

2021 Internal Validation of Proflex Thermal Cyclers

INTRODUCTION

Per the CBI-FS Quality Manual (QP19), Validation studies may be proposed and conducted under the following circumstances: “On any scientifically accepted testing method or instrumentation not currently being utilized by the CBI-FS.” This internal validation must follow the applicable sections of the FBI Quality Assurance Standards for Forensic DNA Testing Laboratories (July 2020); in addition the SWGDAM validation guidelines (2016) will be referenced. The CBI-FS DNA Section currently uses the validated Applied Biosystems GeneAmp® PCR System 9700 Thermal Cycler to amplify samples and controls. Fifteen new thermal cyclers, the ABI ProFlex™ PCR System, were purchased for use by the casework DNA section for replacement of these instruments. One of the thermal cyclers (D17-96) will undergo the full internal validation and a performance check will be completed on the remaining instruments prior to use.

Laboratory	Instrument	Serial Number Base Unit / Sample Block
Arvada	*D17-96	2978019074532 / 297813561
Arvada	D18-96	2978019084606 / 297813562
Arvada	D19-96	2978019084608 / 297813527
Arvada	D20-96	2978019084613 / 297813531
Arvada	D21-96	2978019084624 / 297813547
Grand Junction	W8-96	2978019084611 / 297813556
Grand Junction	W9-96	2978019084602 / 297813549
Grand Junction	W10-96	2978019084614 / 297813555
NCRFL	N3	2978019074535 / 297813525
NCRFL	N4	2978019084612 / 297813543
NCRFL	N5	2978019084581 / 297813554
Pueblo	9	2978019074588 / 297813546
Pueblo	10	2978019084605 / 297813542
Pueblo	11	2978019084607 / 297813502
Pueblo	12	2978019084601 / 297813536

**Validation instrument*

GOALS

This validation is being performed to determine if the Applied Biosystems ProFlex™ PCR System is an appropriate replacement for the Applied Biosystems GeneAmp® PCR System 9700 within the CBI-FS DNA Section.

PROCEDURE

Initial Instrument and Software Setup

Thermal Cycler (D17-96) will be programmed with the GlobalFiler™ (29 cycle) and the Yfiler™ Plus (30 cycle) programs in addition to a denature program. The denature program will be used on every plate that is run during the validation. The programs will be entered by one analyst and checked by another analyst. The programs will then be transferred to each of the three other thermal cyclers using the USB flash drive provided by ABI. The firmware software version is 2.0.0 and each thermal cycler will be updated to the latest firmware prior to starting the validation.

Prior to release for use in casework, each instrument will undergo a performance check using the Driftcon system temperature verification system. This action will be recorded in Resource Manager.

Amplification and Data Analysis Conditions

The Hamilton STARlet instrument will typically be used to prepare samples per the manufacturer's recommendations and applicable DNA DOM for both GlobalFiler™ (DNA 10-17) and Yfiler Plus™ (DNA 10-18) amplifications. Manual amplification will also be performed.

The existing analytical and stochastic thresholds validated in 2016 for these chemistries will be used for analysis. The manufacturer default settings for stutter will be used when samples are analyzed under "GlobalFiler_Routine." For capturing and evaluating stutter, the analysis method "STRmix_Questioned" will be used.

If, during evaluation of the various studies below, the AT, ST, and/or stutter thresholds require further examination that will be undertaken. It is expected that there will be minimal difference between thermal cycler instruments (9700 to ProFlex™) regarding the peak heights detected as the main drivers that influence them are: chemistry, cycle number, and detection platform. None of these three factors are being changed. As a result, no impact to STRmix's Model Maker is anticipated at this time (personal communication with ESR, 2020).

STUDIES

Known and Non-probative Samples

Known and non-probative (mock) samples will be amplified in both GlobalFiler™ and Yfiler Plus™, ideally where there is 9700 data available for comparison. This will include approximately 20 non-probative samples along with their associated references. Target inputs of 500pg (optimum per the internal validations) will be used; if an alternative input target was performed that value will be carried forward.

Precision and Accuracy

Two samples with known profiles will be amplified in duplicate at the following input values: 1000pg, 750pg, 500pg, 250pg, 125pg, 60pg, 30pg, and 15pg both on the existing 9700 thermal cycler and

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Proflex™. A second analyst will repeat the amplification on the same ProFlex™ thermal cycler and the data will be compared for reproducibility.

The RFU height of each detected allele in the replicate amplifications on the ProFlex™ thermal cycler will be examined for precision.

The RFU height of each detected allele from the 9700 thermal cycler will be compared to the RFU height of each detected allele from the ProFlex™ thermal cycler to evaluate precision.

Sensitivity and Stochastic

Using the data from the precision and accuracy study, samples will be evaluated for RFU height, peak height ratio (PHR), and drop-out.

Mixture Samples

Mixture samples will be covered in part by the non-probative samples study and additional samples will be run. Where available, samples will be used that have 9700 data available for comparison.

Two-person ratios will be tested in both GlobalFiler™ and Yfiler Plus™ at: 20:1, 10:1, 5:1, 2:1, 1:1, 1:2, 1:5, 1:10, and 1:20. Yfiler Plus™ must be M:M mixtures.

Contamination Assessment

Contamination will be evaluated using both controls and known samples to detect the presence of exogenous DNA possibly from reagents, consumables, other samples, the operator or the laboratory. Each plate that is amplified on the ProFlex™ PCR Systems will have both amplification blanks and positive controls.

NIST Concordance

To satisfy QAS standard 8.4, the NIST standard 2391C will be run with the samples in the known and non-probative section. The correct GlobalFiler™ and Yfiler™ Plus profiles should be obtained.

PRE-APPROVAL (in Qualtrax)

RESULTS

The correct thermal cycler programs were entered into the thermal cycler by one analyst and checked by another analyst. Based on the GF User Guide and the YFP User Guide it was determined that both kit chemistries would be run in the simulation mode. For GlobalFiler™ the correct mode was determined to be the GeneAmp PCR System 9700 emulation mode and GeneAmp PCR System 9600 emulation mode for Yfiler Plus™. Before using the thermal cycler, the firmware version was checked and found to be the most current version (2.0.0). The self-verification test was completed and passed.

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In an effort to reduce variation in DNA input quantity, all samples were manually amplified using a pooled reaction for each individual GlobalFiler™ and Yfiler Plus™ sample. One half of the pooled reaction was amplified using the Applied Biosystems GeneAmp® PCR System 9700 and the other half was amplified using the Applied Biosystems ProFlex™ PCR System.

A total of 17 samples including single source, two person, three person, and four person mixtures were amplified in GlobalFiler™ on both the Applied Biosystems GeneAmp® PCR System 9700 and the Applied Biosystems ProFlex™ PCR System with target inputs of 500pg. All results were as expected and concordance was verified. See the Appendix under Figure 1.

A total of 15 samples including single source and two person mixtures were amplified in Yfiler Plus™ on both the Applied Biosystems GeneAmp® PCR System 9700 and the Applied Biosystems ProFlex™ PCR System with target inputs of 500pg. All results were as expected and concordance was verified. See the Appendix under Figure 2.

Precision and accuracy was confirmed for both GlobalFiler™ and Yfiler Plus™ with an analyst amplifying two separate samples in duplicate at the following input values: 1000pg, 750pg, 500pg, 250pg, 125pg, 60pg, 30pg, and 15pg both on the existing Applied Biosystems GeneAmp® PCR System 9700 and on the Applied Biosystems ProFlex™ PCR System. The results were concordant with the expected genotypes. The RFU height of each detected allele from the 9700 thermal cycler was compared to the RFU height of each detected allele from the ProFlex™ and all results were as expected and concordance was verified. A second analyst performed a repeat amplification on the same ProFlex™ and all results were as expected and concordance was verified. See the Appendix under Figure 3.

The RFU heights of each detected allele from the 9700 thermal cycler were compared to the RFU heights of each detected allele from the ProFlex™ thermal cycler using the single source samples to further evaluate precision. The peak heights obtained from the ProFlex™ PCR System were compared to the peak heights obtained from the data from the Applied Biosystems' GeneAmp® PCR System 9700 and found to be comparable. The average peak heights for both GlobalFiler™ and Yfiler Plus™ were found to be slightly lower on the ProFlex™ PCR System, however the peaks are within the optimal range and no samples failed due to the observed lower peak heights. See the Appendix under Figure 4.

A sensitivity study was performed utilizing the data from the precision and accuracy study. For the GlobalFiler™ study, samples N04_001 and N04_064 were amplified at the following input target values: 1000pg, 750pg, 500pg, 250pg, 125pg, 60pg, 30pg, and 15pg. Partial drop out was observed in the 15pg, 30pg, and 60pg target samples in both N04_001 and N04_064 as well as in the 125pg N04_064 sample. No drop out was observed in the 250pg, 500pg, 750pg, and 1000pg target samples in both N04_001 and N04_064 and the 125pg N04_001 sample. The results are as expected and concordant.

For the Yfiler Plus™ study, samples Male 1 and N04_004 were amplified at the following input target values: 1000pg, 750pg, 500pg, 250pg, 125pg, 60pg, 30pg, and 15pg. Complete drop out was observed in the 15pg, 30pg, 60pg, and 125pg target samples in both Male 1 and N04_004, and near complete drop out was observed in the 250pg samples. Partial drop out occurred in the 500pg Male 1 sample and

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complete DNA profiles were developed from the 750pg and 1000pg Male 1 samples and the 500pg, 750pg, and 1000pg N04_004 samples. The results are as expected and concordant. See the Appendix under Figure 5.

A stochastic study was conducted in order to compare stutter data between the 9700 PCR system and the ProFlex™. Stutter data was evaluated for all single source samples amplified in both GlobalFiler™ and Yfiler Plus™, respectively and the stutter ratios at each locus were compared across both the 9700 PCR system and the ProFlex. The results indicate no significant difference between the two PCR systems and all stutters fall within CBI's current thresholds. See the Appendix under Figure 6.

A mixture study was conducted in which two person, three person, and four person mixtures were amplified in GlobalFiler™ on both the Applied Biosystems GeneAmp® PCR System 9700 and the Applied Biosystems ProFlex™ PCR System. The mixture samples were deconvoluted using STRmix™ to assist in evaluating contributor breakdown and template RFU. Utilizing STRmix™, the template RFU of each contributor in the mixtures was determined and compared across both PCR systems. All results were as expected and concordance was verified. Additionally, two-person ratios were tested using both GlobalFiler™ and Yfiler Plus™ at the ratios 20:1, 10:1, 5:1, 2:1, 1:1, 1:2, 1:5, 1:10, and 1:20. All results were as expected and concordance was verified. See the Appendix under Figure 7.

All samples (56) and positive controls (6) run with GlobalFiler™ were concordant. All negative controls (5) run with GlobalFiler™ were found to be free from exogenous DNA. All samples (52) and positive controls (4) run with Yfiler™ Plus were concordant. All negative controls (3) run with Yfiler™ Plus were found to be free from exogenous DNA. Concordance was confirmed manually. Positive and negative controls were checked manually by an analyst.

NIST standard 2391C was run with both GlobalFiler™ and Yfiler™ Plus and was found to be concordant.

CONCLUSIONS

The ProFlex™ PCR System performed as expected and was found to be an acceptable replacement for the Applied Biosystems' GeneAmp® PCR System 9700 within the case working CBI DNA Unit. A performance check and a self-verification test was performed on all thermal cyclers prior to use and passed.

COMPETENCY

Each analyst will amplify a plate to include one positive control and one negative control on the ProFlex™ PCR System.

APPENDIX

Figure 1: KNOWN AND NON-PROBATIVE SAMPLES AND CONCORDANCE FOR GLOBALFILER

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GLOBALFILER 29 CYCLES					
SAMPLE NAME	FLUID SOURCE/ SUBSTRATE TYPE	NUMBER OF CONTRIBUTORS	NUMBER OF COMPLETE LOCI	CONCORDANT AT AVAILABLE LOCI	COMMENTS
N04_001	Blood/Stain Card	1	24	YES	Concordant
N04_002	Blood/Cotton Fabric	1	24	YES	Concordant
N04_004	Semen/Stain Card	1	24	YES	Concordant
N04_009	Blood/Cotton Fabric	1	24	YES	Concordant
N04_013	Blood/Stain Card	2	24	YES*	One additional true allele with 9700 at TPOX
N04_014	Blood/Stain Card	2	24	YES	Concordant
N04_016	Blood/Swab	2	24	YES	Concordant
N04_029	Saliva/Cigarette butt	1	24	YES	Concordant
N04_041_E	Blood card/Semen	2	24	YES*	Both profiles concordant with expected profile however variation in minor contributor alleles present at D3S1358, AMEL, FGA, D5S818, and D12S391
N04_041_S	Blood card/Semen	3	24	YES*	Both profiles concordant with expected profile however variation in minor contributor alleles present at D3S1358, D16S539, TH01, FGA, D22S1045, D13S317, D10S1248, D12S391, and D2S138
N04_053	Blood/Cotton Fabric	3	24	YES	Concordant
N04_064	Blood/Stain Card	1	24	YES	Concordant
N04_067	Blood/Swab	3	24	YES*	Both profiles concordant with expected profile however variation in minor contributor alleles present at D3S1358, vWA, D16S539, CSF1P0, D8S1179, D18S51, D22S1045, D7S820, SE33, D10S1248, D1S1656, D2S1338

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1.2.1_E	Semen/swab	2	24	YES*	One additional true allele with ProFlex at SE33
1.2.1_S	Semen/Swab	1	24	YES	Concordant
3.2	Trace/Swab	3	24	YES*	One additional true allele with ProFlex at D8S1179
4.1	Trace/Swab	4	24	YES*	One additional true allele with 9700 at D21S11 and D22S1045
NIST Sample	Liquid Kit Control	1	24	YES	Concordant
Positive Control	Liquid Kit Control	1	24	YES	Concordant
Positive Control	Liquid Kit Control	1	24	YES	Concordant
Positive Control	Liquid Kit Control	1	24	YES	Concordant
Amplification Blank	N/A	N/A	N/A	N/A	No Exogenous DNA
Amplification Blank	N/A	N/A	N/A	N/A	No Exogenous DNA
Amplification Blank	N/A	N/A	N/A	N/A	No Exogenous DNA

*variation in loci obtained for the minor contributor in several samples however all profiles matched the expected genotype

Figure 2: KNOWN AND NON-PROBATIVE SAMPLES AND CONCORDANCE FOR YFILER PLUS

YFILER PLUS 30 CYCLES					
SAMPLE NAME	FLUID SOURCE/ SUBSTRATE TYPE	NUMBER OF CONTRIBUTORS	NUMBER OF COMPLETE LOCI	CONCORDANT AT AVAILABLE LOCI	COMMENTS
N04_004	Semen/Stain Card	1	25	YES	Concordant
N04_013	Blood/Stain Card	2	25	YES*	Both profiles concordant however variation in minor contributor allele present at DYS533
N04_014	Blood/Stain Card	2	25	YES*	Both profiles concordant however variation in minor contributor allele present at DYS448
N04_016	Blood/Swab	1	25	YES	Concordant
N04_041_E	Blood card/ Semen	2	24	YES*	Drop out at YGATAH4 in ProFlex sample.

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					Drop out at DYS533 in 9700 sample. Variation in minor contributor allele present at DYS448, DYS390, DYS392, and DYS481.
N04_041_S	Blood card/ Semen	2	25	YES	Concordant
N04_053	Blood/Cotton Fabric	1	25	YES	Concordant
N04_067	Blood/Swab	2	25	YES*	Both profiles concordant however variation in minor contributor allele present at DYS439
1.2.1_E	Semen/swab	1	25	YES	Concordant
1.2.1_S	Semen/Swab	1	25	YES	Concordant
3.2	Trace/Swab	1	25	YES	Concordant
4.1	Trace/Swab	2	25	YES*	Both profiles concordant however variation in minor contributor alleles present at DYS389II, DYS19, DYS449, DYS439, and DYS387S1
Male 1	Saliva/swab	1	24	YES	Drop out at DYS533 in ProFlex sample
NIST Sample	Liquid Kit Control	1	25	25	Concordant
Positive Control	Liquid Kit Control	1	25	25	Concordant
Positive Control	Liquid Kit Control	1	25	25	Concordant
Amplification Blank	N/A	N/A	N/A	N/A	No Exogenous DNA
Amplification Blank	N/A	N/A	N/A	N/A	No Exogenous DNA

Figure 3: PRECISION AND ACCURACY (REPEATABILITY/REPRODUCIBILITY)

GLOBALFILER 29 CYCLES						
SAMPLE NAME	FLUID SOURCE/ SUBSTRATE TYPE	TARGET CONCENTRATION (pg)	NUMBER OF COMPLETE LOCI RUN	NUMBER OF COMPLETE	CONCORDANT AT AVAILABLE LOCI	COMMENTS

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			#1 (Analyst 1/MK))	LOCI RUN #2 (Analyst 1/HM)		
N04_001	Blood/stain card (F)	15	2	0	N/A*	No profile developed for run #2
N04_001	"	30	12	0	YES*	Concordant at partial locus
N04_001	"	60	8	2	YES	
N04_001	"	125	18	13	YES	
N04_001	"	250	20	16	YES	
N04_001	"	500	22	21	YES	F profile – no male markers
N04_001	"	750	22	22	YES	F profile – no male markers
N04_001	"	1000	22	22	YES	F profile – no male markers
N04_064	Blood/stain card (F)	15	0	0	YES*	Concordant at single allele at TH01
N04_064	"	30	3	0	YES	Concordant at partial locus
N04_064	"	60	10	0	YES	Concordant at partial locus
N04_064	"	125	15	9	YES	
N04_064	"	250	20	20	YES	
N04_064	"	500	22	22	YES	
N04_064	"	750	22	22	YES	
N04_064	"	1000	22	22	YES	

YFILER PLUS 30 CYCLES

SAMPLE NAME	FLUID SOURCE/SUBSTRATE TYPE	TARGET CONCENTRATION (pg)	NUMBER OF COMPLETE LOCI RUN #1 (Analyst 1/MK)	NUMBER OF COMPLETE LOCI RUN #2 (Analyst 2/HM)	CONCORDANT AT AVAILABLE LOCI	COMMENTS
N04_004	Semen/stain card	15	0	0	N/A	No profile developed
N04_004	"	30	0	0	N/A	No profile developed
N04_004	"	60	0	0	N/A	No profile developed

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N04_004	"	125	0	0	N/A	No profile developed
N04_004	"	250	1	0	N/A	No profile developed
N04_004	"	500	25	25	YES	
N04_004	"	750	7	25	YES	
N04_004	"	1000	25	25	YES	
Male 1	Saliva/swab	15	0	0	N/A	No profile developed
Male 1	"	30	0	0	N/A	No profile developed
Male 1	"	60	0	0	N/A	No profile developed
Male 1	"	125	0	0	N/A	No profile developed
Male 1	"	250	2	0	N/A	No profile developed
Male 1	"	500	24	25	YES	
Male 1	"	750	25	25	YES	
Male 1	"	1000	25	25	YES	

Figure 4: Peak height comparison data for GlobalFiler™ and Yfiler Plus™

Sample Name	GlobalFiler RFU Summations	
N04_001	63,891	66,730
N04_002	113,874	99,485
N04_004	88,738	82,410
N04_009	66,598	53,509
N04_029	38,686	44,513
N04_064	44,306	45,427
Overall Average	69,349	65,346

Sample Name	Yfiler Plus RFU Summations	
N04_004	25,090	25,043
N04_016	14,201	13,500

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N04_053	60,419	48,798
Overall Average	33,237	29,114

Figure 5: Sensitivity Study

GLOBALFILER 29 CYCLES						
SAMPLE NAME	FLUID SOURCE/ SUBSTRATE TYPE	TARGET CONCENTRATION (pg)	NUMBER OF COMPLETE LOCI (ProFlex)	NUMBER OF COMPLETE LOCI RUN (9700)	CONCORDANT AT AVAILABLE LOCI	COMMENTS
N04_001	Blood/stain card (F)	15	2	1	YES	
N04_001	"	30	12	11	YES	
N04_001	"	60	8	11	YES	
N04_001	"	125	18	18	YES	
N04_001	"	250	20	19	YES	
N04_001	"	500	22	22	YES	F profile – no male markers
N04_001	"	750	22	22	YES	F profile – no male markers
N04_001	"	1000	22	22	YES	F profile – no male markers
N04_064	Blood/stain card (F)	15	0	1	N/A	No loci available for comparisons
N04_064	"	30	3	3	YES	Concordant at partial loci
N04_064	"	60	10	10	YES	
N04_064	"	125	15	15	YES	
N04_064	"	250	20	20	YES	
N04_064	"	500	22	22	YES	F profile – no male markers
N04_064	"	750	22	22	YES	F profile – no male markers
N04_064	"	1000	22	22	YES	F profile – no male markers

YFILER PLUS 30 CYCLES

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SAMPLE NAME	FLUID SOURCE/SUBSTRATE TYPE	TARGET CONCENTRATION (pg)	NUMBER OF COMPLETE LOCI (ProFlex)	NUMBER OF COMPLETE LOCI (9700)	CONCORDANT AT AVAILABLE LOCI	COMMENTS
N04_004	Semen/stain card	15	0	0	N/A	No profile developed
N04_004	"	30	0	0	N/A	No profile developed
N04_004	"	60	0	0	N/A	No profile developed
N04_004	"	125	0	0	N/A	No profile developed
N04_004	"	250	1	4	N/A	No comparable loci
N04_004	"	500	25	25	YES	
N04_004	"	750	7	10	YES	
N04_004	"	1000	25	25	YES	
Male 1	Saliva/swab	15	0	0	N/A	No profile developed
Male 1	"	30	0	0	N/A	No profile developed
Male 1	"	60	0	0	N/A	No profile developed
Male 1	"	125	0	1	N/A	No profile developed with ProFlex
Male 1	"	250	2	1	N/A	No comparable loci
Male 1	"	500	24	25	YES	
Male 1	"	750	25	25	YES	
Male 1	"	1000	25	25	YES	

Figure 6: SENSITIVITY/STOCHASTIC STUDY

GlobalFiler Locus	Count (9700, ProFlex)	9700 Average (%)	ProFlex Average (%)	% Difference	Panel Stutter Filter
D3S1358	18, 18	8.16	8.65	5.92	10.98
vWA	10, 9	6.42	6.71	4.60	10.73
D16S539	8, 7	5.70	5.67	0.48	9.48
CSF1PO	9, 8	5.40	5.66	4.92	8.77
TPOX	2, 1	3.15	3.80	20.52	5.55
D8S1179	14, 14	5.66	5.73	1.15	9.60
D21S11	10, 9	8.01	7.85	1.98	10.45

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D18S51	6, 4	6.05	5.95	1.71	12.42
DYS391	2, 1	7.30	7.61	4.15	7.43
D2S441	9, 11	4.97	5.40	8.67	8.10
D19S433	11, 11	6.86	6.87	0.06	9.97
TH01	2, 2	2.78	2.74	1.34	4.45
FGA	17, 17	6.68	7.62	14.04	11.55
D22S1045	14, 14	9.79	9.28	5.19	16.26
D5S818	10, 10	5.84	5.39	7.71	9.16
D13S317	10, 9	4.64	5.07	9.10	9.19
D7S820	6, 6	6.15	5.84	5.14	8.32
SE33	9, 8	8.33	8.90	6.77	14.49
D10S1248	10, 10	7.65	7.19	5.97	11.46
D1S1656	17, 17	5.99	6.22	3.89	12.21
D12S391	12, 13	9.90	8.76	11.46	13.66
D2S1338	13, 13	7.78	7.58	2.57	11.73
Alternate Stutter					
D22S1045 (+3 bp)	8, 7	3.71	3.79	2.04	6.69
SE33 (-2 bp)	3, 3	2.95	3.46	17.39	3.97

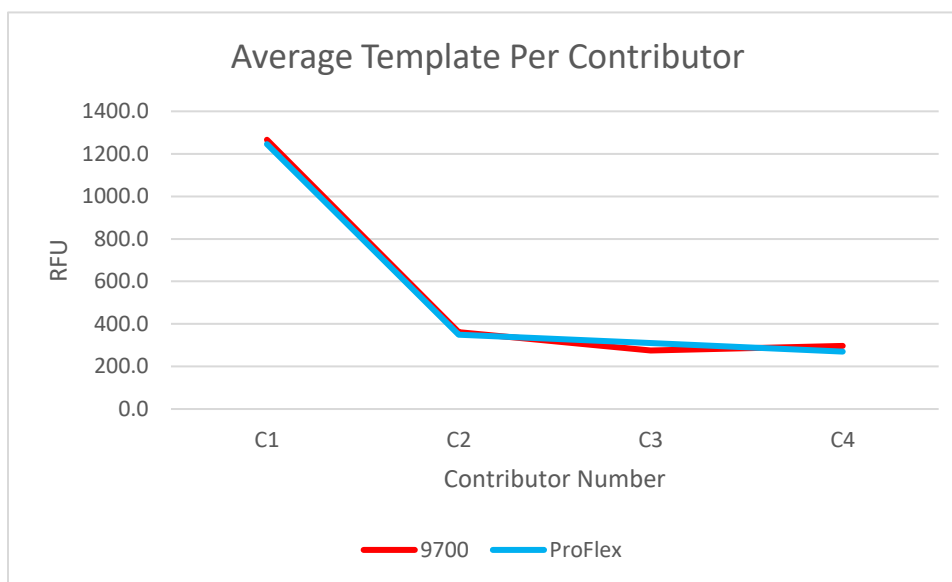
YFP Locus	Count (9700, ProFlex)2	9700 Average (%)	ProFlex Average (%)	% Difference	Panel Stutter Filter
DYS576	9, 9	11.62	10.81	7.00	15.15
DYS389I	7, 6	7.14	5.81	18.62	9.16
DYS635	8, 6	9.46	8.79	7.06	13.38
DYS389II	7, 7	16.25	13.05	19.67	18.79
DYS627	7, 7	9.96	9.83	1.28	15.18
DYS460	2, 2	7.90	6.70	15.30	11.65
DYS458	7, 7	11.72	9.96	14.95	15.31
DYS19	6, 6	8.63	8.28	4.01	12.68
YGATAH4	4, 3	6.89	6.56	4.82	11.53
DYS448	0, 0	No Stutter Above AT	No Stutter Above AT	N/A	4.68
DYS391	3, 4	6.36	7.66	20.36	9.99
DYS456	9, 8	12.60	11.70	7.15	15.36
DYS390	9, 7	9.86	10.05	1.87	13.58
DYS438	4, 4	3.67	3.50	4.66	5.86
DYS392	7, 8	10.43	10.07	3.46	16.94
DYS518	8, 7	18.70	18.20	2.70	25.5

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DYS570	5, 5	12.06	11.96	0.79	15.65
DYS437	4, 3	4.99	5.66	13.28	8.13
DYS385	4, 5	10.83	9.95	8.16	18.32
DYS449	7, 5	17.41	16.29	6.45	23.24
DYS393	3, 4	8.81	9.26	5.03	14.07
DYS439	1, 0	6.39	No Stutter Above AT	N/A	9.89
DYS481	9, 7	23.05	22.17	3.85	28.55
DYF387S1	9, 9	9.85	10.33	4.92	15.71
DYS533	5, 5	9.77	9.81	0.45	12.00
-2 bp stutter					
DYS627 (n-2)	1, 1	1.73	1.82	5.10	2.71
DYS19 (n-2)	2, 3	8.13	6.69	17.65	10.1
DYS481 (n-2)	4, 3	8.01	8.72	8.84	9.55

Figure 7: Mixture Study

Contributor Number	Average Template (RFU)	
	9700	ProFlex
C1	1266.4	1245.8
C2	361.6	348.9
C3	275.2	310.6
C4	296.0	270.0

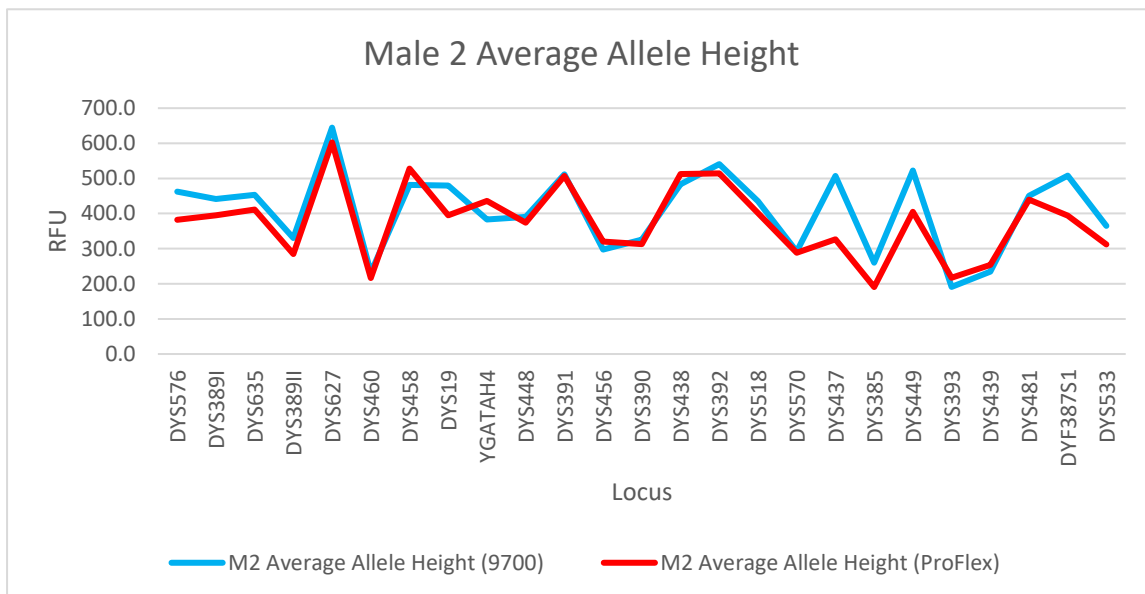
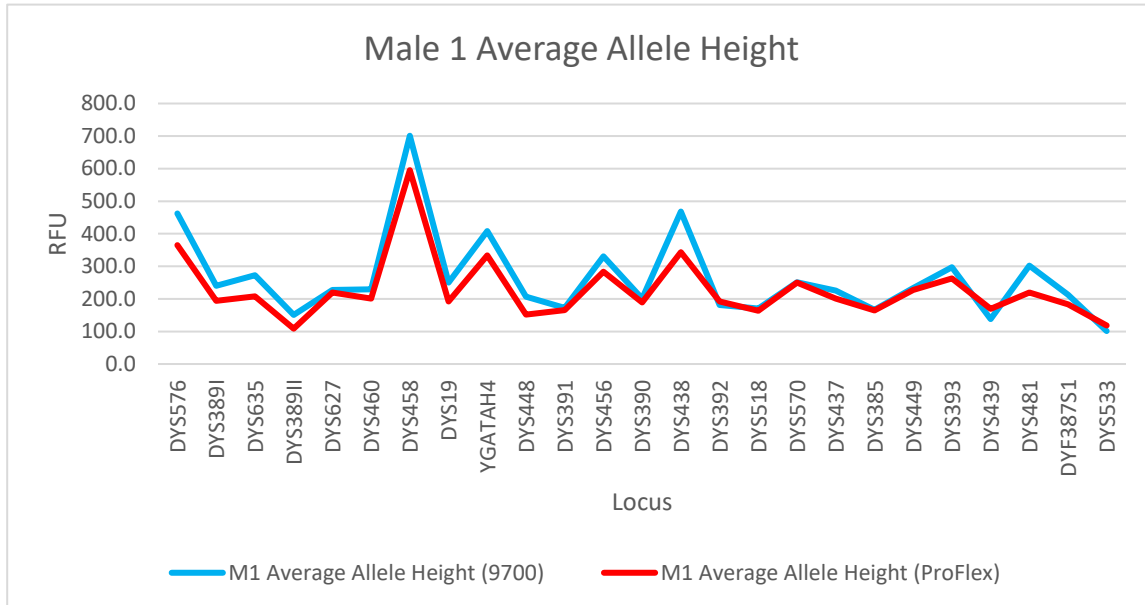


YFP Locus	M1 Average Allele Height (9700)	M1 Average Allele Height (ProFlex)	M2 Average Allele Height (9700)	M2 Average Allele Height (ProFlex)
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2021 Internal Validation of Proflex Thermal Cyclers

DYS576	462.4	364.6	462.2	382.1
DYS389I	240.3	193.9	441.1	395.0
DYS635	272.7	208.0	453.6	411.4
DYS389II	151.2	108.6	330.6	284.6
DYS627	227.6	219.6	644.6	602.6
DYS460	229.8	200.9	232.2	215.9
DYS458	701.0	595.3	481.3	527.8
DYS19	249.8	191.8	479.3	394.8
YGATAH4	407.9	333.5	383.5	436.3
DYS448	206.8	151.3	390.3	374.3
DYS391	172.3	165.5	511.1	506.7
DYS456	330.6	283.6	297.6	319.9
DYS390	200.8	189.3	325.5	312.6
DYS438	468.3	343.3	483.0	512.1
DYS392	181.4	192.1	541.1	514.5
DYS518	170.6	163.2	434.7	401.0
DYS570	251.1	250.1	292.1	288.3
DYS437	225.4	200.4	506.7	326.6
DYS385	166.7	164.7	260.1	190.7
DYS449	233.3	227.3	522.8	405.0
DYS393	297.1	262.9	191.0	217.0
DYS439	138.3	169.6	234.1	253.5
DYS481	302.5	220.0	450.1	439.6
DYF387S1	213.0	182.8	508.3	393.6
DYS533	101.2	118.0	364.6	312.3

2021 Internal Validation of Proflex Thermal Cyclers

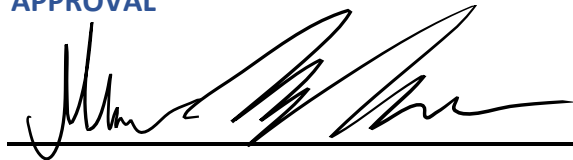


2021 Internal Validation of Proflex Thermal Cyclers

REFERENCES

1. Thermo Fisher Scientific ProFlex™ PCR Systems USER GUIDE
2. Thermo Fisher Scientific GlobalFiler™ PCR Amplification Kit USER GUIDE
3. Thermo Fisher Scientific Yfiler™ Plus PCR Amplification Kit USER GUIDE
4. Thermo Fisher Scientific ProFlex and Veriti Firmware Upgrade Notification (dated July 20,2020)
5. CBI Discipline Operations Manual DNA 05 Equipment
6. CBI Discipline Operations Manual DNA 10-17 DNA Amplification Using GlobalFiler
7. CBI Discipline Operations Manual DNA 10-18 YSTR DNA Amplification Using YFiler Plus

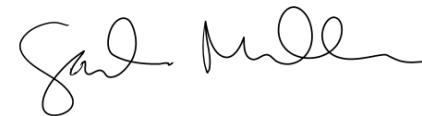
APPROVAL



04/21/2021

Marko Kokotovic, Analyst

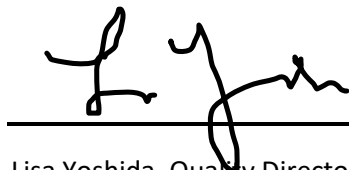
Date



4/20/2021

Sarah Miller, DNA Technical Leader

Date



4/21/2021

Lisa Yoshida, Quality Director

Date