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VALIDATION OF THE VERITIPRO THERMAL CYCLER

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PURPOSE

The purpose of this validation was to assess whether the VeritiPro™ Thermal Cycler (VP) performs similarly to the GeneAmp™ PCR System 9700 (9700) using the PowerPlex® Fusion 5C System (Fusion) and AmpFℓSTR™ Yfiler™ PCR Amplification Kit (Yfiler). Also assessed was whether the product generated by amplification with the VP was sufficiently similar to the 9700 so that no additional STRmix™ validation work would be required for STR profile analysis.

MATERIALS AND METHODS

DNA sample preparation, quantitation, STR amplification, capillary electrophoresis (CE), detection and probabilistic genotyping were performed as described in the VDFS procedure manuals.[1] DNA was purified robotically using the DNA IQ™ System (Promega Corp,. Madison, WI) on the Biomek® NX^P Automation Workstation (Beckman Coulter, Inc., Fullerton, CA). Samples were quantified with the PowerQuant® System (Promega Corp.) using the QuantStudio™ 5 Real-Time PCR System (ThermoFisher Scientific, Waltham, MA). DNA samples were amplified in 0.2mL tubes or 96 well amplification plates using the same cycling parameters as the 9700. For Fusion (Promega): 96°C for 60s; then 94°C for 10s, 59°C for 60s, 72°C for 30s for 28 cycles; then 60°C for 10 min; then 4°C soak and ramp speed set to max. For Yfiler (ThermoFisher Scientific): 95°C for 11 min; then 94°C for 60s, 61°C for 60s, 72°C for 60s for 29 cycles; then 60°C for 80 min; then 4°C soak and ramp speed set to 9600 simulation. Amplified samples (1 µL) were separated on the Applied Biosystems™ 3500xL Genetic Analyzer (ThermoFisher Scientific) and analysis was performed using GeneMapper™ ID-X Software (GMID-X; ThermoFisher Scientific) v1.4 or v1.5.

Sample and substrate types included single source blood samples on fabric, cotton swabs, Whatman® FTA® (FTA) or untreated paper (Cytiva), and buccal and/or saliva samples on Whatman® FTA® paper and buccal collectors (Bode Technology Group Inc.).

Samples amplified on the VP were used to assess sensitivity, reproducibility, and repeatability. Parameters used in casework DNA profile interpretation (stutter, stochastic threshold, peak height ratio) were measured. The results were compared to those observed for half-volume reactions on the on the 3500xL in the VDFS Validation of the Fusion and Yfiler Amplification Systems (9700 data) summary reports.

STRmix™ (ESR, Wellington, NZ) analysis was performed on two-contributor mixtures amplified for Fusion in both the VP and the 9700 thermal cyclers and the subsequent likelihood ratios

[1] http://www.dfs.virginia.gov/documentation-publications/manuals/ (accessed August 23, 2024)

(LR)s for contributors compared. This was designed to provide a quantitative evaluation and comparison of the resulting DNA profiles.

Sensitivity

A dilution series (1 ng-0.0075 ng) was prepared from two DNA sample extracts for Fusion amplification and from two DNA sample extracts for Yfiler amplification. Blood samples on fabric and a buccal sample on FTA paper were used. Diluted samples were quantitated and amplified in quintuplicate using the same master mix in the same VP. Amplified samples were injected for 12s and 24s on the 3500xL and the completeness of the profile was determined.

Reproducibility

One set of dilutions (1 ng-0.0075 ng) for one sample (buccal on FTA) was amplified in two different VPs. Amplified samples were injected for 12s and 24s on the 3500xL and the resulting profiles compared for concordance.

Repeatability

One DNA extract (buccal on FTA) was amplified (0.5ng) in all wells of columns 1, 6 and 12 in the same VP. Amplified samples were injected for 12s and 24s on the 3500xL and the resulting profiles compared for concordance.

Stutter

DNA profiles from 217 Fusion and 28 Yfiler blood and buccal samples representing a range of substrate types were evaluated for N-1, N+1 and N-2 (repeat) stutter (ST), as well as n+/-2 (bp) stutter (Yfiler DYS19 only) using a 24s injection time. A limit of detection of 50 RFU for Fusion and 10 RFU for Yfiler was applied in order to collect sufficient stutter examples. ST proportion was calculated by dividing the peak height of the smaller (apparent stutter) peak by the height of the larger (allele) peak. No off-scale peaks were used to calculate ST, as the height of such peaks cannot be accurately measured. ST was not calculated when two alleles at a locus were one repeat different in size, as the percent N-1 or N+1 ST cannot be decoupled from the height of the allele. ST was also not calculated when alleles were two repeats apart, as the N+1 ST of the shorter allele cannot be decoupled from the N-1 ST of the larger allele, nor was it calculated when alleles were three repeats apart, as the N+1 ST of the shorter allele cannot be decoupled from the N-2 ST of the larger allele. The average ST plus three standard deviations was calculated for each locus.

Stochastic Threshold

DNA extracts from three different samples (blood on fabric or buccal cells on FTA) were diluted over a range of concentrations such that DNA template would be limited during Fusion and Yfiler amplification. The resulting DNA profiles were analyzed for instances of allelic dropout at 12s or 24s, using the limits of detection described in the VDFS procedure manuals.[1] A total of 63 Fusion samples exhibited allelic dropout and these were in the template range of 0.031 ng-0.15 ng. A total of 41 Yfiler samples exhibited allelic dropout at DYS385a/b and these were in the template range of 0.01 ng-0.1 ng. The average RFU value for the

remaining peak where allelic dropout was observed (one allele was above the LOD and one was below or not observed) was tallied. The standard deviation (SD) was measured and 2X the SD was added to the average RFU value to derive the stochastic threshold (STH) for each injection time.

Peak Height Ratios

Peak height ratios were calculated by dividing the peak height of the lower RFU peak by that of the higher RFU peak at heterozygous Fusion loci using seven DNA profiles from 1 ng, 0.5 ng, 0.25 ng and 0.125 ng DNA templates and an additional ten DNA profiles from 0.075 ng and 0.05 ng DNA templates using a 24s injection time. A total of three donors were used and the following samples and substrates included: blood on fabric and buccal samples on FTA paper. The minimum peak height ratios were determined and the average peak height ratios were calculated for each locus.

Concordance

DNA profiles from two NIST-traceable blood samples on untreated paper were developed and compared to those previously developed internally. All DNA profiles used in the sensitivity, reproducibility, repeatability, stochastic, peak height ratio, and STRmix mixture studies, as well as three Fusion and five Yfiler DNA profiles generated for stutter measurements and selected for concordance, were compared to published proficiency test data.

Contamination Assessment

All reagent blanks and amplification controls were evaluated for any instances of unaccounted for alleles.

STRmix

DNA extracts from four different blood samples on fabric were used to generate fifteen two-person mixtures covering ratios that ranged from 1:1 to 1:40. The mixtures were amplified for Fusion in the VP and two different 9700 thermal cyclers. STRmix analysis was performed on the resulting DNA profile data and the subsequent likelihood ratios (LR)s for contributors compared using an unpaired, equal variance, 2-tailed t-test using an $\alpha=0.05$. Microsoft 365 Excel v2406 was utilized for data analysis and t-test comparisons. The following comparisons were set up: VP and two different 9700 instruments, VP and one 9700 instrument, two different 9700 instruments, and consecutive amplifications in the same 9700. For each comparison, the same DNA mixture samples and Fusion amplification master mix were used so as to minimize the introduction of other variables.

RESULTS

Sensitivity and Reproducibility

A complete or nearly complete Fusion profile was obtained with 0.125 ng of template for sample 1 (Table 1). This was similar to the sensitivity obtained with the 9700 (data not shown). Comparable results were observed for sample 2, except that the profiles observed at 0.125 ng were

partial. This dilution appears overestimated when the targeted quantity is compared to that measured (0.125 ng versus 0.093 ng). The results were reproducible when VP 2 was used and are similar to those generated for sample 1 at 0.062 ng. The template quantities at which artifacts were observed were similar to the 9700, with artifacts such as pull-up and raised baseline observed in the 24s data at 1 ng, and to a lesser degree at 0.5 ng. Little to no results were developed for samples in the 0.062 ng or less template range. This differed from the 9700 validation data where it was possible to obtain approximately 50% of the DNA profile at 0.03 ng template and approximately 10% of the profile at a target of 0.015 ng using the positive control, 2800M, measured internally. The dilutions in the 0.062 ng and lower range appear overestimated when the targeted quantities are compared to that measured after dilution. In addition, there is increased variability in quantitation results in this range.

A complete or nearly complete Yfiler profile was obtained with 0.125 ng of template for samples 1 and 2 (Table 1). This was similar to the sensitivity obtained with the 9700 (data not shown). At 0.062 ng of template, allele/locus dropout was observed for sample 1 and increased in severity for each subsequent dilution. For sample 2, little to no results were obtained for 0.062 ng and 0.031 ng DNA templates. This differed from the 9700 validation data where it was possible to obtain a useful partial profile (alleles at 4 or greater Y-STR loci) with as little as 0.031 ng input DNA. The results were reproducible when VP 2 was used. The dilutions for the 0.031 ng and lower range appear overestimated for sample 2 when the targeted quantities are compared to that measured after dilution. The template quantities at which artifacts were observed were similar to the 9700, including the occurrence of pull-up, raised baseline, elevated stutter, and off-scale data at 1ng, and to a much lesser degree at 0.5 ng.

Since the sensitivity observed was less than expected for DNA targets in the 0.062ng and less range for both Fusion and one Yfiler samples, additional sample data (as described in stochastic study) were evaluated. Sample 3 and additional dilutions of sample 2 were amplified in replicate at various target amounts (see Table 2). Quantitation was only performed prior to preparing the dilutions. Partial Fusion profiles were developed at 0.075 ng target template (43-44 out of 44 alleles for sample 2; 39-42 out of 43 alleles for sample 3) and at 0.05 ng target template (41-44 out of 44 alleles for sample 2; 36-41 out of 43 alleles for sample 3) at 1 µL and 24s injection time. The completeness of the profiles for these dilutions were more similar to the 9700 sensitivity observed for template quantities in this range.

Using 1 µL and 24s injection time, a complete or nearly complete Yfiler profile was obtained for both samples when the template amount was 0.075 ng or greater. Allelic dropout was observed at 0.05 ng template and increased in severity for each subsequent dilution, with the exception of sample 2, 0.0125 ng dilution, where the results were similar to those generated for sample 2 at 0.02 ng. These results were more similar to the 9700 sensitivity observed for template quantities in this range, including a useful partial Y-STR profile for both samples with 0.037 ng input DNA.

As expected for both Fusion and Yfiler amplified samples, more complete profiles were obtained at the lower templates when the 24s CE injection conditions were used versus the 12s.

Repeatability

The same Fusion/Yfiler profile was obtained in all 24 VP wells used (Table 1; data not shown).

DNA template quantity (ng)			VP 1				VP 2		DNA template quantity (ng)			VP 1				VP 2	
Target	Measured (Autosomal)		Sample 1		Sample 2		Sample 2		Target	Measured (Y)		Sample 1		Sample 2		Sample 2	
	Sample 1	Sample 2	12s	24s	12s	24s	12s	24s		Sample 1	Sample 2	12s	24s	12s	24s	12s	24s
1.0	1.1	1.2	43		44		44		**1.0**	1.0	1.0	17		17		17	
0.5	0.6	0.7	43		44		44		**0.5**	0.5	0.5	17		17		17	
0.25	0.36	0.33	43		44		44		**0.25**	0.27	0.30	17		17		17	
0.125	0.193	0.093	42-43	43	0-1	1-4	0	3	**0.125**	0.148	0.117	17		16-17	17	17	
0.062	0.049	0.027	0	0-1	0		0		**0.062**	0.082	0.072	16-17	17	0-1	0-2	0	
0.031	0.022	0.013	0	0-1	0		0		**0.031**	0.038	0.025	11-15	16-17	0		0	
0.015	0.008	0.006	0		0		0		**0.015**	0.019	0.010	0-1	0-3	0	0-1	0	
0.0075	0.003	0	0		0		0		**0.0075**	0.0075	0.0070	0	0-1	0	0-1	0	

Table 1. VP Sensitivity and Reproducibility.
Completeness of profile obtained (Left-Fusion, Right-Yfiler) showing number of alleles at various DNA template amounts and 12s and 24s injection times. For samples amplified in quintuplicate (VP1), the range of results obtained is displayed (for example, at the 0.031 target, Sample 1 results ranged from 11 to 15 loci). Full profile=green, partial profile=gray, no results=red.

Target DNA template quantity (ng)	Fusion				Yfiler			
	Sample 2		Sample 3		Sample 2		Sample 3	
	12s	24s	12s	24s	12s	24s	12s	24s
0.15	44		41-43	43	17		14-17	16-17
0.125	43-44	44	33-43	42-43	NT			
0.1	42-44	44	35-38	41-43	17		13-15	14-16
0.075	38-44	43-44	22-36	39-42	17		8-15	15-17
0.05	27-39	41-44	19-26	36-41	14-17	17	2-8	11-13
0.037	NT				15-16	16-17	5-8	7-14
0.025					8-17	14-17	0-2	1-2
0.02					1-7	5-13	NT	
0.0125					5-10	10-15		
0.01					1-5	1-8		

Table 2. Additional VP sensitivity assessment.
Completeness of profile obtained (Left-Fusion, Right-Yfiler) at various DNA template amounts and 12s and 24s injection times is displayed. The range of results obtained is shown as described for Table 1. Fusion: n=5; Yfiler: n=5-13, depending on sample and target. Full profile=green, partial profile=gray, no results=red, NT=not tested.

Stutter

The derived N-1, N+1, and N-2 (repeat) Fusion stutter threshold values (average +3SDs) are shown in Table 3, along with the existing casework stutter thresholds (9700 ST). The vast majority of Fusion stutter values measured with the VP (58/69 measurements) were similar to and did not exceed 9700 ST. Several Fusion loci (D2S441, D16S539, Penta D, TH01, and D8S1179 at N-1; D1S1656 and

FGA at N+1; D18S51, D2S1338, D5S818 and D12S391 at N-2) did display larger ST values, but all were within 1% of 9700 ST. In addition, >99% of the individual ST peaks observed on the VP (4500/4517 for N-1, 577/581 for N+1, and 253/254 for N-2) fell below 9700 stutter thresholds.

	N-2			N-1			N+1		
Locus	**9700**	**VP**	**n**	**9700**	**VP**	**n**	**9700**	**VP**	**n**
D3S1358	2	1.6	37	13	12.5	186	2	1.5	20
D1S1656	2	1.9	18	14	13.3	307	3	3.2	47
D2S441	2*	N/A	0	8	8.5	240	2	1.6	22
D10S1248	2	1.2	17	13	12.3	176	3	2.1	6
D13S317	1	0.7*	2	10	10.0	179	4	2.9	23
Penta E	N/A	N/A	0	7	6.7	145	3	0.9*	1
D16S539	3	1.5	14	11	11.1	171	2	1.9	58
D18S51	2	2.6	40	15	14.3	254	5	4.8	57
D2S1338	2	2.2	22	14	13.7	291	9	3.6	5
CSF1PO	1	0.4*	1	10	9.6	146	4	3.4	21
Penta D	2*	N/A	0	4	4.1	102	4	N/A	0
TH01	2	0.8*	1	5	5.6	133	5	N/A	0
vWA	2	1.3*	4	14	12.9	189	5	1.9	6
D21S11	3	1.6	18	12	12.0	272	4	2.5	65
D7S820	2	0.5*	1	12	9.2	177	5	1.9	5
D5S818	N/A	0.6*	2	10	9.1	144	3	2.4	33
TPOX	2*	N/A	0	6	5.8	148	3	1.0*	2
DYS391	2*	N/A	0	9	8.8	105	2	1.4*	1
D8S1179	2	1.7	37	11	11.1	226	3	1.7	57
D12S391	3	3.2	18	18	16.9	281	5	2.4	3
D19S433	3	3.0	7	12	11.9	212	4	1.4*	1
FGA	3	1.1	6	12	11.1	251	4	4.4	11
D22S1045	3	2.7	9	16	15.4	182	9	8.5	137

Table 3. Fusion stutter measured on VP.
Fusion stutter threshold values (average stutter (%) + 3SDs) measured using 24s injection time (blue). 9700 ST are shown for comparison (green). *=fewer than 5 observations; max. value observed. n=number of observations.

The derived N-1, N+1, and N-2 (repeat) Yfiler stutter threshold values (average +3SDs) are shown in Table 4, along with the existing casework 9700 ST. The vast majority of Yfiler stutter values measured with the VP (40/48 measurements) were similar to and did not exceed 9700 ST. One Yfiler locus, Y_GATA_H4, displayed a larger N-1 ST value, but was within 1% of 9700 ST. N-1 ST values were slightly higher than the 9700 at DYS437 (11.4% vs. 9%) and DYS438 (7.6% vs. 6%); however, >99% of the individual N-1 ST peaks observed on the VP (403/406) fell below 9700 ST.

Two Yfiler loci displayed a slightly larger N+1 stutter threshold value (average +3SDs) on the VP compared to the 9700 ST (3.8% vs. 1% at DYS389I and 5.8% vs. 4% at DYS389II). For two of the samples displaying individual DYS389I N+1 ST peaks above the 9700 ST, both samples also displayed DYS389II N+1 ST above 9700 ST. Greater than 95% of the individual N+1 ST peaks observed on the VP (134/140) fell below 9700 ST.

Two Yfiler loci, DYS458 and DYS19, displayed larger N-2 stutter threshold values (average +3SDs), but both were within 1% of 9700 ST. DYS390 displayed a much larger N-2 stutter threshold value compared to the 9700 ST (7.9% vs. 2%). This was due to one sample where the N-2 stutter peak was 5.9%. Upon reamplification of the sample, the N-2 stutter peak was observed but below 9700 ST. Greater than 95% of the individual N-2 ST peaks observed on the VP (89/92) fell below 9700 ST.

	N-2			N-1			N+1		
Locus	**9700**	**VP**	**n**	**9700**	**VP**	**n**	**9700**	**VP**	**n**
DYS456	2	1.1	23	16	13.3	28	4	3.8	28
DYS389I	1	0.6*	3	10	8.9	28	1	3.8	5
DYS390	2	7.9	6	14	13.2	28	3	0.6*	1
DYS389II	4	2.0	24	23	18.3	28	4	5.8	24
DYS458	2	2.5	18	17	13.2	28	5	2.1	17
DYS19	1	1.2*	3	11	10.4	28	3	2.0	14
DYS385a/b	2	1.5*	4	20	14.4	34	3	2.6	7
DYS393	3	2.3*	4	13	12.6	23	3	2.4	10
DYS391	2	1.1*	3	11	10.2	28	2	1.0*	2
DYS439	1	N/A	0	11	10.7	28	5	1.7*	3
DYS635	3	1.2*	1	13	11.7	28	6	3.3*	1
DYS392	4	1.7*	3	20	17.7	28	11	8.3	26
Y_GATA_H4	2	N/A	0	11	11.8	25	3	N/A	0
DYS437	2	N/A	0	9	11.4	19	3	2.8*	2
DYS438	2	N/A	0	6	7.6	12	2	N/A	0
DYS448	1	N/A	0	6	5.7	13	2	N/A	0

Table 4. Yfiler stutter measured on VP.
Yfiler stutter threshold values (average stutter (%) + 3SDs) measured using 24s injection time (blue). 9700 ST are shown for comparison (green). *=fewer than 5 observations; max. value observed. n=number of observations.

The derived n+/-2 (bp) Yfiler stutter threshold values (average +3SDs) at DYS19 are shown in Table 5, along with the existing casework 9700 ST. Both Yfiler stutter values measured with the VP were similar to and did not exceed 9700 ST and none of the individual n-2 or n+2 (bp) ST peaks observed on the VP displayed a stutter value above the 9700 ST.

Locus	9700	VP	n
DYS19 n-2 (bp)	13	11.9	28
DYS19 n+2 (bp)	4	3.2	27

Table 5. Yfiler n+/-2 (bp) stutter measured on VP.
Yfiler stutter threshold values (average stutter (%) + 3SDs) measured using 24s injection time (blue). 9700 ST are shown for comparison (green). n=number of observations.

Stochastic Threshold

The STH for Fusion was similar to and slightly less than the 9700 STH for both injection times (Table 6).

The Yfiler data indicated a higher STH than the 9700; however, this was due to one sample which exhibited a high RFU at 12s (231) and 24s (481). When this outlier is removed, the Yfiler VP (155-12s and 292-24s) and 9700 STHs for DYS385a/b were very similar, with the 24s injection time value lower than the 9700 value.

	Fusion		Yfiler (DYS385a/b)	
	12s	24s	12s	24s
n	140	43	23	18
VP STH	**191**	**289**	**183 (155)**	**377 (292)**
9700 STH	**210**	**300**	**150**	**315**

Table 6. Stochastic Threshold Measured on VP.
Fusion and Yfiler stochastic threshold values (average peak height of observed allele (RFUs) + 2SDs) for each injection time (blue). 9700 STHs are shown for comparison (green). n=number of observations.

Peak Height Ratios

The average and minimum heterozygous peak height ratios (PHRs) observed on the VP for samples amplified using 0.125 ng-1 ng Fusion template are shown in Figure 1, along with the average and minimum 9700 PHRs observed for the same template quantities and injection time (24s). The average PHRs were approximately ≥80% at all loci for 0.125 ng-1 ng Fusion templates, which is the same as that observed on the 9700. For both thermal cyclers, minimum PHRs fell below 60% when templates were below 0.5 ng, and remained above 60% for 1 ng and 0.5 ng input quantities, with the exception of one VP sample that showed a PHR=56% at Penta D (genotype 2.2,10) at 0.5 ng DNA template. This is not unexpected when a large repeat number difference is observed between alleles.

At DNA template quantities of 0.075 ng and 0.05 ng, average PHRs were all ≥60%, with the exception of PE (54%) and D22 (58%) for the 0.05 ng template quantity. These results were similar to those obtained for the 9700 using 0.062 ng and 0.031 ng DNA templates, where average PHRs were ≥60% (data not shown). The minimum VP PHRs observed at 0.075 ng and 0.05 ng were 22% and 15%, respectively, and comparable to 9700 values observed for similar DNA template quantities in the

stochastic range (28% at 0.062 ng and 25% at 0.031 ng). PHRs are impacted by the number of repeats separating the alleles, particularly at higher MW loci.

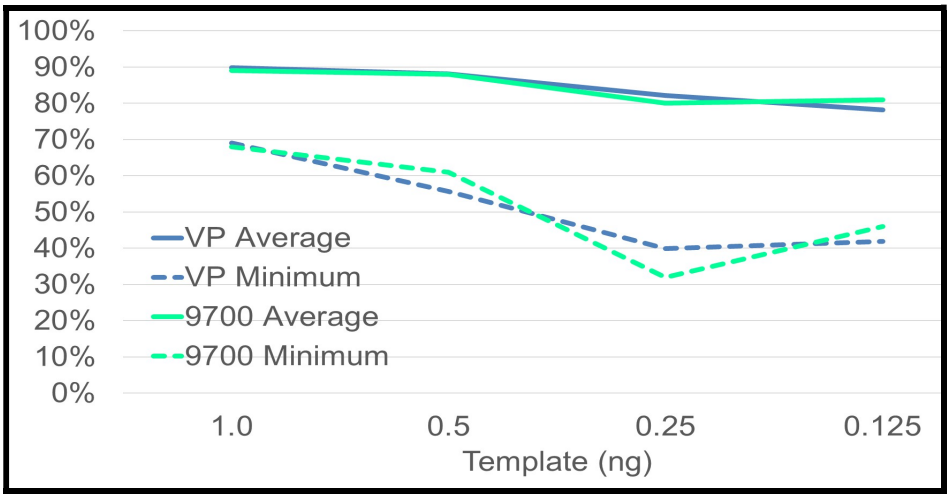

Figure 1. VP peak height ratios. Average and minimum PHRs observed for Fusion templates above the stochastic range and 0.125 ng using a 24s injection time. 9700 PHRs are shown for comparison.

Concordance

Correct profiles were obtained for the NIST-traceable samples and all samples used in the sensitivity, reproducibility, repeatability, stochastic, peak height ratio, and STRmix mixture studies, as well as selected stutter samples.

Contamination Assessment

One reagent blank in the Yfiler stutter set had a single called allele at the 24s injection time that was not reproducible upon reamplification. All remaining reagent blanks, as well as all amplification controls for all data sets, performed as expected with no observable contamination.

STRmix

STRmix analysis was used to quantitatively assess differences between the same two-person mixture samples amplified in the VP and 9700 TCs (Figure 2). Comparisons were made between contributor-specific likelihood ratios (LRs) to evaluate if amplification in the VP generated significantly different LRs when compared to the 9700. Also compared were two different 9700s and consecutive amplifications in the same 9700. No statistically significant differences were observed (VP v 9700.1 and 9700.2, 24s inj. p=0.60 and 0.70, respectively; VP v 9700.1, 12s inj. p=0.97; 9700.1 v 9700.2 p=0.99, 12s inj.; consecutive amplifications in 9700.1 p=0.92, 12s inj.).

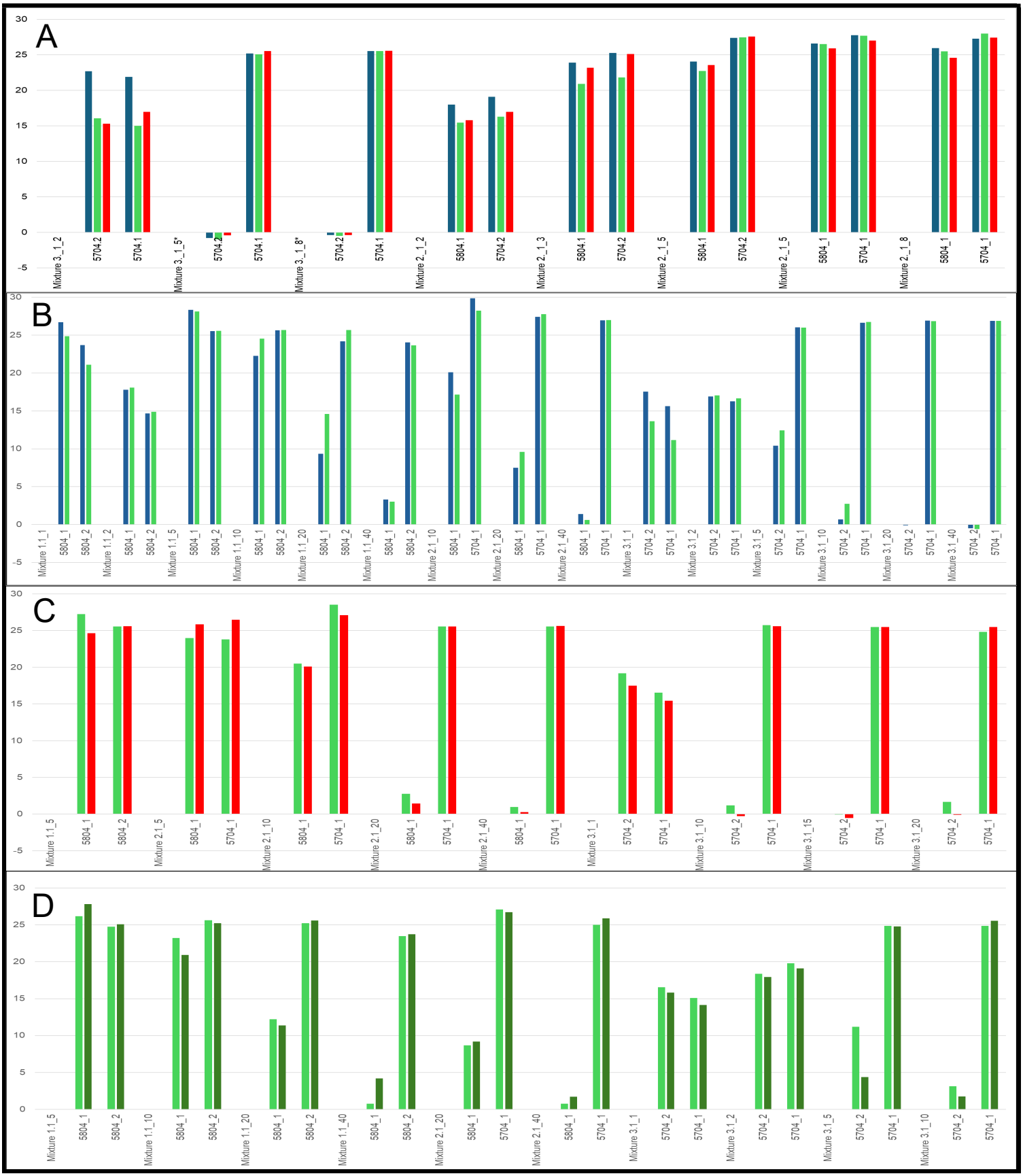

Figure 2. STRmix assessment of two-person mixtures amplified in VP and 9700. Comparison of contributor specific log(LR)s for mixtures amplified in VP, 9700.1 and 9700.2, 24s inj. (Panel A), VP and 9700.1, 12 s inj. (Panel B), 9700.1 and 9700.2, 12 s inj. (Panel C), and consecutively in same 9700, 12 s inj. (Panel D). Different mixtures and contributors are indicated on horizontal axis and log(LR) on vertical axis. blue = VP, green = 9700.1, red = 9700.2

CONCLUSIONS

The results of the sensitivity, reproducibility, repeatability, stochastic threshold, peak height ratio assessments, and STRmix log(LR) comparisons for two-contributor mixtures support an overall conclusion that the VP thermal cycler performs comparably to the 9700 with the Fusion and Yfiler kits. For Yfiler stutter, it is proposed that the following stutter thresholds be increased to reflect the stutter values observed (average plus 3SDs, rounded up): DYS389I (N+1 ST current=1%, proposed=4%); DYS389II (N+1 ST current=4%, proposed=6%); DYS390 (N-2 ST current=2%, proposed=8%). Increasing the stutter threshold at a locus would have the potential to decrease the extent of a minor profile observed. All other Yfiler and all Fusion stutter values measured were comparable to the 9700 and will, therefore, be effectively filtered using current casework stutter thresholds.

The analysis of the STRmix data for the two-person Fusion amplified mixtures supports the conclusion of no statistically significant difference in LRs produced when amplified using the VP and 9700 thermal cyclers. Given this data along with the summarized validation data above, no new validation of the current STRmix software version is needed prior to its use with the VP for casework samples.