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

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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 Northern 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 VDFS 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 an automated or manual PCR setup, and the same master mix for each VP. 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 VDFS 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 VDFS procedures manual were applied, with these exceptions for the 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]

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

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 VDFS 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 in all samples down to 0.125 ng of template. 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, a complete or nearly complete profile was obtained. Allele/locus dropout was observed in less than half of the samples and increased in severity for each subsequent dilution. As few as 4 alleles were detected when less than 0.015 ng was added to the amplification reaction.

A complete Yfiler profile was obtained in all samples down to 0.062 ng of template. Artifacts including pull-up, raised baseline, elevated stutter, and off-scale data were observed at 1ng and 0.5ng, and to a much lesser degree at 0.25 ng. When 0.0075-0.062ng template was used for amplification, elevated N+1 stutter at DYS389I and DYS389II 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 in half of the samples and generally increased in severity for each subsequent dilution. For one of the replicates in VP2, the same number of alleles (12) was observed when both 0.015 ng and 0.0075 ng of DNA were amplified. A useful partial Y-STR profile was obtained with 0.0075 ng input DNA.

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 VDFS 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 58 samples processed, including 6 reagent blanks, 2 negative and 2 positive amplification controls were tallied with each amplification kit. No unaccounted for Fusion alleles were observed in 58 amplified samples. No instances of unaccounted for alleles were observed in 58 Yfiler samples.

Table 1. **Fusion sensitivity.** Percentage 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**				
		Replicate			**Avg.**	**SD**	**Replicate**			**Avg.**	**SD**
		1	**2**	**3**			**1**	**2**	**3**		
1.0	1.2	100	100	100	100	0	100	100	100	100	0
0.5	0.6	100	100	100	100	0	100	100	100	100	0
0.25	0.34	100	100	100	100	0	100	100	100	100	0
0.125	0.175	100	100	100	100	0	100	100	100	100	0
0.062	0.102	98	100	98	98	1.3	100	100	100	100	0
0.031	0.044	95	91	95	94	2.7	93	84	95	91	6.2
0.015	0.021	53	70	47	57	12	58	65	65	63	4.0
0.0075	0.0134	9.3	35	12	19	14	23	30	47	33	12

Table 2. **Yfiler sensitivity.** Percentage 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				
		Replicate			Avg.	SD	Replicate			Avg.	SD
		1	2	3			1	2	3		
1.0	1.2	100	100	100	100	0	100	100	100	100	0
0.5	0.6	100	100	100	100	0	100	100	100	100	0
0.25	0.34	100	100	100	100	0	100	100	100	100	0
0.125	0.175	100	100	100	100	0	100	100	100	100	0
0.062	0.102	100	100	100	100	0	100	100	100	100	0
0.031	0.044	94	94	100	96	3.4	100	100	94	98	3.4
0.015	0.021	53	65	71	63	9.0	71	65	71	69	3.4
0.0075	0.0134	24	53	47	41	16	41	53	71	55	15

CONCLUSIONS

The sensitivity observed in the Northern Laboratory assessment for both Fusion and Yfiler amplifications using the VeritiPro thermal cycler was similar to that obtained in the VDFS 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 Northern Laboratory.