2.8 Test Report

STRmix™ V2.8 Test Report
Issued by Institute of Environmental Science and Research Limited
Date of Issue: 29 September 2020

1. CONTENTS

2. INTRODUCTION

This document discusses the testing and developmental validation carried out for STRmix™ version 2.8 prior to commercial release. Developmental validation is carried out using acceptance/qualification testing to determine whether or not each element of the system satisfies pre-determined acceptance criteria.

2.1. Summary of developmental validation tests

The following functionality and outputs from STRmix™ were verified by hand as part of the developmental validation tasks:

1. Pre checks for missing stutter
2. Smart start
3. Expected allele and stutter heights given mass parameters using stutter regression and exceptions files
4. Expected peak heights of dropout or 'Q' alleles given mass parameters
5. Probabilities given expected and observed peak heights including those below the analytical threshold
6. Locus specific amplification efficiency penalties
7. Summation of probabilities for each allele in a locus and across a profile
8. Summation of probabilities across multiple replicate profiles and multiple kits
9. Informed priors on mixture proportion
10. *LR* values with and without assumed contributors
11. *LR* values with varying theta values
12. *LR* stratified and unified point estimates
13. *LR* HPD interval values including theta beta and allele frequency resampling
14. Gaussian walk
15. Gelman-Rubin statistic, ESS, weight resampling
16. Relatives calculations
17. *LR* Batcher, Batch Mode and *LR* from previous functionality
18. Drop-in function
19. Database search functionalities including familial matching
20. Variable number of contributors function and *LR*s
21. *Hd* true tester function
22. Mix to mix *LR*s
23. Model Maker.

Many calculations including the calculation of expected heights, penalties, and *LR* values were conducted in Excel. A summary of the developmental validation results will be discussed in turn for each comparison undertaken. Note that this is only a small subset of all the testing activities that were undertaken during the STRmix™ development project.

2.2. Summary of extended output tests

Extended output testing is the “by hand” testing of the biological and statistical models within STRmix. The extended outputs contain all the outputs needed to replicate the models. For example for an interpretation these contain the genotype combinations, mass parameters, observed and expected peak heights, peak height and LSAE variances, and all penalties including peak, variances, drop-in, Mx priors, and their sum per iteration.

A list of all the samples and functions tested is given on the next page. All results were as expected. The Excel sheets are saved within the extended outputs folder of each run folder which are saved in the following location:

V:\STRmix v2.8\v2.8 Science\Extendeds 2.8.0.23

The extended outputs were checked in V2.8.0.23 which was the version with the final science changes (defined as where a change affects the seed). Release version STRmix 2.8.0.34 was compared against builld 23 using the standard set of configs and no changes in these results were observed.

Profile	burn-in	log p drop	log p not drop	SLN	smart start	drop-in	TAP + E_a	SR_a	c^2 an k^2	Mx priors	Peaks > s	LSAE var	LSAE penalty	Total penalty
Ex1A drop in_gamma	-	y	y	y	y	y	y	y	y	N/A	N/A	y	y	y
Ex1A drop in_uniform	-	y	y	y	y	y	-	-	y	N/A	N/A	y	y	y
Ex1A GF_extended	-	y	y	y	y	N/A	y	y	y	N/A	N/A	y	y	y
Ex1A saturated_GF 3500 sat -5000	-	y	y	y	y	N/A	y	y	y	N/A	y	y	y	y
Ex1C_assumed extended	-	y	y	y	y	N/A	y	y	y	N/A	N/A	y	y	-
extended_reps (Ex1E)	-	y	y	y	y	N/A	y	y	y	N/A	N/A	-	y	y
multi kit_reps	-	y	y	y	y	N/A	y	y	y	N/A	N/A	y	y	y
NIST case 1_multikit assumed	-	Y	y	y	y	N/A	y	y	y	N/A	N/A	-	y	y
NIST mix 3_conditioned two	-	y	y	y	y	N/A	y	y	y	N/A	N/A	Y	y	y
NIST mix 3_three person	-	y	y	y	y	N/A	-	-	y	N/A	N/A	-	y	y

Profile	burn-in	log p drop	log p not drop	SLN	smart start	drop-in	TAP + E_a	SR_a	c^2 an k^2	Mx priors	Peaks > s	LSAE var	LSAE penalty	Total penalty
NIST 3 3p_mx priors	-	y	y	y	y	N/A	y	y	y	y	N/A	-	y	y
NIST case 1_multikit varNOC	-	y	y	y	y	N/A	Y	Y	y	N/A	N/A	y	y	y
TC 2p_reps assumed VF02	-	N/A	y	y	y	N/A	Y	Y	y	N/A	y	-	y	y
Multikit_multi dropin extended	-	y	y	y	N/A	y	-	-	y	N/A	N/A	-	y	-
Ex1A_stutter proprotion b-2000 AT D22 100 rfu	Y	y	y	y	y	N/A	-	-	y	N/A	N/A	y	y	y
Ex1A no stutter_extended	-	y (CNA log p)	y	y	y	N/A	-	-	y	N/A	N/A	-	y	y
Ex1C missing stutter_extended	y (only)	y	y	N/A	-	N/A	Y	Y	y	N/A	N/A	y	y	y
Ex1E as 2_conditioned	-	Y	Y	-	y	N/A	y	y	y	N/A	N/A	y	y	y

Profile	burn-in	log p drop	log p not drop	SLN	smart start	drop-in	TAP + E_a	SR_a	c^2 an k^2	Mx priors	Peaks > s	LSAE var	LSAE penalty	Total penalty
STR-1485 Ex1E as NOC2_conditioned (different size)	-	Y	Y	-	-	N/A	Y	y	Y	N/A	N/A	Y	Y	y
Ex1A with minor_extended 2 alleles	-	Y	Y	Y	-	N/A	-	-	y	N/A	N/A	Y	Y	y
GF 3500 AT 30 MM rejects on	N/A	y	y	y	N/A	y	y	y	y	N/A	N/A	y	y	y
GF 3500 AT 30 MM rejects off	N/A	y	y	y	N/A	y	y	y	y	N/A	N/A	y	y	y
422_883-E03_1.00_extd_MM	N/A	-	Y	-	N/A	-	-	-	-	N/A	N/A	-	-	y

3. ACCEPTANCE CRITERIA

3.1. Microsoft Excel precision

Microsoft Excel implements the IEEE 754 floating-point standard. Excel stores 15 significant digits of precision. For example, the number 1234567890123456 cannot be exactly represented if 15 digits of precision are used.

The IEEE 754 floating-point standard requires that numbers be stored in binary format. This means a conversion must occur before the numbers can be used in calculations. If the number can be represented exactly in floating-point format, then the conversion is exact. If not, then the conversion will result in a rounded value which will represent the original value. Numbers that appear exact in the decimal format may need to be approximated when converted to binary floating-point. For example, the fraction 1/10 can be represented in the decimal format as the rational number 0.1. However, 0.1 cannot be represented precisely in binary floating-point of finite precision. 0.1 becomes the repeating binary decimal 0.0001100110011', where the sequence 1100 repeats infinitely. This number cannot be represented in a finite amount of space and therefore in Excel, it is rounded down by approximately 2.8E-17 when it is stored.

The discrepancy between the STRmix™ output and the Excel repeat is defined by, at least, the Excel precision.

3.2. Normal density comparison

The unit test for the normal distribution density method within STRmix™ [com.strmix.calculation.STRMath.calculateNormalDistributionDensity] over the range [-20,20] in increments of 0.01 with mean=0, var=1 was obtained. The output was compared with Excel using the norm.s.dist(X,0) function. The results appear Figure 3.1 Comparison of normal density STRmix™ and MS Exceland Table 3.1.

Table 3.1 A comparison of log(STRmix) vs log(STRmix)-log(Excel)

X	log(STRmix)	Log(STRmix)-log(Excel)	X	log(STRmix)	Log(STRmix)-log(Excel)	X	log(STRmix)	Log(STRmix)-log(Excel)	X	log(STRmix)	Log(STRmix)-log(Excel)
-20	-87.26	0	-10	-22.11	-1.4E-14	3.27E-13	-0.40	-8.3E-16	10	-22.11	-2.6E-13
-19.9	-86.39	-7.2E-13	-9.9	-21.68	-3.2E-14	0.1	-0.40	-1E-15	10.1	-22.55	-2.5E-13
-19.8	-85.53	-5.7E-13	-9.8	-21.25	-3.6E-14	0.2	-0.41	-3.9E-16	10.2	-22.99	-2.4E-13
-19.7	-84.67	-4.7E-13	-9.7	-20.83	0	0.3	-0.42	-8.3E-16	10.3	-23.44	-2.3E-13
-19.6	-83.82	-3.3E-13	-9.6	-20.41	-1.8E-14	0.4	-0.43	-2.2E-16	10.4	-23.89	-2.3E-13
-19.5	-82.97	-1.7E-13	-9.5	-20.00	-2.5E-14	0.5	-0.45	-1.2E-15	10.5	-24.34	-2.2E-13
-19.4	-82.12	-7.1E-14	-9.4	-19.59	-2.8E-14	0.6	-0.48	-3.3E-16	10.6	-24.80	-2.1E-13
-19.3	-81.28	-7.5E-13	-9.3	-19.18	0	0.7	-0.51	-1.1E-15	10.7	-25.26	-2E-13
-19.2	-80.45	-6.4E-13	-9.2	-18.78	-1.1E-14	0.8	-0.54	-1.4E-15	10.8	-25.73	-2E-13
-19.1	-79.62	-5E-13	-9.1	-18.38	-1.4E-14	0.9	-0.57	-8.9E-16	10.9	-26.20	-1.8E-13
-19	-78.79	-3.6E-13	-9	-17.99	-2.5E-14	1	-0.62	-3.6E-15	11	-26.67	-1.8E-13

-18.9	-77.97	-2.3E-13	-8.9	-17.60	-2.8E-14	1.1	-0.66	-3.7E-15	11.1	-27.15	-1.7E-13
-18.8	-77.15	-8.5E-14	-8.8	-17.21	0	1.2	-0.71	-5.3E-15	11.2	-27.64	-1.5E-13
-18.7	-76.33	-7.8E-13	-8.7	-16.83	-7.1E-15	1.3	-0.77	-5.2E-15	11.3	-28.13	-1.5E-13
-18.6	-75.52	-6.7E-13	-8.6	-16.46	-1.8E-14	1.4	-0.82	-5.2E-15	11.4	-28.62	-1.4E-13
-18.5	-74.72	-5.1E-13	-8.5	-16.09	-2.5E-14	1.5	-0.89	-5.2E-15	11.5	-29.12	-1.4E-13
-18.4	-73.92	-4E-13	-8.4	-15.72	-3.4E-14	1.6	-0.95	-6.9E-15	11.6	-29.62	-1.2E-13
-18.3	-73.12	-2.8E-13	-8.3	-15.36	0	1.7	-1.03	-6.4E-15	11.7	-30.12	-1.1E-13
-18.2	-72.33	-1.6E-13	-8.2	-15.00	-1.2E-14	1.8	-1.10	-6.4E-15	11.8	-30.63	-9.9E-14
-18.1	-71.54	-4.3E-14	-8.1	-14.65	-1.8E-14	1.9	-1.18	-7.1E-15	11.9	-31.15	-9.2E-14
-18	-70.75	-7E-13	-8	-14.30	-2.5E-14	2	-1.27	-7.1E-15	12	-31.67	-8.9E-14
-17.9	-69.98	-5.5E-13	-7.9	-13.95	-3.4E-14	2.1	-1.36	-6.4E-15	12.1	-32.19	-7.8E-14
-17.8	-69.20	-4.4E-13	-7.8	-13.61	-5.3E-15	2.2	-1.45	-4.4E-15	12.2	-32.72	-5.7E-14
-17.7	-68.43	-3.1E-13	-7.7	-13.27	-1.2E-14	2.3	-1.55	-2E-15	12.3	-33.25	-5E-14
-17.6	-67.66	-2E-13	-7.6	-12.94	-2.1E-14	2.4	-1.65	-1E-14	12.4	-33.79	-5E-14
-17.5	-66.90	-8.5E-14	-7.5	-12.61	-2.8E-14	2.5	-1.76	-8.9E-15	12.5	-34.33	-2.8E-14
-17.4	-66.14	-7E-13	-7.4	-12.29	0	2.6	-1.87	-8.4E-15	12.6	-34.87	-2.1E-14

-17.3	-65.39	-6E-13	-7.3	-11.97	-7.1E-15	2.7	-1.98	-7.1E-15	12.7	-35.42	0
-17.2	-64.64	-4.8E-13	-7.2	-11.66	-1.4E-14	2.8	-2.10	-2.2E-15	12.8	-35.98	-5.5E-13
-17.1	-63.90	-3.6E-13	-7.1	-11.35	-2E-14	2.9	-2.23	-1.2E-14	12.9	-36.53	-5.5E-13
-17	-63.15	-2.4E-13	-7	-11.04	-2.5E-14	3	-2.35	-9.8E-15	13	-37.10	-5.3E-13
-16.9	-62.42	-1.1E-13	-6.9	-10.74	-1.8E-15	3.1	-2.49	-7.1E-15	13.1	-37.66	-5.3E-13
-16.8	-61.69	-7.1E-15	-6.8	-10.44	-7.1E-15	3.2	-2.62	-4.4E-15	13.2	-38.23	-5.2E-13
-16.7	-60.96	-6.2E-13	-6.7	-10.15	-1.2E-14	3.3	-2.76	-1.3E-15	13.3	-38.81	-5E-13
-16.6	-60.24	-4.9E-13	-6.6	-9.86	-2.3E-14	3.4	-2.91	-1.6E-14	13.4	-39.39	-5E-13
-16.5	-59.52	-3.8E-13	-6.5	-9.57	-2.5E-14	3.5	-3.06	-9.8E-15	13.5	-39.97	-4.9E-13
-16.4	-58.80	-2.6E-13	-6.4	-9.29	-1.8E-15	3.6	-3.21	-6.7E-15	13.6	-40.56	-4.8E-13
-16.3	-58.09	-1.5E-13	-6.3	-9.02	-1.1E-14	3.7	-3.37	-3.1E-15	13.7	-41.16	-4.8E-13
-16.2	-57.39	-5E-14	-6.2	-8.75	-1.8E-14	3.8	-3.53	-8.9E-16	13.8	-41.75	-4.6E-13
-16.1	-56.69	-6.2E-13	-6.1	-8.48	-2.1E-14	3.9	-3.70	-1.5E-14	13.9	-42.35	-4.5E-13
-16	-55.99	-5.3E-13	-6	-8.22	0	4	-3.87	-1.2E-14	14	-42.96	-4.5E-13
-15.9	-55.30	-5.4E-13	-5.9	-7.96	-6.2E-15	4.1	-4.05	-7.1E-15	14.1	-43.57	-4.3E-13
-15.8	-54.61	-5.5E-13	-5.8	-7.70	-1.1E-14	4.2	-4.23	-3.6E-15	14.2	-44.18	-4.3E-13

-15.7	-53.92	-5.7E-13	-5.7	-7.45	-1.6E-14	4.3	-4.41	-8.9E-16	14.3	-44.80	-4.1E-13
-15.6	-53.24	-5.8E-13	-5.6	-7.21	-2E-14	4.4	-4.60	-1.3E-14	14.4	-45.43	-4.1E-13
-15.5	-52.57	-5.8E-13	-5.5	-6.97	-2.7E-15	4.5	-4.80	-1.2E-14	14.5	-46.05	-3.8E-13
-15.4	-51.90	-5.9E-13	-5.4	-6.73	-6.2E-15	4.6	-4.99	-7.1E-15	14.6	-46.69	-3.8E-13
-15.3	-51.23	-6E-13	-5.3	-6.50	-1.1E-14	4.7	-5.20	-8.9E-16	14.7	-47.32	-3.8E-13
-15.2	-50.57	-6.2E-13	-5.2	-6.27	-1.7E-14	4.8	-5.40	-2E-14	14.8	-47.96	-3.6E-13
-15.1	-49.91	-6.3E-13	-5.1	-6.05	-2.1E-14	4.9	-5.61	-1.7E-14	14.9	-48.61	-3.5E-13
-15	-49.26	-6.4E-13	-5	-5.83	-5.3E-15	5	-5.83	-9.8E-15	15	-49.26	-3.3E-13
-14.9	-48.61	0	-4.9	-5.61	-8E-15	5.1	-6.05	-5.3E-15	15.1	-49.91	-3.3E-13
-14.8	-47.96	-1.4E-14	-4.8	-5.40	-1.2E-14	5.2	-6.27	-8.9E-16	15.2	-50.57	-3.1E-13
-14.7	-47.32	-2.8E-14	-4.7	-5.20	-1.5E-14	5.3	-6.50	-1.9E-14	15.3	-51.23	-2.8E-13
-14.6	-46.69	-4.3E-14	-4.6	-4.99	-2.4E-14	5.4	-6.73	-1.5E-14	15.4	-51.90	-2.8E-13
-14.5	-46.05	-5.7E-14	-4.5	-4.80	-6.2E-15	5.5	-6.97	-1.2E-14	15.5	-52.57	-2.8E-13
-14.4	-45.43	-7.1E-14	-4.4	-4.60	-8.9E-15	5.6	-7.21	-4.4E-15	15.6	-53.24	-2.7E-13
-14.3	-44.80	-7.8E-14	-4.3	-4.41	-1.2E-14	5.7	-7.45	0	15.7	-53.92	-2.6E-13
-14.2	-44.18	-8.5E-14	-4.2	-4.23	-1.5E-14	5.8	-7.70	-2.1E-14	15.8	-54.61	-2.4E-13

-14.1	-43.57	-1.1E-13	-4.1	-4.05	-8.9E-16	5.9	-7.96	-1.5E-14	15.9	-55.30	-2.2E-13
-14	-42.96	-1.3E-13	-4	-3.87	-4.4E-15	6	-8.22	-8.9E-15	16	-55.99	-2.3E-13
-13.9	-42.35	-1.4E-13	-3.9	-3.70	-9.8E-15	6.1	-8.48	-1.8E-15	16.1	-56.69	-3.2E-13
-13.8	-41.75	-1.4E-13	-3.8	-3.53	-1.2E-14	6.2	-8.75	-2.5E-14	16.2	-57.39	-4.5E-13
-13.7	-41.16	-1.4E-13	-3.7	-3.37	-1.5E-14	6.3	-9.02	-2E-14	16.3	-58.09	-5.5E-13
-13.6	-40.56	-1.7E-13	-3.6	-3.21	-1.8E-15	6.4	-9.29	-1.4E-14	16.4	-58.80	-6.8E-13
-13.5	-39.97	-1.7E-13	-3.5	-3.06	-4.9E-15	6.5	-9.57	-7.1E-15	16.5	-59.52	-7.1E-14
-13.4	-39.39	-1.8E-13	-3.4	-2.91	-9.8E-15	6.6	-9.86	-3.6E-15	16.6	-60.24	-1.8E-13
-13.3	-38.81	-1.9E-13	-3.3	-2.76	-1.2E-14	6.7	-10.15	-2.7E-14	16.7	-60.96	-3.1E-13
-13.2	-38.23	-2.1E-13	-3.2	-2.62	-1.4E-14	6.8	-10.44	-2E-14	16.8	-61.69	-4.2E-13
-13.1	-37.66	-2.3E-13	-3.1	-2.49	-1.8E-15	6.9	-10.74	-1.4E-14	16.9	-62.42	-5.2E-13
-13	-37.10	-2.3E-13	-3	-2.35	-4.9E-15	7	-11.04	-5.3E-15	17	-63.15	-6.5E-13
-12.9	-36.53	-2.4E-13	-2.9	-2.23	-8E-15	7.1	-11.35	0	17.1	-63.90	-2.8E-14
-12.8	-35.98	-2.5E-13	-2.8	-2.10	-1E-14	7.2	-11.66	-2.3E-14	17.2	-64.64	-1.7E-13
-12.7	-35.42	-2.5E-13	-2.7	-1.98	-2.4E-15	7.3	-11.97	-1.8E-14	17.3	-65.39	-2.7E-13
-12.6	-34.87	-2.7E-13	-2.6	-1.87	-4.2E-15	7.4	-12.29	-1.1E-14	17.4	-66.14	-3.8E-13

-12.5	-34.33	-2.8E-13	-2.5	-1.76	-6E-15	7.5	-12.61	-7.1E-15	17.5	-66.90	-5.1E-13
-12.4	-33.79	-2.8E-13	-2.4	-1.65	-7.1E-15	7.6	-12.94	-3.2E-14	17.6	-67.66	-6.4E-13
-12.3	-33.25	-3E-13	-2.3	-1.55	-9.5E-15	7.7	-13.27	-2.5E-14	17.7	-68.43	-7.7E-13
-12.2	-32.72	-3.1E-13	-2.2	-1.45	-8.9E-16	7.8	-13.61	-1.8E-14	17.8	-69.20	-1.1E-13
-12.1	-32.19	-3.2E-13	-2.1	-1.36	-2.4E-15	7.9	-13.95	-1.2E-14	17.9	-69.98	-2.1E-13
-12	-31.67	-3.3E-13	-2	-1.27	-4.7E-15	8	-14.30	0	18	-70.75	-3.6E-13
-11.9	-31.15	-3.3E-13	-1.9	-1.18	-4.2E-15	8.1	-14.65	-3.2E-14	18.1	-71.54	-4.7E-13
-11.8	-30.63	-3.4E-13	-1.8	-1.10	-3.6E-15	8.2	-15.00	-2.5E-14	18.2	-72.33	-6E-13
-11.7	-30.12	-3.4E-13	-1.7	-1.03	-3.1E-15	8.3	-15.36	-1.2E-14	18.3	-73.12	-7.2E-13
-11.6	-29.62	-3.6E-13	-1.6	-0.95	-4.7E-15	8.4	-15.72	-7.1E-15	18.4	-73.92	-7.1E-14
-11.5	-29.12	-3.7E-13	-1.5	-0.89	-2.9E-15	8.5	-16.09	0	18.5	-74.72	-1.7E-13
-11.4	-28.62	-3.7E-13	-1.4	-0.82	-3.2E-15	8.6	-16.46	-2.8E-14	18.6	-75.52	-3.1E-13
-11.3	-28.13	-3.8E-13	-1.3	-0.77	-2.2E-15	8.7	-16.83	-2.1E-14	18.7	-76.33	-4.3E-13
-11.2	-27.64	-3.8E-13	-1.2	-0.71	-4.2E-15	8.8	-17.21	-1.8E-14	18.8	-77.15	-5.8E-13
-11.1	-27.15	-3.9E-13	-1.1	-0.66	-3.3E-15	8.9	-17.60	-7.1E-15	18.9	-77.97	-7.1E-13
-11	-26.67	-4E-13	-1	-0.62	-8.9E-16	9	-17.99	0	19	-78.79	-1.4E-14

-10.9	-26.20	-4.1E-13	-0.9	-0.57	-3.3E-16	9.1	-18.38	-2.8E-14	19.1	-79.62	-1.6E-13
-10.8	-25.73	-4.1E-13	-0.8	-0.54	-8.9E-16	9.2	-18.78	-2.1E-14	19.2	-80.45	-2.7E-13
-10.7	-25.26	-4.1E-13	-0.7	-0.51	-1.1E-15	9.3	-19.18	-1.8E-14	19.3	-81.28	-4E-13
-10.6	-24.80	-4.3E-13	-0.6	-0.48	-1.3E-15	9.4	-19.59	0	19.4	-82.12	-5.4E-13
-10.5	-24.34	-4.3E-13	-0.5	-0.45	-5.6E-17	9.5	-20.00	-3.9E-14	19.5	-82.97	-6.5E-13
-10.4	-23.89	-4.3E-13	-0.4	-0.43	-6.1E-16	9.6	-20.41	-2.8E-14	19.6	-83.82	-8.2E-13
-10.3	-23.44	-4.4E-13	-0.3	-0.42	-5.6E-16	9.7	-20.83	-2.5E-14	19.7	-84.67	-8.5E-14
-10.2	-22.99	0	-0.2	-0.41	-5E-16	9.8	-21.25	-7.1E-15	19.8	-85.53	-2.1E-13
-10.1	-22.55	-7.1E-15	-0.1	-0.40	-8.3E-16	9.9	-21.68	0	19.9	-86.39	-3.6E-13

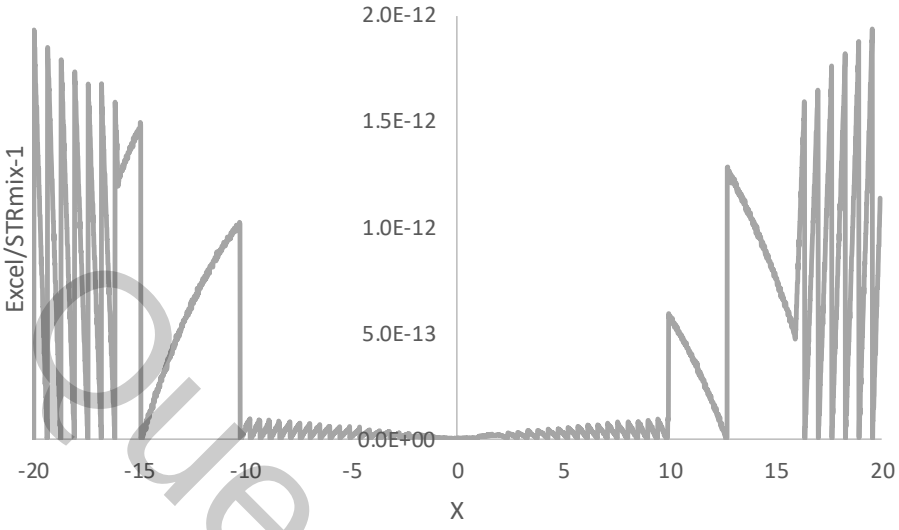

Figure 3.1 Comparison of normal density STRmix™ and MS Excel

3.3. Cumulative Normal approximation

A cumulative normal approximation is used in the calculation of the probability of dropout. Within STRmix™ V2.8, a cumulative normal approximation function via the Normal Distribution from the Apache Commons Math library is implemented. This is slow, therefore a hybrid approach is applied where if X < -7, the Apache method is used in order to obtain a cumulative probability and a programmed approximation is used otherwise. The value of -7[1] is implemented as it appears to be the best balance of speed (as most X values will be greater than this) and accuracy.

In Table 3.2 we give a comparison of the implemented method and the Excel norm.s.dist function. This defines how similar the repeat calculation should be when a cumulative normal distribution probability is used.

The maximum absolute difference was 1.64E-02 and the minimum 0.

Table 3.2 Comparison of log probabilities using STRmix™ and the standard normal distribution in MS Excel

X	Apache [-∞,-7] then programmed approximation (-7,∞]	Excel LOG(NORM.DIST(X,0,1,1))	Difference
-20	-88.5600953	-88.5600953	0.00E+00
-19.5	-84.2604976	-84.2604976	0.00E+00
-19	-80.0691923	-80.0691923	0.00E+00

[1] This setting is controlled within the default.ini file by the CnaSwitchOverPoint parameter (default -7). Note that this parameter should not be less than -8, otherwise a –Infinity/NaN error will arise.

-18.5	-75.9861647	-75.9861647	0.00E+00
-18	-72.0113987	-72.0113987	0.00E+00
-17.5	-68.1448772	-68.1448772	0.00E+00
-17	-64.3865815	-64.3865815	0.00E+00
-16.5	-60.7364910	-60.7364910	0.00E+00
-16	-57.1945838	-57.1945838	1.21E-13
-15.5	-53.7608355	-53.7608355	1.07E-13
-15	-50.4352196	-50.4352196	1.21E-13
-14.5	-47.2177070	-47.2177070	0.00E+00
-14	-44.1082654	-44.1082654	0.00E+00
-13.5	-41.1068595	-41.1068595	0.00E+00
-13	-38.2134498	-38.2134498	0.00E+00
-12.5	-35.4279927	-35.4279927	0.00E+00
-12	-32.7504392	-32.7504392	6.39E-14
-11.5	-30.1807344	-30.1807344	4.62E-14
-11	-27.7188167	-27.7188167	4.26E-14
-10.5	-25.3646162	-25.3646162	0.00E+00
-10	-23.1180534	-23.1180534	8.17E-14
-9.9	-22.6816496	-22.6816496	0.00E+00
-9.8	-22.2495470	-22.2495470	6.04E-14
-9.7	-21.8217446	-21.8217446	3.20E-14
-9.6	-21.3982418	-21.3982418	3.91E-14
-9.5	-20.9790376	-20.9790376	8.88E-14
-9.4	-20.5641312	-20.5641312	0.00E+00
-9.3	-20.1535216	-20.1535216	3.20E-14
-9.2	-19.7472079	-19.7472079	4.26E-14
-9.1	-19.3451892	-19.3451892	0.00E+00
-9	-18.9474644	-18.9474644	9.24E-14
-8.9	-18.5540326	-18.5540326	9.59E-14
-8.8	-18.1648926	-18.1648926	4.26E-14
-8.7	-17.7800435	-17.7800435	0.00E+00
-8.6	-17.3994840	-17.3994840	7.46E-14
-8.5	-17.0232130	-17.0232130	7.11E-14
-8.4	-16.6512293	-16.6512293	9.95E-14
-8.3	-16.2835317	-16.2835317	0.00E+00
-8.2	-15.9201190	-15.9201190	5.51E-14
-8.1	-15.5609897	-15.5609897	9.41E-14
-8	-15.2061426	-15.2061426	5.68E-14
-7.9	-14.8555761	-14.8555761	2.13E-14
-7.8	-14.5092890	-14.5092890	2.13E-14
-7.7	-14.1672796	-14.1672796	9.95E-14
-7.6	-13.8295465	-13.8295465	7.28E-14
-7.5	-13.4960879	-13.4960879	9.06E-14
-7.4	-13.1669023	-13.1669023	7.82E-14
-7.3	-12.8419879	-12.8419879	4.09E-14
-7.2	-12.5213429	-12.5213429	0.00E+00

-7.1	-12.2049655	-12.2049655	5.33E-14
-7.0	-11.8900569	-11.8928536	2.80E-03
-6.9	-11.5823408	-11.5850054	2.66E-03
-6.8	-11.2788847	-11.2814188	2.53E-03
-6.7	-10.9796960	-10.9820916	2.40E-03
-6.6	-10.6847594	-10.6870215	2.26E-03
-6.5	-10.3940710	-10.3962062	2.14E-03
-6.4	-10.1076326	-10.1096434	2.01E-03
-6.3	-9.8254399	-9.8273305	1.89E-03
-6.2	-9.5474908	-9.5492648	1.77E-03
-6.1	-9.2737821	-9.2754437	1.66E-03
-6	-9.0043113	-9.0058643	1.55E-03
-5.9	-8.7390753	-8.7405237	1.45E-03
-5.8	-8.4780710	-8.4794187	1.35E-03
-5.7	-8.2212951	-8.2225463	1.25E-03
-5.6	-7.9687444	-7.9699029	1.16E-03
-5.5	-7.7204152	-7.7214850	1.07E-03
-5.4	-7.4763040	-7.4772892	9.85E-04
-5.3	-7.2364068	-7.2373114	9.05E-04
-5.2	-7.0007198	-7.0015477	8.28E-04
-5.1	-6.7692387	-6.7699939	7.55E-04
-5	-6.5419592	-6.5426457	6.86E-04
-4.9	-6.3188768	-6.3194983	6.22E-04
-4.8	-6.0999866	-6.1005471	5.60E-04
-4.7	-5.8852837	-5.8857870	5.03E-04
-4.6	-5.6747629	-5.6752126	4.50E-04
-4.5	-5.4684185	-5.4688184	4.00E-04
-4.4	-5.2662449	-5.2665986	3.54E-04
-4.3	-5.0682360	-5.0685469	3.11E-04
-4.2	-4.8743855	-4.8746570	2.72E-04
-4.1	-4.6846865	-4.6849221	2.36E-04
-4	-4.4991321	-4.4993349	2.03E-04
-3.9	-4.3177148	-4.3178879	1.73E-04
-3.8	-4.1404268	-4.1405732	1.46E-04
-3.7	-3.9672598	-3.9673823	1.23E-04
-3.6	-3.7982051	-3.7983064	1.01E-04
-3.5	-3.6332533	-3.6333360	8.27E-05
-3.4	-3.4723948	-3.4724613	6.65E-05
-3.3	-3.3156192	-3.3156717	5.25E-05
-3.2	-3.1629155	-3.1629561	4.05E-05
-3.1	-3.0142722	-3.0143027	3.05E-05
-3	-2.8696768	-2.8696990	2.23E-05
-2.9	-2.7291163	-2.7291318	1.56E-05
-2.8	-2.5925767	-2.5925869	1.03E-05
-2.7	-2.4600433	-2.4600494	6.17E-06
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-2.3	-1.9696399	-1.9696387	-1.17E-06
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-2.1	-1.7480126	-1.7480111	-1.54E-06
-2	-1.6430174	-1.6430161	-1.34E-06
-1.9	-1.5418686	-1.5418676	-1.01E-06
-1.8	-1.4445396	-1.4445389	-6.48E-07
-1.7	-1.3510019	-1.3510016	-3.02E-07
-1.6	-1.2612251	-1.2612251	-1.83E-08
-1.5	-1.1751765	-1.1751767	1.78E-07
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-1.3	-1.0141222	-1.0141225	2.91E-07
-1.2	-0.9390389	-0.9390391	2.32E-07
-1.1	-0.8675287	-0.8675288	1.29E-07
-1	-0.7995455	-0.7995455	1.54E-08
-0.9	-0.7350404	-0.7350403	-7.99E-08
-0.8	-0.6739606	-0.6739605	-1.33E-07
-0.7	-0.6162500	-0.6162499	-1.33E-07
-0.6	-0.5618485	-0.5618484	-8.42E-08
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-0.3	-0.4178359	-0.4178359	7.29E-08
-0.2	-0.3759859	-0.3759859	2.24E-08
-0.1	-0.3370797	-0.3370797	-5.56E-08
0	-0.3010300	-0.3010300	4.56E-10
0.1	-0.2677447	-0.2677447	4.74E-08
0.2	-0.2371267	-0.2371267	-1.62E-08
0.3	-0.2090738	-0.2090738	-4.51E-08
0.4	-0.1834792	-0.1834792	-2.95E-08
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0.7	-0.1203099	-0.1203100	4.24E-08
0.8	-0.1033941	-0.1033941	3.58E-08
0.9	-0.0883418	-0.0883418	1.80E-08
1	-0.0750260	-0.0750260	-2.91E-09
1.1	-0.0633185	-0.0633184	-2.03E-08
1.2	-0.0530909	-0.0530909	-3.01E-08
1.3	-0.0442163	-0.0442163	-3.12E-08
1.4	-0.0365695	-0.0365695	-2.45E-08
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1.6	-0.0244760	-0.0244760	1.06E-09
1.7	-0.0197990	-0.0197991	1.41E-08
1.8	-0.0158916	-0.0158916	2.42E-08
1.9	-0.0126540	-0.0126540	2.99E-08
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2.4	-3.57481E-03	-3.57482E-03	3.09E-09
2.5	-2.70524E-03	-2.70523E-03	-6.35E-09
2.6	-2.02908E-03	-2.02906E-03	-1.47E-08
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2.8	-1.11113E-03	-1.11110E-03	-2.63E-08
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3.3	-2.10025E-04	-2.09999E-04	-2.54E-08
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3.6	-6.91216E-05	-6.91055E-05	-1.61E-08
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3.8	-3.14321E-05	-3.14215E-05	-1.06E-08
3.9	-2.08968E-05	-2.08885E-05	-8.33E-09
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5.4	-1.45038E-08	-1.44709E-08	-3.29E-11
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5.6	-4.66702E-09	-4.65459E-09	-1.24E-11
5.7	-2.60909E-09	-2.60159E-09	-7.51E-12
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6.1	-2.31208E-10	-2.30325E-10	-8.83E-13
6.2	-1.23110E-10	-1.22608E-10	-5.02E-13
6.3	-6.49149E-11	-6.46329E-11	-2.82E-13
6.4	-3.38963E-11	-3.37397E-11	-1.57E-13
6.5	-1.75272E-11	-1.74413E-11	-8.60E-14
6.6	-8.97480E-12	-8.92818E-12	-4.66E-14

6.7	-4.55081E-12	-4.52578E-12	-2.50E-14
6.8	-2.28507E-12	-2.27176E-12	-1.33E-14
6.9	-1.13617E-12	-1.12923E-12	-6.94E-15
7	-5.59406E-13	-5.55838E-13	-3.57E-15
7.1	-2.72760E-13	-2.70928E-13	-1.83E-15
7.2	-1.31679E-13	-1.30763E-13	-9.16E-16
7.3	-6.29706E-14	-6.24884E-14	-4.82E-16
7.4	-2.97977E-14	-2.95566E-14	-2.41E-16
7.5	-1.39827E-14	-1.38381E-14	-1.45E-16
7.6	-6.50921E-15	-6.41278E-15	-9.64E-17
7.7	-2.98942E-15	-2.94120E-15	-4.82E-17
7.8	-1.35006E-15	-1.35006E-15	3.94E-30
7.9	-6.26813E-16	-6.26813E-16	0.00E+00
8	-2.89298E-16	-2.89298E-16	0.00E+00

3.4. Unit test exponential function

Code

```
Random randomNumberGenerator = new Random(12345);

System.out.println("Exponential unit test: generating 10000 random exponential densities with lambda chosen between 0.0001 and 10 and x-value between -5 and 2 (on log scale))");

System.out.println("lambda,xval,density");

for (int i = 0; i < 10000; i++) {

    double lambda = (10-0.0001)*randomNumberGenerator.nextDouble()+0.0001;

    double xval = 7*randomNumberGenerator.nextDouble()-5;

    double density = STRMath.exponentialProbability(xval, lambda);

    System.out.println(lambda + "," + xval + "," + density);

}
```

Exponential unit test: generating 10000 random exponential densities with lambda chosen between 0.0001 and 10 and x-value between -5 and 2 (on log scale). Figure 3.2 is a comparison of the exponential density for STRmix™ and MS Excel.

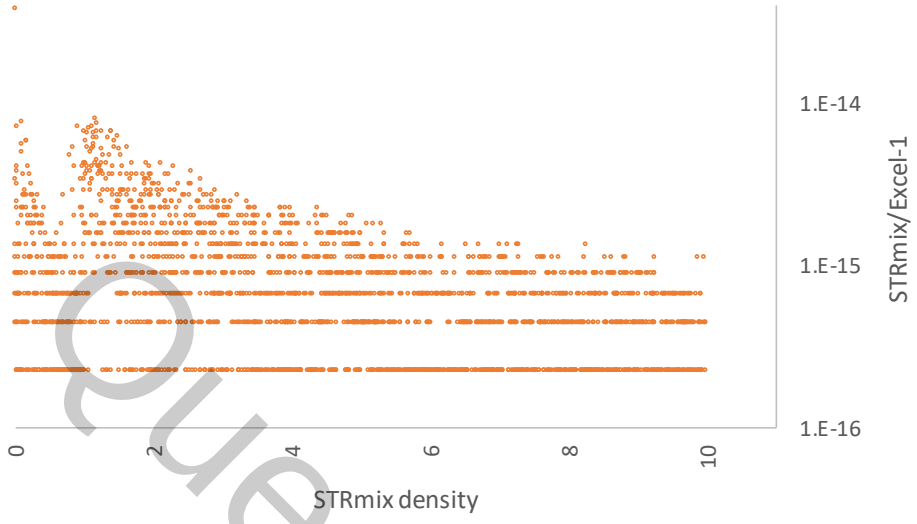


Figure 3.2 Comparison of exponential density STRmix™ and MS Excel

Examination of Figure 3.2 suggests that we should expect precision to 14 sig fig most of the time.

3.5. Unit test Gamma distribution

Code

System.out.println("Gamma unit test: generating 10000 random gamma densities with alpha nd beta chosen between 0.1 and 10 and x-value between 0 and 200");

System.out.println("alpha,beta,xval,density");

```
for (int i = 0; i < 10000; i++) {

    double alpha = (10-0.1)*randomNumberGenerator.nextDouble()+0.1;

    double beta = (10-0.1)*randomNumberGenerator.nextDouble()+0.1;

    GammaParameters gammaPair = new GammaParameters(alpha, beta);

    double xval = 200*randomNumberGenerator.nextDouble();

    double Log10density = STRMath.gammaPenalty(xval,gammaPair);

    System.out.println(alpha + "," + beta + "," + xval + "," + Math.pow(10,Log10density));

}
```

Gamma unit test: generating 10000 random gamma densities with alpha and beta chosen between 0.1 and 10 and x-value between 0 and 200. Figure 3.3 is a comparison of the gamma density for STRmix and MS Excel.

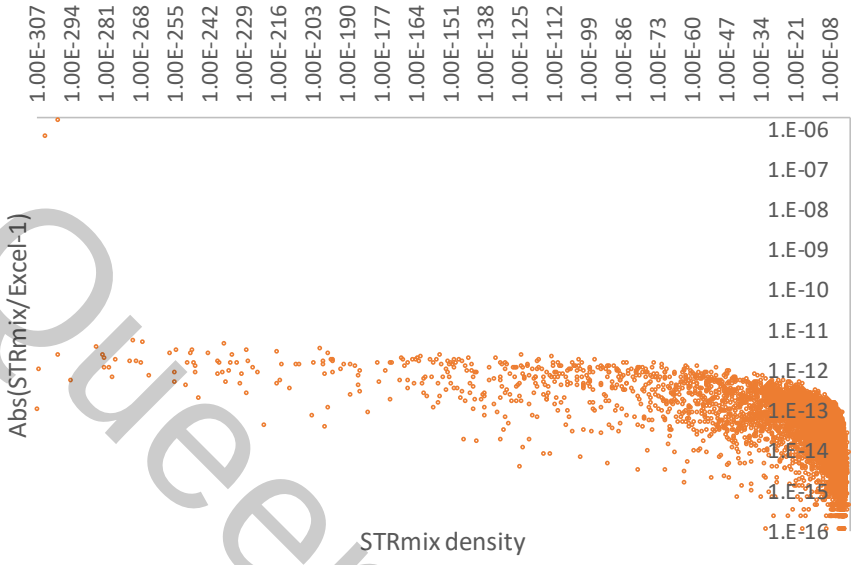


Figure 3.3 Comparison of gamma density STRmix™ and MS Excel

Examination of Figure 3.3 suggests that we should expect precision to 11 sig fig most of the time.

3.6. Variables with stochastic elements

When a stochastic element is present in the calculation the acceptance criterion must be calculated using sampling theory. This includes the random walk, and HPD processes.

4. RESULTS OF DEVELOPMENTAL VALIDATION

4.1. Pre checks for missing stutter peaks

Acceptance criterion: Peaks flagged as missing when dropout penalty less than threshold. Expected peak height in error message rounded to 0 dp. Precision expected to 0 dp for each.

Stutter peaks were artificially deleted from input files to simulate dropout. Within Excel, the dropout probability for back and forward stutter peaks were calculated using the following formula:

=LOG(NORM.DIST(LOG(Z/E_{a-1}),0,SQRT(k^2/(1000/O_a+O_a)),1)) back stutter

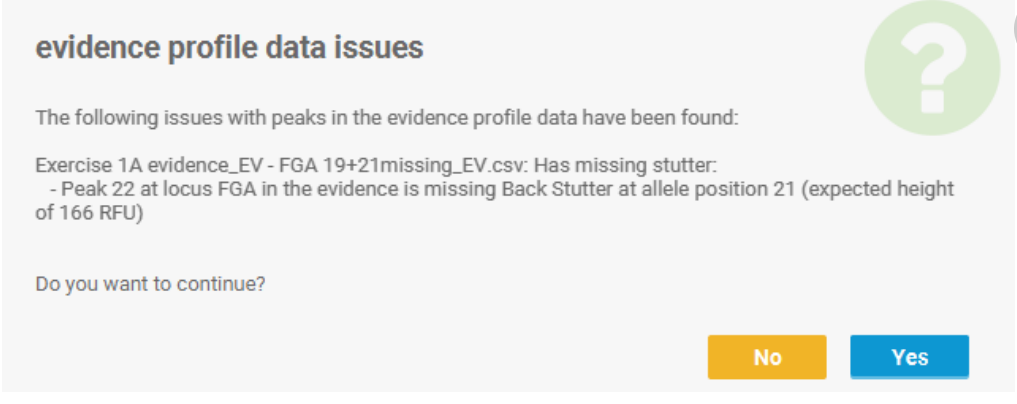
=LOG(NORM.DIST(LOG(Z/E_{a+1}),0,SQRT(k^2/(1000/E_{a+1}+E_{a+1})),1)) forward stutter

Where back stutter variance is proportional to parent peak height and forward stutter variance is proportional to the expected height of the forward stutter, z is the analytical threshold, E_{a-1} the expected height of the back stutter peak, E_{a+1} the expected height of the forward stutter peak, O_a the observed height of the presumed parent peak and k^2 the mode of the relevant stutter variance prior.

Where the dropout penalty was less than the threshold of -8 a warning was given. An example is given below for a single source GlobalFiler™ profile.

Locus	Stutter	Allele	O_a	E_{a-1}	Mode of k^2	Probability of dropout
FGA	19	20	2538	136	6.7944	-6.59179
FGA	21	22	2482	166	6.7944	-10.6692

As expected, a message was given for the missing 21 stutter peak (and not for the 19 peak).



These tests satisfy the acceptance criteria.

4.2. Modelling drop-in peaks in stutter positions

Acceptance criterion: stutter penalties are written to an extended output (labelled in 'part peak designations') and are rounded to 4 dp. Drop-in penalties are written to the same file also rounded to 4 dp. Precision is expected to 4 dp.

A back stutter penalty is assigned for one example by the equation:

LOG(NORM.DIST(LOG(O/E),0,SQRT(k^2/(1000/$O_{17.3}$+$O_{17.3}$)),0))

Peak	O	E	O_{16}	Drop-in penalty STRmix	Drop-in penalty Excel	Stutter penalty STRmix	Stutter penalty Excel
15	1552	386.4461	5174	-3.8028	-3.8028	-59.2468	-59.2468

From the peak designation file:

> expected stutter height (386.4461) and < drop-in cap (2000) and <= stutter max (1,552.2) but drop-in penalty (-3.8028) > stutter balance penalty (-59.2468).

These tests satisfy the acceptance criteria.

4.3. Smart start

Acceptance criterion: Smart start is rounded to 0 dp within the STRmix™ extended output and log. Precision expected to 0 dp.

The values necessary to replicate smart start are written to a text file as part of the extended output from STRmix™. The smart start algorithm was checked in Excel for 17 different interpretations including; multiple numbers of contributors, assumed contributors, replicate PCRs, multi-kit analysis, drop-in, and combinations of each. A summary of the results from STRmix™ and Excel™ for a number of profiles is given in the table below.

Sample	STRmix™	Excel
Single source	4695	4695
Two person with assumed contributor	17403; 404	17403; 404
Three person with two assumed contributors	256; 987; 50	256; 987; 50
Three person multi kit	662; 225; 50	662; 225; 50
Two person multi kit with replicates	295; 50	295; 50

The values are rounded up to the nearest whole number. These tests satisfy the acceptance criteria.

4.4. Expected peak heights given mass parameters

In STRmix™, the amount of DNA for each contributor is modelled by an exponential equation using mass parameters proposed at each MCMC iteration. The total allelic product (TAP) of allele a at locus l for replicate number y, kit k is:

$$T^{l}_{anyk} = A^{l}_{k} R_{y} t_{n} B_{k} \times e^{-d_n \times \left(m^{l}_{ak} - \min\left(m_{ak}\right)\right)} X^{l}_{an}$$

The amplification efficiency (A^{l}_{k}), replicate efficiency (R_y), kit efficiency (B_k), template DNA (t_n), and slope (d_n), are collectively referred to as the mass parameters. The allele molecular weight (m^{l}_{a}) and the minimum molecular weight across the profile are taken from the input file. Molecular weight values are truncated. The expected height of an allele is calculated from the TAP using the following general formula (considering back and forward stutter only):

$$E_a = \frac{T^{l}_{anyk}}{1 + SR_a + FSR_a}$$

Per allele stutter ratios are determined from the stutter files. If a value is present in the stutter exceptions file, this is used as the per allele stutter ratio otherwise a value is calculated from the stutter regression file. A minimum stutter ratio of 0.001 is applied to all stutter types and all loci where the stutter is enabled.

Expected stutter peaks heights are calculated using the following general formula (considering back and forward stutter only):

$$E_{a-1} = SR_a O_a$$
$$E_{a+1} = FSR_a O_a$$

Where:

$E_{a\text{-}1}$ and E_a are the expected heights of the stutter and allele respectively, and O_a the observed height of allele, a. SR_a and FSR_a are the stutter and forward stutter ratios for the allele, respectively.

Note that total allelic product is not output within STRmix™ extended outputs.

4.4.1. Expected peak heights using stutter regression file

Acceptance criterion: Expected allele height is output to 15 sig fig and stutter height to 5 dp in STRmix™. Precision is expected to 10 sig fig for expected allele height and 5 dp for stutter peak height.

Expected allele and stutter peak heights were calculated in Excel for selected loci from 14 different interpretations including; single source profiles, mixed DNA profiles, assumed contributors, replicate PCRs, multi-kit analysis. The extended output for a single source profile was analysed and the expected height of various peaks using a stutter regression file is given below.

A summary of the genotype combination at one locus and the mass parameters for five iterations is provided in the following table:

Genotype	t_1	d_1	A^l
9,9	4297.75181663863	0.000677705280678254	0.862441558791565
9,9	4326.12830893682	0.000594011278317268	0.854594074489788
9,9	4308.13636117358	0.000600868570364307	0.862632285566135
9,9	4204.82077296148	0.000566925558660158	0.859444700195893
9,9	4219.69644998446	0.000544719478243954	0.839947111416278

The observed peak heights for the double back stutter, back stutter, allele, and forward stutter from the STRmix™ extended output, and their expected height calculated in Excel are in the following table:

E_{a-2} STRmix	E_{a-1} STRmix	E_a STRmix	E_{a+1} STRmix	E_{a-2} Excel	E_{a-1} Excel	E_a Excel	E_{a+1} Excel
32.82240	227.56864	6397.771771	39.59202	32.82240	227.56864	6397.771771	39.59202
32.82240	227.56864	6464.196892	39.59202	32.82240	227.56864	6464.196892	39.59202
32.82240	227.56864	6491.003262	39.59202	32.82240	227.56864	6491.003262	39.59202
32.82240	227.56864	6345.009245	39.59202	32.82240	227.56864	6345.009245	39.59202
32.82240	227.56864	6244.320076	39.59202	32.82240	227.56864	6244.320076	39.59202

Where O_9 = 6838 rfu, total allelic product = $t_1 * A^l$*exp(-d_1*(243-89))*2 and where the truncated size of the 9 allele from the input file was 243 bp and the minimum allele size in the input file was 89 bp.

E_9 = TAP/(1+SR_9+FSR_9+$DBSR_9$)

From the back stutter regression file:

Locus	Intercept	Slope
D16S539	-0.05042	0.0093

SR_9 = -0.05042+0.0093*9 = 0.03328

From the forward stutter regression file:

Locus	Intercept	Slope
D16S539	0.00579	0

FSR_9 = 0.00579+0*9 = 0.00579

From the double back stutter regression file

Locus	Intercept	Slope
D16S539	0.0048	0

FSR9 = 0.0048+0*9 = 0.0048

These tests satisfy the acceptance criteria.

4.4.2. Expected peak heights using stutter exceptions file

Acceptance criterion: Expected allele height is output to 15 sig fig and stutter height to 5 dp fig in STRmix™. Precision is checked to 10 sig fig for expected allele height and 5 dp for stutter peak height.

Expected allele and stutter peak heights were calculated in Excel for selected loci from 14 different interpretations including; single source profiles, mixed DNA profiles, assumed contributors, replicate PCRs, multi-kit analysis. The extended output for a single source profile was analysed and the expected height of various peaks using a stutter exceptions file was calculated.

A summary of the genotype combination at one locus for five iterations and the mass parameters is provided in the following table:

Genotype	t_1	d_1	A^l
16,17.3	4297.75181663863	0.000677705280678254	1.47854746472871
16,17.3	4326.12830893682	0.000594011278317268	1.48990557727800
16,17.3	4308.13636117358	0.000600868570364307	1.50722565299489
16,17.3	4204.82077296148	0.000566925558660158	1.50312903983602
16,17.3	4219.69644998446	0.000544719478243954	1.51072359454794

The observed peak heights for the double back stutter, back stutter (using exceptions file), two base pair stutter, allele, and forward stutter from the STRmix™ extended output for 17.3 allele, and their expected height calculated in Excel are in the following table:

	Iteration	E_{a-2}	E_{a-1}	$E_{a-0.2}$	E_a	E_{a+1}
1	STRmix	40.35028	353.78048	90.80216	5412.441936	35.075
	Excel	40.35028	353.78048	90.80216	5412.441936	35.075
2	STRmix	40.35028	353.78048	90.80216	5538.952747	35.075
	Excel	40.35028	353.78048	90.80216	5538.952747	35.075
3	STRmix	40.35028	353.78048	90.80216	5575.984523	35.075
	Excel	40.35028	353.78048	90.80216	5575.984523	35.075
4	STRmix	40.35028	353.78048	90.80216	5447.035046	35.075
	Excel	40.35028	353.78048	90.80216	5447.035046	35.075
5	STRmix	40.35028	353.78048	90.80216	5506.870953	35.075
	Excel	40.35028	353.78048	90.80216	5506.870953	35.075

These tests satisfy the acceptance criteria.

4.5. Log(likelihood) values

4.5.1. Taylor quantum effect, no dropout

Acceptance criterion: log(likelihood) is output to 15 sig fig in STRmix™. Precision is expected in Excel to 14 sig fig.

Log(likelihood) values were calculated in Excel for selected loci from 20 different profiles including; single source profiles, mixed DNA profiles, assumed contributors, replicate PCRs, multi-kit analysis.

The peak designation, expected and observed peak heights and log(likelihood), or log(p), for each peak for each locus may be recovered from the extended output. They are provided in the following format:

Peak	O	E	E Back	E Forward	E 2BP	E Double back	log(p)

The number of columns per peak and the column titles will depend on the number of stutter types modelled and their labels. The log(likelihood) for a range of peak types and for five iterations is replicated below, where *b*=1000. Note that the log(likelihood) is only calculated where O>*z* or if O = 0 rfu, where E > 1/2*z*.

Log(likelihood) values for back stutter peaks (where back stutter variance is proportional to observed allele height)

Log(p)=LOG(NORM.DIST(LOG(O/E),0,SQRT(k^2/(1000/$O_{17.3}$+$O_{17.3}$)),0)), where $O_{17.3}$ = 5612 rfu

Peak	O	E STRmix	k^2_{a-1}	log(p) STRmix	log(p) Excel
16.3	389	353.78048	4.44207231525283	0.6856397209	0.6856397209
16.3	389	353.78048	4.41008369263630	0.6838286641	0.6838286641
16.3	389	353.78048	4.44204914141806	0.6856384224	0.6856384224
16.3	389	353.78048	4.50591579181502	0.6891442771	0.6891442771
16.3	389	353.78048	4.62206500969138	0.6951631890	0.6951631890

There are no differences in the 10th significant figure. These tests satisfy the acceptance criteria.

Log(likelihood) values for alleles

Log(p)=LOG(NORM.DIST(LOG(O/E),0,SQRT(c^2/(1000/E+E)),0))

Peak	O	E	c^2	log(p) STRmix	log(p) Excel
17.3	5612	5412.44193661015	15.1477137911941	0.8582554553	0.8582554553
17.3	5612	5538.95274770251	15.1353361900925	0.8800620020	0.8800620020
17.3	5612	5575.98452399238	15.1408686264031	0.8833771962	0.8833771962
17.3	5612	5447.03504672663	15.1157058294766	0.8661448485	0.8661448485
17.3	5612	5506.87095368646	15.0870371828278	0.8767214594	0.8767214594

There are no differences in the 10th significant figure. These tests satisfy the acceptance criteria.

Log(likelihood) values for forward stutter peaks (where forward stutter variance is inversely proportional to expected height)

Log(p)=LOG(NORM.DIST(LOG(O/E),0,SQRT(k_{a+1}^2/(1000/E+E)),0))

Peak	O	E	k_{a+1}^2	log(p) STRmix	log(p) Excel
18.3	160	35.075	7.29505631979703	-0.7511961539	-0.7511961539
18.3	160	35.075	7.33617167573560	-0.7478081824	-0.7478081824
18.3	160	35.075	7.26994719134881	-0.7532874358	-0.7532874358
18.3	160	35.075	7.25507448069025	-0.7545341997	-0.7545341997
18.3	160	35.075	7.31469120565082	-0.7495726006	-0.7495726006

To obtain the expected precision we need to convert the normal to the standard normal. The standard deviation is $\sqrt{\frac{k_{a+1}^2}{1000/O_a + O_a}} \approx 0.18$. After multiplying the densities by the sd the difference between log(p) STRmix and log(p) Excel is less than 2 x 10^{-16}.

The precision for a log density at about these X values is about 7 or 8 x 10^{-15}. Hence this passes the acceptance criterion.

4.5.2. Taylor quantum effect, peaks below z (dropped peaks)

Acceptance criterion: log(likelihood) is output to 15 sig fig in STRmix™. Precision is expected in Excel depending on the magnitude of X as discussed in Section 3.3 Cumulative Normal approximation.

The log(likelihood) for a range of peak types and for five iterations is replicated below, where *b*=1000. Note that the log(likelihood) is only calculated where O>*z* or if O = 0 rfu, where E > 1/2*z*.

Expected peak heights of dropped alleles

E=t_1* A^l *EXP(-d_1*(180-89)) where the average size of the peaks present within the input file at this locus = 180 bp and the minimum size of all peaks = 89 bp.

t_1	d_1	A^l	E STRmix	E Excel
37.1018888544871	0.000393288488845183	1.140624695068760	41.77363718	41.77363718

36.7548324832931	0.000366787940226078	1.133357982788490	41.15521305	41.15521305
38.1070866793617	0.000306483714594276	1.131144032422140	42.67084256	42.67084256
34.2766578008051	0.000351912621406127	1.138931875598040	38.58803859	38.58803859
36.4061185511573	0.000369398065685203	1.116414797954950	40.15187720	40.15187720

There are no differences in the 10th significant figure. These tests satisfy the acceptance criteria.

Log(likelihood) values for dropped alleles

Log(p)=LOG(NORM.DIST(LOG(75/E),0,SQRT(c2/(1000/E+E)),1)), where z=75 rfu and E is the expected height of the peak as calculated above

E	X	c^2	log(p) STRmix	log(p) Excel
41.7736371828539	0.550316	14.0162763290725	-0.1493852071	-0.1493852269
41.1552130576283	0.562488	14.0532298013709	-0.1468445153	-0.1468445384
42.6708425609767	0.531052	14.0621270978156	-0.1534719551	-0.1534719692
38.5880385905585	0.619481	14.0004125872925	-0.1353700746	-0.1353701099
40.1518772040832	0.586299	13.9358788054211	-0.1419666585	-0.1419666874

X is calculated in Excel using the formula:

=-LOG10(E/75)/SQRT(k2/(1000/Oa+Oa)) (for stutter peaks with missing data where Z=75 rfu and b=1000)

There are differences of 3.5 x 10^{-8}. At X values for cumulative probabilities in this region the STRmix™ log cumulative probability is within about 3.18 x 10^{-8} and 4.24 x 10^{-8} of the Excel one. Hence these differences are within the acceptance criterion.

Log(likelihood) values for dropped two base pair stutter peaks (where double back stutter variance is proportional to its expected height)

Log(p)=LOG(NORM.DIST(LOG(75/E),0,SQRT($k^2_{a-0.2}$ /(1000/E+E)),1)), where z=75 rfu, E is the expected height of the stutter peak.

Peak	O	E	X	$k^2_{a-0.2}$	log(p) STRmix	log(p) Excel
17.1	0	90.80216	-0.785	1.13840043346852	-0.6652500046	-0.6652498681

17.1	0	90.80216	-0.782	1.14810583375804	-0.6632931004	-0.6632929633
17.1	0	90.80216	-0.784	1.14195938321898	-0.6645290834	-0.6645289467
17.1	0	90.80216	-0.775	1.16922560903803	-0.6591316384	-0.6591315002
17.1	0	90.80216	-0.779	1.15584515134752	-0.6617529342	-0.6617527966

There are differences of about 1.38 x 10^{-7}. It is expected that values for cumulative probabilities for log(p) from STRmix™ are about 2.3 x 10^{-9} to -2.8 x 10^{-7} of the Excel values given the precision of the expected stutter. This is within the expected precision.

Log(likelihood) values for dropped double back stutter peaks (where double back stutter variance is inversely proportional to expected height)

Log(p)=LOG(NORM.DIST(LOG(75/E),0,SQRT(k_{a-2}^2 /(1000/E+E)),1)), where z = 75 rfu.

Peak	O	E	X	k_{a-2}^2	log(p) STRmix	log(p) Excel
15.3	0	40.35028	1.01459	4.58585705891935	-0.07322092095	-0.07322091516
15.3	0	40.35028	1.01214	4.60804357284515	-0.07352113438	-0.07352112906
15.3	0	40.35028	1.00985	4.62895253220254	-0.07380294564	-0.07380294077
15.3	0	40.35028	1.00529	4.67106866110201	-0.07436732801	-0.07436732405
15.3	0	40.35028	1.01361	4.59469771834702	-0.07334069346	-0.07334068785

There are differences of about 5.8 x 10^{-9}. It is expected that values for cumulative probabilities for log(p) from STRmix™ are about 1.7 x 10^{-8} of the Excel one given the precision of the expected stutter. This is within the expected precision. These tests satisfy the acceptance criteria.

4.6. Probabilities and expected stutter peak heights given saturated alleles

Acceptance criterion: log(likelihood) is output to 15 sig fig in STRmix™. Precision is checked in Excel to 10 sig fig. Expected back stutter peak height is output to 5 dp and precision is expected to 5 dp in STRmix™. For saturated alleles, expected back stutter height is output to 15 sig fig and precision is checked to 10 sig fig.

The log(likelihood) and expected peak heights for two profiles with observed and expected saturated alleles were replicated in Excel from the extended output.

The following tables replicate one profile analysed on a CE 3500 that was interpreted with the saturation setting artificially low at s=5000. The log(likelihood) and expected peak heights for a range peak types for five iterations is replicated below.

Log(likelihood) for alleles when O_a < s

When E<s, log(p)=LOG(NORM.DIST(LOG(O/E),0,SQRT(c^2/(1000/E+E)),0))

When E >= s, log(p)=LOG(NORM.DIST(LOG(O/5000),0,SQRT(c^2/(1000/5000+5000)),0))

Allele	O	E	c^2	log(p) STRmix	log(p) Excel
16	4614	5457.97190970947	14.5973718712686	0.7777079518	0.7777079518
16	4614	5450.60722423450	14.6979162814592	0.7768368887	0.7768368887
16	4614	5450.65600358263	14.6112390254063	0.7775877118	0.7775877118
16	4614	5400.36513172157	14.6263675177696	0.7774565720	0.7774565720
16	4614	5470.21487602310	14.6079473365208	0.7776162506	0.7776162506

There are no differences in the 10th significant figure. These tests satisfy the acceptance criteria.

Expected height and log(likelihood) values for back stutter peaks when Oa < s

Log(p)=LOG(NORM.DIST(LOG(O/E),0,SQRT(k^2_{a-1}/(1000/O_{16}+O_{16})),0))

Where O_{16} = 4614 rfu and SR_{16} = 0.09351.

Peak	O	E STRmix	k^2_{a-1}	log(p) STRmix	E Excel	log(p) Excel
15	460	431.45514	7.92737048732550	0.8855569253	431.45514	0.8855569253
15	460	431.45514	7.81140930154187	0.8873043970	431.45514	0.8873043970
15	460	431.45514	7.75784407225460	0.8881130083	431.45514	0.8881130083
15	460	431.45514	7.95319255985181	0.8851684089	431.45514	0.8851684089
15	460	431.45514	7.90429767203593	0.8859042695	431.45514	0.8859042695

There are no differences in the 10th significant figure for log(p) values. The expected heights are the same to 5 dp. Hence these pass the acceptance criterion.

Log(likelihood) values for alleles when Oa > s

When E<s, log(p)=LOG(NORM.DIST(LOG(O/E),0,SQRT(c^2/(1000/E+E)),0))

When E >= s, log(p)=LOG(NORM.DIST(LOG(O/5000),0,SQRT(c^2/(1000/5000+5000)),0))

Peak	O	E	c^2	log(p) STRmix	log(p) Excel
15	5079	5046.552683849510	14.7168046299834	0.8630772453	0.8630772453
15	5079	5022.356145983540	14.7071510373387	0.8632174866	0.8632174866
15	5079	5005.710029338570	14.7465562768069	0.8626456007	0.8626456007
15	5079	4901.811784821820	14.7310133251947	0.8447959226	0.8447959226
15	5079	4913.943178152170	14.7515704348481	0.8473230332	0.8473230332

There are no differences in the 10th significant figure. These tests satisfy the acceptance criteria.

Expected height of stutter peaks when Oa>s

If O_a > s, E_{a-1} = SR_a * E_a, otherwise, E_{a-1} = SR_a * O_a

Peak	O_{16}	E_{16} STRmix	$E_{16.3}$ STRmix	$E_{16.3}$ Excel
15	5174	5731.45453209985	426.0989833	426.0989833
15	5174	5757.93526666577	428.0768294	428.0768294
15	5174	5733.92140341930	426.2832340	426.2832340
15	5174	5652.13940162810	420.1749363	420.1749363
15	5174	5705.61608453316	424.1691097	424.1691097

Where SR_{16} = 0.07469

There are no differences in the 10th significant figure. These tests satisfy the acceptance criteria.

Log(likelihood) values of stutter peaks when Oa > s

=LOG(NORM.DIST(LOG(O/E),0,SQRT(k^2_{a-1}/(1000/$E_{17.3}$+$E_{17.3}$)),0))

Peak	O	E	k^2_{a-1}	log(p) STRmix	log(p) Excel

15	400	426.098983397538	7.92737048732550	0.9117190326	0.9117190326
15	400	428.076829462266	7.81140930154187	0.8953842479	0.8953842479
15	400	426.283234016387	7.75784407225460	0.9122292728	0.9122292728
15	400	420.174936302603	7.95319255985181	0.9555829672	0.9555829672
15	400	424.169109748782	7.90429767203593	0.9278379128	0.9278379128

There are no differences in the 10th significant figure. These tests satisfy the acceptance criteria.

4.7. Shifted log normal for composite peaks

Acceptance criterion: log(likelihood) is output to 15 sig fig in STRmix™. Precision is expected in Excel to 13 sig fig and less for dropped alleles depending on the magnitude of X as discussed in Section 3.3 Cumulative Normal approximation. Precision if checked to 10 sig fig.

The log(likelihood) using the shifted log normal (SLN) model from the post burn-in extended output was replicated in Excel for 17 profiles containing composite alleles, back, forward, double back, and exotic stutter types such as 2 bp stutter at SE33.

In the following tables, the log(likelihood) is replicated for a composite allele and forward stutter peak

a	O_a	E_a	E_{a+1}	c^2	k^2
12	3687	3745.84	39.22497	15.29999120742390	7.42248582823617

Allelic distribution			
$\mu^i_{\log_{10}}$	$\mu^i_{\ln}$	$\mathrm{var}^i_{\log_{10}}$	$\mathrm{var}^i_{\ln}$
3.57354886354320	8.22840034228039	0.00408424105865942	0.02165422995164470

Forward stutter distribution			
$\mu^i_{\log_{10}}$	$\mu^i_{\ln}$	$\mathrm{var}^i_{\log_{10}}$	$\mathrm{var}^i_{\ln}$
1.59356262010246	3.66931353380045	0.114688009996105	0.608064143492875

Moments

Allele	Double back stutter

$E[E^i]$	$VAR[E^i]$	$E[E^i]$	$VAR[E^i]$
3786.613856853140	313874.060228181000	53.162093040873	2365.174551699180

Combined	
$E[E]$	$VAR[E]$
3839.7759498940	316239.23477988

Shifted LN approximation parameters			
τ	μ	σ^2	Pr(*O*|*E*,SLN)
1540.98091900597	7.7110798499248	0.0581210301903956	0.000760708718226549

log(p)	
STRmix™	Excel
0.810107220	0.810107220

The log(likelihood) calculation using the SLN model for one composite back and double back stutter peak for one post burn-in iteration when the observed peak height is 0 is given below.

a	O_a	O_{a+1}	E_{a-1}	E_{a-2}	k_{a-1}^2	k_{a-2}^2
13	0	2840	173.2968	26.537	47.0454136111740	5.09159310850694

Back stutter distribution			
$\mu_{\log_{10}}^i$	$\mu_{\ln}^i$	$\mathrm{var}_{\log_{10}}^i$	$\mathrm{var}_{\ln}^i$
2.23879054335343	5.15500573146164	0.0165632329179250	0.0878165733109604

Double back stutter distribution			
$\mu_{\log_{10}}^i$	$\mu_{\ln}^i$	$\mathrm{var}_{\log_{10}}^i$	$\mathrm{var}_{\ln}^i$
1.42385182444069	3.27853998558951	0.0792833151420747	0.2974635355438830

Moments

Back stutter		Double back stutter	
$E[E^i]$	$VAR[E^i]$	$E[E^i]$	$VAR[E^i]$
181.0754903582340	3009.5703031133600	30.7925184609700	328.4865144150490

Combined	
$E[E]$	$VAR[E]$
211.8680088192040	3338.0568175284100

Shifted LN approximation parameters			
τ	μ	σ^2	Pr(*O*|*E*,SLN)
0	5.3200990346478700	0.0717288924378393	0.0000907138508489173

log(p)	
STRmix™	Excel
-4.042193828	-4.042326396

These tests satisfy the acceptance criteria.

4.8. Expected peak heights for replicate profiles

Acceptance criterion: Expected allele height is output to 15 sig fig and stutter height to 5 dp in STRmix™. Precision is checked to 10 sig fig for expected allele height and 5 dp for stutter peak height.

The expected peak heights for four replicate interpretations were calculated in Excel from the extended output.

In the following tables, the expected peak heights for peaks from two inputs from replicate PCR of the same extract interpreted assuming two contributors, assuming the presence of one individual are given. The extended output for each peak within a replicate analysis is in the form:

Peak	O	E	E Back	E Forward	log(p)

The number of columns per peak and the column titles will depend on the number of stutter types modelled and their labels.

At CSF1PO, the following alleles were present in the input files:

REP1	Allele	Height	Size

CSF1PO	12	42	100

REP2	Allele	Height	Size
CSF1PO	12	30	306

Note the sizes are truncated to an integer (whole base pairs). The sizes for the 12 allele were deliberately edited to be disparate to demonstrate that an average is used within the calculation of total allelic product. The truncated average size for the 12 allele = 203 bp. The truncated minimum size across both replicates = 50 bp. The reference at CSF1PO for the assumed contributor is 12,12.

In the following tables the masss parameters for the CSF1PO locus is reproduced:

C1	C2	t_1	t_2	d_1	d_2
12.0,12.0	-1.0,-1.0	69.1948660741329	15.4207059682330	0.00275215129535	0.00320287534391
12.0,12.0	-1.0,-1.0	65.1320439369875	13.4396100559091	0.00279456705228	0.00323987532790
12.0,12.0	-1.0,-1.0	71.9197613892744	13.9764345793742	0.00271672687702	0.00331881538199
12.0,12.0	-1.0,-1.0	67.8653376376408	14.0764588358648	0.00271771011286	0.00326439928352
12.0,12.0	-1.0,-1.0	67.7397706397475	13.8723750374247	0.00275929288135	0.00320878099001

A^{CSF}	R_1	R_2
0.905006756472633	1.074484034783070	0.930679254068109
0.899379226505035	1.069603337934040	0.934926027747367
0.902102967100105	1.060018817984670	0.943379478773043
0.894086759179492	1.058915258208140	0.944362631710640
0.893622128364227	1.067144803732010	0.937079950633505

The total allelic product was calculated using the formula $T^{l}_{any} = A^{l}_{k} t_n R_y \times e^{-d_n \times \left(m^{l}_{a} - \min\left(m_a\right)\right)} X^{l}_{an}$

Total allelic product = t_1 * A^{CSF} *R_1*EXP(-d_1 *(203-50))*2

E=TAP/(1+SR_{12}+FSR_{12}), where SR_{12} = 0.06182 and FSR_{12} = 0.00691

The expected peak height for the 12 allele for contributor 1, replicate 1, is given in the table below:

Peak	O	E STRmix	TAP Excel	E Excel
12	42	82.64475683	88.3249309684726	82.64475683
12	42	76.45951720	81.7145798276304	76.45951720
12	42	84.93005732	90.7673001620110	84.93005732
12	42	79.33540506	84.7881274535081	79.33540506
12	42	79.25672041	84.7040348128612	79.25672041

There are no differences in the 10th significant figure. These tests satisfy the acceptance criteria.

The expected back and forward stutter peaks for the 12 allele for contributor 1, replicate 1, is given in the table below:

Stutter	E STRmix™	E Excel
Back	2.59644	2.59644
Forward	0.29022	0.29022

These numbers are the same to 5 dp. Hence these tests pass the acceptance criterion.

The expected peak height for the -1 (dropped allele) for contributor 2, replicate 1, is given in the table below E= $t_2 * A^{CSF}$ *R_1*EXP(-d_2 *(203-50)), where the average size for the 12 allele = 203 bp and the minimum size across both replicates = 50 bp.

Peak	O	E STRmix	E Excel
-1	0	16.96818105	16.96818105
-1	0	17.52566261	17.52566261
-1	0	17.79693489	17.79693489
-1	0	17.30576420	17.30576420
-1	0	18.98568938	18.98568938

There are no differences in the 10th significant figure. These tests satisfy the acceptance criteria.

4.8.1. Replicates conditioning on an individual whose alleles has 'dropped out' of the profile

Acceptance criterion: Expected allele height is output to 15 sig fig in STRmix™. Precision is checked to 10 sig fig.

Replicate analysis was undertaken for a profile where the assumed contributor was dropping out of the profile. Note the size of the 15 allele was deliberately edited to 50 bp.

REP1	Allele	Height	Size
D22S1045	15	67	50

The assumed contributor is a 15,17 at this locus. There is no profiling information at D22S1045 for replicate 2 and no 17 allele present in replicate 1. From the size regression file:

Locus	Intercept	Slope
D22S1045	64.66242	2.986364

The expected size of a 17 allele at D22S1045 is:

64.66242+17*2.986364=115 bp

The truncated average of the 15 and 17 alleles = 82 bp. The minimum size across both replicates is 50 bp. In the following tables the mass parameter information for the D22S1045 locus is reproduced:

C1	C2	t_1	t_2	d_1	d_2
15.0,17.0	-1.0,17.0	61.0049782822864	24.2206638791544	0.00279051611587667	0.00334788500742973
15.0,17.0	-1.0,17.0	62.6064927040726	24.6900162420881	0.00274189043810030	0.00327577388864725
15.0,17.0	-1.0,17.0	59.9694063736518	28.9075195936333	0.00275795102315941	0.00334134956135675
15.0,17.0	-1.0,17.0	56.2602821011490	28.5733034489033	0.00272091403365052	0.00345935157950035
15.0,17.0	-1.0,17.0	55.8464923952199	33.7996322498695	0.00263403638606275	0.00336544763984819

A^{CSF}	R_1	R_2
1.194852671427460	0.970139090556726	1.030780029105040
1.209568325940900	0.946377388364466	1.056660918038420
1.207952666474240	0.931028149369584	1.074081380543770
1.215290276466970	0.931011251799601	1.074100874792910
1.224243289216440	0.948867930345718	1.053887446312630

Total allelic product 17 allele C1 = $t_1 * A^{D22}$ *R_1*EXP(- d_1 *(115-50))

E_{17} C1 = TAP C1/(1+SR_{17}+FSR_{17})

Where SR_{17} = 0.10167 and FSR_{17} = 0.04783

The expected peak height for the 17 allele for replicate 1, is given in the table below:

Peak	O	E STRmix	TAP C1	TAP C2	E Excel
17	0	70.96136195	58.9847531506033	22.5853324167460	70.96136195
17	0	72.03966455	59.9670828938541	22.8425115175653	72.03966455
17	0	71.80420316	56.3751732355576	26.1637582998248	71.80420316
17	0	68.86123500	53.3369025942188	25.8190870424085	68.86123500
17	0	75.00144286	54.6654360283063	31.5487225497124	75.00144286

There are no differences in the 10th significant figure. These tests satisfy the acceptance criteria.

Total allelic product -1 allele C2 = $t_2 * A^{D22}$ *R_2*EXP(- d_1 *(50-50)), where 50 is the average of the observed peak sizes within the input file that represent a Q (or -1 or dropped allele) and also the minimum observed size across both replicates.

The expected peak height for the -1 (dropped allele) for contributor 2, replicate 2, is given in the table below:

Peak	O	E STRmix	E Excel
-1	0	29.83090282	29.83090282
-1	0	31.55639809	31.55639809
-1	0	37.50575683	37.50575683
-1	0	37.29800019	37.29800019
-1	0	43.60878014	43.60878014

There are no differences in the 10th significant figure. These tests satisfy the acceptance criteria.

4.9. Summation of probabilities

Acceptance criterion: Expected log(likelihood) is output to 15 sig fig. Precision is checked to 10 sig fig.

The expected peak heights for 15 interpretations from post burn-in extended output were summed in Excel. Profiles included single source and mixed, replicates, multikits, drop-in peaks, saturated peaks, assumed contributors, Mx priors.

The probability densities (allele and stutter penalties, drop-in penalties, individual allele and stutter log(p) values, LSAE variance probabilites and penalties) were extracted from the extended output and summed. These values were compared to the total MCMC probability, also within the extended output.

A summary of the typical results for four iterations is provided in the table below.

Iteration	1	2	3	4
Allele variance penalty	-1.3407707629600700	-1.3363142511696200	-1.3392784620858000	-1.3382755282301500
Back stutter variance penalty	-1.2987129622276200	-1.2984025502479400	-1.2986669015400100	-1.2979458552627500
Forward stutter variance penalty	-1.2018873666276500	-1.2015454289562200	-1.2023791465223500	-1.2018219247822500

Locus amplification probability	7.4907105824075700	7.5417767085782000	7.3883971604947200	7.5126935200450300
LSAE variance penalty	1.3792633655887200	1.4399249001969100	1.3816601064907900	1.4181193157074100
log(p)	0.0776511966624886	0.0940821241490230	0.0744838285450870	0.0887141433415545
log(p)	0.1046075398286740	0.0910948487236711	0.1097837751373630	0.1009170453459900
log(p)	0.0228836374590745	-0.0042297436195270	0.0370631356888559	0.0085911088858639
log(p)	-0.1559587891395780	-0.1436246969683320	-0.1729553769423160	-0.1555171418369840
log(p)	0.0900597829587922	0.0879646722992457	0.0902620690144070	0.0900903468704862
log(p)	0.0708937181083023	0.0413928057135613	0.0711638679966473	0.0628924184158468
log(p)	0.0713405309182403	0.0721864785870541	0.0701611928386212	0.0725972715595952
log(p)	-0.1304537287640520	-0.1122351413166880	-0.1393332268632940	-0.1254529113142270
log(p)	0.0731090562348370	0.0713957237954703	0.0722194565278112	0.0729134870283290
log(p)	-0.1218258125058230	-0.1045133510548020	-0.1303013727167410	-0.1171629818875430
log(p)	-0.0850664554196270	-0.0372439439829755	-0.1048654590875510	-0.0583332988982263
log(p)	-0.2213971111688410	-0.1651328199256550	-0.2724780680309890	-0.2107309679562680

log(p)	-0.1064617665962880	-0.0429297414207318	-0.1320119084889190	-0.0767016099335090
log(p)	-0.2830662552802010	-0.2085784491747760	-0.3505420187323680	-0.2773541463325860
log(p)	-0.1088276010953260			
log(p)	-0.2987542286766990			
log(p)	0.0747549971585841	-0.2939420251430460	-0.2239705039799200	-0.2548736189059160
log(p)	0.1099934565373140	-0.1977970865169090	-0.2386100575951140	-0.2230741712987460
log(p)		0.0927313765457170	0.0743205213202413	0.0825733203168214
log(p)		0.0850011312078612	0.1166059709215130	0.1079999721018140
log(p)	0.0259676830742868	-0.0067622308767940	0.0424207345928612	
log(p)	-0.1486067423369150	-0.1332922843679800	-0.1669245257967070	
log(p)				0.0186039409894540
log(p)				-0.1520664714817070
log(p)	0.0774709944783207	0.0305982164153785	0.0760221250878223	

log(p)	-0.2492241568870150	-0.2154766083043580	-0.2598193959760260	
log(p)				-0.0151950257790526
log(p)				-0.1912539666090180
log(p)	0.1351603741310150	0.1238152973725630	0.1376539909058620	0.1290261823689960
log(p)	0.0838258359005377	0.0878306516634439	0.0737052084906049	0.0846201313223828
log(p)	0.1091660256320190	0.0899949805649791	0.1166383530440600	0.0979998747557283
log(p)	0.0452601413150780	0.0536597314330319	0.0308478902356558	0.0462830708365047
log(p)	0.0926991622390662	0.1030110064776640	0.0907367008802467	0.1005208064156970
log(p)	0.1135655333193090	0.0923569915922598	0.1221066293224440	0.1067861754845510
log(p)	-0.0727057717177849	-0.1101956376463990	-0.0441914302500985	-0.0844168371688653
log(p)	0.0227747996216209	0.0406240319511316	-0.0091434542000112	0.0186313112839509
log(p)	0.1771443005878370	0.1577228330618420	0.1774433348457140	0.1680807678780860
log(p)	-0.2622321711812550	-0.3187671318122690	-0.2369007958069560	-0.2719416935480780
log(p)	-0.4852968595350810	-0.5370886137855010	-0.4660609766477010	-0.4925405793038970

log(p)	0.00273654287962 67	-0.02350388204873 61	0.02245980124360 51	0.00928164897170 71
log(p)	-0.17393615108603 40	-0.21839545031865 60	-0.15872809711909 00	-0.18084225671815 50
log(p)	-0.07940120194502 12	-0.11197369587214 40	-0.05354981829472 76	-0.07043789896141 30
log(p)	-1.03183917829908 00	-1.09059119724857 00	-1.00608705433918 00	-1.04421374513849 00
log(p)	-0.00820782277198 99	-0.05069255626170 69	0.02368044821871 45	-0.00260525038507 03
log(p)	0.12664963690540 70	0.12507146048425 90	0.12454597279344 00	0.12635190873343 10
log(p)	-0.26598590474049 90	-0.24263015065217 20	-0.29298602326214 40	-0.26654490448658 90
log(p)	-0.23714968203082 30	-0.21236461456583 10	-0.25041448471267 00	-0.22730069998735 60
log(p)	-0.18686089047348 20	-0.17006217915709 80	-0.20634157716074 60	-0.18809152909207 90
log(p)	0.17219149393516 30	0.17657033213745 50	0.17186828346236 80	0.17556023286864 40
log(p)	0.17921172310023 50	0.16039014130058 50	0.18390998635335 70	0.17625066417474 30
log(p)	0.01897561395463 92	0.04266323191053 39	0.00602509104443 76	0.03457359900108 14
log(p)	0.09857794395215 51	0.07766347327584 54	0.11565002863923 80	0.09400808593142 70
log(p)	0.02925310186986 76	0.01202442225298 31	0.03923861908990 97	0.03283253348291 89

log(p)	0.0599117398321706	0.0570578260717088	0.0589281413496906	0.0590375573370327
log(p)	0.0049206208927753	-0.0212321630573009	0.0136300647844153	-0.0036278122971623
log(p)	0.0150621309097576	-0.0128867367827191	0.0182687358275940	0.0079086115591390
log(p)	0.0113328859401320	0.0351787852712603	-0.0010101639673520	0.0323612418240804
log(p)	0.0051768283058560	0.0231808165503585	-0.0175312409413609	0.0140738385310730
log(p)	-0.1369786404238060	-0.1254987329755230	-0.1497521105671950	-0.1362987117205170
log(p)	0.0664858937966010	0.0617567040692072	0.0691578215599386	0.0669611602756727
log(p)	0.0884122778988330	0.0800686404752821	0.0898403167351098	0.0859572082446891
log(p)	0.0609221851346066	0.0624269886816710	0.0555896681546720	0.0592463768113736
log(p)			0.0732765189555010	0.0716232221094932
log(p)			0.0604101235488521	0.0490049607539302
log(p)	0.0661041486530194	0.0714855559544557		
log(p)	0.0786560363722775	0.0650740604350356		
log(p)			0.0913861309274417	0.0874423225658637
log(p)			-0.1507623723833590	-0.1411100109739540

log(p)	0.03797303655525 50	0.01399123544029 29		
log(p)	0.10463733858903 50	0.07085220741141 83	0.10268384217559 90	0.09113419458164 14
log(p)	-0.00887962894599 99	-0.05419627241688 52	0.00163263532894 70	-0.01479110267463 79
Total probability STRmix	2.975006250	2.730521024	2.600282258	2.841740925
Total probability Excel	2.975006250	2.730521024	2.600282258	2.841740925

There are no differences in the 10th significant figure. These tests satisfy the acceptance criteria.

4.10. Locus specific amplification efficiency penalties

Acceptance criterion: Expected log(likelihood) is output to 15 sig fig. Precision is checked to 10 sig fig.

The locus amplification variance penalty was calculated in Excel for 20 interpretations from post burn-in extended output. Profiles included single source and mixed, replicates, multikits, drop-in peaks, saturated peaks, assumed contributors, Mx priors.

From version 2.7 onwards, σ_A^2 is sampled during the MCMC process from an exponential distribution and a penalty applied. The parameter used to describe an exponential distribution is lambda, λ, and the mean of this distribution is $1/\lambda$. Lambda is determined during Model Maker analysis and the mean is used as the Locus Amplification Variance parameter within STRmix™ Settings. The penalty can be calculated in Excel using the following formula:

=LOG(EXPON.DIST(LSAE var, λ,0)) where λ is the LSAE variance parameter sampled within the MCMC process in STRmix™. The penalty is smaller the further the proposed LSAE variance strays from the mean. In the following table five iterations (five chains) of the penalty applied during the MCMC are calculated where $1/\lambda$=0.019:

LSAE variance	STRmix penalty	Excel penalty
0.0137523898943288	1.406899712	1.406899712
0.0150988934454997	1.376121867	1.376121867

0.0118746972505151	1.449819267	1.449819267
0.0181837156343168	1.305610222	1.305610222
0.0216516826163686	1.226340805	1.226340805

Figure 4.1 shows a zoom of the exponential density unit test.

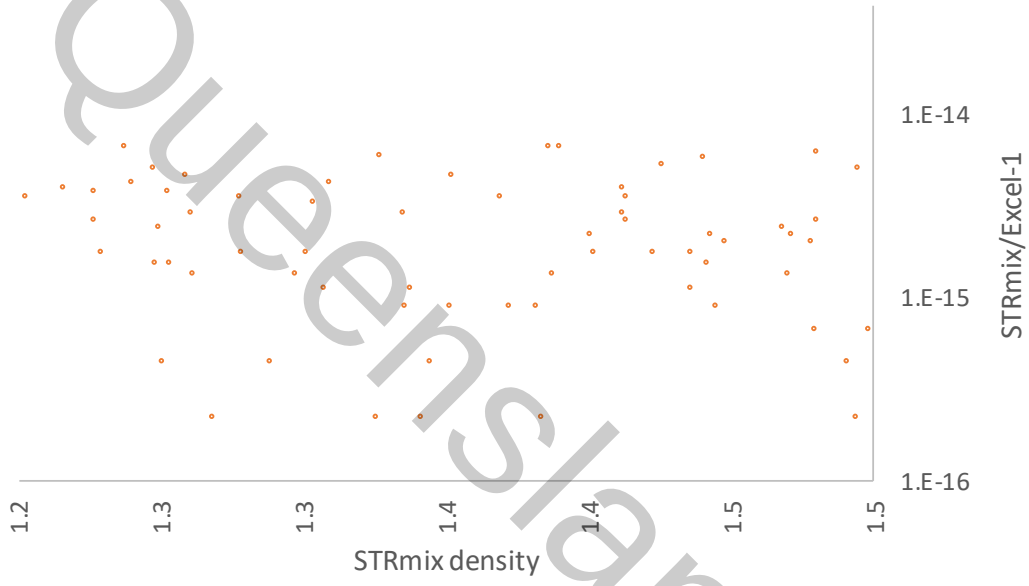


Figure 4.1 Zoom of comparison of exponential density STRmix™ and MS Excel

There are no differences in the 10th significant figure. These tests satisfy the acceptance criteria.

A penalty is applied to the sampled per locus LSAE parameters. The penalty can be calculated in Excel using the following formula:

=LOG(NORM.DIST(LOG(A^l),0,SQRT(σ_A^2),0)) where σ_A^2 is the sampled LSAE variance per iteration. In the following table one iteration of the per locus penalty are calculated for a 21 locus profile where σ_A^2 = 0.0137523898943288 (the first chain in the table above):

Locus	A^l	Penalty
LSAE_Locus 1 (D3S1358)	1.387069370419550	0.212895381584714
LSAE_Locus 2 (vWA)	1.046227652875870	0.525638950535388
LSAE_Locus 3 (D16S539)	0.862441558791565	0.466498525881671
LSAE_Locus 4 (CSF1PO)	0.888979476867885	0.490477201791106
LSAE_Locus 5 (TPOX)	0.927922190808752	0.515054883256491
LSAE_Locus 7 (D8S1179)	1.280915855888240	0.349180362017557
LSAE_Locus 8 (D21S11)	0.819401670019595	0.413569260661096

LSAE_Locus 9 (D18S51)	0.990669308378352	0.531459255357885
LSAE_Locus 11 (D2S441)	0.980599242876516	0.530577897419838
LSAE_Locus 12 (D19S433)	0.778047132074599	0.344142905082050
LSAE_Locus 13 (TH01)	0.628756882197346	-0.109489466305029
LSAE_Locus 14 (FGA)	0.909069680156644	0.504654226545927
LSAE_Locus 15 (D22S1045)	1.450987997192760	0.119052009602909
LSAE_Locus 16 (D5S818)	1.027934043726750	0.529460398706086
LSAE_Locus 17 (D13S317)	0.878956604944536	0.482146612356248
LSAE_Locus 18 (D7S820)	0.857925004088643	0.461788154110071
LSAE_Locus 19 (SE33)	1.096610658425770	0.506391012674211
LSAE_Locus 20 (D10S1248)	0.862102064281114	0.466151016251318
LSAE_Locus 21 (D1S1656)	1.478547464728710	0.076280196257713
LSAE_Locus 22 (D12S391)	1.112630244413250	0.497798172782929
LSAE_Locus 23 (D2S1338)	1.453755567055920	0.114816220006709
STRmix penalty	8.028543176	
Sum penalties Excel	8.028543176	

There are no differences in the 10th significant figure. These tests satisfy the acceptance criteria.

4.11. Penalties

4.11.1. Allele and stutter variance penalties

Acceptance criterion: allele and stutter variance penalties are output to 15 sig fig. Precision is checked to 10 sig fig.

The allele and stutter variance penalties were calculated in Excel for 20 interpretations from post burn-in extended output. Profiles included single source and mixed, replicates, multikits, drop-in peaks, saturated peaks, assumed contributors, Mx priors.

Extended outputs were analysed to check the penalties (or probabilities) for allele and stutter variance. The variances and their penalties are contained within the extended output. A summary of some typical results for allele, back stutter and forward stutter is provided in the following table:

c^2	c^2 penalty	k^2_{a-1}	k^2_{a-1} penalty
15.1477137911941	-1.121575494	4.44207231525283	-1.327824120
15.1353361900925	-1.121141618	4.41008369263630	-1.328791308

15.1408686264031	-1.121335281	4.44204914141806	-1.327824813
15.1157058294766	-1.120457948	4.50591579181502	-1.325953028
15.0870371828278	-1.119469307	4.62206500969138	-1.322745162

k^2_{a+1}	k^2_{a+1} penalty	$k^2_{a-0.2}$	$k^2_{a-0.2}$ penalty
7.29505631979703	-1.146842273	1.13840043346852	-0.6587723352
7.33617167573560	-1.148011831	1.14810583375804	-0.6573341392
7.26994719134881	-1.146135775	1.14195938321898	-0.6582406274
7.25507448069025	-1.145720092	1.16922560903803	-0.6543311902
7.31469120565082	-1.147398844	1.15584515134752	-0.6562137631

The allele variance penalty (or probability) is calculated using the following formula:

=LOG(GAMMA.DIST(c^2, α, β, 0))

Where α and β are in the STRmix™ settings for each peak type. The calculated values for the above five iterations is provided in the following tables:

c^2 parameters	c^2 penalty Excel	k^2_{a-1} parameters	k^2_{a-1} penalty Excel
$\Gamma(8.45,1.746)$	-1.121575494	$\Gamma(1.884,7.686)$	-1.327824120
	-1.121141618		-1.328791308
	-1.121335281		-1.327824813
	-1.120457948		-1.325953028
	-1.119469307		-1.322745162

k^2_{a+1} parameters	k^2_{a+1} penalty Excel	$k^2_{a-0.2}$ parameters	$k^2_{a-0.2}$ penalty Excel
	-1.146842273		-0.6587723352

$\Gamma(2.144, 4.507)$	-1.148011831	$\Gamma(2.189,1.431)$	-0.6573341392
	-1.146135775		-0.6582406274
	-1.145720092		-0.6543311902
	-1.147398844		-0.6562137631

Figure 4.2 below shows a zoom of the gamma density unit test below.

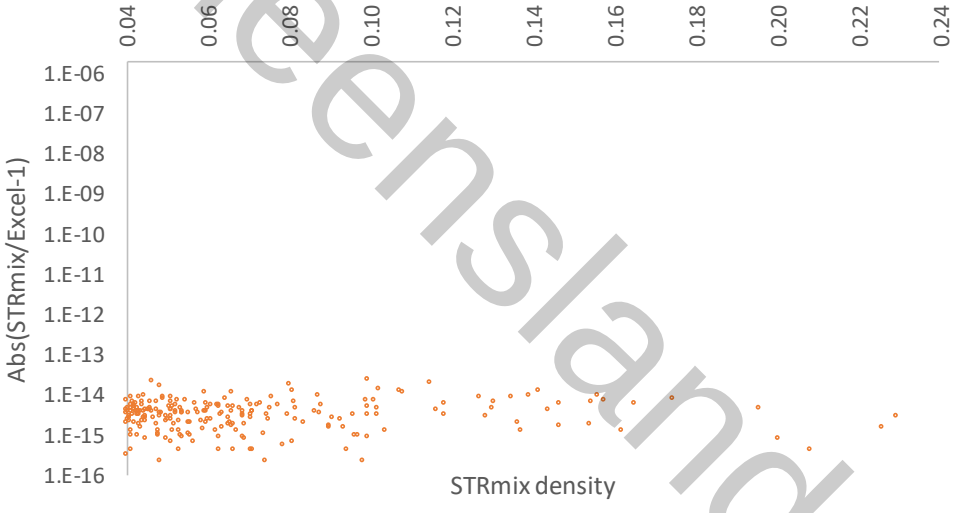


Figure 4.2 Zoom of comparison of gamma density STRmix™ and MS Excel

There are no differences in the 10th significant figure. These tests satisfy the acceptance criteria.

4.11.2. Drop-in penalty

Acceptance criterion: drop-in penalties are output to 15 sig fig. Precision is checked to 10 sig fig.

The drop-in penalty was calculated in Excel for three interpretations from post burn-in extended output including multiple drop-in peaks, multi kits, and replicates.

The drop-in penalty is replicated in the table below where *O*=80, Z =75, c^2= 14.7537 (mean of $\Gamma(8.45,1.746)$), b =1000, f_{di} =0.0001. The drop-in penalty has been calculated for both a uniform and gamma drop-in prior (with prior parameters $\Gamma(5,3)$).

$E=e$	Variance	$p(O=o \mid E=e)$	$p(E=e \mid C)$ Gamma prior	$p(E=e \mid C)$ Uniform prior

	$\left(\frac{b}{E_a}+E_a\right)$	NORM.DIST(LOG(O/E_a),0, SQRT(c^2/var),0)	GAMMA.DIST(E_a,5,3,0)	1/(Z-1)
1	1001	1.43837395361617E-53	0.000122862022	0.010101010101
2	502	2.53209549679704E-19	0.001408551767	0.010101010101
3	336.3333333	1.63742241322308E-10	0.005109436683	0.010101010101
…				
97	107.309278350515	1.048861888977	0.000000000138	0.010101010101
98	108.204081632653	1.050051066692	0.000000000103	0.010101010101
99	109.101010101010	1.051044763657	0.000000000077	0.010101010101

The drop-in penalty for a gamma prior is the log of the sum of products of column $p(O=o \mid E=e)$ and $p(E=e \mid C)$ for the gamma prior multiplied by f_{di}. The drop-in penalty for a uniform prior is the log of the sum of products of column $p(O=o \mid E=e)$ and $p(E=e \mid C)$ multiplied by f_{di} for the uniform prior. The following values were obtained from STRmix™ and Excel:

Model	STRmix™ drop-in penalty	Excel
Gamma prior	-4.677289093	-4.677289093
Uniform prior	-4.164180358	-4.164180358

There are no differences in the 10th significant figure. These tests satisfy the acceptance criteria.

4.11.3. Mx prior penalties

Acceptance criterion: Mx prior penalties are output to 15 sig fig. Precision is expected to 14 sig fig.

The extended output for a three-person mixture was analysed to check the mixture proportion (Mx) priors. The penalty is calculated for each contributor by the following formula:

=LOG(NORM.DIST(Mx,Mx mean,SQRT(Mx var),0))

Where Mx is taken from the extended output and Mx mean and variance from the interpretation set up. The calculated results for five iterations are provided in the following table:

Mx C1	Mx C2	Mx C3

0.6876289227405140	0.2460821338953720	0.0662889433641133
0.6909319420431470	0.2445278560075910	0.0645402019492624
0.6943718796422450	0.2472549174583470	0.0583732028994076
0.6912375207929320	0.2503470588350160	0.0584154203720522
0.6812439308910470	0.2548074087091730	0.0639486603997798
Mx penalty C1 Excel	Mx penalty C2 Excel	Mx penalty C1 Excel
1.07202946731256	-0.03627948278756	-2.86941392257372
1.08777555433683	-0.11189146070745	-2.97408047062838
1.09901666568106	0.01935137081752	-3.35404553702406
1.08898710575129	0.16016191760807	-3.35138691132720
1.02783642222174	0.34829712367788	-3.00979353991426

Where means of the informed prior = 0.7, 0.3, and 0.2 and the variance = 0.0009765625, 0.00048828125, and 0.0009765625for contributor 1, 2 and 3, respectively.

Mx penalty STRmix™	Mx penalty sum Excel
-1.833663938	-1.833663938
-1.998196376	-1.998196376
-2.235677500	-2.235677500
-2.102237887	-2.102237887
-1.633659994	-1.633659994

There are no differences in the 10th significant figure. These tests satisfy the acceptance criteria.

4.12. Replicating Genotype Weights

Acceptance criterion: Precision is expected to at least 10 significant figures.

The weight for a given genotype set at a locus is calculated as the number of times the genotype set was accepted during the post burn-in MCMC across all chains, divided by the total number of post burn-in iterations. For each post burn-in chain, the genotype set producing the largest log(likelihood) is selected as the starting genotype set. The log(likelihood) for each genotype set is calculated using the mass parameters that were accepted in the final burn-in accept of the corresponding chain. The starting genotype set at each locus is written out to the post burn-in extended output file for each chain (labelled as iteration zero).

A low-level single-source GlobalFiler™ profile and a two-person unresolvable GlobalFiler™ mixture were each deconvoluted in STRmix™ V2.8. A further interpretation of the single-source profile with the GR auto extend function enabled was also performed (500 additional accepts with a GR threshold of 1.2). The profiles were interpreted under the following conditions: 2 chains, 1000 burn-in accepts per chain, 1000 post burn-in accepts per chain (single-source profile) or 500 post burn-in accepts per chain (mixture), extended output enabled. For each interpretation, the weights were replicated across all loci within MS Excel. These were then compared with the STRmix™ result.

By way of example, the genotype weights at two loci within the mixed profile are reproduced in the table below. Values are presented to 10 significant figures.

Locus	Contributor 1	Contributor 2	MS Excel	STRmix™
D3S1358	15,16	15,15	0.1769994632	0.1769994632
	16,16	15,15	0.004159957058	0.004159957058
	15,16	15,16	0.04039184111	0.04039184111
	15,15	16,16	0.7784487385	0.7784487385
vWA	18,18	15,16	0.09178743961	0.09178743961
	16,18	15,18	0.09205582393	0.09205582393
	15,18	16,18	0.6155394524	0.6155394524
	15,16	18,18	0.2006172839	0.2006172839

The weights calculated in MS Excel match the STRmix™ result to 10 significant figures and therefore pass the acceptance criterion. While not reproduced here, all other weights calculated in MS Excel also passed the acceptance criterion.

4.13. Likelihood ratios

4.13.1. Posterior mean allele frequencies

Acceptance criterion: Allele frequencies are output to 15 sig fig in the *LR* extended results text file. Precision is checked to 10 sig fig.

The posterior mean frequencies of the alleles including Q were calculated using the following formula:

$$f_i^{'} = \frac{x_i + \frac{1}{k+1}}{N+1}$$

Where x_i are the observed allele counts from the database, k the number of possible alleles and N the size of the population database.

The posterior mean of allele Q is calculated using the following formula:

$$f_Q = 1 - \sum_{i=1}^{n} f_i^{'}$$

Posterior mean frequencies were calculated for each *LR* check described below. An example of a locus is given in the table below for the FBI Caucasian allele frequency database [1].

Locus	Allele	STRmix™	Excel
D3S1358	15	0.288999783	0.288999783
	16	0.305706226	0.305706226
	18	0.055109568	0.055109568
	Q	0.350184421	0.350184421

The results are the same at 10 significant figures, hence these tests pass the acceptance criterion.

4.13.2. *LR* point estimates (sub-sub-source *LR*)

Acceptance criterion: Per locus *LR*s are output to 15 sig fig in the STRmix™ extended results text file. Precision is checked to 10 sig fig.

The per locus likelihoods $\Pr(E \mid H_p)$, $\Pr(E \mid H_d)$, and their ratio (*LR*) are output in the STRmix™ interpretation log. The product of these (profile *LR*) are output in the results text files. The individual likelihoods and per locus sub-sub-source *LR* were calculated in Excel for two loci from the weights and posterior mean allele frequencies.

In the examples below, the population database used for the STRmix™ analysis was the FBI extended African American subpopulation[2] and $\theta = 0.03$ within the Balding and Nichols formula, $\frac{x\theta + (1-\theta)\Pr(A)}{(1+(n-1)\theta)}$. The first example presented here is for a two person mixture (C1 and C2) at locus D3S1358 where the POI=15,15. The *LR* considering the POI is in contributor position 2 is given and there is some dropout (-1 allele) considered.

C1		C2		Weight	$\Pr(E \mid H_p)$	$\Pr(E \mid H_d)$
16	18	15	15	0.058262871	0.001691818	0.000174662
16	18	-1	15	0.039974372	0	0.000219835
15	18	15	16	0.003480337	0	2.08669E-05
16	18	15	16	0.303890141	0	0.001606547
18	18	15	16	0.045187905	0	3.0528E-05
-1	18	15	16	0.001760387	0	9.68108E-06
15	18	16	16	0.080841834	0	0.000213689
15	18	-1	16	0.004994618	0	2.74674E-05
15	16	15	18	0.00318752	0	1.91112E-05
16	16	15	18	0.124562866	0	0.000329257
-1	16	15	18	0.002325105	0	1.27867E-05
16	18	15	18	0.162268605	0	0.000219251
15	15	16	18	0.001415978	0	4.24485E-06
-1	15	16	18	0.001119676	0	6.15755E-06
15	16	16	18	0.065844037	0	0.000348091
15	18	16	18	0.034702275	0	4.68883E-05
15	16	18	18	0.060002343	0	4.05363E-05
15	16	-1	18	0.006179131	0	3.39815E-05

The locus likelihoods and *LR* is given in the table below

	$\Pr(E \mid H_p)$	$\Pr(E \mid H_d)$	*LR*
STRmix	0.001691818	0.003363583	0.502980829
Excel	0.001691818	0.003363583	0.502980829

[2] Moretti TR, Moreno LI, Smerick JB, Pignone ML, Hizon R, Buckleton JS, et al. Population data on the expanded CODIS core STR loci for eleven populations of significance for forensic DNA analyses in the United States. Forensic Science International: Genetics. 2016;25:175-81.

The results are the same at 10 significant places. Hence these tests pass the acceptance criterion.

The second example is for the same two person mixture (C1 and C2) at locus vWA where the POI=15, 19 and is considered to have dropped out. The *LR* considering the POI is in contributor position 2 is given and there is some drop-in considered.

#	C1		C2		Drop-in	weight
1	14	14	14	14	18	1.20E-04
2	14	18	14	14		0.250961415
3	18	18	14	14		0.020935006
4	-1	18	14	14		0.015950846
5	14	14	-1	14	18	8.51E-05
6	14	18	-1	14		0.040353639
7	18	18	-1	14		0.002247717
8	-1	18	-1	14		0.00119776
9	14	14	14	18		0.339486901
1	-1	14	14	18		0.018004049
1	14	18	14	18		0.035300458
1	18	18	14	18		0.002099217
1	-1	18	14	18		0.002095732
1	-1	-1	14	18		0.000957232
1	14	14	18	18		0.144605478
1	-1	14	18	18		0.004290463
1	14	18	18	18		0.004691343
1	14	14	-1	18		0.100031792
1	-1	14	-1	18		0.002014858
2	14	18	-1	18		0.007146821

2	14	18	-1	-1		0.0074243

#	$\Pr(E \mid H_p)$	$\Pr(E \mid H_d)$	
1	0	1.4957135080E-09	
2	0	3.9165455767E-05	
3	0	2.1489300310E-06	
4	0	1.6530951627E-05	
5	0	1.1364263951E-08	
6	0	8.3642467861E-05	
7	0	4.0315089877E-06	
8	0	2.2473616623E-05	
9	0	5.2980890356E-05	
10	0	3.7317653290E-05	
11	0	1.4494042129E-05	
12	0	8.8186203697E-07	
13	0	7.5178139064E-06	
14	0	8.9802897622E-06	
15	0	1.4843418591E-05	
16	0	7.6953803692E-06	
17	0	1.9707903178E-06	
18	0	1.0366978187E-04	
19	0	3.7804861490E-05	
20	0	2.5637095926E-05	
21	1.1899817106E-04	6.9651205883E-05	*LR*
Excel	1.1899817106E-04	5.5145087680E-04	0.215791063
STRmix™	1.1899817106E-04	5.5145087680E-04	0.215791063

The results are the same at 10 sig figs. Hence these tests pass the acceptance criterion.

4.13.3. Sub-source *LR*

Acceptance criterion: Sub-source *LR*s are output to 15 sig fig in the STRmix™ results text file. Precision is checked to 10 sig fig.

The sub-source *LR* was calculated in Excel for a two person mixture and is given in the table below.

	Contributor order 1		Contributor order 2	
Locus	$\Pr(E \mid H_p)$	$\Pr(E \mid H_d)$	$\Pr(E \mid H_p)$	$\Pr(E \mid H_d)$
D3S1358	4.1116701786E-05	3.3635827753E-03	1.6918176522E-03	3.3635827753E-03
vWA	1.5342707190E-05	5.5145087680E-04	1.1899817106E-04	5.5145087680E-04
D16S539	7.6236604313E-03	1.4271711960E-02	9.4882877236E-03	1.4271711960E-02
CSF1PO	1.6392640801E-03	1.3843815552E-02	4.2303924846E-03	1.3843815552E-02
TPOX	7.7840377093E-02	7.2785251599E-02	7.7630925479E-02	7.2785251599E-02
D8S1179	2.8835870833E-03	5.7313007386E-03	1.1230327970E-02	5.7313007386E-03
D21S11	2.2395293463E-03	1.7591151013E-04	1.8711779565E-03	1.7591151013E-04
D18S51	2.0005874569E-03	5.6163742982E-03	3.0505975409E-03	5.6163742982E-03
D2S441	1.0751094210E-04	5.8661418394E-04	1.3652411922E-03	5.8661418394E-04
D19S433	9.1990833196E-03	3.3940941804E-03	8.1042663523E-03	3.3940941804E-03
TH01	1.9677404674E-03	2.0352547356E-03	3.4351913935E-03	2.0352547356E-03
FGA	1.4833199495E-02	1.6362797472E-02	1.9119124086E-02	1.6362797472E-02
D22S1045	1.7397073671E-02	4.4192526701E-03	1.2890782596E-02	4.4192526701E-03
D5S818	2.2652392040E-02	1.7359800095E-02	2.7227980432E-02	1.7359800095E-02
D13S317	3.7244779103E-03	2.3356552256E-02	9.1999237730E-03	2.3356552256E-02

D7S820	1.1808079152E-01	5.4542882221E-02	9.4906868348E-02	5.4542882221E-02
SE33	3.0749291758E-03	7.4932565371E-03	3.9797124856E-03	7.4932565371E-03
D10S1248	2.9986329596E-02	1.7743060480E-02	3.1880978779E-02	1.7743060480E-02
D1S1656	4.9362431191E-05	2.7258033348E-04	1.6818828677E-04	2.7258033348E-04
D12S391	1.3144820197E-03	1.6038290718E-04	2.1681839774E-03	1.6038290718E-04
D2S1338	2.2759341533E-03	3.7878202855E-03	3.4932323926E-03	3.7878202855E-03
Product	5.4011862364E-55	2.1548744037E-50	1.4474743086E-48	2.1548744037E-50

The sub-source *LR* is replicated below

	$\Pr(E \mid H_p)$	$\Pr(E \mid H_d)$	*LR*
Excel	7.2373742436E-49	2.1548744037E-50	3.3586060660E+01
STRmix			3.3586060660E+01

The results are identical to 10 sig fig. Hence these tests satisfy the acceptance criteria.

4.13.4. *LR* point estimates with varying theta values

Acceptance criterion: Precision is expected to 10 significant figures.

A full, single-source GlobalFiler™ profile was interpreted in STRmix™ V2.8. An *LR* was then assigned for the known donor using the FBI extended Caucasian allele frequencies with θ = 0, θ = 0.01, and θ = 1.0b(0.8, 110.3). *LRs* were calculated under the same conditions in MS Excel and compared with the STRmix™ result. A summary of the locus *LR* results for STRmix™ and MS Excel are provided in Table 4.1 below. All results matched those of STRmix™ to 10 significant figures and therefore meet the acceptance criteria.

Table 4.1 Unrelated LRs assigned in STRmix™ V2.8 and using MS Excel. Values are displayed to 10 significant figures.

Locus	STRmix™: θ = 0 STRmix™: θ = 0.01 STRmix™ θ = 1.0b(0.8, 110.3)	MS Excel: θ = 0 MS Excel: θ = 0.01 MS Excel: θ = 1.0b(0.8, 110.3)
D3S1358	13.17678247 12.50011024 12.68206194	13.17678247 12.50011024 12.68206194
vWA	8881.032754 235.2591507 324.0749731	8881.032754 235.2591507 324.0749731
D16S539	92.49465682 63.03538569 69.57941153	92.49465682 63.03538569 69.57941153
CSF1PO	5.127380908 5.056742323 5.076180837	5.127380908 5.056742323 5.076180837
TPOX	3.594355093 3.567991610 3.575205421	3.594355093 3.567991610 3.575205421
D8S1179	14.08861398 13.12725824 13.38102231	14.08861398 13.12725824 13.38102231
D21S11	11.92934759 11.37956620 11.52792910	11.92934759 11.37956620 11.52792910

D18S51	81596.19289 1140.307952 1661.130752	81596.19289 1140.307952 1661.130752
D2S441	38.47709640 32.35432331 33.85951335	38.47709640 32.35432331 33.85951335
D19S433	10.01102340 9.553223002 9.676109745	10.01102340 9.553223002 9.676109745
TH01	17.42299764 16.31012062 16.60747312	17.42299764 16.31012062 16.60747312
FGA	18.56194631 17.29678327 17.63375736	18.56194631 17.29678327 17.63375736
D22S1045	48.57263764 36.95056179 39.70542178	48.57263764 36.95056179 39.70542178
D5S818	3.494594125 3.484138310 3.487043516	3.494594125 3.484138310 3.487043516
D13S317	15.93786153 14.75448152 15.06581635	15.93786153 14.75448152 15.06581635
D7S820	15.11246939 14.24914738 14.48065258	15.11246939 14.24914738 14.48065258
SE33	408.4053112 253.1744378 285.5431956	408.4053112 253.1744378 285.5431956
D10S1248	13.28868260 11.71568772 12.12150512	13.28868260 11.71568772 12.12150512
D1S1656	29.80028375 26.91385029 27.66920896	29.80028375 26.91385029 27.66920896
D12S391	242.0812645 153.7952357	242.0812645 153.7952357

	171.8786379	171.8786379
D2S1338	21.39871316 19.72850549 20.17018096	21.39871316 19.72850549 20.17018096
LR total	1.167305498E+34 3.256835914E+29 1.279597303E+30	1.167305498E+34 3.256835914E+29 1.279597303E+30

4.13.5. Relatives *LR*s

Acceptance criterion: Precision is expected to 10 significant figures.

A single-source GlobalFiler™ profile was interpreted within STRmix™ V2.8. The profile is low level and includes instances of dropout and drop-in within the accepted genotypes. An *LR* was then assigned for one of the known donors. This was done using the FBI extended Caucasian population with $\theta = 0$, $\theta = 0.01$, and $\theta = 1.0$b(0.8, 110.3). Relatives *LR*s were replicated in MS Excel across all loci and are summarised in the tables below. All results matched those of STRmix™ to 10 significant figures and therefore meet the acceptance criteria.

Table 4.2: Full sibling *LR*s assigned in STRmix™ V2.8 and using MS Excel. Values are displayed to 10 significant figures.

Locus	STRmix™: $\theta = 0$ STRmix™: $\theta = 0.01$ STRmix™ $\theta = 1.0$b(0.8, 110.3)	MS Excel: $\theta = 0$ MS Excel: $\theta = 0$ MS Excel: $\theta = 1.0$b(0.8, 110.3)
D3S1358	2.596913938 2.571889152 2.578802879	2.596913938 2.571889152 2.578802879
vWA	2.896550596 2.860520683 2.870462329	2.896550596 2.860520683 2.870462329
D16S539	1.940914304 1.909367902 1.918056275	1.940914304 1.909367902 1.918056275
CSF1PO	Not calculated – no peak information at locus	Not calculated – no peak information at locus
TPOX	2.104454157 2.064549747	2.104454157

	2.075519013	2.064549747 2.075519013
D8S1179	2.387221962 2.368806432 2.373896015	2.387221962 2.368806432 2.373896015
D21S11	2.838514707 2.804988375 2.814243925	2.838514707 2.804988375 2.814243925
D18S51	Not calculated – no peak information at locus	Not calculated – no peak information at locus
D2S441	Not calculated – no peak information at locus	Not calculated – no peak information at locus
D19S433	Not calculated – no peak information at locus	Not calculated – no peak information at locus
TH01	2.765132872 2.691362316 2.711588311	2.765132872 2.691362316 2.711588311
FGA	2.480158558 2.422472198 2.438313328	2.480158558 2.422472198 2.438313328
D22S1045	2.482992765 2.460643596 2.466811549	2.482992765 2.460643596 2.466811549
D5S818	1.956183218 1.950804272 1.952296688	1.956183218 1.950804272 1.952296688
D13S317	2.879051769 2.841634892 2.851940996	2.879051769 2.841634892 2.851940996
D7S820	1.554287263 1.540884576 1.544582577	1.554287263 1.540884576 1.544582577
SE33	1.893997374 1.849927030 1.862006491	1.893997374 1.849927030 1.862006491

D10S1248	2.983758910 2.940525819 2.952414548	2.983758910 2.940525819 2.952414548
D1S1656	3.663951950 3.598226926 3.616347578	3.663951950 3.598226926 3.616347578
D12S391	2.564683299 2.502443326 2.519527643	2.564683299 2.502443326 2.519527643
D2S1338	3.362307791 3.308211964 3.323124432	3.362307791 3.308211964 3.323124432
LR total	5.541071255E+06 4.278883243E+06 4.596451005E+06	5.541071255E+06 4.278883243E+06 4.596451005E+06

Table 4.3: Parent/child *LR*s assigned in STRmix™ V2.8 and using MS Excel. Values are displayed to 10 significant figures.

Locus	STRmix™: $\theta = 0$ STRmix™: $\theta = 0.01$ STRmix™ θ = 1.0b(0.8, 110.3)	MS Excel: $\theta = 0$ MS Excel: $\theta = 0$ MS Excel: θ = 1.0b(0.8, 110.3)
D3S1358	4.518828452 4.408875955 4.438894133	4.518828452 4.408875955 4.438894133
vWA	6.034768212 5.802950773 5.865572672	6.034768212 5.802950773 5.865572672
D16S539	2.278360155 2.222109536 2.237462456	2.278360155 2.222109536 2.237462456
CSF1PO	Not calculated – no peak information at locus	Not calculated – no peak information at locus
TPOX	2.453663358 2.385009864 2.403701986	2.453663358 2.385009864 2.403701986
D8S1179	3.712500000 3.650602410	3.712500000 3.650602410

	3.667597334	3.667597334
D21S11	5.738419619 5.533598689 5.589042071	5.738419619 5.533598689 5.589042071
D18S51	Not calculated – no peak information at locus	Not calculated – no peak information at locus
D2S441	Not calculated – no peak information at locus	Not calculated – no peak information at locus
D19S433	Not calculated – no peak information at locus	Not calculated – no peak information at locus
TH01	4.923346113 4.568425069 4.661827435	4.923346113 4.568425069 4.661827435
FGA	3.665701873 3.481905403 3.531111826	3.665701873 3.481905403 3.531111826
D22S1045	3.946940985 3.872296114 3.892757118	3.946940985 3.872296114 3.892757118
D5S818	2.636289666 2.619785287 2.624351523	2.636289666 2.619785287 2.624351523
D13S317	5.576592083 5.385868760 5.437554273	5.576592083 5.385868760 5.437554273
D7S820	1.589527625 1.576308078 1.579960028	1.589527625 1.576308078 1.579960028
SE33	1.882305782 1.829437612 1.843830749	1.882305782 1.829437612 1.843830749
D10S1248	5.903790087 5.684092046 5.743494141	5.903790087 5.684092046 5.743494141
D1S1656	21.81328546 18.23592350 19.10680733	21.81328546 18.23592350 19.10680733
D12S391	3.981673721	3.981673721

	3.759690169 3.818862607	3.759690169 3.818862607
D2S1338	11.07056622 10.15827327 10.39638295	11.07056622 10.15827327 10.39638295
LR total	5.776549040E+10 2.717675641E+10 3.331493416E+10	5.776549040E+10 2.717675641E+10 3.331493416E+10

Table 4.4: Half sibling, uncle/aunt, niece/nephew, grandparent/grandchild *LR*s assigned in STRmix™ V2.8 and using MS Excel. Values are displayed to 10 significant figures.

Locus	STRmix™: $\theta = 0$ STRmix™: $\theta = 0.01$ STRmix™ $\theta = 1.0$b(0.8, 110.3)	MS Excel: $\theta = 0$ MS Excel: $\theta = 0$ MS Excel: $\theta = 1.0$b(0.8, 110.3)
D3S1358	6.269720539 6.088988110 6.138246028	6.269720539 6.088988110 6.138246028
vWA	9.291673806 8.848747106 8.967909879	9.291673806 8.848747106 8.967909879
D16S539	3.215577675 3.101201581 3.132293968	3.215577675 3.101201581 3.132293968
CSF1PO	Not calculated – no peak information at locus	Not calculated – no peak information at locus
TPOX	4.055342999 3.859640516 3.912333668	4.055342999 3.859640516 3.912333668
D8S1179	4.923349203 4.822917746 4.850416728	4.923349203 4.822917746 4.850416728
D21S11	8.513402857 8.152728647 8.250165983	8.513402857 8.152728647 8.250165983
D18S51	Not calculated – no peak information at locus	Not calculated – no peak information at locus

D2S441	Not calculated – no peak information at locus	Not calculated – no peak information at locus
D19S433	Not calculated – no peak information at locus	Not calculated – no peak information at locus
TH01	8.214523873 7.481044559 7.673359481	8.214523873 7.481044559 7.673359481
FGA	5.882334625 5.494404834 5.597829492	5.882334625 5.494404834 5.597829492
D22S1045	5.592865931 5.444454619 5.484876150	5.592865931 5.444454619 5.484876150
D5S818	3.005361933 2.990762953 2.994808192	3.005361933 2.990762953 2.994808192
D13S317	9.522660496 9.010254767 9.147149473	9.522660496 9.010254767 9.147149473
D7S820	2.117726258 2.080044803 2.090368655	2.117726258 2.080044803 2.090368655
SE33	3.444285025 3.248697315 3.301032215	3.444285025 3.248697315 3.301032215
D10S1248	11.68168555 10.84767327 11.06725698	11.68168555 10.84767327 11.06725698
D1S1656	43.59791601 35.19775305 37.20860795	43.59791601 35.19775305 37.20860795
D12S391	6.483064205 6.015789631 6.139845423	6.483064205 6.015789631 6.139845423
D2S1338	20.13501218 18.07167294 18.60479038	20.13501218 18.07167294 18.60479038
LR total	1.194226052E+14 4.289408634E+13	1.194226052E+14 4.289408634E+13

	5.655106310E+13	5.655106310E+13

Table 4.5: First cousin *LR*s assigned in STRmix™ V2.8 and using MS Excel. Values are displayed to 10 significant figures.

Locus	STRmix™: $\theta = 0$ STRmix™: $\theta = 0.01$ STRmix™ θ = 1.0b(0.8, 110.3)	MS Excel: $\theta = 0$ MS Excel: $\theta = 0$ MS Excel: θ = 1.0b(0.8, 110.3)
D3S1358	7.776233287 7.522260633 7.591353567	7.776233287 7.522260633 7.591353567
vWA	12.72562049 11.99724596 12.19216519	12.72562049 11.99724596 12.19216519
D16S539	4.048204670 3.865898496 3.915199617	4.048204670 3.865898496 3.915199617
CSF1PO	Not calculated – no peak information at locus	Not calculated – no peak information at locus
TPOX	6.020271549 5.586764106 5.701566149	6.020271549 5.586764106 5.701566149
D8S1179	5.882681162 5.745430780 5.782929250	5.882681162 5.745430780 5.782929250
D21S11	11.22828923 10.68029558 10.82793004	11.22828923 10.68029558 10.82793004
D18S51	Not calculated – no peak information at locus	Not calculated – no peak information at locus
D2S441	Not calculated – no peak information at locus	Not calculated – no peak information at locus
D19S433	Not calculated – no peak information at locus	Not calculated – no peak information at locus
TH01	12.33860268	12.33860268

	10.98178825 11.33434147	10.98178825 11.33434147
FGA	8.431613098 7.727648870 7.913737656	8.431613098 7.727648870 7.913737656
D22S1045	7.066217543 6.831192171 6.894858141	7.066217543 6.831192171 6.894858141
D5S818	3.231566509 3.218653492 3.222235546	3.231566509 3.218653492 3.222235546
D13S317	14.73654509 13.57930965 13.88264017	14.73654509 13.57930965 13.88264017
D7S820	2.539695560 2.475606392 2.493062593	2.539695560 2.475606392 2.493062593
SE33	5.886772438 5.307419065 5.457658615	5.886772438 5.307419065 5.457658615
D10S1248	22.87556404 19.87530052 20.62709734	22.87556404 19.87530052 20.62709734
D1S1656	87.08143793 65.79835551 70.69851597	87.08143793 65.79835551 70.69851597
D12S391	9.452087071 8.594451129 8.820147503	9.452087071 8.594451129 8.820147503
D2S1338	34.09212053 29.60167995 30.74014869	34.09212053 29.60167995 30.74014869
LR total	5.352123921E+16 1.324779743E+16 1.925501490E+16	5.352123921E+16 1.324779743E+16 1.925501490E+16

As a further test, a two-person unresolvable mixture was deconvoluted in STRmix™ V2.8. Peak heights in the evidence input file were reduced to one tenth of their original values to ensure that genotype sets considering allelic dropout and/or drop-in were accepted.

Relatives *LR*s were then assigned at two loci (D16S539 and TPOX) for one of the known donors using the FBI extended Caucasian allele frequencies with θ = 0.01. The locus *LR*s, sub-sub-source *LR*s, and sub-source *LR* were all replicated in MS Excel. The results are summarised in the tables below. All results matched those of STRmix™ to 10 significant figures and therefore meet the acceptance criteria.

Table 4.6: Full sibling *LR*s assigned in STRmix™ V2.8 and using MS Excel. Values are displayed to 10 significant figures.

LR	STRmix™: θ = 0.01	MS Excel: θ = 0.01
Contributor order 1 D16S539 *LR*	1.396278882	1.396278882
Contributor order 1 TPOX *LR*	0.7251356073	0.7251356073
Contributor order 1 Sub-sub-source *LR*	1.012491535	1.012491535
Contributor order 2 D16S539 *LR*	1.570563861	1.570563861
Contributor order 2 TPOX *LR*	0.7261279642	0.7261279642
Contributor order 2 Sub-sub-source *LR*	1.140430339	1.140430339
Sub-source *LR*	1.080390646	1.080390646

Table 4.7: Parent/child *LR*s assigned in STRmix™ V2.8 and using MS Excel. Values are displayed to 10 significant figures.

LR	STRmix™: θ = 0.01	MS Excel: θ = 0.01
Contributor order 1 D16S539 *LR*	1.528189960	1.528189960

Contributor order 1 TPOX *LR*	0.6770140500	0.6770140500
Contributor order 1 Sub-sub-source *LR*	1.034606074	1.034606074
Contributor order 2 D16S539 *LR*	1.802152200	1.802152200
Contributor order 2 TPOX *LR*	0.6782779461	0.6782779461
Contributor order 2 Sub-sub-source *LR*	1.222360093	1.222360093
Sub-source *LR*	1.132013345	1.132013345

Table 4.8: Half sibling, uncle/aunt, niece/nephew, grandparent/grandchild *LR*s assigned in STRmix™ V2.8 and using MS Excel. Values are displayed to 10 significant figures.

LR	STRmix™: θ = 0.01	MS Excel: θ = 0.01
Contributor order 1 D16S539 *LR*	1.652362299	1.652362299
Contributor order 1 TPOX *LR*	0.6580819044	0.6580819044
Contributor order 1 Sub-sub-source *LR*	1.087389729	1.087389729
Contributor order 2 D16S539 *LR*	2.016202374	2.016202374
Contributor order 2 TPOX *LR*	0.6591199490	0.6591199490
Contributor order 2 Sub-sub-source *LR*	1.328919206	1.328919206

Sub-source *LR*	1.210655309	1.210655309

Table 4.9: First cousin *LR*s assigned in STRmix™ V2.8 and using MS Excel. Values are displayed to 10 significant figures.

LR	STRmix™: θ = 0.01	MS Excel: θ = 0.01
Contributor order 1 D16S539 *LR*	1.722336094	1.722336094
Contributor order 1 TPOX *LR*	0.6490074229	0.6490074229
Contributor order 1 Sub-sub-source *LR*	1.117808910	1.117808910
Contributor order 2 D16S539 *LR*	2.143499160	2.143499160
Contributor order 2 TPOX *LR*	0.6499411377	0.6499411377
Contributor order 2 Sub-sub-source *LR*	1.393148283	1.393148283
Sub-source *LR*	1.256980023	1.256980023

4.13.6. Unified *LR*

Acceptance criterion: Precision is expected to 10 significant figures.

Formula for the prior proportions used when assigning a unified *LR* are given in the following table for a population of size *N*=1,000,000 with average number of children per family *n*=4.

Table 4.10: Relationship prior proportions calculated in STRmix™ and MS Excel. Values are displayed to 10 significant figures.

Relationship	Prior	STRmix™ priors	MS Excel priors
Siblings	*n*-1	3.000000000E-6	3.00000000000000E-06
Children	2n/3	2.666666667E-6	2.666666667E-06
Parents	4/3	1.333333333E-6	1.333333333E-06

Uncle/Aunt	4(n-1)/3	4.000000000E-6	4.000000000E-06
Niece/Nephew	2*n*(*n*-1)/3	8.000000000E-6	8.000000000E-06
Grandparent	4/3	1.333333333E-6	1.333333333E-06
Grandchild	$n^2/3$	5.333333333E-6	5.333333333E-06
Cousins	2n(n-1)	2.400000000E-5	2.400000000E-05
Unrelated	1-∑(relatives proportions)	0.9999503333	0.9999503333

The MS Excel priors matched those of STRmix™ to 10 significant figures. The results therefore pass the acceptance criterion.

Unified point estimate *LR*s were assigned for the single-source and mixed DNA profiles examined within Section 4.13.5. *LR*s were assigned using the FBI extended Caucasian allele frequencies with θ = 0.01. For the single-source profile, unified *LR*s were also assigned with θ = 0 and θ = 1.0b(0.8,110.3). Unified *LR*s were manually assigned in MS Excel and compared with the STRmix™ result. All results matched those of STRmix™ to 10 significant figures and therefore pass the acceptance criterion.

Table 4.11: Point estimate unified *LR*s assigned for the single-source and mixed profiles examined in Section 4.13.5. Values are displayed to 10 significant figures.

	STRmix™ unified *LR*	MS Excel unified *LR*
Single-source profile, *θ*=0	1.846787017E+12	1.846787017E+12
Single-source profile, *θ* = 0.01	1.425994166E+12	1.425994166E+12
Single-source profile, *θ* = 1.0b(0.8, 110.3)	1.531867755E+12	1.531867755E+12
Mixed profile (D16S539 and TPOX only) *θ* = 0.01	1.308981876	1.308981876

4.13.7. Stratified *LR*

Acceptance criterion: Stratified *LR* are output to 15 sig fig in the STRmix™ extended results text file. Precision is checked to 10 sig fig.

A stratified *LR* is calculated from the individual sub population *LR*s using the following formula:

$$= \frac{\sum_{pop} \Pr(O \mid pop, H_p) P_{pop}}{\sum_{pop} \Pr(O \mid pop, H_d) P_{pop}}$$

A stratified sub-source *LR* for the two person mixed DNA profile described above is summarised below (unrelated, related and unified propositions) where the African American and Caucasian allele frequencies were used in the proportions 0.3 and 0.7, respectively.

Relationship	STRmix	Excel
Unrelated	1.9407763285E+01	1.9407763285E+01
Siblings	1.5923454030E-01	1.5923454030E-01
Parent Child	1.1307943494E-01	1.1307943494E-01
Half sibs, etc	5.8994241320E-01	5.8994241320E-01
Cousins	2.2995226541E+00	2.2995226541E+00
Unified	1.9407686155E+01	1.9407686155E+01

These results are identical and hence these tests satisfy the acceptance criteria.

4.14. Highest posterior density

4.14.1. HPD for unrelated and related *LR*s

Acceptance criterion: HPD *LR* are output to 15 sig fig in the STRmix™ results text file. Precision is checked to 10 sig fig.

The sub-source 99% 1-sided lower HPD array is available in the extended output text files for unrelated, related and unified propositions. The HPD *LR* for the two person mixed DNA profile described above is summarised below (unrelated, related and unified propositions).

Relationship	STRmix	Excel
Unrelated	6.909201843E+18	6.909201843E+18
Siblings	6.281831087E+06	6.281831087E+06
Parent Child	7.487164996E+09	7.487164996E+09
Half sibs, etc	1.241465534E+13	1.241465534E+13
Cousins	2.339834912E+15	2.339834912E+15

Unified	3.136910754E+10	3.136910754E+10

There are no differences in the 10th significant figure. These tests satisfy the acceptance criteria.

4.14.2. Stratified HPD for unrelated and related *LR*s

Acceptance criterion: HPD *LR* are output to 15 sig fig in the STRmix™ results text file. Precision is checked to 10 sig fig.

The stratified sub-source 99% 1-sided lower HPD array is available in the extended output text files for unrelated, related and unified propositions. The HPD *LR* for the two person mixed DNA profile described above is summarised below (unrelated, related and unified propositions) where the African American and Caucasian allele frequencies were used in the proportions 0.7 and 0.3, respectively.

Relationship	STRmix	Excel
Unrelated	3.812452983E+19	3.812452983E+19
Siblings	1.329238672E+07	1.329238672E+07
Parent Child	2.493543181E+10	2.493543181E+10
Half sibs, etc	4.806028524E+13	4.806028524E+13
Cousins	1.021602848E+16	1.021602848E+16
Unified	3.946603851E+13	3.946603851E+13

There are no differences in the 10th significant figure. These tests satisfy the acceptance criteria.

4.14.2.1. HPD unit test – allele frequency resampling

Acceptance criterion: The STRmix 99 percentiles are within the range of the simulated 99 percentiles from Excel.

A single source, single locus (D16S539), profile was run that produced split weights for a previously unobserved allele and Q, and for the 12 allele and Q. Theta was set at 0.01. The minimum resampled count (MRC) was either turned off or set to 1. 100 sets of the 99% lower bound on 10,000 replications of the allele frequency resampling process (gamma.inv) were run in Excel using the 'whatif' function. The STRmix 99% lower bound was compared with these. The results were:

	Unobserved MRC Off	Unobserved MRC = 1	12 allele MRC = Off	12 allele MRC = 1
STRmix 99%	115.872684	115.872684	3.9869096	3.985682
Excel 99% summary				
Min	113.49947	113.200714	3.951802	3.958924
0.05%ile	116.843362	114.2515769	3.966958	3.977519
Average	115.585158	115.7030782	3.986512	4.002212
0.95%ile	114.2587297	117.0724748	4.004792	4.020871
Max	117.6452517	117.2563573	4.01282	4.029905

These tests satisfy the acceptance criteria.

4.14.2.2. HPD unit test – weight resampling

Acceptance criterion: The STRmix 99 percentiles are within the range of the simulated 99 percentiles from Excel.

A single source, single locus (D16S539), profile was run that produced split weights for a previously unobserved allele and Q, and for the 12 allele and Q. Theta was set at 0.01. The weight sampling was enabled and allele frequency resampling disabled. 100 sets of the 99% lower bound on 10,000 replications of the allele frequency resampling process (gamma.inv) were run in Excel using the 'whatif' function. The STRmix 99% lower bound was compared with these. The results were:

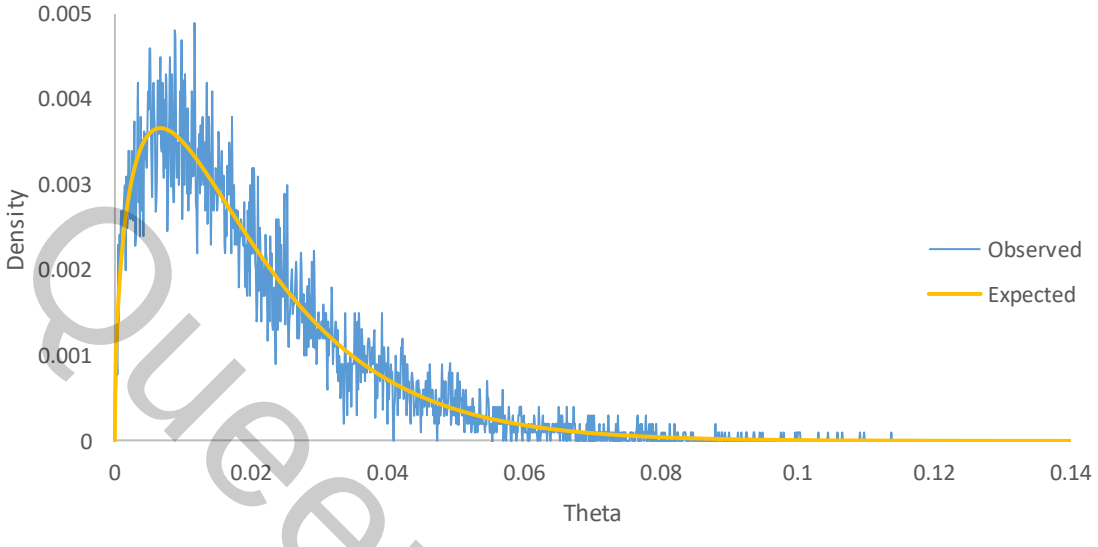
STRmix 99%	4.485382
Excel Min	4.472459
Excel 0.05%ile	4.475923
Excel Average	4.48632
Excel 0.95%ile	4.494006
Excel Max	4.499031

These tests satisfy the acceptance criteria.

4.14.3. Sampling theta values during HPD

The extended output for a *LR* calculation for a single source profile was enabled. The prior used for the theta beta distribution within the HPD was 1.0b(1.5,75). Theta values for ten thousand HPD iterations were recovered from the extended output and imported into Excel. The theta values sampled from the posterior distribution during the HPD are plotted in Figure 4.3 with the expected values sampled from a beta distribution.

Figure 4.3 Observed versus expected theta values drawn from β(1.5,75) during the HPD



Acceptance test	
Mean of beta	0.019608
Average of sample	0.019625
Count of sample	10000
sd of binomial estimate	0.001386
Accept/reject $diff \leq 1.96 \times \sqrt{\frac{p(1-p)}{N}}$	yes

These tests satisfy the acceptance criteria.

4.15. Gaussian walk

In a Gaussian walk the size of the step for any given variable is sampled randomly from $\sim N(\text{current parameter state}, sd^2)$. The sd values for the mass and variance parameters optimised during a STRmix™ interpretation are given in column 2 of

Table 4.12. Within this table, RWSD refers to the random walk standard deviation parameter, which is located within the STRmix™ run settings (and also within the default settings). A RWSD value of 0.005 is used by default; this has previously been found to provide the best performance in terms of precision and run-time.

Table 4.12 Summary of step size in the Gaussian walk

Variable	Standard deviation (*sd*)
t_n (template)	Maximum of: *RWSD* x (highest peak - Z) or *RWSD* x 10 x Z Where Z is the detection threshold
d_n (degradation)	*RWSD* x degradation max
A^l (locus amplification efficiency)	2 × *RWSD*
Allele and stutter variance constants (c^2 and k^2)	$RWSD \times 1.96\sqrt{\alpha\beta^2}$
LSAE variance	100 × *RWSD* × λ
R_y and B_k (replicate and kit efficiencies)	5 × *RWSD*

Single-source and mixed DNA profiles were interpreted within STRmix™ V2.8. The default run settings were changed to: 2 chains, 1000 burn-in accepts per chain, 1000 post burn-in accepts per chain, and extended output was enabled. The option to include rejected iterations within the extended output files was also enabled. Following interpretation, the distribution of step sizes taken by STRmix™ for each parameter was examined and compared with expectation. The test set included interpretations with multiple kits, replicates, and conditioned profiles. One interpretation was carried out using a reduced RWSD of 0.0005 to confirm that the step sizes adjusted accordingly. Stepping during burn-in was also examined.

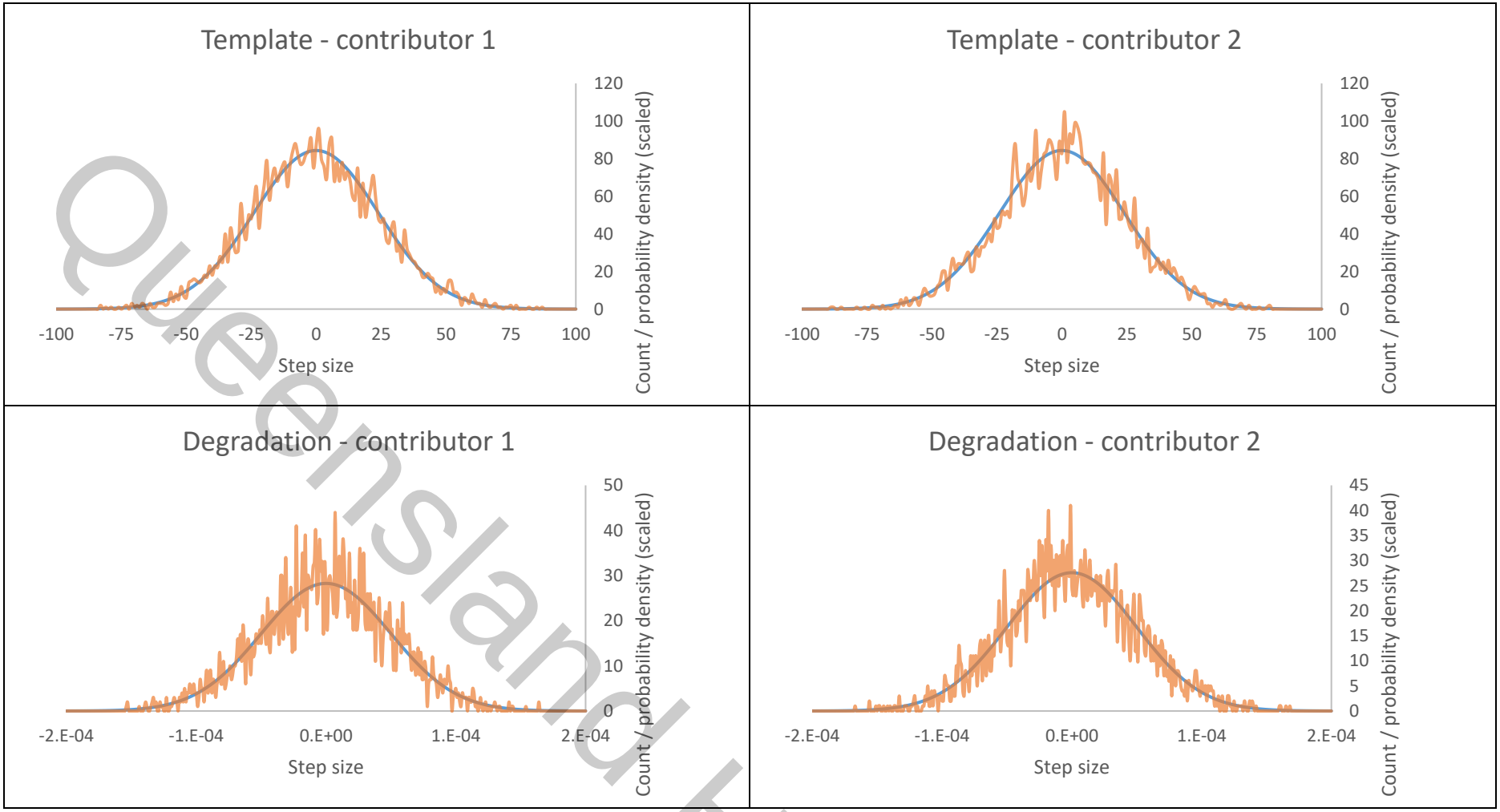
By way of example, plots of observed versus expected step size for all mass and variance parameters following interpretation of a two-person GlobalFiler™ mixture are provided in Figure 4.4. As can be seen in the plots below, the distributions of observed step size correspond with expectation.

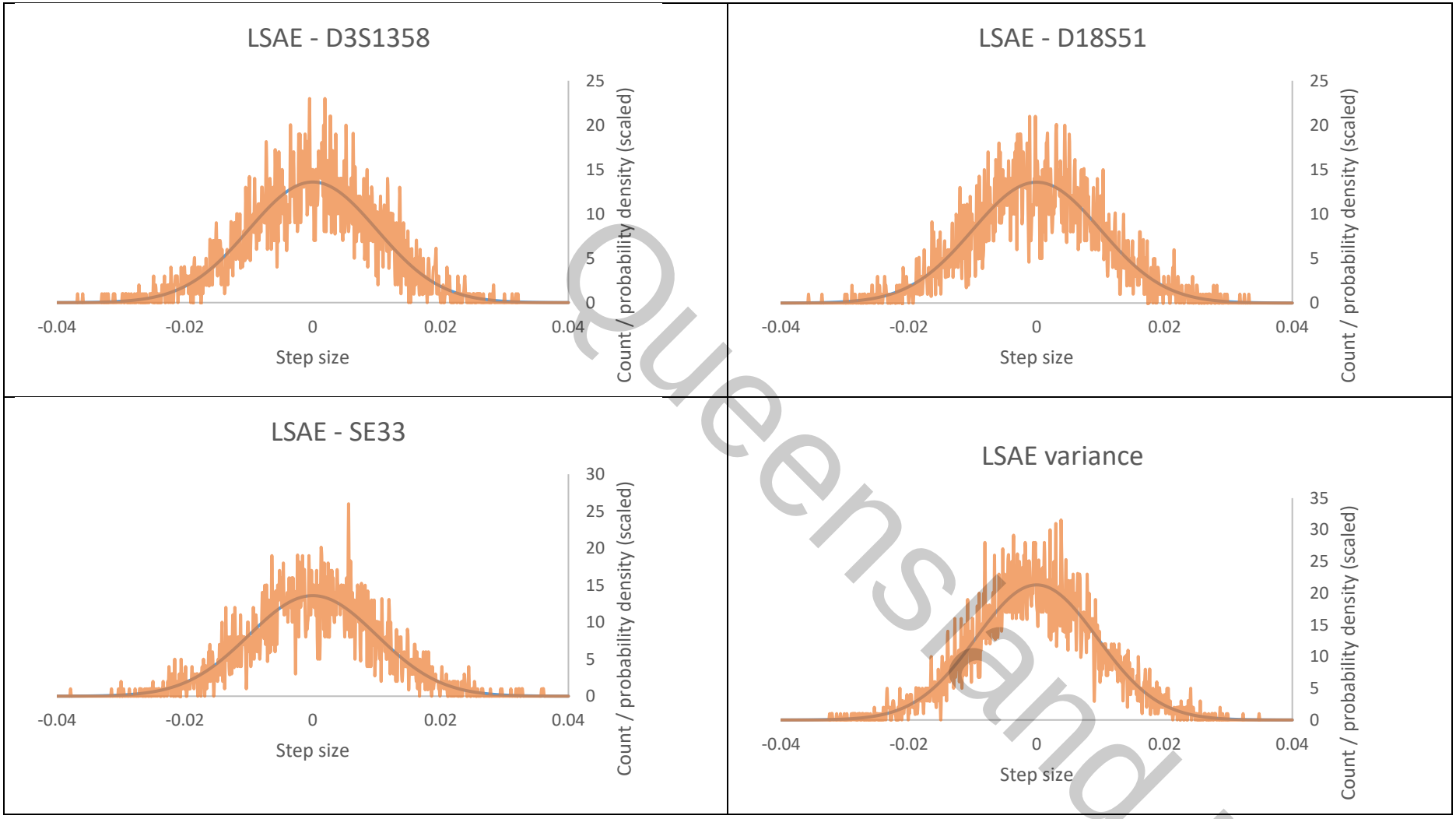
Within the extended output, the parameter values for replicate efficiency (R_y) and kit efficiency (B_k) are post-processed so that the sum of their logs is zero. The pre-processed values are not output within the extended output results. As such, stepping for these parameters was unable to be verified.

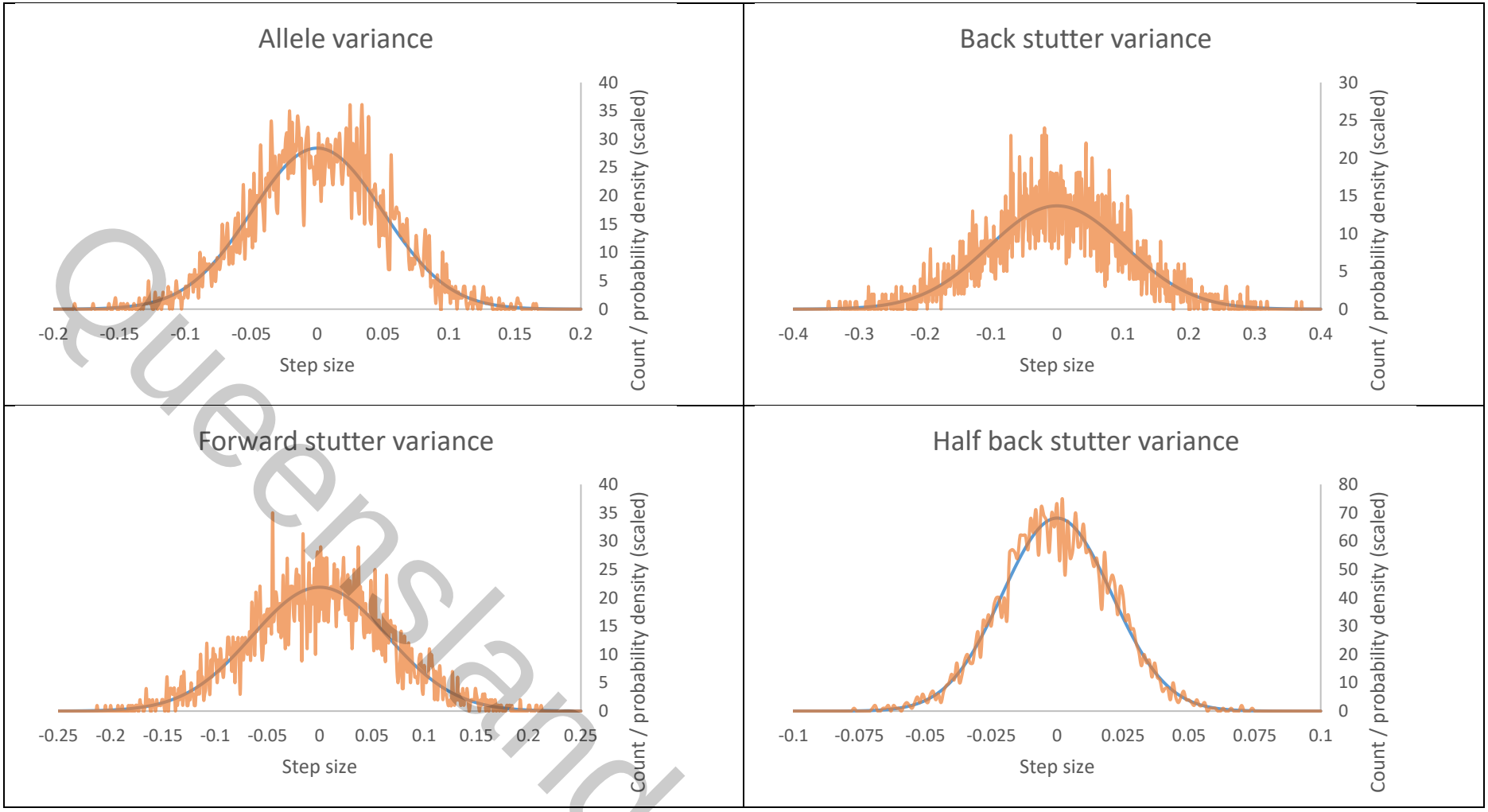
Acceptance criterion: Average step size $\leq 2.58 \times \frac{sd}{\sqrt{N}}$

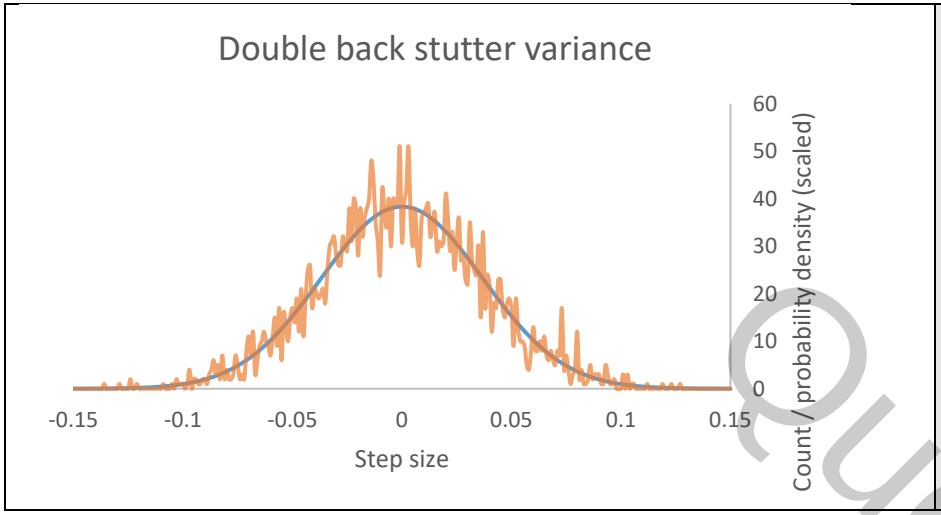
All profiles and parameters examined satisfied the above acceptance criterion.

Figure 4.4 Observed (orange) versus expected (blue) step size distributions following interpretation of a two-person GlobalFiler™ mixture within STRmix™.









	Template – Contributor 1	Template – Contributor 2	Degradation – Contributor 1	Degradation – Contributor 2	LSAE – D3S1358	LSAE – D18S51	LSAE – SE33
Average step size	-0.114505321	0.237879385	-2.58256E-07	-3.25231E-07	-9.23371E-05	0.000112096	0.000190517
Count	5074	5074	3543	3456	3406	3406	3406
Standard deviation	24.005	24.005	0.00005	0.00005	0.01	0.01	0.01
Accept/reject	ACCEPT	ACCEPT	ACCEPT	ACCEPT	ACCEPT	ACCEPT	ACCEPT
	LSAE variance	Allele variance	Back stutter variance	Forward stutter variance	Half back stutter variance	Double back stutter variance	
Average step size	-7.85968E-05	0.000809201	-0.002683601	-0.00067457	-0.000287045	-0.00027886	
Count	5074	3543	3543	3543	3543	3543	
Standard deviation	0.0095	0.049739186	0.103387231	0.064673449	0.02074859	0.036875127	
Accept/reject	ACCEPT	ACCEPT	ACCEPT	ACCEPT	ACCEPT	ACCEPT	

4.16. Post burn-in diagnostics

There are a number of post burn-in diagnostics included in the STRmix™ report. These may be used to assess the reliability of an interpretation and are discussed further in the STRmix™ User's Manual. A number of profiles were interpreted in STRmix™ V2.8 and all post burn-in diagnostics replicated 'by hand' in MS Excel. The profiles examined include single-source profiles, mixtures, mixtures interpreted using a conditioning profile, replicates, multi-kit interpretations (with/without degradation fixed), saturated profiles (single-source and mixtures), and profiles interpreted with the GR auto-extend function enabled. Representative results for each of the diagnostics are reproduced below. Values have been provided to 10 significant figures.

4.16.1. Gelman-Rubin convergence diagnostic

Acceptance criterion: Precision is expected to at least 10 significant figures.

A three-person PowerPlex® Fusion mixture was interpreted in STRmix™ V2.8 conditioning on one of the known donors. The default run settings were changed to: 2 chains, 1000 burn-in accepts per chain, 50 post burn-in accepts per chain, and extended output was enabled. The GR convergence diagnostic was calculated using the method described in the STRmix™ User's Manual.

	MS Excel	STRmix™
GR	1.552123832	1.552123832

The values calculated match to 10 significant figures and pass the acceptance criterion.

The same profile was reinterpreted with the GR auto continue function enabled (50 additional post burn-in accepts per chain with GR threshold = 1.2). The GR was replicated in MS Excel and compared with the STRmix™ result. The intial GR (prior to extension) as well as the final GR post-extension were both replicated.

	MS Excel	STRmix™
GR (initial)	1.552123832	1.552123832
GR (post-extension)	1.021655494	1.021655494

The values calculated match to 10 significant figures and pass the acceptance criterion.

GR diagnostics calculated for all other profiles in the test set matched the STRmix™ result to a minimum of 10 significant figures and therefore pass the acceptance criterion.

4.16.2. Effective sample size

Acceptance criterion: Precision is expected to at least 10 significant figures.

A three-person PowerPlex® Fusion mixture was interpreted in STRmix™ V2.8 conditioning on one of the known donors. The default run settings were changed to: 2 chains, 1000 burn-in accepts per chain, 50 post burn-in accepts per chain, and extended output was enabled. ESS was calculated in MS Excel using the method described in the *STRmix™ V2.8 User's Manual*. The results were then compared with the STRmix™ output and are summarised in the table below.

	MS Excel	STRmix™
Total ESS	44.75003171	44.75003171

The values calculated match to 10 significant figures and pass the acceptance criterion.

Total ESS calculated for all other profiles in the test set matched the STRmix™ result to a minimum of 10 significant figures and therefore pass the acceptance criterion.

4.16.3. Effective sample size thinning

Acceptance criterion: ESS thinning values are given to 2 decimal places and are therefore expected to be precise to 2 decimal places. Precision for ESS array files and Total ESS is expected to at least 10 significant figures.

A single-source multi-kit sample (GlobalFiler™ and Identifiler™ Plus) was interpreted in STRmix™ V2.8 with ESS thinning amount set to 500 for testing purposes (note: by default ESS thinning amount in the STRmix™ software is set to 100,000 and is not customisable).

Both input files were analysed at 50 rfu with the D12S391 locus ignored due to an unresolved back stutter peak in the GlobalFiler™ profile. The default run settings were changed to: 2 chains, 1000 burn-in accepts per chain, 50 post burn-in accepts per chain, and extended output was enabled.

When ESS thinning amount was set to 500, MCMC probability values were thinned to every 3.6th iteration within chain 1 and to every 2.22nd iteration within chain 2. These thinning values were replicated within MS Excel and compared to the STRmix output. The results are summarised in the table below.

	MS Excel	STRmix™
ESS thinning Chain1	3.5960	3.60
ESS thinning Chain2	2.2160	2.22

The thinned ESS value calculated for each chain matches that used by STRmix when rounded to 2 decimal places and passes the acceptance criterion.

The contents of the STRmix ESS array files after thinning were were replicated within MS Excel. While not reproduced here, all values calculated matched the STRmix™ result to 10 significant figures and therefore pass the acceptance criterion.

Total ESS was calculated in MS Excel using the method described in the *STRmix™ V2.8 User's Manual*. The results were then compared with the STRmix™ output and are summarised in the table below.

	MS Excel	STRmix™
Total ESS	12.12428779	12.12428779

The total ESS value calculated matches to 10 significant figures and passes the acceptance criterion.

4.16.4. Total iterations

Acceptance criterion: Total iterations is output as an integer in STRmix™. Precision is expected to zero decimal places.

A three-person PowerPlex® Fusion mixture was interpreted in STRmix™ V2.8 conditioning on one of the known donors. The default run settings were changed to: 2 chains, 1000 burn-in accepts per chain, 50 post burn-in accepts per chain, and extended output was enabled. The total number of iterations performed is equal to the sum of the post burn-in iterations across all chains. This was calculated in MS Excel and compared with the STRmix™ result as summarised in the table below.

	MS Excel	STRmix™
Chain 1	741	741
Chain 2	1192	1192
Total iterations	1933	1933

The values calculated match to 0 decimal places and pass the acceptance criterion.

Total iterations calculated for all other profiles in the test set matched the STRmix™ result to 0 decimal places and therefore pass the acceptance criterion.

4.16.5. Acceptance rate

Acceptance criterion: Acceptance rate is output to 2 decimal places in STRmix™. Precision is expected to 2 decimal places.

A three-person PowerPlex® Fusion mixture was interpreted in STRmix™ V2.8 conditioning on one of the known donors. The default run settings were changed to: 2 chains, 1000 burn-in accepts per chain, 50 post burn-in accepts per chain, and extended output was enabled. The acceptance rate is calculated as the total number of post burn-in accepts divided by the total iterations. The reciprocal is then taken and is presented within the STRmix™ report as an acceptance rate of 1 in *x*. The acceptance rate was calculated in MS Excel and compared with the STRmix™ result as summarised in the table below.

	MS Excel	STRmix™
Acceptance rate	1 in 19.33	1 in 19.33

The values calculated match to 2 decimal places and pass the acceptance criterion.

The acceptance rate calculated for all other profiles in the test set matched the STRmix™ result to 2 decimal places and therefore pass the acceptance criterion.

4.16.6. Average log(likelihood)

Acceptance criterion: Precision is expected to a minimum of 10 significant figures.

A three-person PowerPlex® Fusion mixture was interpreted in STRmix™ V2.8 conditioning on one of the known donors. The default run settings were changed to: 2 chains, 1000 burn-in accepts per chain, 50 post burn-in accepts per chain, and extended output was enabled. The extended output files were used to calculate the average log(likelihood) in MS Excel.

	MS Excel	STRmix™
Average log(likelihood)	56.08947047	56.08947047

The values calculated match to 10 significant figures and pass the acceptance criterion.

Average log(likelihood) values calculated for all other profiles in the test set matched the STRmix™ result to a minimum of 10 significant figures and therefore pass the acceptance criterion.

4.16.7. Template

Acceptance criterion: Template is output as an integer in STRmix™. Precision is expected to 0 decimal places.

In STRmix™ V2.8, per contributor template is calculated by first finding the average per contributor template within each chain, then taking the average of the per chain averages.

This is the value reported within the PDF report. Prior to STRmix™ V2.8 per contributor template was instead calculated by finding the mode within each chain, then taking the average of the per chain modes. STRmix™ V2.8 still performs these calculations and outputs the results in the Results.txt file. Prior to calculating the per chain modes, accepted template values are allocated into bins of width equal to the saturation threshold divided by 1000. These bins span from 0 rfu to the saturation threshold. If a multi kit interpretation is performed where the kits have different saturation threshold values, the maximum saturation threshold is used.

A three-person PowerPlex® Fusion mixture was interpreted in STRmix™ V2.8 conditioning on one of the known donors. The default run settings were changed to: 2 chains, 1000 burn-in accepts per chain, 50 post burn-in accepts per chain, and extended output was enabled. The extended output files were used to calculate per contributor template using both approaches. The results were then compared with the STRmix™ output as summarised in the tables below.

		MS Excel	STRmix™
Contributor 1	Chain 1 average	1057.280862	
	Chain 2 average	1107.735453	
	Average of averages	1083	1083
Contributor 2	Chain 1 average	14153.56092	
	Chain 2 average	14004.18052	
	Average of averages	14079	14079
Contributor 3	Chain 1 average	1320.491768	
	Chain 2 average	1147.189429	
	Average of averages	1234	1234

		MS Excel	STRmix™
Contributor 1	Chain 1 mode	1080	
	Chain 2 mode	1050	
	Average of modes	1065	1065
Contributor 2	Chain 1 mode	14250	
	Chain 2 mode	14100	

	Average of modes	14175	14175
Contributor 3	Chain 1 mode	1260	
	Chain 2 mode	1170	
	Average of modes	1215	1215

The values calculated match to 0 decimal places and pass the acceptance criterion.

Per contributor template values were calculated using both approaches for all other profiles in the test set. All calculated values matched the corresponding STRmix™ result to 0 decimal places and therefore pass the acceptance criteria.

As a further test, several saturated profiles were examined. Posterior template values calculated as the average of the per chain averages will remain unchanged for saturated contributors. However, where the mode of a contributor's template falls within the last bin, STRmix™ will instead use the average template amount for that contributor calculated within the affected chain. The results for one of the saturated profiles examined are provided below. The saturation threshold was reduced to 3000 rfu so that contributor 1 would be 'saturated' whilst contributors 2 and 3 would be on-scale.

		MS Excel	STRmix™
Contributor 1	Chain 1 average	10241.45692	
	Chain 2 average	10958.78434	
	Average of averages	10600	10600
Contributor 2	Chain 1 average	2147.191815	
	Chain 2 average	1382.160294	
	Average of averages	1765	1765
Contributor 3	Chain 1 average	106.8476310	
	Chain 2 average	127.8852240	
	Average of averages	117	117

		MS Excel	STRmix™
Contributor 1	Chain 1 mode	3000*	

		(10241.45692)	
	Chain 2 mode	3000* (10958.78434)	
	Average of modes	10600	10600
Contributor 2	Chain 1 mode	2208	
	Chain 2 mode	1410	
	Average of modes	1809	1809
Contributor 3	Chain 1 mode	102	
	Chain 2 mode	129	
	Average of modes	116	116

The values calculated match to 0 decimal places and pass the acceptance criterion. Note that both of the per chain modes for contributor 1 fall within the last bin (in this case, 3000 rfu). The relevant values have been indicated in the table above using an asterisk. STRmix™ will replace these values with the per chain averages. These values have been reproduced here in parentheses. The average of these values is 10,600 rfu and matches the STRmix™ result. These results demonstrate that STRmix™ is performing as expected.

4.16.8. Mixture proportions

Acceptance criterion: Precision is expected to a minimum of 10 significant figures.

Mixture proportions are calculated using the per contributor template values described above. The mixture proportions included in the STRmix™ PDF report use the per contributor template values calculated as the average across the per chain averages. An additional set of mixture proportions are included in the Results.txt file; these are calculated using template values found as the average across the per chain modes. For each approach, per contributor template values are summed within each chain. Per chain mixture proportions are then calculated for each contributor as a proportion of their template out of the total amount. For each contributor, the per chain mixture proportions are then averaged across all chains to produce the reported values.

A three-person PowerPlex® Fusion mixture was interpreted in STRmix™ V2.8 conditioning on one of the known donors. The default run settings were changed to: 2 chains, 1000 burn-in accepts per chain, 50 post burn-in accepts per chain, and extended output was enabled. The extended output files were used to calculate the mixture proportions using both approaches. The results were then compared with the STRmix™ output as summarised in the tables below.

Calculated using template - average of per chain averages:

	MS Excel	STRmix™
Contributor 1 *Mx*	6.604316751	6.604316751
Contributor 2 *Mx*	85.87394061	85.87394061
Contributor 3 *Mx*	7.521742636	7.521742636

Calculated using template – average of per chain modes:

	MS Excel	STRmix™
Contributor 1 *Mx*	6.471884640	6.471884640
Contributor 2 *Mx*	86.14608818	86.14608818
Contributor 3 *Mx*	7.382027178	7.382027178

The values calculated match to at least 10 significant figures and pass the acceptance criterion.

Mixture proprotions calculated for all other mixtures in the test set match the STRmix™ result to a minimum of 10 significant figures and therefore pass the acceptance criterion.

4.16.9. Degradation

Acceptance criterion: Precision is expected to a minimum of 10 significant figures.

A three-person PowerPlex® Fusion mixture was interpreted in STRmix™ V2.8 conditioning on one of the known donors. The default run settings were changed to: 2 chains, 1000 burn-in accepts per chain, 50 post burn-in accepts per chain, and extended output was enabled. The extended output files were used to calculate per contributor degradation (exponential parameter as well as the linear approximation). The results were then compared with the STRmix™ output as summarised in the table below.

		MS Excel	STRmix™
Contributor 1	Exponential parameter	0.001653107154	0.001653107154
	Linear approximation	1.519887062	1.519887062
Contributor 2	Exponential parameter	0.003172007947	0.003172007947

	Linear approximation	33.06194547	33.06194547
Contributor 2	Exponential parameter	8.979763817E-04	8.979763817E-4
	Linear approximation	1.025475000	1.025475000

The values calculated match to a minimum of 10 significant figures and pass the acceptance criterion.

Per contributor degradation parameters calculated for all other profiles in the test set matched the STRmix™ result to a minimum of 10 significant figures and therefore pass the acceptance criterion. Of note, a multi-kit interpretation where degradation was fixed between kits produced identical degradation parameters for each kit, as expected.

4.16.10. Allele, stutter, and LSAE variance constants

Acceptance criterion: Precision is expected to a minimum of 10 significant figures.

The allele, stutter, and LSAE variance diagnostics output in the STRmix™ report are each calculated as the average across all accepted values within the post burn-in MCMC. These parameters are kit specific and are described further in the STRmix™ User's Manual.

A three-person PowerPlex® Fusion mixture was interpreted in STRmix™ V2.8 conditioning on one of the known donors. The default run settings were changed to: 2 chains, 1000 burn-in accepts per chain, 50 post burn-in accepts per chain, and extended output was enabled. The extended output files were used to calculate the average posterior variance parameters. The results were then compared with the STRmix™ output as summarised in the table below.

	MS Excel	STRmix™
Allele variance	24.54215850	24.54215850
Back stutter variance	16.19567554	16.19567554
Forward stutter variance	17.45763414	17.45763414
LSAE variance	0.01702957894	0.01702957894

The values calculated match to a minimum of 10 significant figures and pass the acceptance criterion.

Posterior variance parameters calculated for all other profiles in the test set matched the STRmix™ result to a minimum of 10 significant figures and therefore pass the acceptance criterion.

4.16.11. Locus Specific Amplification Efficiencies

Acceptance criterion: LSAE is output as a percentage to 0 decimal places in STRmix™. Precision is expected to 0 decimal places.

The posterior locus specific amplification efficiencies output in the STRmix™ report are each calculated as are the average across all accepted values in the post burn-in MCMC. These parameters are kit and locus specific as described in User's Manual.

A three-person PowerPlex® Fusion mixture was interpreted in STRmix™ V2.8 conditioning on one of the known donors. The default run settings were changed to: 2 chains, 1000 burn-in accepts per chain, 50 post burn-in accepts per chain, and extended output was enabled. The extended output files were used to calculate the average posterior LSAE at three loci. The results were then compared with the STRmix™ output as summarised in the table below.

	MS Excel	STRmix™
Penta D LSAE	78%	78%
D7S820 LSAE	119%	119%
D22S1045 LSAE	88%	88%

The values calculated match to 0 decimal places and pass the acceptance criterion.

The posterior LSAE at three loci was replicated manually for all other profiles in the test set. All posterior LSAE values calculated matched the STRmix™ result to 0 decimal places and therefore pass the acceptance criterion.

4.16.12. Replicate efficiency

Acceptance criterion: Replicate efficiency is output as a percentage to 0 decimal places in STRmix™. Precision is expected to 0 decimal places.

The replicate efficiency value is the average of the accepted post burn-in values and scale all peaks up or down between replicates (where appropriate) as described in the STRmix™ User's Manual.

A single-source GlobalFiler™ profile with replicate evidence inputs was interpreted in STRmix™ V2.8. The default run settings were changed to: 2 chains, 1000 burn-in accepts per chain, 50 post burn-in accepts per chain, and extended output was enabled. The extended output files were used to calculate the posterior replicate efficiency for both PCR replicates. The results were then compared with the STRmix™ output as summarised in the table below.

	MS Excel	STRmix™
Replicate 1 efficiency	65%	65%
Replicate 2 efficiency	154%	154%

The values calculated match to 0 decimal places and pass the acceptance criterion.

4.16.13. Kit efficiency

Acceptance criterion: Kit efficiency is output as a percentage to 0 decimal places in STRmix™. Precision is expected to 0 decimal places.

The kit efficiency values are the average of the accepted post burn-in values and scale all peaks up or down between kits (where appropriate) as described in the STRmix™ User's Manual.

A multi kit interpretation of a single-source profile tested using the Identifiler™ Plus and GlobalFiler™ multiplex kits was undertaken in STRmix™ V2.8. The default run settings were changed to: 2 chains, 1000 burn-in accepts per chain, 50 post burn-in accepts per chain, and extended output was enabled. The extended output files were used to calculate the posterior replicate efficiency for both PCR replicates. The results were then compared with the STRmix™ output as summarised in the table below.

	MS Excel	STRmix™
Identifiler™ Plus kit efficiency	54%	54%
GlobalFiler™ kit efficiency	186%	186%

The values calculated match to 0 decimal places and pass the acceptance criterion.

4.17. Mix to Mix function

Acceptance criterion: Precision is expected to at least 10 significant figures.

Test 1

As an initial test of the Mix to Mix function, an unambiguous single-source GlobalFiler™ profile was compared with a two-person GlobalFiler™ major/minor mixture. The donor to the single-source profile is the known minor donor to the mixed profile. The STRmix™ Mix to Mix function was used to compare prior deconvolutions of the relevant profiles. Mix to Mix *LR*s were assigned using the FBI extended Caucasian allele frequencies with an *LR* threshold of zero. Extended output was also enabled.

Mix to Mix *LR*s (pairwise *LR*s and average *LR*) were then calculated within MS Excel across all loci. As part of the workup, the prior genotype probabilities and the posterior marginal

probabilities written to the STRmix™ extended output file were verified. The Mix to Mix *LR*s calculated manually and those assigned within STRmix™ are reproduced in the table below. Values have been provided to 10 significant figures.

Comparison	MS Excel	STRmix™
Single-source C1 vs Mix C1	0	0
Single-source C1 vs Mix C2	1.116575335E+29	1.116575335E29
Average *LR*	5.582876676E+28	5.582876676E28

The Mix to Mix *LR*s calculated in MS Excel match those assigned by STRmix™ to 10 significant figures and therefore pass the acceptance criterion.

As an additional check, the *LR* from Previous function of STRmix™ was used to assign an *LR* for the known minor donor to the mixed profile. Under these circumstances (i.e. Mix to Mix comparison of an unambiguous single-source profile with a mixed profile), the pairwise Mix to Mix *LR*s are expected to be equal to the sub-sub-source *LR*s for corresponding contributor orders. The average Mix to Mix *LR* is expected to be equal to the sub-source *LR*. The results obtained are summarised in the table below and indicate that the Mix to Mix function is performing as expected.

Comparison	STRmix™ - Mix to Mix	STRmix™ - *LR* from Previous
Single-source C1 vs Mix C1 / *LR* Prev. contributor order 1	0	0
Single-source C1 vs Mix C2 / *LR* Prev. contributor order 2	1.116575335E+29	1.116575335E29
Average Mix to Mix *LR* / *LR* Prev. sub-source *LR*	5.582876676E+28	5.582876676E+28

Test 2

A second test was then performed whereby a two-person GlobalFiler™ major/minor mixture was compared to the same profile with peak heights reduced by half. The STRmix™ Mix to Mix function was used to compare prior deconvolutions of the relevant profiles. Mix to Mix *LR*s were assigned at two loci (D3S1358 and FGA) using the FBI extended Caucasian allele frequencies with an *LR* threshold of zero. Extended output was also enabled. The loci examined include instances of allelic dropout and/or drop-in.

As before, Mix to Mix *LR*s (pairwise *LR*s and average *LR*) were calculated within MS Excel. This included verification of the prior genotype frequencies and posterior marginal probabilities written to the STRmix™ extended output file. The Mix to Mix *LR*s calculated

manually and those assigned within STRmix™ are reproduced in the table below. Values have been provided to 10 significant figures.

Comparison	MS Excel	STRmix™
Mix 1, C1 vs Mix 2, C1	2.399680107E+03	2.399680107E+03
Mix 1, C1 vs Mix 2, C2	1.036066244E-02	1.036066244E-02
Mix 1, C2 vs Mix 2, C1	8.383930514E-03	8.383930514E-03
Mix 1, C2 vs Mix 2, C2	7.589432532E+04	7.589432532E+04
Average *LR*	1.957350604E+04	1.957350604E+04

The Mix to Mix *LR*s calculated in MS Excel match those assigned by STRmix™ to 10 significant figures and therefore pass the acceptance criterion.

Test 3

A third test was performed whereby a two-person unresolvable GlobalFiler™ mixture was compared with a three-person GlobalFiler™ mixture with an approximate mixture ratio of 4:4:1. The two donors to the two-person mixture are the two major contributors to the three-person mixture. The STRmix™ Mix to Mix function was used to compare prior deconvolutions of the relevant profiles. Mix to Mix *LR*s were assigned using the FBI extended Caucasian allele frequencies with an *LR* threshold of zero. Extended output was also enabled.

Mix to Mix *LR*s (pairwise *LR*s and average *LR*) were calculated within MS Excel across all loci. The prior genotype frequencies and posterior marginal probabilities written to the STRmix™ extended output file were used in these calculations. These values had previously been verified in the preceding two tests as described above. The Mix to Mix *LR*s calculated manually and those assigned within STRmix™ are reproduced in the table below. Values have been provided to 10 significant figures.

Comparison	MS Excel	STRmix™
Mix 1, C1 vs Mix 2, C1	3.178939538E+19	3.178939538E+19
Mix 1, C1 vs Mix 2, C2	2.468184053E+19	2.468184053E+19
Mix 1, C1 vs Mix 2, C3	3.608969743E+08	3.608969743E+08
Mix 1, C2 vs Mix 2, C1	1.925156112E+17	1.925156112E+17
Mix 1, C2 vs Mix 2, C2	4.898637197E+17	4.898637197E+17
Mix 1, C2, vs Mix 2, C3	7.923178106E+07	7.923178106E+07
Average *LR*	9.525602539E+18	9.525602539E+18

The Mix to Mix *LR*s calculated in MS Excel match those assigned by STRmix™ to 10 significant figures and therefore pass the acceptance criterion.

Test 4

The performance of the Mix to Mix function when used to compare varNOC deconvolutions was then examined. The height reduced input file from Test 2 was deconvoluted assuming a range of 2-3 contributors. The STRmix™ Mix to Mix function was then used to compare this interpretation with that of the unedited profile. Mix to Mix *LR*s were assigned using the FBI extended Caucasian allele frequencies with an *LR* threshold of zero. Extended output was also enabled.

Mix to Mix *LR*s (pairwise *LR*s and average *LR*) were calculated within MS Excel across all loci. The average contributor stratified *LR* was also calculated. The prior genotype frequencies and posterior marginal probabilities written to the STRmix™ extended output file were used in these calculations. The Mix to Mix *LR*s calculated manually and those assigned within STRmix™ are reproduced in the table below. Values have been provided to 10 significant figures.

Comparison	**MS Excel**	**STRmix™**
Mix 1, C1 vs Mix 2, C1 Mix 2 NoC = 2	3.675025497E+40	3.675025497E+40
Mix 1, C1 vs Mix 2, C2 Mix 2 NoC = 2	2.562159406E-15	2.562159406E-15
Mix 1, C2 vs Mix 2, C1 Mix 2 NoC = 2	1.000722088E-22	1.000722088E-22
Mix 1, C2 vs Mix 2, C2 Mix 2 NoC = 2	2.986561507E+23	2.986561507E+23
Average *LR*, Mix 2 NoC = 2	9.187563742E+39	9.187563742E+39
Mix 1, C1 vs Mix 2, C1 Mix 2 NoC = 3	4.539045089E+40	4.539045089E+40
Mix 1, C1 vs Mix 2, C2 Mix 2 NoC = 3	1.168455542E+08	1.168455542E+08
Mix 1, C1 vs Mix 2, C3 Mix 2 NoC = 3	3.717317095E+04	3.717317095E+04
Mix 1, C2 vs Mix 2, C1 Mix 2 NoC = 3	4.313139308E-27	4.313139308E-27
Mix 1, C2 vs Mix 2, C2 Mix 2 NoC = 3	7.203840318E+19	7.203840318E+19
Mix 1, C2 vs Mix 2, C3 Mix 2 NoC = 3	1.145037269E+13	1.145037269E+13
Average *LR*, Mix 2 NoC = 3	7.565075148E+39	7.565075148E+39
Contributor stratified *LR*	7.565075148E+39	7.565075148E+39

The Mix to Mix *LR*s calculated in MS Excel match those assigned by STRmix™ to 10 significant figures and therefore pass the acceptance criterion. In this example, the

contributor stratified *LR* closely aligned with the average Mix to Mix *LR* where three contributors were assumed to mix 2 (displayed in the second last row of the table above). This was due to the fact that the varNOC weights used in the assignment of the contributor stratified *LR* (i.e. Z_n) heavily favoured an explanation of three contributors to this mixture (Z_n two contributors = 1.1731099947664E-20, Z_n three contributors = 1.0).

Test 5

As a final test, two varNOC deconvolutions were compared with each other using the STRmix™ Mix to Mix function. Specifically, a single-source Identifiler™ profile interpreted assuming a range of 1-2 contributors was compared with a three-person GlobalFiler™ mixture interpreted assuming a range of 2-3 contributors. To better demonstrate that the contributor stratified *LR* is being calculated correctly, the adjusted varNOC weights (Z_n) were modified to be equal to 0.5 for each value of *N* in both deconvolutions. Mix to Mix *LR*s were assigned using the FBI extended Caucasian allele frequencies with an *LR* threshold of zero. Extended output was also enabled.

Mix to Mix *LR*s (pairwise *LR*s and average *LR*) were calculated within MS Excel across all loci. The average contributor stratified *LR* was also calculated. The prior genotype frequencies and posterior marginal probabilities written to the STRmix™ extended output file were used in these calculations. The average Mix to Mix *LR*s and the average contributor stratified *LR* calculated manually and assigned within STRmix™ are reproduced in the table below. Values have been provided to 10 significant figures.

Comparison	MS Excel	STRmix™
Average Mix to Mix *LR* Left mix NoC = 1 Right mix NoC = 2	2.415697655E+08	2.415697655E+08
Average Mix to Mix *LR* Left mix NoC = 1 Right mix NoC = 3	6.235550567E+07	6.235550567E+07
Average Mix to Mix *LR* Left mix NoC = 2 Right mix NoC = 2	7.282741000E+07	7.282741000E+07
Average Mix to Mix *LR* Left mix NoC = 2 Right mix NoC = 3	1.851961784E+07	1.851961784E+07
Contributor stratified *LR*	9.881807476E+07	9.881807476E+07

The Mix to Mix *LR*s calculated in MS Excel match those assigned by STRmix™ to 10 significant figures and therefore pass the acceptance criterion. While not reproduced here, all pairwise Mix to Mix *LR*s calculated manually also matched the corresponding STRmix™ result to 10 significant figures.

4.18. Top-down approach

Acceptance criterion: Where full precision is output in STRmix, precision is expected to 10 sig fig. DNA amounts are given as a whole number and precision is expected to the full number given. Allele and stutter variances, degradation, and locus efficiencies are given to three decimal places and precision is expected to 3dp.

A three-person mixture was analysed using a top-down approach with Top N=2. Nineteen of the possible twenty steps were completed. The results from each step of the top-down approach were duplicated by manually running an interpretation and database search with the same settings (eg. the same seed, detection thresholds, Fst value and kit settings). The interpretation Results.txt and DBSearchResults.txt files from the manual runs where compared with their corresponding files in the top-down Steps results subfolder, using BeyondCompare. Where rounding is necessary, numerical values are displayed to at least 10 significant figures in both the Results.txt and DBSearchResults.txt files. The results for Step 5 of the top-down approach and the corresponding manual run are given in the table below. The detection threshold values used for each run are also displayed.

Parameter	Top-down approach (Step 5)	Manual run
Seed	807471	807471
Total iterations	2437743	2437743
Effective Sample Size	6951.289384	6951.289384
Average log(likelihood)	14.37478793	14.37478793
Gelman-Rubin convergence diagnostic	1.152482575	1.152482575
DNA Amounts: Contributor 1	287	287
DNA Amounts: Contributor 2	177	177
DNA Amounts (mode): Contributor 1	246	246
DNA Amounts (mode): Contributor 2	181	181
Mixture Proportions (%): Contributor 1	61.83933661	61.83933661
Mixture Proportions (%): Contributor 2	38.16066338	38.16066338
Mixture Proportions (%) (mode): Contributor 1	57.56386754	57.56386754
Mixture Proportions (%) (mode): Contributor 2	42.43613245	42.43613245

LSAE Variance		0.03059442890	0.03059442890
Variance		2.948	2.948
Back Stutter Variance		11.342	11.342
Forward Stutter Variance		3.080	3.080
Degradation (rfu/bp), starting at 122.0bp: Contributor 1		0.805	0.805
Degradation (rfu/bp), starting at 122.0bp: Contributor 2		0.403	0.403
Degradation curve d values: Contributor 1		0.004	0.004
Degradation curve d values: Contributor 2		0.003	0.003
Efficiencies (%)	D8S1179	71	71
	D21S11	116	116
	D7S820	115	115
	CSF1PO	89	89
	D3S1358	103	103
	TH01	109	109
	D13S317	164	164
	D16S539	132	132
	D2S1338	115	115
	D19S433	129	129
	vWA	69	69
	TPOX	187	187
	D18S51	125	125
	D5S818	132	132
	FGA	109	109
ource *LR*	Suspect01A	2.712962496E-4	2.712962496E-4
	Victim01A	0.002624427661	0.002624427661
	Suspect02A	1.080178660E8	1.080178660E8

	Suspect02B	0.5046853411	0.5046853411
	Suspect02C	0.003291804394	0.003291804394
	Suspect02D	1.282176332E-4	1.282176332E-4
	Victim03A	0.01825567560	0.01825567560
	Consensual partner03	1.519103863E-4	1.519103863E-4
	Suspect03A	2.173796841E-4	2.173796841E-4
	Suspect03B	0.1215428090	0.1215428090
	Victim04A	0.009972502188	0.009972502188
	Suspect04A	0.009505451861	0.009505451861
	Suspect05A	0.001797692221	0.001797692221
	Suspect05B	2.317439023E-4	2.317439023E-4
	Suspect05C	0.002088474065	0.002088474065
on Thresholds (rfu)	D8S1179	232	232
	D21S11	394	394
	D7S820	321	321
	CSF1PO	173	173
	D3S1358	317	317
	TH01	303	303
	D13S317	671	671
	D16S539	411	411
	D2S1338	299	299
	D19S433	659	659
	vWA	155	155
	TPOX	468	468
	D18S51	367	367
	D5S818	578	578
	FGA	351	351

The differences are 0 in the 10th sig fig. Hence, these tests satisfy the acceptance criteria.

4.19. H_d true tester

Acceptance criterion: Precision is expected to 10 sig fig.

A one locus single source profile with ambiguity and one locus two person mixed DNA profile were analysed using the H_d True Tester module. 10,000 profiles were generated and importance sampling enabled. The *LR*, bias, effective iterations and average *LR* were calculated. The results are given in the table below.

Single source example, where $f_{12}^{`} = 0.3141424619640 39$

D16S539, S_y	Weight, w_y	Fraction in database	Pr(S_y)
12,12	0.8264563264180106	0.8234	0.0986854864088275
12,Q	0.173543673581989	0.1766	0.430913951110422

STRmix™			
Database profile	*LR*	Bias $b_y = \frac{\Pr(S_y)}{w_y}$	Effective iterations
12,12	5.286220323	0.1194079871	0.5396468233
12,Q	1.110028521	2.483028866	11.22168350
Average *LR* $\overline{LR} = \frac{1}{y}\sum_y LR_y b_y$	1.006494742	# effective iterations $I = \frac{\sum_y LR_y}{\overline{LR}}$	45193.52819
Excel			
Database profile	*LR*	Bias $b_y = \frac{\Pr(S_y)}{w_y}$	Effective iterations
12,12	5.286220323	0.1194079871	0.5396468233
12,Q	1.110028521	2.483028866	11.22168350
Average *LR* $\overline{LR} = \frac{1}{y}\sum_y LR_y b_y$	1.006494742	# effective iterations $I = \frac{\sum_y LR_y}{\overline{LR}}$	45193.52819

The differences are 0 in the 10th sig fig. Hence, these tests satisfy the acceptance criteria.

Mixture example

D18S51, S_y	Weight, w_y
16,20 and 12,16	0.425666776424102
16,20 and 12,20	0.224604873230161
16,20 and 12,12	0.19250082318077
16,20 and 12,19	0.112734606519591
16,20 and 12,15	0.0363845900559762
16,20 and 12,Q	0.00810833058939743

STRmix™			
Database profile	*LR*	$b_y = \frac{\Pr(S_y)}{w_y}$ Bias	Effective iterations
[16,20]	94.32535321	0.01060160355	0.5370423171
[12,16]	10.15810311	0.1564660955	7.926057040
[12,20]	5.359966032	0.03652037839	1.850002080
[12,19]	2.690296309	0.1618839739	8.200508917
[12,12]	4.593835648	0.1338475101	6.780274006
[12,15]	0.8682811018	2.123911358	107.5903538
Average *LR* $\overline{LR} = \frac{1}{y}\sum_y LR_y b_y$	1.004951937	# effective iterations $I = \frac{\sum_y LR_y}{\overline{LR}}$	506567.0625
Excel			
	LR	Bias	Effective iterations
[16,20]	94.32535321	0.01060160355	0.5370423171
[12,16]	10.15810311	0.1564660955	7.926057040
[12,20]	5.359966032	0.03652037839	1.850002080
[12,19]	2.690296309	0.1618839739	8.200508917
[12,12]	4.593835648	0.1338475101	6.780274006
[12,15]	0.8682811018	2.123911358	107.5903538
Average *LR*:	1.004951937	# effective iterations	506567.0625

These numbers are the same to the 10th sig fig. Hence, these tests satisfy the acceptance criteria.

4.20. Model Maker

Acceptance criterion: MM results output to 15 sig fig in STRmix™ except per sample template and LSAE values that are rounded to the nearest whole number. Precision is expected to 10 sig fig.

Model Maker works by using a component-wise MCMC. In component 1, each profile has its mass parameters optimised, using a stable gamma distribution when sampling allele and stutter constants and a stable exponential distribution when sampling the LSAE variance constant. In component 2, the mass parameters for each profile are held constant and the hyper-parameters for each distribution (for allele, each stutter type, and LSAE variance) are varied. A hyper-parameter is a parameter of a prior distribution. Within Model Maker, the hyper-parameters relate to the shape and scale parameters within the gamma distribution for allele and stutter and the mean of the exponential LSAE variance. The likelihood of the total dataset is recalculated at each iteration.

Components are 1000 accepts long and they cycle through a number of times depending on the user input number of accepts (default = 100,000). For example, if 100,000 accepts are chosen then there will be 100 cycles of the two components, if 200,000 is chosen there will be 200 cycles.

Each MCMC is a Gaussian walk MCMC with step-sizes as per a normal STRmix™ calculation. This way they adjust in larger or smaller increments as the hyper-parameter becomes larger or smaller. In doing this the process has some memory, but doesn't require specific settings to do with step-sizes.

The final gamma distributions chosen are the set at the posterior mode of the posterior sample within the last 25% of the Model Maker iterations i.e. the set that gave the highest likelihood (versus being the last or an average value).

The extended output for two Model Maker runs was analysed in Excel. A summary of the component cycle log(likelihood) values from the report is given in the table below.

Component cycle	STRmix	Excel
1	-79.6436652212192	-79.6436652212192
2	-79.6326367530189	-79.6326367530189
3	-78.3041519933175	-78.3041519933175
4	-79.3585682507529	-79.3585682507529
5	-86.2530742186945	-86.2530742186945
6	-82.7768477687874	-82.7768477687874
7	-80.8892117226486	-80.8892117226486

8	-87.2366956340459	-87.2366956340459
9	-85.7081860522184	-85.7081860522184
10	-85.6989939063051	-85.6989939063051

These numbers are the same to 10 sig fig. Hence, they pass the acceptance criterion.

A summary of the allele, stutter, and LSAE variances are in the table below.

Type	Alpha - STRmix	Beta – STRmix	Alpha - Excel	Beta – Excel
Allele	3.19830509012194	5.61810201194136	3.19830509012194	5.61810201194136
Back Stutter	3.33346506249546	4.25748402418060	3.33346506249546	4.25748402418060
Forward Stutter	3.25503804759332	6.92949977966533	3.25503804759332	6.92949977966533
LSAE variance	98.8766115735246	0.0101136151824579	98.8766115735246	0.010113615182457

These numbers are the same to 10 sig fig. Hence, they pass the acceptance criterion.

A summary of the mass parameters for one profile are given in the table below (5 LSAE only).

	Template	Degradation	Locus 1	Locus 2	Locus 3	Locus 4	Locus 5
STRmix	1264	6.01053343431042E-04	100%	117%	83%	118%	76%
Excel	1264	6.01053343431041E-04	100%	117%	83%	118%	76%

These numbers are the same to 10 sig fig. Hence, they pass the acceptance criterion.

4.21. Variable number of contributors function

Note that the developmental validation of the range of contributors (or varNOC) function within STRmix™ has been restricted to a difference in *n* of only 1.

A varNOC interpretation starts with the deconvolution of the profile under the differing assignments of *n*. This deconvolution follows the same MCMC process as a typical deconvolution with the generation of genotype combinations within pre burn-in, followed by the determined number of burn-in accepts, and then post burn-in accepts. Setting the seed within a single *n* deconvolution will return the same deconvolution results as the *n* component of a varNOC deconvolution with the same seed. For varNOC, the seed is conserved between the different *n* interpretations to facilitate this check.

The primary output from varNOC are the contributor number probabilities i.e. weights, Z_n, used within the calculation of the varNOC *LR*. The validation of these outputs, following the

algorithms described in the STRmix™ User's Manual was undertaken across nine interpretations, including but not limited to examples using replicate PCR inputs generated in the same or different kits, where drop-in and dropout were variously modelled, and where autocontinuation was triggered based on GR value.

Described below is an example of the calculations replicated from the extended output from a profile analysed using replicates and (unless otherwise specified) a range of contributors (*n*=1 and 2), with two chains, each containing 5000 burn-in and post burn-in accepts, and with hyper-rectangle set to contain 2.5 percent of the total MCMC accepts.

4.21.1. Hyper-rectangle

For each chain the hyper-rectangle is drawn around the MCMC iteration with the highest posterior probability. In an extended output the posterior probabilities (thinned to every 10th accept) are written to the file labelled in part "Max_Posterior_Probability". These values are the inverse log of the total MCMC probability for each accepted MCMC iteration recalculated to sum across all accepted genotype combinations. For each *n* the accepted genotype combinations per locus are listed in the corresponding GenotypePdf file.

The following steps describe a modified Weinberg algorithm[3] for the calculation of a hyper-rectangle from an extended output of the varNOC replicates example described above. This is repeated for each chain and each *n* within the contributor range. For varNOC multikit scenarios, the kit efficiency parameters are also included in these calculations. The method to determine the hyper-rectangle is broken up into 6 steps that are describe below.

Step 1. Acceptance criterion: Precision is expected to 10 significant figures to the maximum in the extended output "1C_Max_Posterior_Probability_1".

The posterior sample with the highest likelihood (defined as θ_{peak}) from the entire posterior MCMC sample (Ω) of chain 1 was identified from the extended output "1C_Max_Posterior_Probability_1". For *n*=1 chain 1, the maximum was identified as 7.033393771E-4 for accept 1271. From the log, this was confirmed as the "Maximum posterior probability of 7.0333937719E-4 in chain 1 at position 1271".

These tests satisfy the acceptance criteria.

Step 2. Acceptance criteria: The squared distances for each (11) mass parameter values and their sum are output per iteration to 15 sig fig in the extended output file labelled in part "Q0_distances". Parameter values for theta peak, minimum and maximum mass parameters are output to the Interpretation.txt to 15 sig fig. Precision is expected to 10 sig fig.

The squared distance for each peak variance parameter, LSAE variance parameter, replicate efficiency, template, degradation and LSAE parameter within the 5,000 post burn-in accepts from the corresponding parameter values at θ_{peak} identified in step 1 were

[3] Weinberg, M.D. Yoon, I. Katz, N A remarkably simple and accurate method for computing the Bayes Factor from a Markov chain Monte Carlo Simulation of the Posterior Distribution in high dimension. arXiv:1301.3156v1 [astro-ph.IM] 14 Jan 2013

calculated. These squared distances (and their sum) are printed to the extended output "Q0_distances". The parameter values for θ_{peak}, minimum mass parameters and maximum mass parameters are printed to the log. The values for chain 1 *n*=1 are replicated below:

STRmix™			
Parameter	θ_{peak}	Min value	Max value
Template	146.0227269	85.17521810	194.9547979
Degradation	0.005404250766	0.004763308227	0.009999411039
σ_A^2	0.005413234625	0.003800381771	0.04658405605
LSAE D3S1358	1.087506025	0.6375913459	1.783865504
LSAE vWA	1.041038174	0.5849071934	1.461332569
LSAE CSF1PO	1.011147176	0.6325915338	1.251410747
PCR1	1.979409485	0.8465530441	3.304798482
PCR2	0.50520117611	0.3025903107	1.181260887
c^2	7.247820263	6.479049025	7.639948860
k_{a-1}^2	7.669425682	6.292885184	9.031396254
k_{a+1}^2	5.702647873	5.023280953	6.139612456
Excel			
Parameter		Min value	Max value
Template		85.17521810	194.9547979
Degradation		0.004763308227	0.009999411039
σ_A^2		0.003800381771	0.04658405605
LSAE D3S1358		0.6375913459	1.783865504
LSAE vWA		0.5849071934	1.461332569
LSAE CSF1PO		0.6325915338	1.251410747
PCR1		0.8465530441	3.304798482

PCR2		0.3025903107	1.181260887
c^2		6.479049025	7.639948860
k^2_{a-1}		6.292885184	9.031396254
k^2_{a+1}		5.023280953	6.139612456

These numbers are the same to the 10th sig fig. Hence they pass the acceptance criterion.

Step 3. Acceptance criterion: The top 125th accept is expected to be the identical iteration detailed in the Interpretation.txt output. The initial sample distance (d bar) is output to 16 sig fig in the Interpretation.txt. Precision is expected to 10 sig fig.

The summed distances were sorted from lowest to highest and the top 125 accepts (2.5 percent of the total MCMC accepts for chain 1 *n*=1 for this example) were identified. The top 125th accept was the 317th iteration. This was confirmed within the log for this sample; "125 accepted samples resulted in 317 MCMC iterations".

Excel	STRmix
0.3354336864	0.3354336864

These numbers are the same to the 10th sig fig. Hence they pass the acceptance criterion.

Step 4. Acceptance criteria: The updated squared distances for each mass parameter values and their sum are output per iteration to 15 sig fig in the extended output file labelled in part "Q1_distances". Precision is expected to 10 sig fig.

The root mean variance of each of the parameters for each iteration of the post burn-in accepts was calculated. This is the square root of the summed differences of the parameter to the corresponding θ_{peak} parameter value identified in step 1, divided by the total number of iterations represented by the top 125 accepts for this chain. The squared distances were then recalculated as in step 2 using the root mean variance. These updated values and their sum were confirmed by comparison to extended output "Q1_distances". The values for chain 1 *n*=1 are replicated below:

Parameter	Excel	STRmix
Template	7.374405137	7.374405137
Degradation	9.864714794	9.864714794

σ_A^2	2.158712793	2.158712793
LSAE D3S1358	36.90325530	36.90325530
LSAE vWA	18.93335569	18.93335569
LSAE CSF1PO	0.0002162115253	0.0002162115253
PCR1	0.04988464719	0.04988464719
PCR2	0.05493124769	0.05493124769
c^2	38.96944339	38.96944339
k_{a-1}^2	121.9621485	121.9621485
k_{a+1}^2	0.4708947576	0.4708947576
Total	236.7419624	236.7419624

These numbers are the same to the 10th sig fig. Hence they pass the acceptance criterion.

Step 5. Acceptance criterion: The revised top 125th accept is expected to be the identical iteration detailed in the Interpretation.txt output. The updated sample distance (d bar) is output to 17 sig fig in the Interpretation.txt. Precision is expected to 10 sig fig.

The updated summed distances were sorted from lowest to highest and the top 125 accepts identified. The top 125th accept was identified as the 362nd iteration. This was confirmed within the log for this sample; “125 accepted samples resulted in 362 MCMC iterations”.

Excel	STRmix
3.817499653	3.817499653

These numbers are the same to the 10th sig fig. Hence they pass the acceptance criterion.

Step 6. Acceptance criteria: The extrema of each mass parameter within the hyper-rectangle are output to 15 sig fig in the Interpretation.txt and the extended output file labelled in part “1C_HR_shape_1”. Precision is expected to 10 sig fig.

The hyper-rectangle is drawn around the top 125 accepted iterations. The extrema of each parameter within these iterations were determined. These values are written to the extended output file named in part 1C_HR_shape_1. The volume, *V*, of the hyper-rectangle is calculated by taking the product of the range (difference) for each parameter. *V* is replicated for *n*=1 chain 1 below:

STRmix™		
Parameter	Minimum	Maximum
Template	101.1109928	148.7622312
Degradation	0.005175907701	0.006176379061
σ_A^2	0.0038073628	0.01476719248
LSAE D3S1358	1.034461994	1.169056219
LSAE vWA	0.9725499324	1.120733226
LSAE CSF1PO	1.000951381	1.180689626
PCR1	1.699550678	2.246809917
PCR2	0.4450754789	0.5883908099
c^2	7.212786887	7.360963129
k_{a-1}^2	7.555787759	7.970356417
k_{a+1}^2	5.584899986	5.753800267
Excel		
Parameter	Minimum	Maximum
Template	101.1109928	148.7622312
Degradation	0.005175907701	0.006176379061
σ_A^2	0.0038073628	0.01476719248
LSAE D3S1358	1.034461994	1.169056219
LSAE vWA	0.9725499324	1.120733226
LSAE CSF1PO	1.000951381	1.180689626
PCR1	1.699550678	2.246809917
PCR2	0.4450754789	0.5883908099
c^2	7.212786887	7.360963129
k_{a-1}^2	7.555787759	7.970356417
k_{a+1}^2	5.584899986	5.753800267

These numbers are the same in the 10th sig fig. Hence they pass the acceptance criterion.

4.21.2. MC average

Acceptance criteria: The per locus dropout frequencies are output to 15 sig fig in the Interpretation.txt file.

The per locus genotype set probabilities, mass parameter penalties, and log(profile probability) values per iteration are output to 15 sig fig in the extended output file labelled in part “1C_Naive_MC”.

Under each *n* the per chain MC average and Unadjusted MC average values are output to 15 sig fig in the Interpretation.txt. Precision is expected to 10 sig fig.

For each chain and each *n*, once the HR is defined, a naïve MC sampling of the parameters is conducted drawing 10,000 new values uniformly from the range enveloped by the hyper-rectangle. For each MC iteration, the posterior likelihoods, log(*p*), are recalculated for all peaks, for each proposed genotype set. The inverse log of the sum of the log(*p*) values are each multiplied by the probability of the corresponding genotype set, Pr(S_j), calculated (F_{ST}=0) using the posterior mean allele frequencies for the chosen Population for Range and incorporating Pr(C) where drop-in is proposed. Where dropout is proposed the per locus dropout frequencies are obtained by subtracting from 1 the sum of the posterior mean allele

frequencies for all alleles present in the posterior burn-in MCMC sample at that locus under the corresponding *n*. These dropout frequencies are written to the log for each *n*. The Locus Probability value per locus is then obtained by summing across the genotype combinations.

The log(product) of the locus probabilities is added to the sum of the mass parameter penalties for all peak variances, LSAEs and LSAE variances and a combined template/degradation penalty across all contributors to obtain the unadjusted log(profile probability) value. These penalties can be calculated using the formulae in the table below, where *x* is the sampled parameter value for the MC iteration, and we obtain from the kit file α,β describing the corresponding variance's prior gamma distribution, the mean LSAE variance 1/λ, saturation and deg max.

Parameter type	Penalty formula
Peak variance	=log(GAMMA.DIST(*x*,α,β,0)
LSAE	=log(product(L)) Where L is calculated per locus by =NORM.DIST(log(*x*),0,SQRT(*y*),0) And where *y* is the LSAE variance value sampled for the MC iteration
Template and degradation	=log(product(C)) Where C is calculated as per contributor by (1/minimum saturation per kit)^n for template, and (1/minimum deg max per kit)^n for degradation
LSAE variance	=EXPON.DIST(x,λ,0) Where λ is the reciprocal of the mean LSAE variance

The Adjusted log(profile probability) value is calculated by adding the log(product) of the per locus adjustments to the sum of the penalties. These values are replicated below for chain 1 *n*=1 Iteration 1 for a 3-locus example where generalised stutter was enabled and a double drop-in event was proposed (modelled U[0,0] with frequency = 0.0029) at SE33. The chosen Population for Range was FBI_extended_Cauc.

varNOC extended output value	STRmix™	Excel
Pr(S_j) SE33 [29.2,30]	4.264886417E-05	4.264886417E-05

Pr(S_j) D22S1045 [15,16]	2.298011632E-01	2.298011632E-01
Pr(S_j) FGA [21,23]	5.553288257E-02	5.553288257E-02
Locus probability SE33	1.722603204E-09	1.722603204E-09
Locus probability D22S1045	1.931449339E+02	1.931449339E+02
Locus probability FGA	4.373730062E+01	4.373730062E+01
LSAE penalty	0.6703954923	0.6703954923
LSAE variance penalty	1.641603480	1.641603480
Allele variance penalty	-1.140829899	-1.140829899
Back Stutter variance penalty	-1.910237172	-1.910237172
Forward Stutter variance penalty	-0.8099411461	-0.8099411461
Template and degradation penalty	-2.477121254	-2.477121254
log(profile probability)	-2.599019861	-2.599019861
log(profile probability) unadjusted	-8.863209951	-8.863209951

These numbers are the same in the 10th sig fig. Hence they pass the acceptance criterion.

The average of the inverse log of each of the 10,000 calculations for both the adjusted and unadjusted profile probabilities are printed to the Log as the “MC average” and “Unadjusted MC average” per chain and *n*. These were replicated as shown below.

STRmix™		
N=1	Chain 1	Chain 2
Marginal Likelihood	7.042094814E-12	1.445465161E-11
Unadjusted Marginal Likelihood	5.421674180E-15	1.290061869E-14
N=2	Chain 1	Chain 2

Marginal Likelihood	4.591872433E-13	4.369723161E-13
Unadjusted Marginal Likelihood	2.744245475E-18	2.743538057E-18
Excel		
N=1	Chain 1	Chain 2
Marginal Likelihood	7.042094814E-12	1.445465161E-11
Unadjusted Marginal Likelihood	5.421674180E-15	1.290061869E-14
N=2	Chain 1	Chain 2
Marginal Likelihood	4.591872433E-13	4.369723161E-13
Unadjusted Marginal Likelihood	2.744245475E-18	2.743538057E-18

These numbers are the same in the 10th sig fig. Hence they pass the acceptance criterion.

4.21.3. F(Omega)

Acceptance Criterion: F(omega) is output per chain to 16 sig fig in the Interpretation.txt file. Precision is expected to 10 sig fig.

For each chain and *n*, Ω (omega) is the posterior burn-in sample from the MCMC deconvolution for that chains. The number of total iterations is $|\Omega|$. Ω_s is a subset of the sample that falls within the HR and $|\Omega_s|$ is the count of iterations within this subset. $|\Omega_s|$ is determined by counting all iterations (including rejects) of the extended PostBurninResults for the given chain and *n* where the parameter values fall between the corresponding minimum and maximum determined in step 6 of the HR process above. For chain 1 *n*=1, $|\Omega_s|$=686 and $|\Omega|$=9,857. $F(\Omega_s) = \frac{|\Omega_s|}{|\Omega|}$ = 0.06959521152 (Excel) and 0.06959521152 in the extended output.

These numbers are the same to 10 sig fig and hence these tests satisfy the acceptance criteria.

4.21.4. Marginal likelihoods

Acceptance Criterion: The per chain marginal likelihoods and normalized values are output to 16 sig fig in the Interpretation.txt file. They also appear as the Transdimensional Parameters in the Results.txt file. Precision is expected to 10 sig fig.

The marginal likelihoods are calculated using the following formula:

$$p(\boldsymbol{O}|N_n) = \frac{V}{F(\Omega_s)Y'}\sum_{i=1}^{Y'}\sum_{j} p(\boldsymbol{O} \mid N_n, \boldsymbol{M_n^{(i)}}, \boldsymbol{S_j})\, p(\boldsymbol{M_n^{(i)}} \mid N_n)\, \Pr(\boldsymbol{S_j} \mid N_n)$$

where *V* is the volume of the HR described above, $F(\Omega_s)$ is also described above, *Y'* is the number of MC iterations performed, and the final term is the summation across *Y'* of the corresponding profile probability. It is this summation divided by *Y'* that gives the MC average per chain described above. Within the Interpretation log they are reported as "marginal likelihood" and "unadjusted marginal likelihood" for each chain.

These values are averaged across chains then normalised across *n*. The normalised marginal likelihood using the adjusted profile probabilities is Z_n used within the calculation of the varNOC *LR*. The normalised marginal likelihood using the unadjusted profile probabilities is the $p(N \mid O)$. Both sets of normalised values appear in the Trans-dimensional parameters section of the raw Results.txt file. The normalised values from the STRmix™ log were replicated in Excel and are given for the example described here in the table below:

	STRmix™	Excel
N=1	Normalised Average	Normalised Average
Marginal Likelihood	0.9599802026	0.9599802026
Unadjusted Marginal Likelihood	0.9997005756	0.9997005756
N=2	Normalised Average	Normalised Average
Marginal Likelihood	0.04001979733	0.04001979733
Unadjusted Marginal Likelihood	0.0002994243349	0.0002994243349

These numbers are the same in the 10th sig fig . Hence these tests satisfy the acceptance criterion.

4.22. varNOC likelihood ratios

4.22.1. varNOC sub-source *LR*S

Acceptance criterion: Precision is expected in Excel to 10 sig fig.

There are three methods for assigning a Likelihood Ratio for a varNOC interpretation; a stratified *LR*, a Maximum Likelihood Estimate (MLE) *LR* and an *LR* using user defined *N* under H_p and user defined *N* under H_d.

The stratified likelihood ratio treats *N* as a nuisance parameter and integrates *N* out by stratifying the *LR* across the variable contributor numbers assigned in the deconvolution. The MLE method of assigning the *LR* uses the most probable number of contributors under each hypothesis i.e. the *N* that produces the maximum posterior probability of the epg under H_p and under H_d. The user defined assigns an *LR* using the user selected *N* under H_p and the user selected *N* under H_d. Note that the choice between *LR*s only affects the values of $\Pr(N_n \mid H_x)$ terms in the calculation of the *LR*.

As part of the V2.8 validation, 127 varNOC *LR*s were replicated in Excel. These included single population and stratified population, mixed DNA profiles, sub-sub source and sub-source *LR*s, unrelated, related, and unified propositions, and the HPD.

Stratified LR replication

The stratified *LR*s are assigned using the formula:

$$LR = \frac{\sum_n Z_n \sum_j w_j \Pr(S_j \mid N_n, H_p)}{\sum_{n'} Z_{n'} \sum_{j'} w_{j'} \Pr(S_{j'} \mid N_{n'}, H_d)}$$

Where Z_n is the adjusted probability of *n* given the profile and is available from the deconvolution results.

The sub-source *LR* uses the average of all $\Pr(E \mid H_p)$ across contributor orders and the average of all $\Pr(E \mid H_d)$ across contributor orders for each *n* assigned, and the sub-sub-source *LR* uses the maximum $\Pr(E \mid H_p)$ and $\Pr(E \mid H_d)$ across the contributor orders and for each *n* assigned. These probabilities are then weighted using the adjusted probability of *n* given the profile. The replication of the sub-source stratified varNOC unrelated and related *LR*s for profile interpreted assuming 2 and 3 contributors is given in the table below. The FBI extended African American allele frequencies were applied with a theta value of 3%. The population was set to 1 million and the average number of children was set to 4 (these values are used to determine the priors on the relationship type in H_d used in the unified *LR*).

LR type	Relationship	Excel	STRmix
Stratified	Sibling	0.256409647	0.256409647
	Parent child	0.216130973	0.216130973
	Half sibs, etc	1.090975980	1.090975980
	Cousin	4.243243038	4.243243038
	Unrelated	33.58608354	33.58608354
	Unified	33.52809658	33.52809658

These numbers match and hence these tests satisfy the acceptance criteria.

Maximum Likelihood Estimate LR replication

The MLE *LR*s are replicated in Excel using the formula:

$$LR = \frac{Z_n \sum_j w_j \Pr(S_j \mid N_n, H_p)}{Z_{n'} \sum_{j'} w_{j'} \Pr(S_{j'} \mid N_{n'}, H_d)}$$

Where Z_n is the adjusted probability of *n* given the profile available from the deconvolution results and where *n* and *n'* are the number of contributors that produce the maximum posterior probability of the profile under H_p and H_d, respectively.

For the GlobalFiler™ example above, the *n* that produced the maximum posterior probability of the profile under H_p and H_d .for the sub-source *LR* was calculated by taking the average $\Pr(E \mid H_p)$ and $\Pr(E \mid H_d)$ across the contributor orders for each *n* assigned and then weighting them using the adjusted probability *n* given the profile. . In this example, *n* and *n*' are both 2.

The replication of the sub-source MLE varNOC unrelated and related *LR*s for the same profile as above is given in the table below. The FBI extended African American allele frequencies were applied with a theta value of 3%. The population was set to 1 million and the average number of children was set to 4 (these values are used to determine the priors on the relationship type in H_d used in the unified *LR*).

LR type	Relationship	Excel	STRmix
MLE	Sibling	0.256409377	0.256409377
	Parent child	0.216130747	0.216130747
	Half sibs, etc	1.090974896	1.090974896
	Cousin	4.243239152	4.243239152
	Unrelated	33.58606065	33.58606065
	Unified	33.52807371	33.52807371

These numbers match and hence these tests pass the acceptance criterion

User defined n LR replication

User defined sub-source *LR*s and sub-sub-source *LR*s were generated from the example above using the *LR* from previous function, and enabling the extended output.

The *LR*s were replicated in Excel using the formula:

$$LR = \frac{Z_n \sum_j w_j \Pr(S_j \mid N_n, H_p)}{Z_{n'} \sum_{j'} w_{j'} \Pr(S_{j'} \mid N_{n'}, H_d)}$$

Where Z_n is the adjusted probability of *n* given the profile available from the deconvolution results and where *n* and *n'* are user defined.

The sub-source *LR* uses the average of all $\Pr(E \mid H_p)$ across contributor orders and the average of all $\Pr(E \mid H_d)$ across contributor orders for the user defined *n*, and the sub-sub-

source *LR* uses the maximum $\Pr(E \mid H_p)$ and $\Pr(E \mid H_d)$ across the contributor orders for the user defined *n*. These probabilities were then weighted using the adjusted probability of *n* given the profile given the user defined *n*.

The replication of the point estimate sub-source *LR* assuming under H_p N=3 and under H_d N=2 for the same profile as above, are in the table below. The FBI extended African American allele frequencies were applied with a theta value of 3%. The population was set to 1 million and the average number of children was set to 4 (these values are used to determine the priors on the relationship type in H_d used in the unified *LR*).

LR type	Relationship	Excel	STRmix
User defined H_p=3 H_p=2	Sibling	$3.15085386 \times 10^{-07}$	$3.15085386 \times 10^{-07}$
	Parent child	$2.65589507 \times 10^{-07}$	$2.65589507 \times 10^{-07}$
	Half sibs, etc	$1.34063055 \times 10^{-06}$	$1.34063055 \times 10^{-06}$
	Cousin	$5.21425020 \times 10^{-06}$	$5.21425020 \times 10^{-06}$
	Unrelated	$4.12718014 \times 10^{-05}$	$4.12718014 \times 10^{-05}$
	Unified	$4.12005448 \times 10^{-05}$	$4.12005448 \times 10^{-05}$

These numbers match and hence these tests pass the acceptance criterion.

Population stratified varNOC LR replication

The population stratified; varNOC stratified, MLE and user defined *n*=3 under H_p and *n*=2 under H_d, sub-source, point estimate *LR* for the same profile where the population proportion was set to 0.7 for the Caucasian population, 0.3 for the African American population and the African American population size was set to 1000000 with four children while the Caucasian population was kept at 0 children, are below:

LR type	Relationship	Excel	STRmix
Stratified	Sibling	$1.592347924 \times 10^{-01}$	$1.592347924 \times 10^{-01}$
	Parent child	$1.130796131 \times 10^{-01}$	$1.130796131 \times 10^{-01}$
	Half sibs, etc	$5.899433127 \times 10^{-01}$	$5.899433127 \times 10^{-01}$
	Cousin	2.299525972	2.299525972
	Unrelated	19.40778646	19.40778646
	Unified	19.40770933	19.40770933
MLE	Sibling	$1.592345403 \times 10^{-01}$	$1.592345403 \times 10^{-01}$
	Parent child	$1.130794349 \times 10^{-01}$	$1.130794349 \times 10^{-01}$
	Half sibs, etc	$5.899424131 \times 10^{-01}$	$5.899424131 \times 10^{-01}$
	Cousin	2.299522654	2.299522654
	Unrelated	19.40776328	19.40776328
	Unified	19.40768615	19.40768615
User defined	Sibling	$2.818009490 \times 10^{-07}$	$2.818009490 \times 10^{-07}$
	Parent child	$2.001192204 \times 10^{-07}$	$2.001192204 \times 10^{-07}$
	Half sibs, etc	$1.044034363 \times 10^{-06}$	$1.044034363 \times 10^{-06}$
	Cousin	$4.069516983 \times 10^{-06}$	$4.069516983 \times 10^{-06}$
	Unrelated	$3.434635539 \times 10^{-05}$	$3.434635539 \times 10^{-05}$
	Unified	$3.434621889 \times 10^{-05}$	$3.434621889 \times 10^{-05}$

These numbers macth and hence these tests pass the acceptance criterion.

4.22.2. varNOC HPD *LR*

Precision is expected to 10 sig fig.

The HPD *LR* was replicated for all of the the varNOC scenarios described previously. The sub-source population stratified varNOC *LR*s are provided in the table below:

LR type	Relationship	Excel	STRmix
Stratified	Sibling	8.5677353428E-02	8.5677353428E-02
	Parent child	5.3526548635E-02	5.3526548635E-02
	Half sibs, etc	2.6627819890E-01	2.6627819890E-01
	Cousin	9.5301325797E-01	9.5301325797E-01
	Unrelated	6.5452395741E+00	6.5452395741E+00
	Unified	6.5452357799E+00	6.5452357799E+00
MLE	Sibling	8.5677045386E-02	8.5677045386E-02
	Parent child	5.3526484692E-02	5.3526484692E-02
	Half sibs, etc	2.6627740020E-01	2.6627740020E-01
	Cousin	9.5300983557E-01	9.5300983557E-01
	Unrelated	6.5452185896E+00	6.5452185896E+00
	Unified	6.5452147954E+00	6.5452147954E+00
User selected	Sibling	1.3439858261E-07	1.3439858261E-07
	Parent child	9.4744093553E-08	9.4744093553E-08
	Half sibs, etc	5.1023167679E-07	5.1023167679E-07
	Cousin	1.8708679380E-06	1.8708679380E-06
	Unrelated	1.3665088039E-05	1.3665088039E-05
	Unified	1.3665083025E-05	1.3665083025E-05

These numbers match and hence these tests pass the acceptance criterion.

4.23. CODIS report

Acceptance criterion: alleles within the xml file and CODIS summary report as expected based on the settings logic.

The STRmix™ CODIS report logic has been tested using 40 single source and mixed profiles with respect to the correct alleles being present on the report. This included changing the Genotype Weight Threshold (default 0.99), Max alleles (default 4), and the

option to force a single genotype if the threshold is reached. A three-person mixture was interpreted in STRmix™ and CODIS reports run under a range of different settings. A summary of the component interpretation for contributor one (0.65 mixture proportion) for three loci is given below.

Locus	Genotype	Weight
D3S1358	14, 16	97.78%
	15, 16	1.90%
	14, 15	0.33%
D1S1656	11, 17	99.97%
	17, 17	0.03%
D2S441	10, 14	99.62%
	11, 14	0.27%
	10, 11	0.11%

The alleles present within the CODIS file and CODIS summary report given different settings are in the table below where * indicates an obligate allele.

Max alleles	4	4	2	4	2
Threshold	0.99	0.999	0.99	0.99	0.99
Force single genotype ?	Enabled	Enabled	Enabled	Disabled	Disabled
D3S1358	15,16,17	15,16,17	-	15,16,17	-
vWA	15,16,18	-	-	15,16,18	-
D8S1179	11,12,13,14	11,12,13,14	-	11,12,13,14	-
D21S11	29,31,31.2	29,31,31.2	-	29,31,31.2	-
D2S441	10,11,13	10,11,13	11	10,11,13	11
TH01	7,9,9.3	7,9,9.3	-	7,9,9.3	-
D22S1045	11,15,17	11,15,17	-	11,15,17	-
D5S818	10,11,13	10,11,13	-	10,11,13	-
D10S1248	12,14,15,16	12,14,15,16	-	12,14,15,16	-
D2S1338	17,18,19,22	-	-	17,18,19,22	-

All the alleles within the xml file and CODIS summary report were as expected based on the logic. These tests satisfy the acceptance criteria.

4.23.1. CODIS match threshold

Acceptance criterion: Match statistic precision is expected to 10 sig fig. CODIS inverse match statistic precision is expected to 5 sig fig.

The CODIS match threshold logic follows the formulae:

$p_i(2-p_i)$, for a single allele present,

$\left(\sum p_i\right)^2$, for more than one allele, with no obligates,

$p_i\left(p_i+2\sum_{j,i<>j}p_j\right)$, for more than one allele, one of which is obligate (p_i)

Where p is the posterior mean frequency of the allele.

A two person mixed GlobalFiler™ profile was interpreted in STRmix™. The inverse match statistic was replicated for contributor one and compared to the CODIS report inverse match statistic. The settings used in the CODIS report were: Max alleles = 4, Genotype Weight Threshold = 99, Match Statistic Threshold = 0, Force Single Genotype = Y. The FBI extended Caucasian allele frequencies were used. The alleles present in the report, the corresponding posterior mean frequencies, locus match statistic and overall profile inverse match statistic are in the table below.

	Alleles				Posterior Mean Frequencies				
Locus	a	b	c	d	f` a	f` b	f` c	f` d	Match statistic
D3S1358	15	16	17		0.2472	0.2324	0.2102		0.4758444787
vWA	15	18			0.1139	0.2176			0.1098345066
D16S539	12	13			0.3410	0.1632			0.2542704835
CSF1PO	10	11	12	13	0.2521	0.2990	0.3262	0.0718	0.9006883399
TPOX	7	11			0.0004	0.2547			0.06509847754
D8S1179	10	15			0.1089	0.1064			0.04633848146
D21S11	28	30	32.2	33.2	0.1681	0.2323	0.1088	0.0298	0.2905547845
D18S51	16	20			0.1039	0.0249			0.01657599482

D2S441	11	14			0.3089	0.2620			0.3258346780
D19S433	11	12	16.2		0.0002	0.0965	0.0199		0.01359054276
TH01	6	7			0.2250	0.1731			0.1585219479
FGA	19				0.0545				0.1060504018
D22S1045	11	17	19		0.1435	0.0966	0.0003		0.05775813308
D5S818	11				0.4077				0.6491997218
D13S317	11	12			0.3114	0.3090			0.3848593964
D7S820	8	12			0.1632	0.1410			0.09253613778
SE33	14.2	21.2			0.0025	0.0124			0.0002234511232
D10S1248	15	16			0.1731	0.1311			0.09253613778
D1S1656	11	14			0.0693	0.0915			0.02586405189
D12S391	17	18			0.1038	0.1754			0.07800105750
D2S1338	21	22	25		0.0200	0.0298	0.0965		0.02140260631

STRmix™ match statistic	1.543451464E-23
Excel match statistic	1.543451464E-23
STRmix™ inverse match statistic	6.4789E+22
Excel inverse match statistic	6.4789E+22

These numbers satisfies the acceptance criterion.

4.24. Precision

To demonstrate the precision in the STRmix™ v2.8 likelihood ratio, a number of different samples were deconvoluted in STRmix™ v2.8 twenty times each and an *LR* was generated for each deconvolution. Due to MCMC variability, results are expected to be reproducible to within one order of magnitude difference.

The samples including the following:

- a fully resolved single source sample (Exercise 1A)
- a partial single source sample (Exercise 1B)
- a 2 person mixture in equal proportions with one assumed contributor (Exercise 1C)
- a 2 person mixture in equal proportions without assumed contributors (Exercise 1C)
- a major:minor 2 person mixture (Exercise 1D)
- a low level single source sample with replicate inputs (Exercise 1E)
- a 2 person mixture run as a variable 2-3 person mixture (varNOC 2-3p)
- a single source profile with replicates from two different DNA profiling kits (Multikit replicates)

The results are plotted below in Figure 4.5 to Figure 4.12. *LR* results are reproducible to within one order of magnitude and hence these samples satisfy the acceptance criteria.

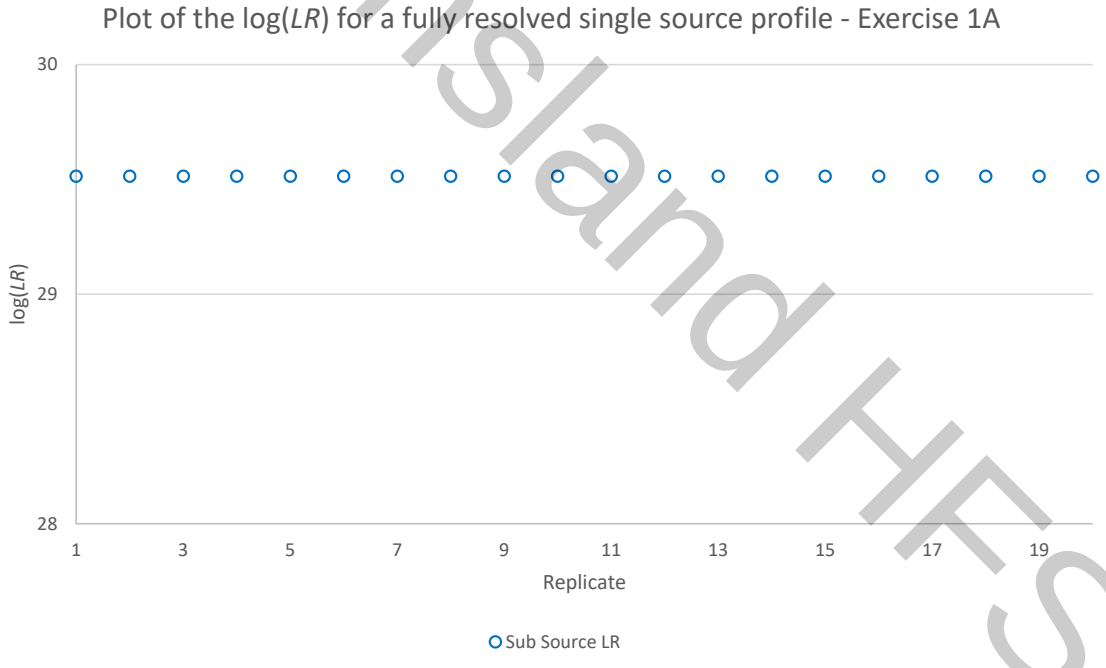


Figure 4.5 Plot of the log(*LR*) for a fully resolved single source sample (Exercise 1A). As expected there is no variability in the *LR* as this profile is full resolved.

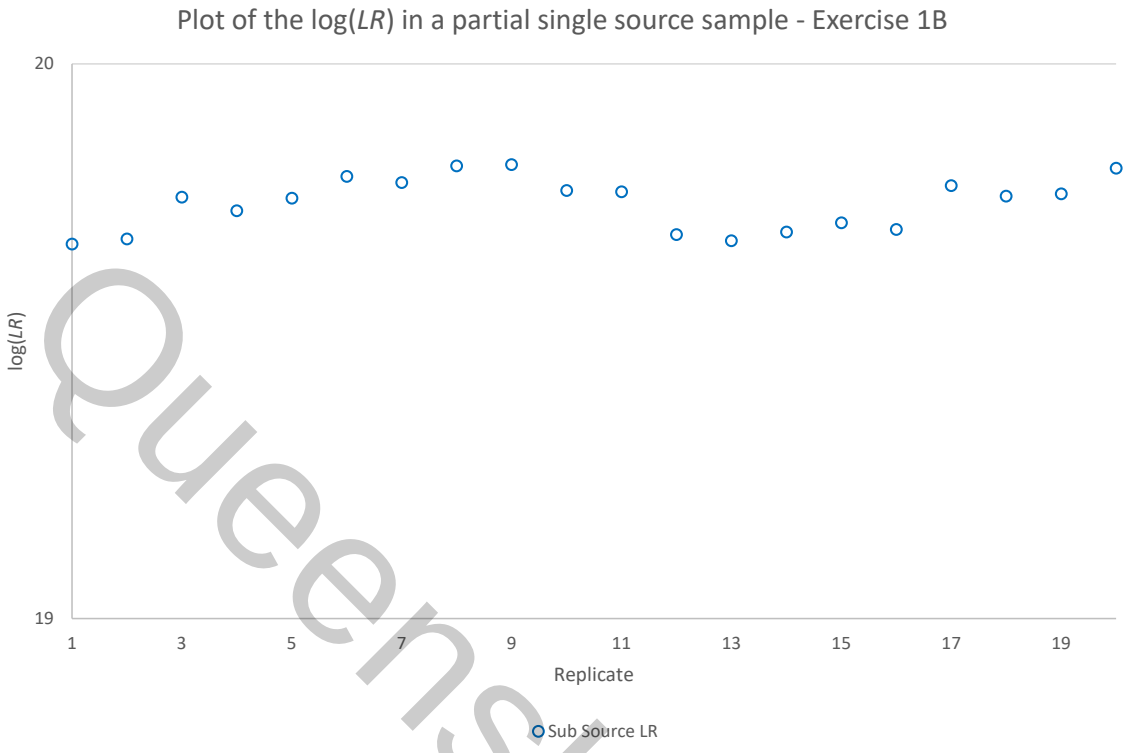


Figure 4.6 Plot of log(*LR*) of a partial single source sample (Exercise 1B). As expected there is some variability within the *LR* as this is a partial profile, however the variability is within one order of magnitude.

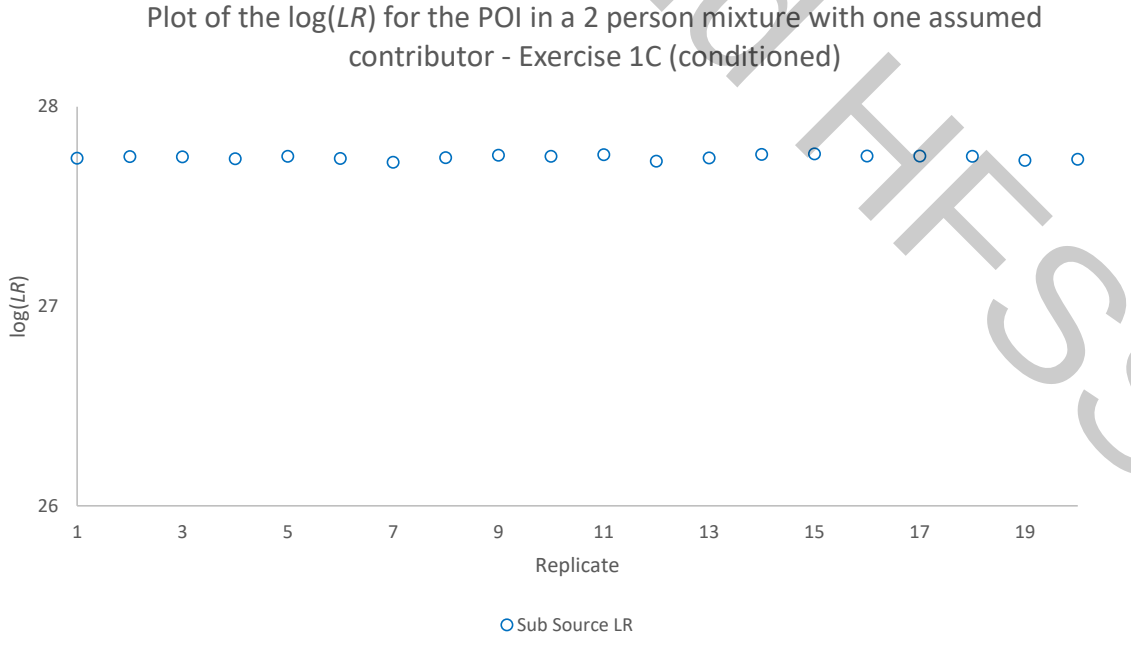


Figure 4.7 Plot of log(*LR*) for the person of interest in an equal proportion mixture with one assumed contributor (Exercise 1C - conditioned). As expected there is very little variability within the *LR* when conditioning on one of the contributors to this two person mixture.

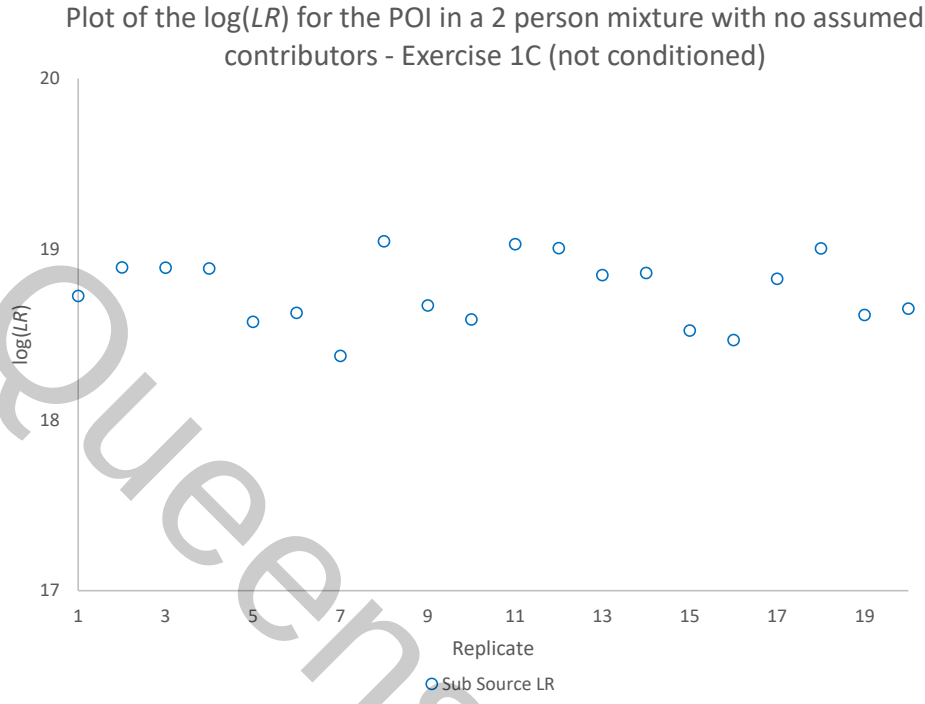


Figure 4.8 Plot of log(*LR*) for the person of interest in an equal proportion mixture with no assumed contributors (Exercise 1C - not conditioned). As expected there is more variability in the *LR* when there is no assumed contributor in this two person mixture and the mixture proportions are nearly equal. The variability is still within the expected one order of magnitude difference.

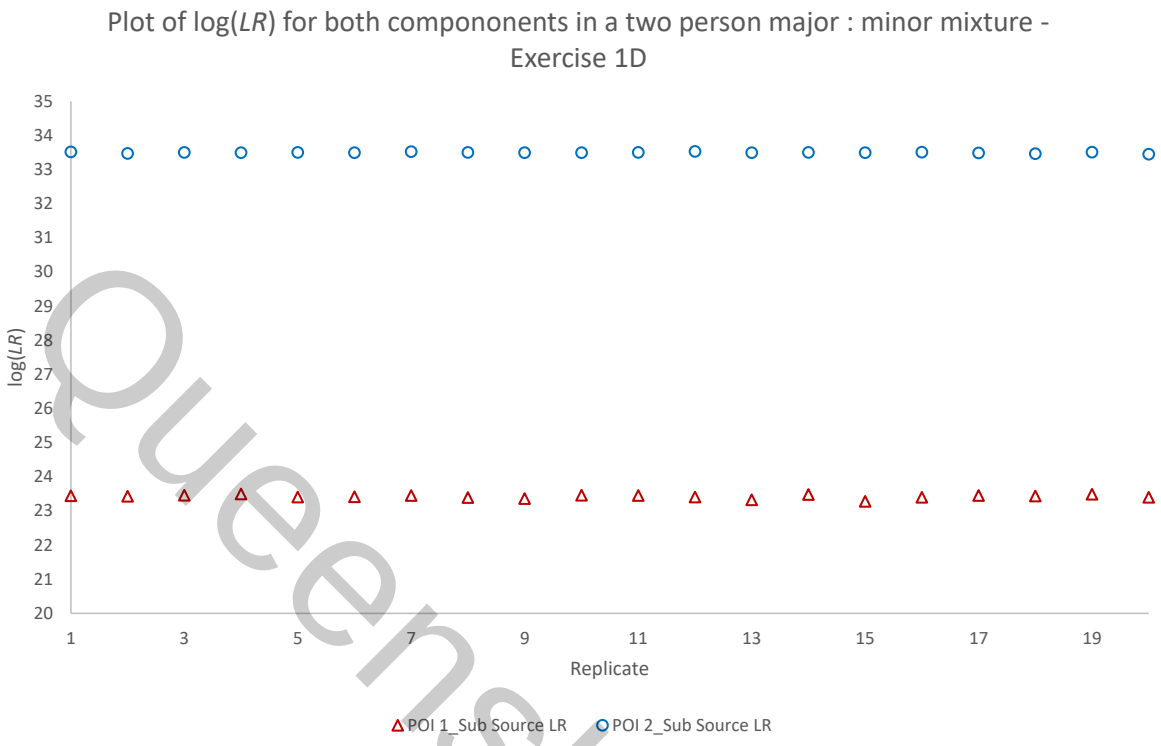


Figure 4.9 Plot of log(*LR*) for each component in a major:minor 2 person mixture (Exercise 1D). As this mixture can be resolved into clear major and minor components, there is very little variability in the *LR* and it is well within one order of magnitude difference.

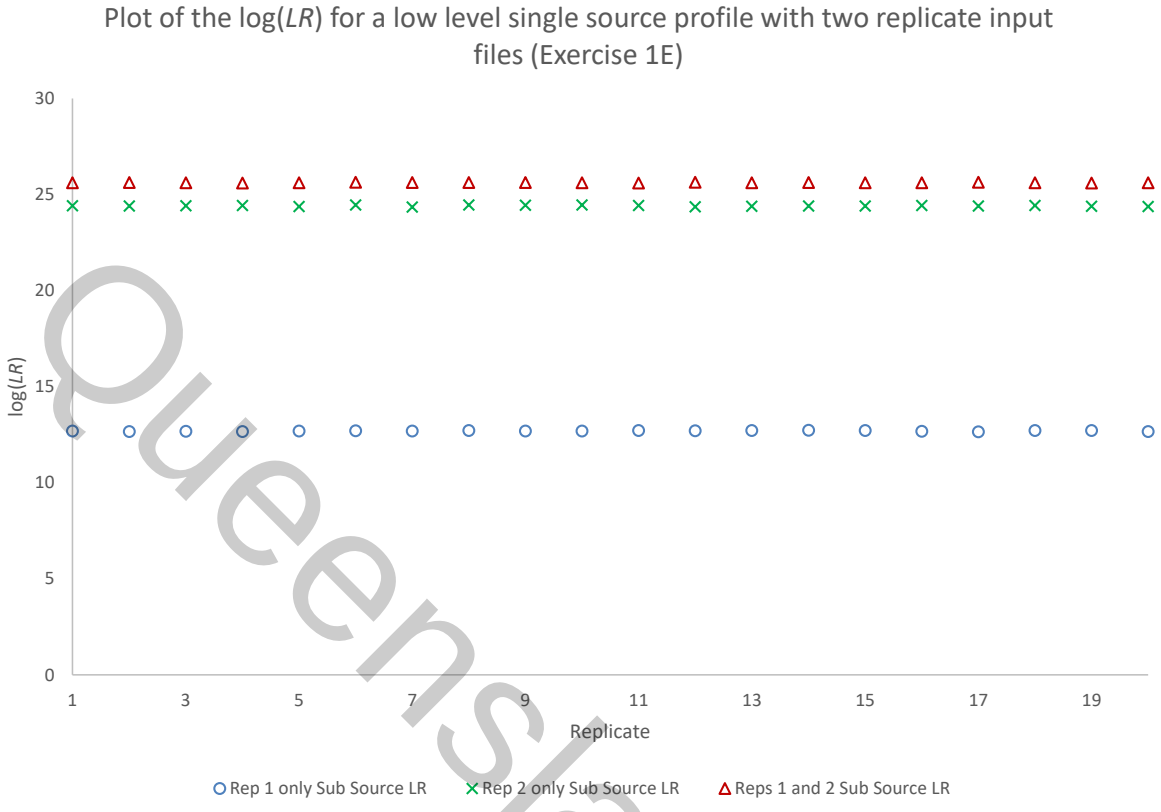


Figure 4.10 Plot of log(*LR*) for the first and second replicates individually and combined for a low level single source DNA profile - Exercise 1E. There is very little variability in the *LR* and the results are well within one order of magnitude difference.

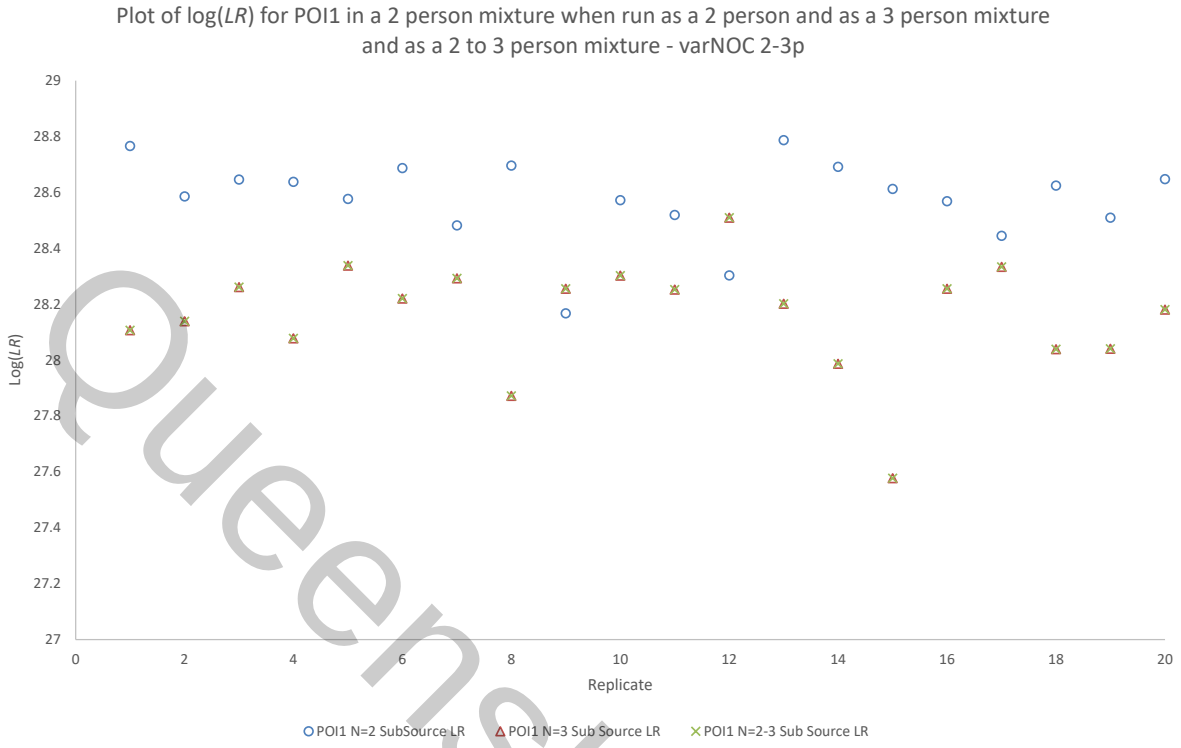


Figure 4.11 Plot of log(*LR*) for one person of interest in a 2 person mixture where the sample was run in STRmix™ v2.8 with a variable number of contributors (varNOC 2-3p). Variability within each of the three plotted *LR*s is within one order of magnitude difference.

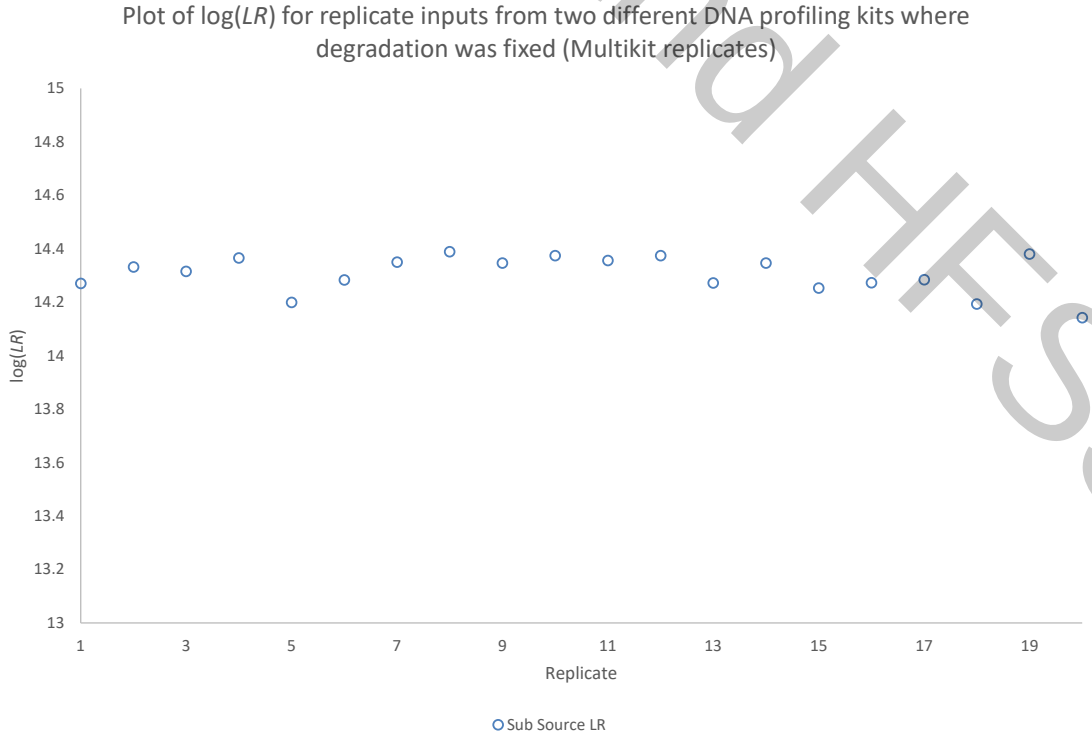


Figure 4.12 Plot of the log(*LR*) for a single source DNA profile generated in two different DNA profiling kits - Multikit replicates. As expected, the *LR* is well within one order of magnitude of difference.

4.25. Sensitivity and Specificity

A demonstration of the sensitivity and specificity for a range of mixtures interpreted in STRmix™ V2.8 was undertaken as per Taylor [2]. With respect to interpretation methods, sensitivity is defined as the ability of the software to reliably resolve the DNA profile of true contributors within a mixed DNA profile for a range of starting DNA templates. The log(*LR*) for contributors (H_p true) should be high and should trend to 0 as less information is present within the profile. Information includes the amount of DNA from the contributor of interest, conditioning profiles (for example the complainant's profile on intimate samples), replicates and decreasing numbers of contributors (i.e. reduced profile complexity).

Specificity is defined as the ability of the software to reliably exclude non-contributors (H_d true) within a mixed DNA profile for a range of starting DNA templates. The log(*LR*) should be low and trend upwards to 0 as less information is present within the profile.

Specificity and sensitivity were tested by assigning the *LR* for a number of one-, two-, three-, four-, and five-person profiles for both true contributors and non-contributors. The plots in [2] have been reproduced for these interpreted profiles. The contributors include homozygote and heterozygote alleles and there is varying amounts of allele sharing across the different loci. Given the template amounts allele and/or locus dropout was expected to occur within the profiles containing the lower DNA amounts. A summary of the 323 profiles interpreted is given in the table below.

Each profile was interpreted in STRmix™ and the results were compared to a database containing the true contributors and non-contributors using the Database Search function within STRmix™. The non-contributors were artificially generated.

Using the FBI Extended Caucasian allele frequencies and an F_{ST} of 0, a sub-source point estimate *LR* was assigned where the propositions considered were:

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

H_d: The DNA originated from *N* unknown unrelated individuals

Where *N* is the experimental number of contributors to the profile.

Table 4.13: Counts of the profiles interpreted.

Dataset	Database size (n_{db})	Number of samples for each experimental design *N*					Number of Samples (*n*)	Number of *LR*s		
		1	2	3	4	5		H_p	H_d	All
ProvedIt_GF_A	250	0	6	6	6	6	24	84	5916	6000
ProvedIt_GF_B	1050	0	10	10	10	10	40	140	41860	42000
ProvedIt_GF_Conditioned	250	0	7	11	12	17	47	180	11570	11750
ProvedIt_GF_SS	250	145	0	0	0	0	145	145	36105	36250
QIAGEN_Investigator	5	0	35	0	0	0	35	70	105	175
Fusion	230	0	11	11	10	0	32	95	7265	7360
Total		145	69	38	38	33	323	714	102821	103535

Plots of the log(*LR*) versus the average peak height (*APH*) per contributor for the single-source profiles and the two-, three-, four-, and five contributor mixtures are given in the figures below. *APH* was calculated using unmasked, unshared and non-stutter affected alleles for each contributor in the crime scene profile. Where the contributor had completely dropped out of the mixture, an *APH* of half the analytical threshold was applied. The per contributor amount of DNA for H_d true contributors is taken as the lowest APH of the known contributors.

Exclusions (*LR* = 0) are plotted as log(*LR*) = -40. The results of all comparisons are provided below for each *N*.

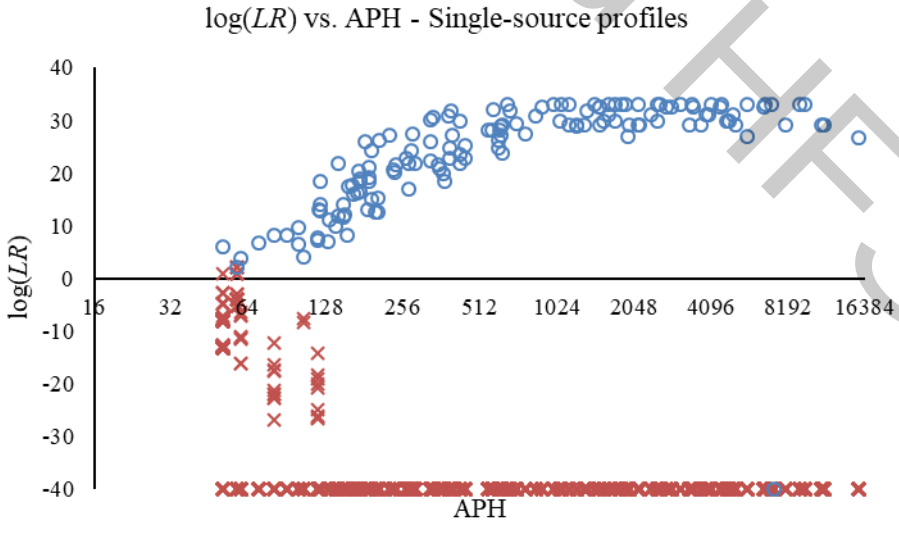


Figure 4.13: Scatter plot of the log(*LR*) vs. APH for single-source profiles. *LR*s for true donors are plotted as blue circles, *LR*s for non-contributors are plotted as red crosses.

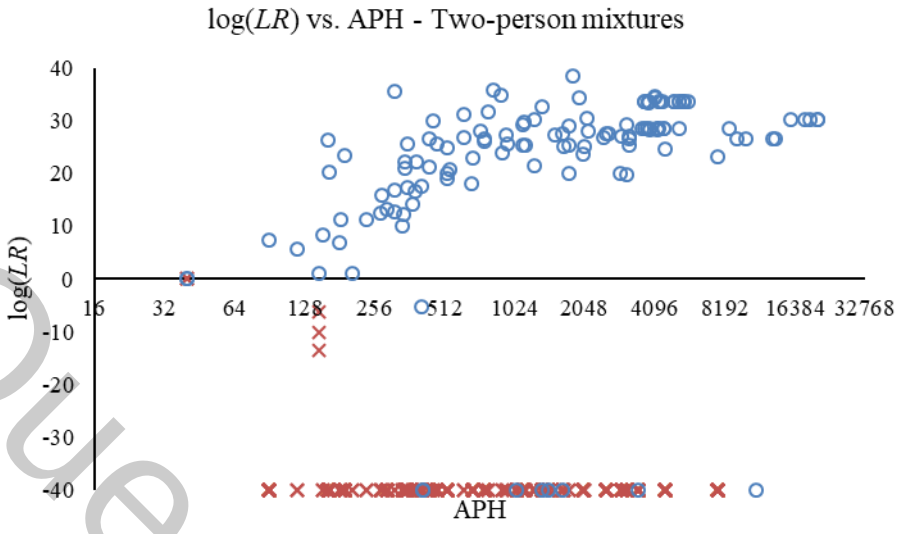


Figure 4.14: Scatter plot of the log(*LR*) vs. APH for two-person profiles. *LR*s for true donors are plotted as blue circles, *LR*s for non-contributors are plotted as red crosses.

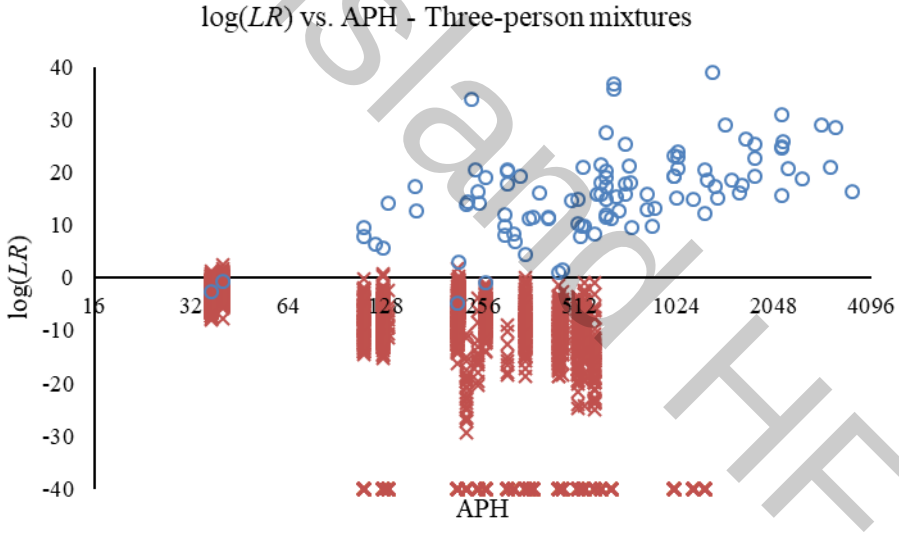


Figure 4.15: Scatter plot of the log(*LR*) vs. APH for three-person profiles. *LR*s for true donors are plotted as blue circles, *LR*s for non-contributors are plotted as red crosses.

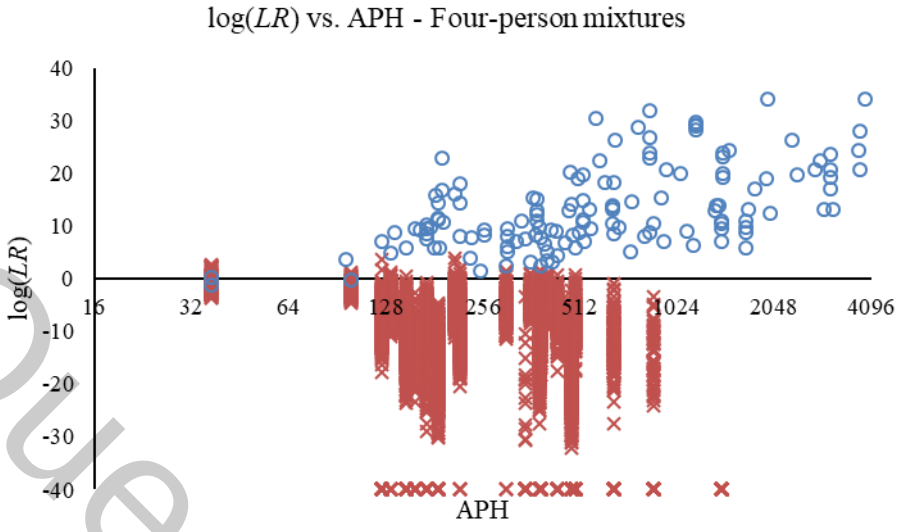


Figure 4.16: Scatter plot of the log(*LR*) vs. APH for four-person profiles. *LR*s for true donors are plotted as blue circles, *LR*s for non-contributors are plotted as red crosses.

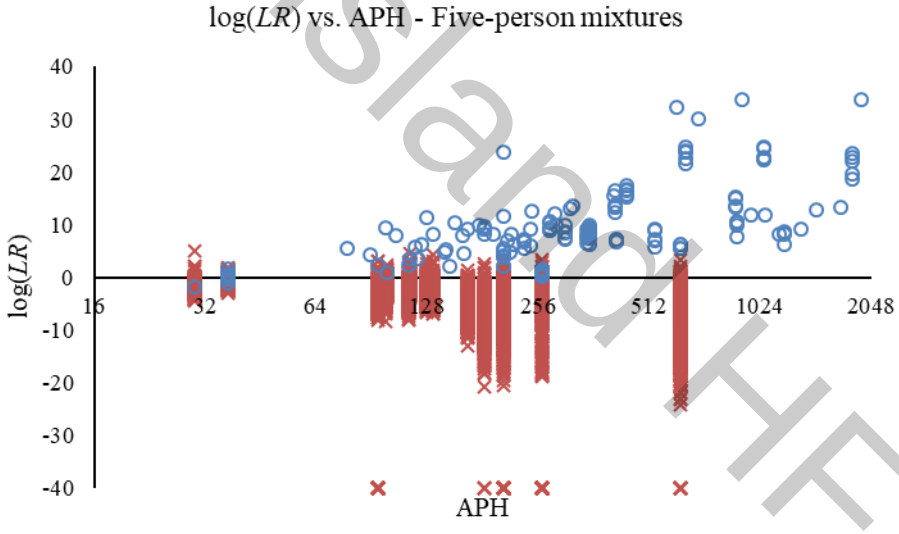


Figure 4.17: Scatter plot of the log(*LR*) vs. APH for five-person profiles. *LR*s for true donors are plotted as blue circles, *LR*s for non-contributors are plotted as red crosses.

Inspection of the plots shows that as template increases the log(*LR*) distributions for H_p true and H_d true are well separated for the single-source profiles and the two-, three-, four-, and five-person mixtures. As the amount of template decreases, H_p true and H_d true converge on log(*LR*) = 0. This is intuitive because as profile information is reduced (i.e. template) we would expect our statistic to reliably inform us of such (i.e. tend towards an *LR* of 1 = uninformative). We also see that as the number of contributors to the profile increases (in the plots with the scaled X axes), the log(*LR*)s for H_p true and H_d true tend towards 0. This is also intuitive because as profiles become more complex we (generally) expect there to be more genotype combinations possible, leading to a spread of the genotype weights and resulting in lower *LR*s compared to simpler profiles with less ambiguity.

At high template STRmix™ correctly and reliably gave a high *LR* for true contributors and a low or exclusionary *LR* for false contributors. At low template and high contributor number STRmix™ correctly and reliably reported that the analysis of the sample tends towards uninformative or inconclusive.

There were nine exclusions (*LR*=0) assigned to known contributors. Six of these exclusions are expected, as they have been identified and diagnosed in other validations. These were due peaks in the profile approaching the saturation threshold which resulted in elevated posterior mean stutter variances.

One of these exclusions was due to a saturated peak that was not labelled in the input file. When this peak was added back into the input file and reinterpreted the *LR* increased to 8.089×10^{30}.

The two remaining exclusions were due to a single-locus exclusion from the interpretation of the same profile and same contributor. These exclusions were a result of the reference alleles dropping out of the profile. This meant that the dropout of two alleles is needed to be accepted during the deconvolution to produce an inclusionary *LR*.

5. REFERENCES

[1] T.R. Moretti, L.I. Moreno, J.B. Smerick, M.L. Pignone, R. Hizon, J.S. Buckleton, J.-A. Bright, A.J. Onorato, Population data on the expanded CODIS core STR loci for eleven populations of significance for forensic DNA analyses in the United States, Forensic Science International: Genetics 25 (2016) 175-181.
[2] D. Taylor, Using continuous DNA interpretation methods to revisit likelihood ratio behaviour, Forensic Science International: Genetics 11 (2014) 144-153.