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Eastern Laboratory Site-Specific Validation and Performance Check of the VeritiPro™ Thermal Cycler

Prepared in August 2025

PURPOSE

This study was designed to determine sensitivity and repeatability, as well as perform a contamination assessment of the Applied Biosystems VeritiPro™ Thermal Cycler (VP) in the Eastern Laboratory. These assessments also serve as the performance check of the instrument.

MATERIALS AND METHODS

Sensitivity series

Single-source male human genomic DNA (2800M) was quantitated in duplicate at the Central Laboratory using the PowerQuant® System (PQ; Promega, Madison, WI) and the QuantStudio™ 5 Real-Time PCR System (QS5; Thermo Fisher Scientific, Waltham, MA), as described in the VADFS procedures manual.[1] Dilutions were made in the following series (ng/5µL): 1.0, 0.5, 0.25, 0.125, 0.062, 0.031, 0.015, and 0.0075 using PowerQuant® Dilution buffer. The dilution series was re-quantitated using PQ and QS5 to confirm accuracy of the dilutions. The dilutions and reagent blank were then aliquoted and provided to all four labs for testing.

Prior to amplification, each VP instrument (firmware v1.2.1) passed the Self Verification and Block (Cycle) Performance Tests, as specified by the manufacturer. The sensitivity series was amplified in triplicate using a manual PCR setup, and the same master mix was used for each replicate for all three VPs. For example, a single master mix was made and used for Replicate 1 across all three VPs. DNA samples were amplified using the same half-volume reaction cycling parameters as the GeneAmp™ PCR System 9700 (Applied Biosystems, Foster City, CA), defined in the VADFS procedures manual.[1] For the PowerPlex® Fusion 5C System (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 (no simulation). For the AmpFℓSTR™ Yfiler™ PCR Amplification Kit (Yfiler; Thermo Fisher 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.

A 1 µL volume of amplified DNA in the loading cocktail for all samples was separated on the Applied Biosystems 3500xL Genetic Analyzer using a 24s injection time and analysis was performed using Applied Biosystems GeneMapper™ ID-X Software v1.5. The interpretation methods described in the VADFS procedures manual were applied, with these exceptions for the

[1] http://www.dfs.virginia.gov/documentation-publications/manuals/ (accessed March 31, 2025)

stutter filters applied to the Yfiler amplification: DYS389I N+1 stutter=4.0, DYS389II N+1 stutter=6.0, and DYS390 N-2 stutter=8.0.[1]

The percentage of alleles detected in each replicate, as well as the average percentage of alleles and standard deviation at each template target, were calculated for each instrument.

Repeatability

The sensitivity samples were used to assess repeatability. The DNA profiles of all 3 replicates of samples with sufficient template (250 pg and above) amplified in the same instrument were compared for concordance.

Contamination assessment

All samples, reagent blanks and amplification controls were evaluated. Any instances of unaccounted for alleles observed in reagent blanks and amplification controls were handled according to the VADFS procedures manual.[1] The total number of samples and controls exhibiting unexpected results versus total tested was tallied.

RESULTS

Sensitivity

All DNA profiles generated using 2800M produced results concordant with the manufacturer's published data. Tables 1 and 2 display the measured amount of autosomal DNA and the percentage profile obtained for each sample based on the total number of alleles possible. From these data, the average percentage profile obtained and standard deviation at each template target were calculated for each instrument.

A complete Fusion profile was obtained with 0.125 ng of template for all three instruments. Artifacts such as pull-up and raised baseline were observed at 1 ng, and to a lesser degree at 0.5 ng. At 0.062 ng of template, one instance of allelic dropout was observed for one instrument and increased in severity and across all three instruments for each subsequent dilution. An average of 15 alleles (out of 43) were detected when less than 0.015 ng was added to the amplification reaction.

A complete Yfiler profile was consistently obtained across all three instruments with as little as 0.062 ng of template. Artifacts including pull-up, raised baseline, elevated stutter and off-scale data were observed at both 1 ng and 0.5 ng, and to a lesser degree at 0.25 ng. When 0.0075 ng-0.015 ng template was used for amplification, elevated N+1 stutter at DYS389I and DYS389II, including preferential amplification of the N+1 stutter peak at DYS389I in one 0.015 ng replicate for one VP, was observed in the 2800M sample. When amplification of the same samples was repeated on the 9700 thermal cycler, the same artifacts were observed (Central Laboratory, data not shown). At 0.031 ng of template, allele/locus dropout was observed for only one replicate for one VP and generally increased in severity for the subsequent dilutions.

For one of the replicates in VP1, 10 and 12 alleles were observed when 0.015 ng and 0.0075 ng of DNA were amplified, respectively. A useful partial Y-STR profile (ranging from 7-16 called alleles) was obtained for all samples at or below 0.031 ng input DNA, except for one replicate with 0.0075 ng of template DNA for which only 3 alleles were detected above LOD.

The dilutions appear slightly underestimated when the targeted quantities are compared to that measured after dilution (Tables 1 and 2). Fusion and Yfiler sensitivity results were similar to those obtained for template quantities in this range in the VADFS Validation of the VeritiPro™ Thermal Cycler (Central laboratory-VP 1, data not shown).

Repeatability

For DNA quantities that exceed the stochastic range (250 pg and above), concordant Fusion and Yfiler profiles were obtained in replicate amplifications of the same template quantity in the same instrument (data not shown).

Contamination assessment

Contamination was assessed in all samples, reagent blanks and amplification controls. A total of 99 samples processed, which includes 9 reagent blanks and 9 negative and positive amplification controls, were tallied with each amplification kit. No unaccounted-for Fusion alleles were observed in 99 amplified samples. No unaccounted-for alleles were observed in 99 Yfiler samples.

Table 1. **Fusion sensitivity.** Percentage of 2800M profile obtained, average and standard deviation at different template quantities using the VP (24s injection time). Post-dilution DNA estimate was based on autosomal value measured.

DNA amplified-target (ng)	DNA estimate post-dilution (ng)	VP 1					VP 2					VP 3				
		Replicate			Avg.	SD	Replicate			Avg.	SD	Replicate			Avg.	SD
		1	2	3			1	2	3			1	2	3		
1.0	1.2	100	100	100	100	0	100	100	100	100	0	100	100	100	100	0
0.5	0.6	100	100	100	100	0	100	100	100	100	0	100	100	100	100	0
0.25	0.34	100	100	100	100	0	100	100	100	100	0	100	100	100	100	0
0.125	0.175	100	100	100	100	0	100	100	100	100	0	100	100	100	100	0
0.062	0.102	100	100	100	100	0	100	100	97.7	99	1.3	100	100	100	100	0
0.031	0.044	90.7	97.7	97.7	95	4.0	95.3	100	97.7	98	2.3	95.3	97.7	97.7	97	1.3
0.015	0.021	76.7	72.1	74.4	74	2.3	62.8	74.4	90.7	76	14	67.4	83.7	65.1	72	10
0.0075	0.0134	48.8	27.9	44.2	40	11	32.6	34.9	34.9	34	1.3	23.3	41.9	39.5	35	10

Table 2. **Yfiler sensitivity.** Percentage of 2800M profile obtained, average and standard deviation at different template quantities using the VP (24s injection time). Post-dilution DNA estimate was based on autosomal value measured.

DNA amplified-target (ng)	DNA estimate post-dilution (ng)	VP 1					VP 2					VP 3				
		Replicate			Avg.	SD	Replicate			Avg.	SD	Replicate			Avg.	SD
		1	2	3			1	2	3			1	2	3		
1.0	1.2	100	100	100	100	0	100	100	100	100	0	100	100	100	100	0
0.5	0.6	100	100	100	100	0	100	100	100	100	0	100	100	100	100	0
0.25	0.34	100	100	100	100	0	100	100	100	100	0	100	100	100	100	0
0.125	0.175	100	100	100	100	0	100	100	100	100	0	100	100	100	100	0
0.062	0.102	100	100	100	100	0	100	100	100	100	0	100	100	100	100	0
0.031	0.044	94	100	100	98	3.5	100	100	100	100	0	100	100	100	100	0
0.015	0.021	71	59	71	67	6.9	82	88	76	82	6.0	88	82	71	80	8.6
0.0075	0.0134	65	71	65	67	3.5	53	41	53	49	6.9	65	18	59	47	26

CONCLUSIONS

The sensitivity observed in the Eastern Laboratory assessment for both Fusion and Yfiler amplifications using the VeritiPro thermal cycler was similar to that obtained in the VADFS Validation of the VeritiPro™ Thermal Cycler (conducted using VP 1 in the Central laboratory). Repeated amplifications in the same VP thermal cycler produced concordant results at DNA template amounts that exceed the stochastic range. The contamination assessment demonstrated no observable contamination events for both amplification systems. The VeritiPro™ thermal cycler was demonstrated to be suitable for casework applications in the Eastern Laboratory.