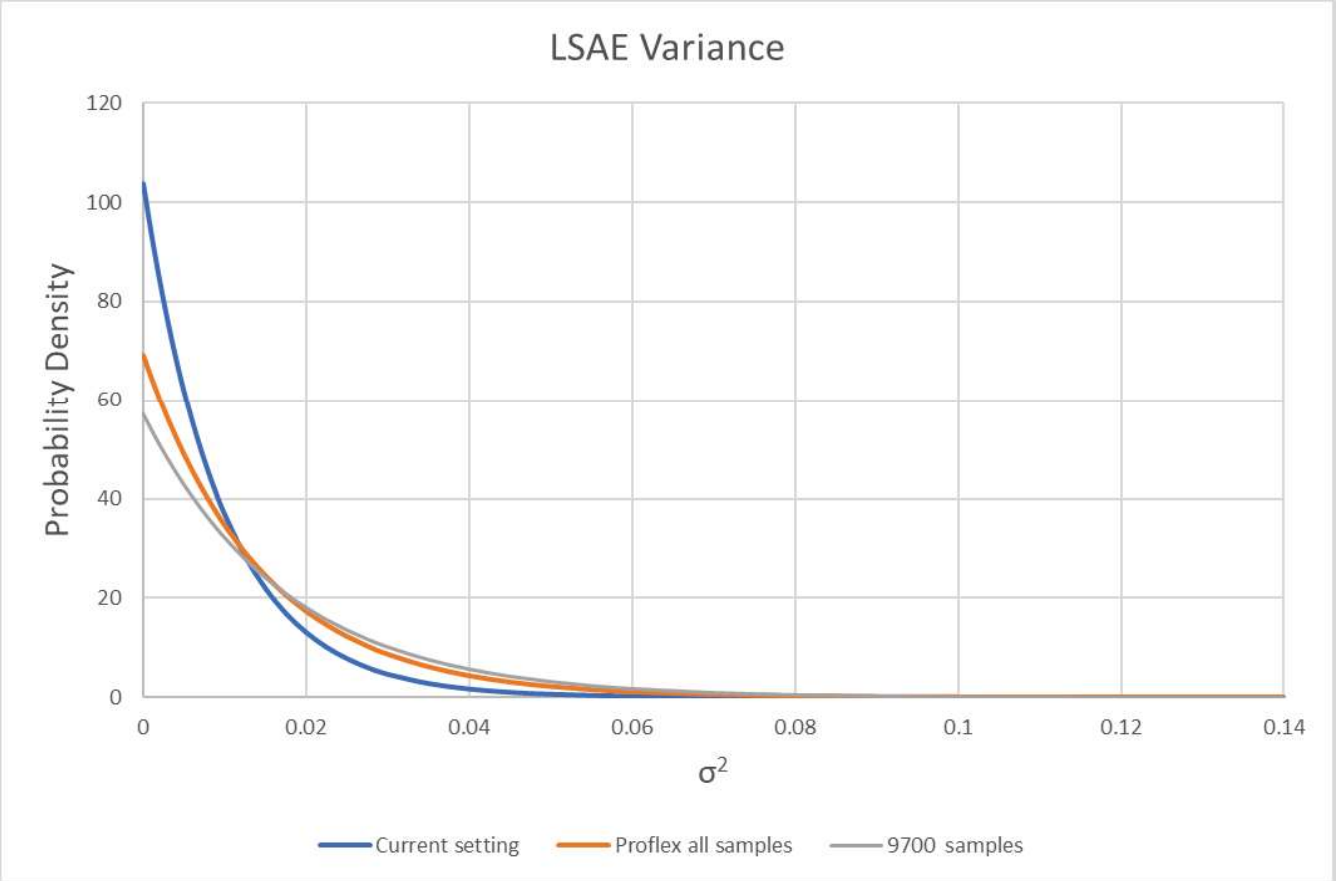

Figure 4 – LSAE variance

The LSAE variance value for the Proflexes is higher than that of the current LSAE variance. This difference could have a significant effect on profile modelling as it may allow for more profile variations than the current settings. It was also considered that this observation could be due to the lower quality of samples in Proflex model maker dataset.

The 9700 variances and the Proflex variances differed from each other and from the current settings. The graphs demonstrated that there are differences observed with the back stutter variance and +1rpt stutter variance. These differences may have also resulted from the limitations of the data used or could indicate a drift in the settings over time. In order assess these differences further a decision was made to conduct further experimentation using a full sample set.

Summary of further work undertaken:

A batch of 10 single source samples were amplified at input templates of 0.025ng, 0.078ng, 0.131ng, 0.183ng, 0.236ng, 0.289ng, 0.342ng, 0.394ng, 0.447ng, 0.125ng,0.25ng, 0.5ng, 0.6ng and 0.7ng across two Proflex instruments. Samples were read at 80 rfu with -1 rpt stutter and +1 rpt stutter left labelled as per standard operating procedures. The resulting files were analysed using the Model Maker function of STRmix™ v2.8.0.

Summary of findings

A summary of each variance value calculated by Model Maker is included in Table 2 below, along with the values currently in place for routine analysis (sourced from Project#219 - Verification STRmix™ 2.7 for 3500xL).

Table 2 – Model Maker output comparison

		Current Settings	Proposed Settings
Allele Variance c^2	**α**	10.197	9.288
	β	1.801	1.974
	MODE	16.564	16.361
Back (-1rpt) Stutter Variance k^2	**α**	1.703	1.875
	β	14.134	12.316
	MODE	9.936	10.777
+1rpt stutter Variance k^2	**α**	5.519	4.780
	β	28.11	24.405
	MODE	127.029	92.251
LSAE Variance	**λ**	103.756*	54.096
	MEAN	0.010	0.018

***Note:** Current setting used in STRmix™ v2.8 is 100.00

In order to visualise the above data, graphical representations comparing the current values to those generated from the full Model Maker analysis are shown in Figures 5-8 below.

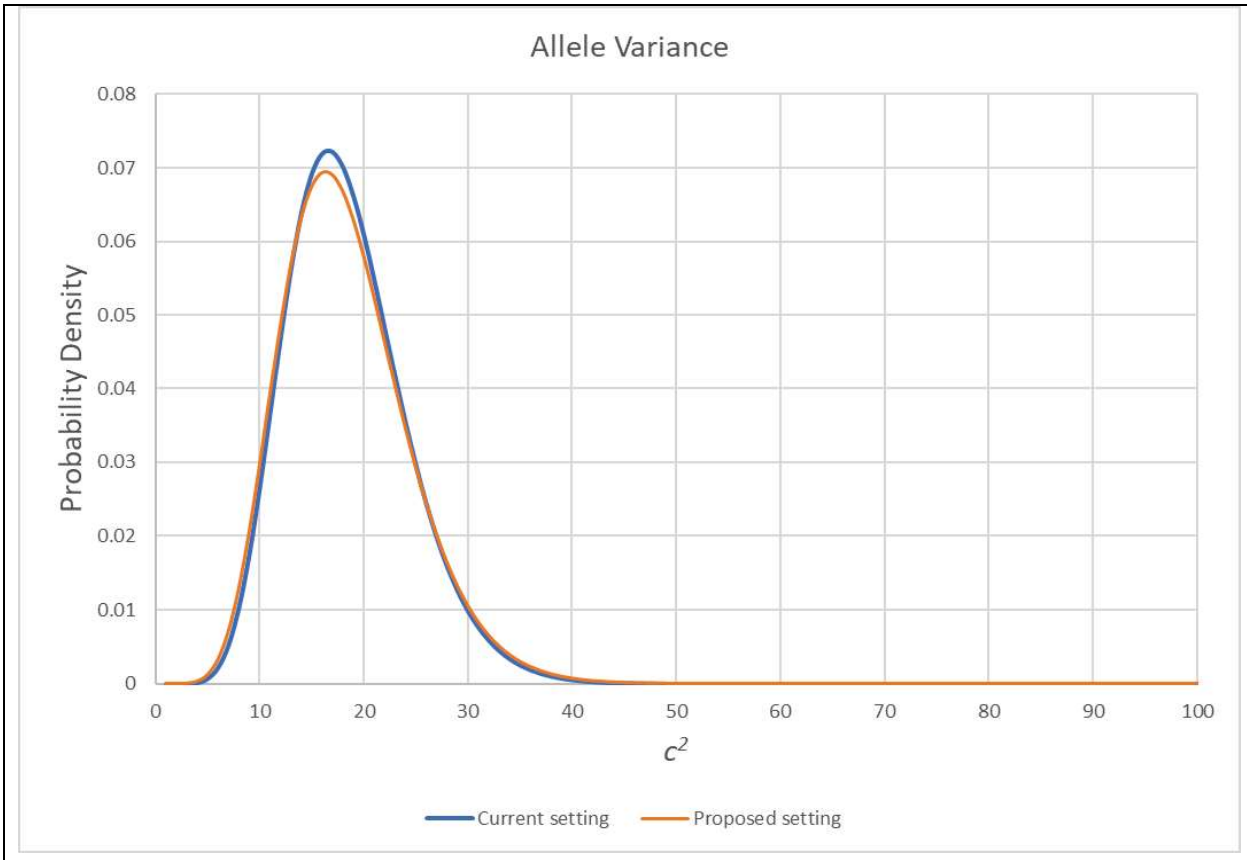

Figure 5 - Allele Variance distribution

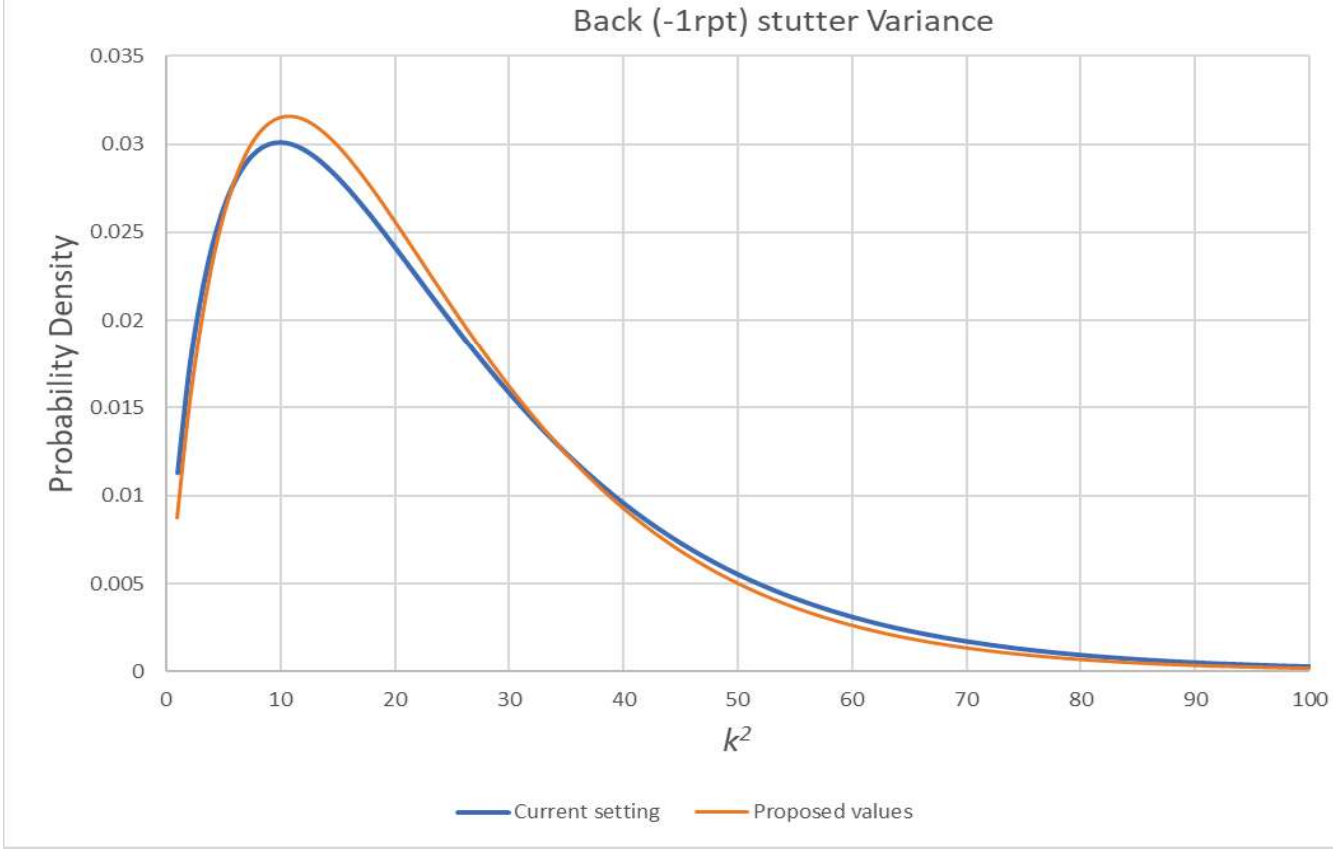

Figure 6 - Back (-1rpt) stutter Variance distribution

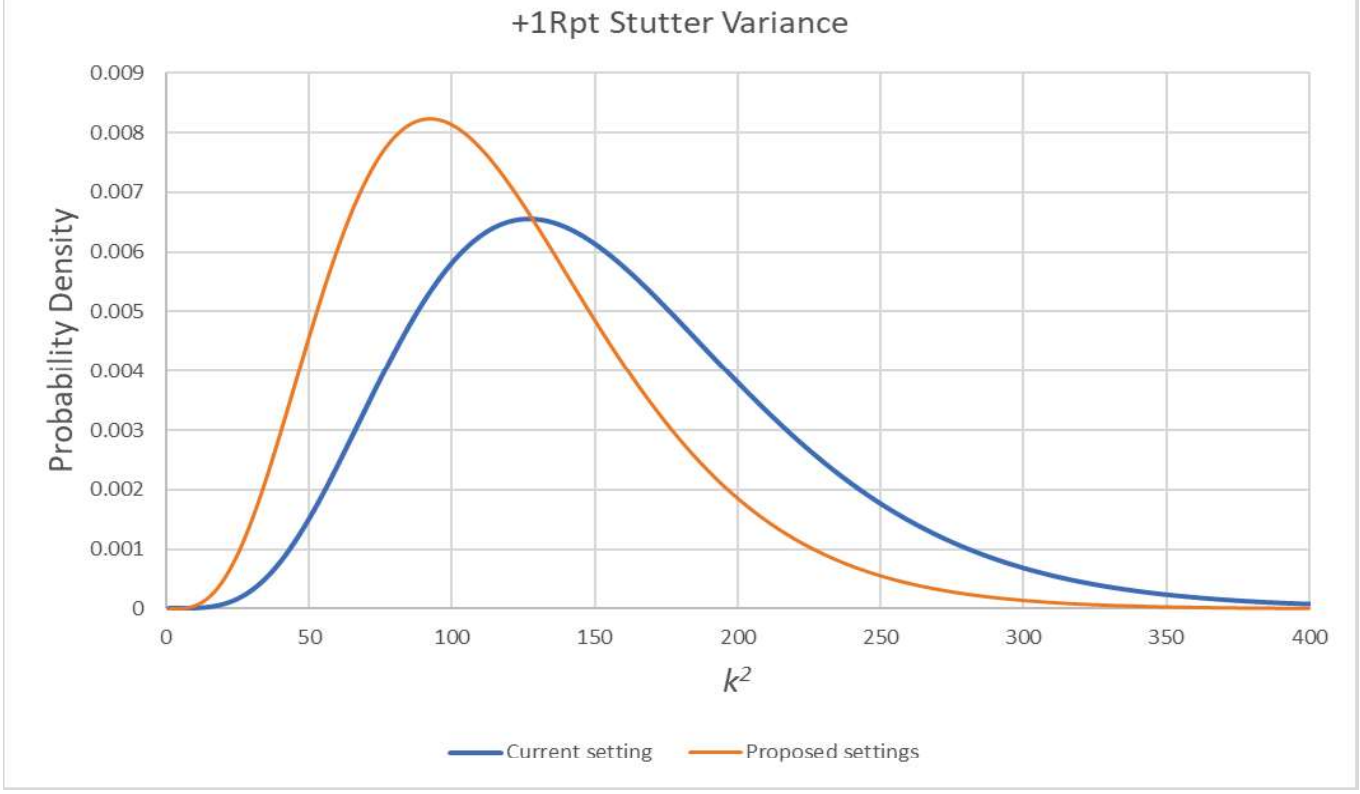

Figure 7 - +1rpt Stutter Variance distribution

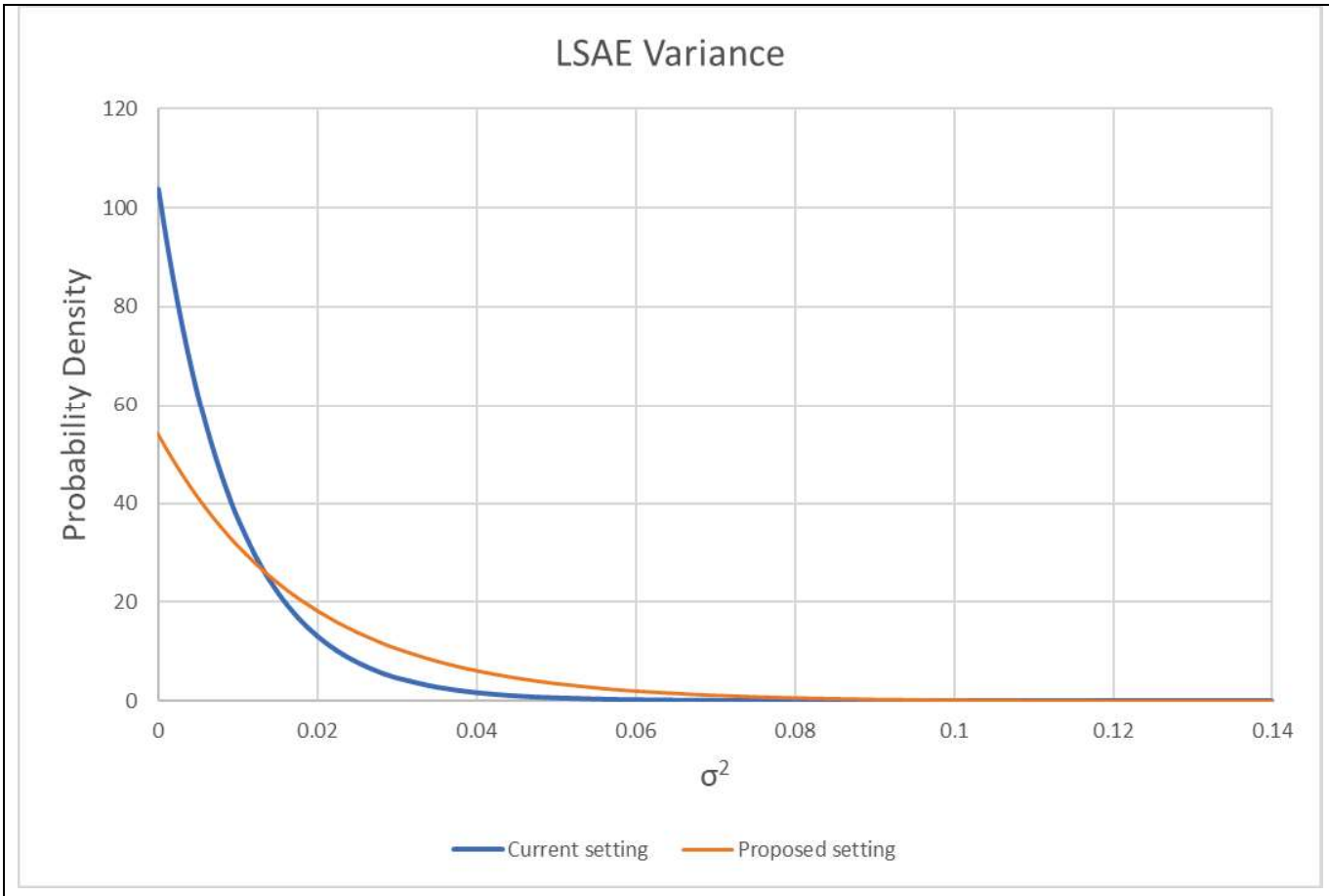

Figure 8 - LSAE Variance distribution

The input data from the full Proflex Model Maker analysis described above was entered into the Model Maker check spreadsheet (provided by STRmix™ technical support), this showed that the data provided a 98.5% coverage which is above the required 95%. This is represented in Figure 9 below.

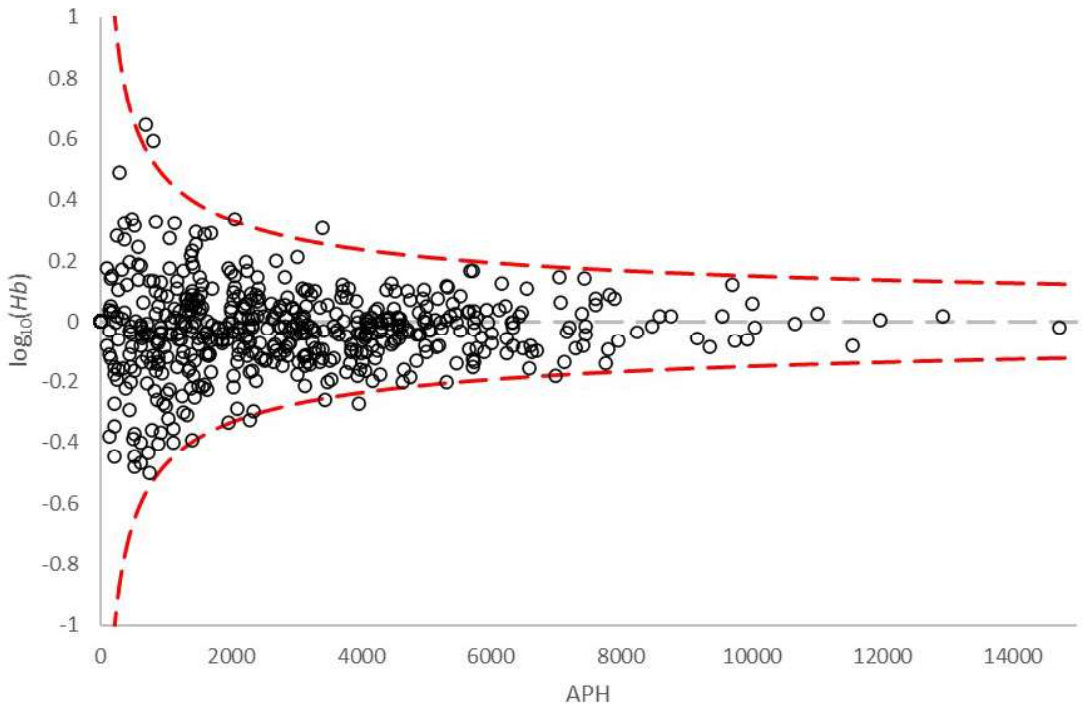

Figure 9 - Model Maker check output (full Proflex Model Maker analysis)

Hypothesis testing

The proposed Model Maker data was compared with the Model Maker data used in the current STRmix™ settings. Differences were noted within the comparisons of the two sets of data. Hypothesis tests were conducted to assess the significance of the differences found.

The hypothesis tests involved conducting an analysis of data obtained from the 9700 (current settings) and Proflex (proposed settings) systems to determine if there was a significant difference between the variances observed for the allele height, the +1 rpt stutter, the -1 rpt stutter, and the LSAE. Testing was undertaken using a process known as bootstrapping. In this process, a simulated sampling is undertaken from an estimated distribution to simulate real data when that data is not available for analysis. In this case, the distributions and their defining parameters (rate and shape) have been obtained from Model Maker. Data was modelled for each of the allele height, +1 rpt stutter, -1 rpt stutter, and the LSAE using n=100, 200, 300, and 500. The Model Maker data is based on a 10x12 matrix (120 samples), and so will have at most 4800 alleles, -1 rpt stutter peaks, +1 rpt stutter peaks, and 2400 loci upon which the data is based. Allowing that at lower dilutions many peaks will not be observed and hence the true number of peaks in the original Model Maker analysis will be much lower. As such, the values for n used are considered to be conservative and would likely be much higher. It should be noted that as n increases, the probability of a significant difference being observed between two groups increases.

The distributions from Model Maker were modelled in R at the various n-values using standard sampling methodology (Crawley, 2007). Hypothesis testing (examining the distributions obtained from the two analysers for significant differences) was undertaken using the following three tests:

Welch Two Sample t-test: this test is the least ideal as it requires an assumption of normality in the distributions. However, it can be employed as an indicator because it will work asymptotically due to the constraints of the Central Limit Theorem. That is, essentially, that if a distribution is sampled enough times

the mean of the means will tend towards the true mean of the distribution. Though, it should be stated, that the t-test is not ideal for highly skewed distributions (Crawley, 2007).

Wilcoxon rank sum test: The unpaired two-samples Wilcoxon test (also known as Wilcoxon rank sum test or Mann-Whitney test) can be used to compare two independent groups that are non-parametric (ie. is not normally distributed). This is the most ideal test for this analysis (Crawley, 2007).

Kolmogorov-Smirnov Test - this test makes no assumption about the distributions. This test is most ideal when it is not known what distributions are involved. Even though we are using gamma and exponential distributions, it must be noted that there is no definitive "distribution" for a set of data, and so while data might fit a particular distribution it is possible for it to also fit other types of distribution (Crawley, 2007).

The null hypothesis for all these tests is that the data is all from the same population. The alternative hypothesis is that the data likely comes from two different populations. The significance level was set at $p \leq 0.05$.

Discussion

The significantly different results for the -1 rpt stutter at the n=100 and n=200 levels were unexpected. However, as these groups were not significantly different at n=300 and n=500, the effect is likely to be due to the small sample size not reflecting the full gamut of the respective distributions. This is supported by the observation that when reanalysed using a different seed for the modelling, the results for the n=100 and n=200 analyses were not significantly different ($p>0.05$).

The results obtained are illustrated in Table 3. The values of $p \leq 0.05$ have been highlighted in orange.

Table 3 Hypothesis Tests

	n	t-test	Wilcoxon	KS
Allele Variance	100	0.60917	0.585	0.81275
	200	0.95202	0.90876	0.99719
	300	0.63267	0.83527	0.78704
	500	0.37253	0.19716	0.25743
n-1 Stutter	100	0.11885	0.015298	0.054103
	200	0.037185	0.21057	0.3275
	300	0.40401	0.39405	0.51755
	500	0.15476	0.24195	0.41315
n+1 Stutter	100	0.00019131	0.000791	0.015814
	200	8.6668E-08	7.95E-08	9.91E-08
	300	1.0302E-11	1.83E-11	8.7E-09
	500	2.22E-16	2.22E-16	2.22E-16
LSAE	100	8.1796E-05	5.45E-05	3.73E-05
	200	4.0082E-06	7.18E-07	1.22E-05
	300	5.365E-11	4.42E-12	5.22E-09
	500	2.22E-16	3.83E-16	3.84E-13

The hypothesis tests indicated a significant difference in the data obtained. In order to determine the impact of changing the STRmix™ variance settings for casework, a comparison between the two sets of variances was made. For this comparison seven mixed DNA profiles consisting of two- and three-contributors from *Project #219 – Verification of STRmix™ v2.7.0 for 3500xL Part B* were used.

Comparison of LR of current settings vs proposed settings

The seven mixtures were deconvoluted in STRmix™ v2.8.0 using both the current Model Maker settings and the proposed Model Maker settings and LRs calculated for the true contributors. The LRs obtained using both sets were compared to each other to assess the differences between them. 21 sets of LRs obtained were all within the same order of magnitude indicating little impact on the LRs with the proposed settings additionally the result lines used to report these samples would not change. One LR set did change by one order of magnitude but the result would still be reported within the greater than 100 billion result line so it would not change the final result line. The comparison of the $\log_{10}$(LR) is represented in Figure 10 below.

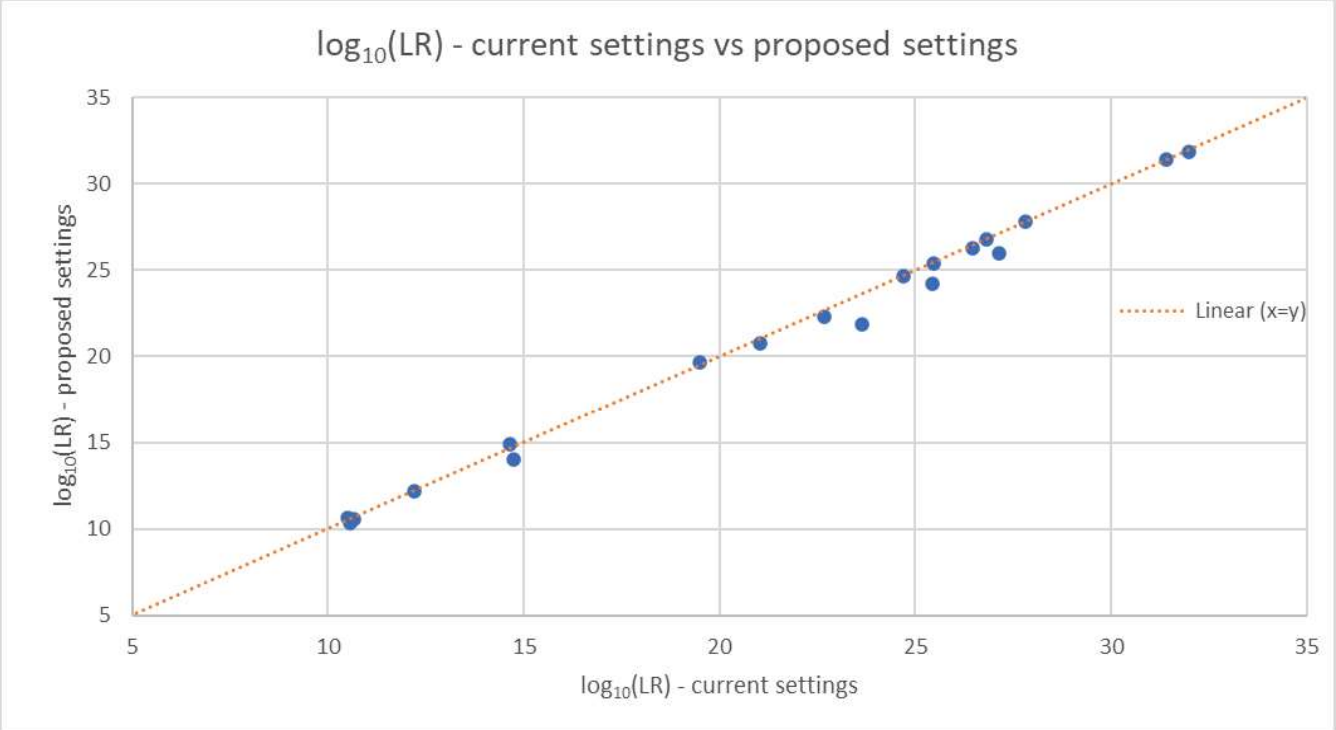

Figure 10 – Comparison of $\log_{10}$(LR)

The number of alleles resolved to ≥99% were also compared to detemine whether there are any differences in the number of uploads to NCIDD using the proposed settings. The results (Figure 11) show that there is little difference in the number of resolved alleles between the current and proposed settings.

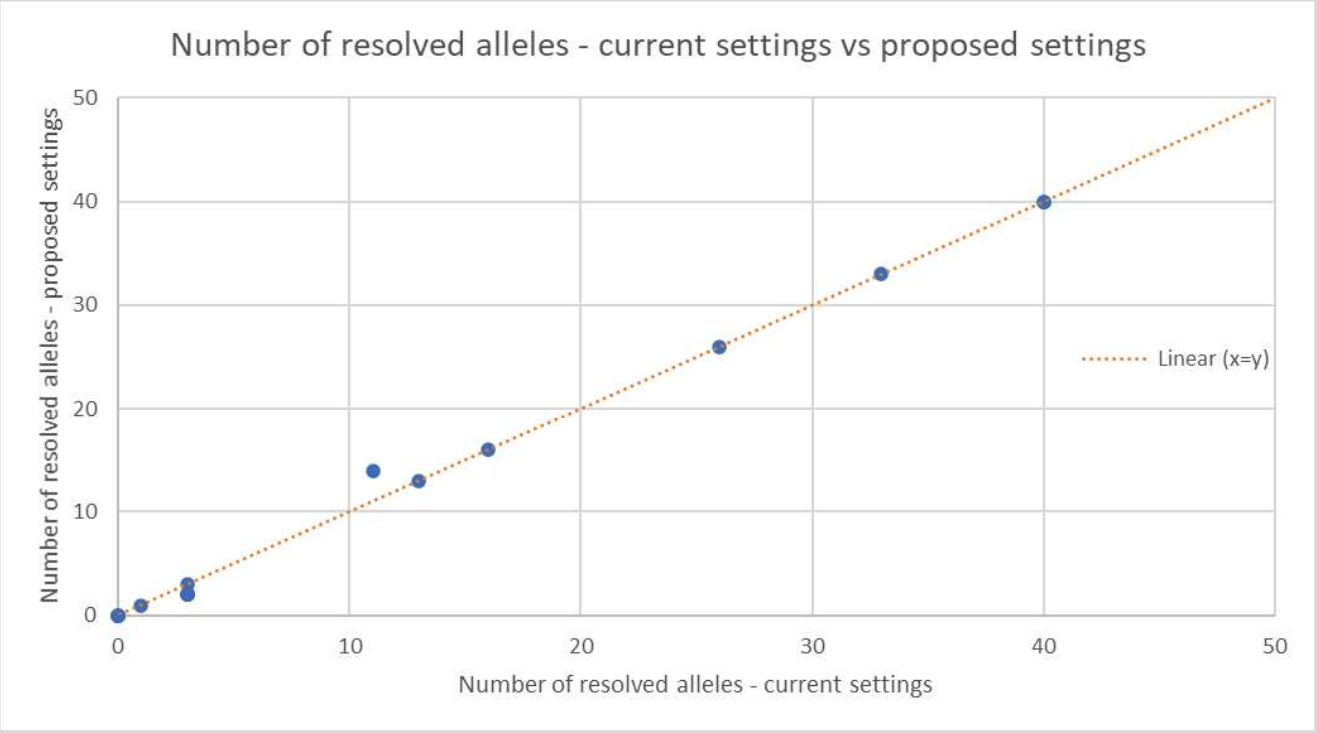

Figure 11 – Comparison of resolved alleles to ≥99%

Conclusion:

Based on the findings of the further testing and comparisons made using the current and proposed model maker settings it demonstrates that there would be minimal risk with the introduction of the model maker settings created using the latest sample set. The result lines for all samples compared would not have changed, this indicates that STRmix™ can be updated to the proposed model maker settings and continue with casework without having to reanalyse samples already processed using the current settings.

Recommendations:

- It is recommended that all computers with STRmix™ v2.8 be updated with the new model maker settings by the STRmix™ team members.
- It is acceptable to have a mix of model maker settings in one case.
- Samples run on Proflex using 9700 settings do not require re-analysis with Proflex settings.

Acknowledgment:

We would like to acknowledge Allan McNevin for his assistance with data analysis.

References:

1. STRmix™ support - ticket 3422
2. Crawley, Michael J (2007) The R Book. John Wiley & Sons Ltd. p293-4; p317-8.

Line Manager Signature:	[redacted] Digitally signed by Justin Howes - Team Leader, Forensic DNA Analysis Date: 2022.04.29 10:39:13 +10'00'	**Comments:**
Quality & Projects Signature:	[redacted] Digitally signed by Kirsten Scott Date: 2022.04.29 10:54:51 +10'00	**Comments:**

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