STRmix™ 2.6.2 Internal Functional and Reliability Testing

October 2019

Introduction

STRmix version 2.6.2 validation was conducted in conjunction with PBSO's validation of PowerPlex® Fusion 6C. The validation for STRmix was conducted as a collaboration between the Palm Beach County Sheriff's Office Forensic Biology Unit (FBU) and the Institute of Environmental Science & Research (ESR) in New Zealand. As part of the validation, the FBU conducted internal function and reliability testing of STRmix v2.6.2. Functional testing was conducted to confirm that the software performs the tasks as expected whereas; reliability testing was performed to ensure that the software works appropriately in the laboratory environment. Supporting data may be found in the STRmix validation companion binder.

Methods

Thirteen two person mixture samples from five female donors and four male donors with Auto:Y ratios ranging between 3.2- 27.6 were amplified and typed with PowerPlex Fusion 6C, analyzed with GeneMapper ID-X version 1.5, and STRmix version 2.6.2 (Windows 10). The samples selected for analysis were extracted with the Casework Direct Kit in 400 µl, 200 µl, and 100 µl and amplified with a DNA target of 0.8 ng.

One sample previously amplified with PowerPlex Fusion 5C (YP vag swab 5 PC) was analyzed with GeneMapper IDX v1.5, and deconvoluted in STRmix version 2.4.08. The corresponding donor reference samples (one male and one female) were amplified and typed with both Fusion 5C and 6C using the Forensic Biology Unit's validated methods. The LR from previous function was used in both STRmix version 2.4.08 and STRmix version 2.6.2 (Windows 10) to calculate likelihood ratios (LR). Likelihood ratios were calculated using the following hypotheses:

$$\frac{H1: Female\ Reference\ and\ one\ unknown\ contributor}{H2: Two\ unknown\ contributors}$$

$$\frac{H1: Male\ Reference\ and\ one\ unkown\ contributor}{H2\ Two\ unknown\ contributoirs}$$
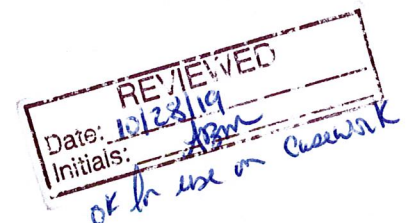
The parameters for analysis with Fusion 6C developed during internal validation and the STRmix 2.6.2 parameters developed during internal validation were used.

The male and female donor profiles were compared to the contributor profiles developed from STRmix. The weights and the likelihood ratios (LR) developed by STRmix for the contributor genotypes were evaluated. The 99%1-sided HPD is reported for all likelihood ratios in the evaluation summary.

Results and Conclusions

Table one summarizes the data analysis from the 13 two person mixtures. The software utilizing was able to deconvolute the mixtures and conduct likelihood ratios. As expected, likelihood ratios increased when all alleles from each contributor were. Low Auto:Y ratios produced a higher number of male alleles detected than the larger Auto:Y ratios. As the Auto:Y ratios increased, the male contributor proportion decreased. Likelihood ratios obtained from STRmix ranged from seven hundred sixty quadrillion for the 27.6 Auto:Y ratio to one nonillion eight hundred octillion for the 3.2 Auto:Y ratio.

Table 1. Summary of Auto:Y ratio evaluation using STRmix version 2.6.2

Sample Name	Auto:Y Ratio	STRMix Proportions	Unambigous Male alleles-IDX	Male alleles> 99% STRmix	LR**
YP_Vag Swab-5PC_1	3.2	69%:31%	32/32	32/32	1.80E+30
YP Vag_1_1_2	4.07	76%:24%	32/32	32/32	1.90E+30
D3_4_24HRS_19	11.15	90%:10%	32/32	31/32	2.15E+25
Neat_ys_17	11.329	89%:11%	32/32	32/32	2.94E+29
*NP_07 Boxers_3ys_17	12.7	82%:18%	19/21	17/21	7.00E+27
SSM1_64_8_K100	13.6	92%:8%	31/31	25/31	7.00E+28
TP_12_24hr_0.2_ys_16	17.27	93%:7%	28/33	21/33	2.00E+25
Swab_4_ys_18	18.4	94%:6%	23/27	21/27	9.24E+28
YP_Vag_1_64_8	22.04	94%:6%	30/32	23/30	2.49E+26
Swab_1_ys_17	27.61	96%:4%	20/27	15/27	7.60E+17
Swab_2_YS_15	13.45	92%:8%	27/27	21/27	5.40E+20
Swab_8_YS_13	11.0	90%:10%	26/27	24/27	1.69E+27
YP_vag 1_16_6	11.2	91%:9%	32/32	28/32	4.12E+27

Includes amelogenin
* References have PP16 data only-
no stats calculated for Penta E and
Penta D
** Lowest LR reported

STRmix version 2.6.2 was also able to calculate likelihood ratios from samples previously typed with Fusion 5C and deconvoluted with STRmix version 2.4.08. Table 2 summarizes the results of the likelihood ratios calculated for the sample YP vag swab 5 PC. The results show that similar likelihood ratios are calculated between the two software versions as well all between references analyzed with Fusion 5C or 6C in STRmix version 2.6.2. The likelihood ratios calculated were all within one order of magnitude.

Table 2. Comparison of likelihood ratios from STRmix version 2.4.08 and STRmix version 2.6.2

YP vag swab 5C	Caucasion 2.4.08	African American 2.4.08	Hispanic 2.4.08	Caucasion 2.6.2 5C reference	African American 2.6.2 5C reference	Hispanic 2.6.2 5 C Reference	Caucasion 2.6.2 reference re-run with 6C	Reference re-run with 6C African American 2.6.2	Reference re-run with 6CHispanic 2.6.2
HP: Male reference and one Unknown HD: two unknowns	1.61E+25	6.10E+25	1.17E+25	1.18E+25	4.90E+25	7.55E+24	9.93E+24	5.00E+25	7.42E+24
HP: Female reference and one Unknown HD: two unknowns	1.65E+26	1.05E+29	4.78E+26	7.24E+25	1.10E+29	1.60E+26	7.60E+25	9.84E+28	1.80E+26

Conclusion

Internal testing of demonstrates that the STRmix version 2.6.2 performs the tasks as expected and works appropriately in the laboratory environment. STRmix version 2.6.2 is appropriate for use on casework.