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Applied Biosystems™ SeqStudio™ Flex Genetic Analyzers for Human Identification - a fluorescence-based benchtop capillary electrophoresis system with intuitive operation and plate loading flexibility

Jill Muehling, Liansen Liu, Jimmy Ly, Nick Andrews, Haoning Fu, Stephanie Schneider, Yoke Peng Lim, Siva Samsani, Angie Lackey, Joe Chang, Pranapda Katie Aumsuwan

Thermo Fisher Scientific, 6055 Sunol Blvd, Pleasanton, CA, USA, 94566

INTRODUCTION

Forensic and Human Identification customers have come to expect gold-standard fragment analysis and Sanger sequencing technology over the last 20+ years. The SeqStudio Flex instrument is an 8 or 24 capillary genetic analyzer that builds on our legacy capillary electrophoresis instruments. Key features include an onboard, simplified instrument software with an interactive touch screen and a 4-plate deck with continuous plate loading data for ease of plate/sample/run setup and prioritization. The system uses the same consumables as the Applied Biosystems™ 3500 Genetic Analyzer with the addition of an easier-to-install capillary array. The instrument software has a decreased burden for routine spectral calibrations or manual spatial calibrations along with the algorithms for extended dynamic range and pull-up reduction to aid in data analysis with Applied Biosystems™ GeneMapper™ ID-X v1.7.

In the developmental validation study, we evaluated the performance of the SeqStudio Flex Genetic Analyzer for human identification (HID) analyses using Applied Biosystems™ STR Amplification kits for both databasing and casework. The results demonstrate that the performance of the SeqStudio Genetic Analyzer meets the rigorous HID validation standards set forth by SWGDAM and DAB.

Materials and Method

Casework Amplification Kits

- Applied Biosystems™ GlobalFiler™ IQC PCR Amplification Kit
- Applied Biosystems™ NGM Detect™ PCR Amplification Kit
- AmpFℓSTR™ NGM SElect™ PCR Amplification Kit
- Applied Biosystems™ Verifiler™ Plus PCR Amplification Kit
- Applied Biosystems™ Yfiler™ Plus PCR Amplification Kit

Database Amplification Kits

- Applied Biosystems™ Verifiler™ Express(VFE) PCR Amplification Kit
- Applied Biosystems™ GlobalFiler™ Express PCR Amplification Kit
- AmpFℓSTR™ NGM SElect™ Express PCR Amplification Kit

Instruments

- Three SeqStudio™ 8 Flex Genetic Analyzers
- Five SeqStudio™ 24 Flex Genetic Analyzers
- SeqStudio Flex Series Instrument Software v 1.1.0 with off-scale recovery and internal size standard normalization enabled
- SeqStudio Flex Series Instrument Software v 1.1.1 with off-scale recovery enabled and auto-spectral calibration both enabled and disabled
- One 3500xL Genetic Analyzer with 3500 Series HID Data Collection Software v 4.0.1

Summary of Studies and Results

Instrument Setup – Calibrations and Install Run

Spatial calibrations were completed using the onboard wizards and passed. Twenty-four cap instruments utilized signal optimization to reduce peak height variation across capillaries in an injection.

Spectral calibrations were run for G5, J6 and J6T dye sets on all instruments. A manual spectral calibration is required initially for each dye set and a representative dye matrix is created. Subsequent sample runs will use auto-spectral calibration, if applicable. Auto-spectral calibration enables the instrument to optimize and update the dye matrix per capillary, by using the sample spectral data within. The result is a reduction in instrument-specific and spectral overlap pull-up artifacts.

An HID Install Run, consisting of the GlobalFiler Allelic Ladder and GeneScan™ 600 LIZ™ Size Standard v2.0, was used to verify the instrument meets human identification specifications, including sizing precision and peak height. An install run is required to access HID modules after installation or software upgrade.

Instrument and Analysis Functional Testing

Table 1: On-instrument analysis settings

Parameter	Kit settings					
	G5 dye set (5-dye)		J6 dye set (6-dye)		J6-T dye set (6-dye)	
	SeqStudio™ 8 Flex	SeqStudio™ 24 Flex	SeqStudio™ 8 Flex	SeqStudio™ 24 Flex	SeqStudio™ 8 Flex	SeqStudio™ 24 Flex
Size standards	GS600_LIZ_(60-460)	GS600_LIZ_(60-460)	GS600_LIZ_(60-460)	GS600_LIZ_(60-460)	GS600_LIZ_(60-460)	GS600_LIZ_(60-460)
Run module						
Run module name	HID_G5_36_POP4	HID_G5_36_POP4xl	HID_J6_36_POP4	HID_J6_36_POP4xl	HID_J6T_36_POP4	HID_J6T_36_POP4xl
Injection time	15 sec	24 sec	15 sec	24 sec	15 sec	24 sec
Injection voltage	1,200 V	1,200 V	1,200 V	1,200 V	1,200 V	1,200 V
Run time	1,210 sec	1,210 sec	1,550 sec	1,550 sec	1,550 sec	1,550 sec
Run voltage	15,000 V	15,000 V	13,000 V	13,000 V	13,000 V	13,000 V
Analysis settings[1]						
Analysis settings name	HID_5Dye_Default	HID_5Dye_Default	HID_6Dye_Default	HID_6Dye_Default	HID_6Dye_Default	HID_6Dye_Default
Size calling method	Local Southern	Local Southern	Local Southern	Local Southern	Local Southern	Local Southern
Analysis range	Full	Full	Full	Full	Full	Full
Sizing range	80,400	80,400	60,460	60,460	60,460	60,460
Peak amplitude threshold	175	175	175	175	175	175
Primer peak	Present	Present	Present	Present	Present	Present

Table 2: Summary of the Studies, Samples and Results for the Developmental Validation with SeqStudio Flex Instrument Software 1.1.0 and the verification with Instrument Software 1.1.1

					Casework (v1.1.0)					Database (v1.1.0)			Casework (v1.1.1)		
Study	Test Cases	Samples	Passing Criteria	Result	GlobalFiler™ IQC	NGM Detect™	NGM Select™	VeriFiler™ Plus	Yfiler™ Plus	Verifiler™ Express	NGM Select™ Express	GlobalFiler™ Express	GlobalFiler™ IQC	VeriFiler™ Plus	NGM Select™
Minimum Detection Threshold and Contamination	Contamination	No-template controls (NTC)	No allelic data should be observed using minimum peak amplitude thresholds in the NTCs.	Pass	x	x	x	x	x	x	x	x	x	x	x
Genotyping	Concordance	Kit Positive Control (0.5 ng or 1 ng) 23 gDNA samples (0.5 ng or 1 ng) 23 blood FTA 1.2 mm punches	All allele calls are 100% concordant with the 3500xl genotype results	Pass	x	x	x	x	x	x	x	x	x	x	x
	Reproducibility		STR profiles from the same samples or comtrols run on different instruments, and across injections, are 100% concordant	Pass	x	x	x	x	x	x	x	x	x	x	x
	Pull-Up		Mean pull-up call is <3%	Pass	x	x	x	x	x	x	x	x	x	x	x
Sizing	Precision	Kit Allelic Ladder	The sizing precision does not exceed a standard deviation of 0.15 bp within an injection (8 or 24 capillary) for all alleles	Pass	x	x	x	x	x	x	x	x	NA	NA	NA
	Accuracy		The size difference does not exceed 0.5 bp per injection9 8 or 24 capillary for all alleles	Pass	x	x	x	x	x	x	x	x	NA	NA	NA
	Allelic ladder passing rate		Allelic ladders should pass more than 95% of the time	Pass	x	x	x	x	x	x	x	x	NA	NA	NA
Sensitivity	Lower end limits	Kit positive control samples (31 pg and 125 pg)	Full, on-scale profiles are observed at 125 pg of DNA input. Partial profiles are observed at the lowest amplified input of 31 pg	Pass	x	x	x	x	x	NA	NA	NA	NA	NA	NA
Mixture Analysis	Male:Female	DNA Control 007:9947A 1:2, 2:1, 1:7 and 7:1	Minor contributor alleles are 100% detectable and concordant in a 1:2, 2:1, 1:7 and 7:1 mixture	Pass	x	x	x	x	x	NA	NA	NA	x	x	x
	Male:Male	DNA Control 007:IB-0996 1:2, 2:1, 1:7 and 7:1		Pass	x	x	x	x	x	NA	NA	NA	NA	NA	NA
	Male:Female	NIST SRM 2391d Component D	Minor contributor alleles are 100% detectable and concordant	Pass	NA	NA	NA	NA	NA	NA	NA	NA	x	x	x
Resolution	Resolution	Data from the Genotyping and Sensitivity Studies	The instrument shall detect and resolve alleles that differe in length by a single base pair from 60-470 bp .	Pass	x	x	x	x	x	x	x	x	x	x	x
Crosstalk and Carryover	Crosstalk	DNA Control 007 with Hi-Di Formamide (crosstalk and	Carryover observed should be ≤0.1% in NTC blanks and 15 in Hi-Di Formamide blanks.	Pass	x	x	x	x	x	x	x	x	NA	NA	NA
	Carryover			Pass	x	x	x	x	x	x	x	x	NA	NA	NA

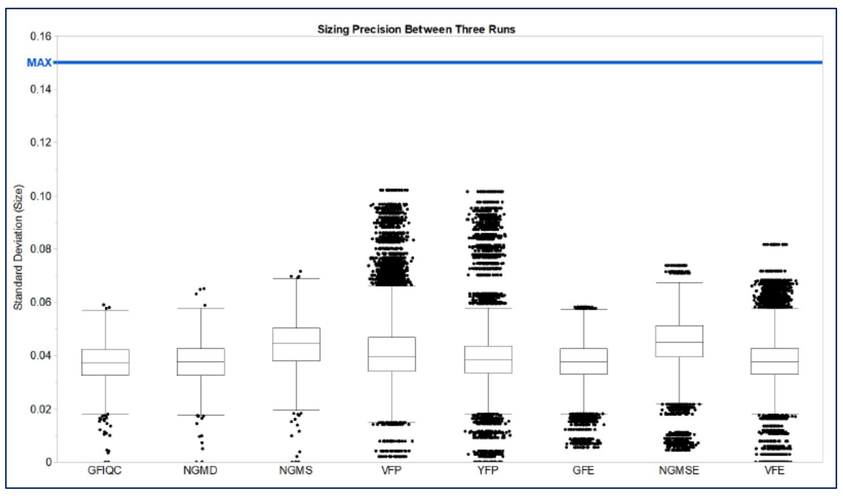

Figure 1: Allelic ladder was in eight wells, injected three times, for a total of 24 data points across five instruments. Precision across the three injections was calculated for each of the STR kits. The blue line represents 0.15 bp, or the standard deviation in which the study results must fall below.

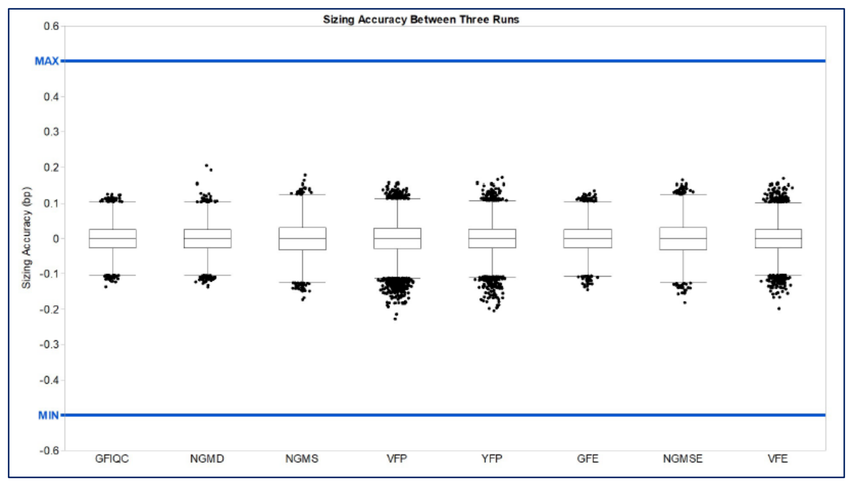

Figure 2: Allelic ladder was in eight wells, injected three times, for a total of 24 data points across three total injections. Precision was calculated across the three injections. The blue lines represent 0.5 bp, or the value which the study must results must be between to pass.

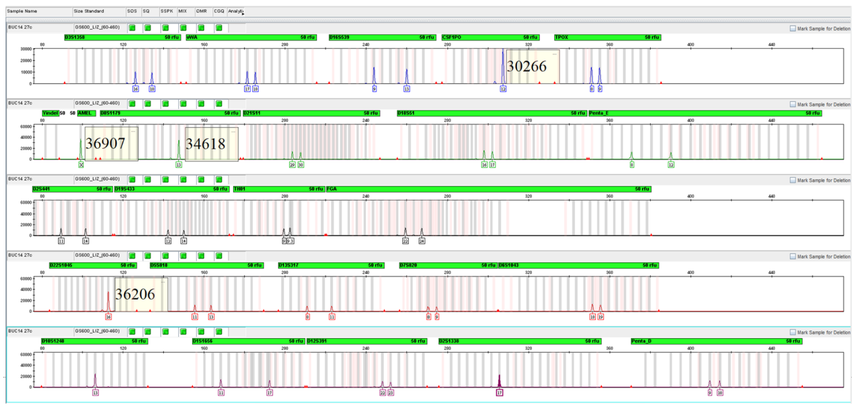

Figure 3: The SeqStudio Flex Instrument Software enables Off-Scale Recovery (OSR) by default to help increase first pass success rate for single source samples.
Direct Amplification of a Buccal Swab with Verifiler Express kit (27 cycles)

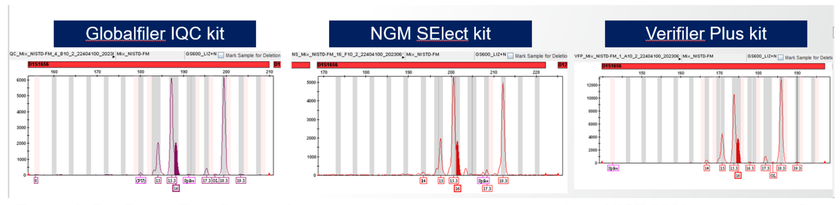

Figure 4: SeqStudio Flex Genetic Analyzer resolves the minor allele of the NIST 2391d component D material. The sample was injected in 14 times total for each of the three kits and resolved 100% of the time, when using study calculated minimum thresholds and no filters.

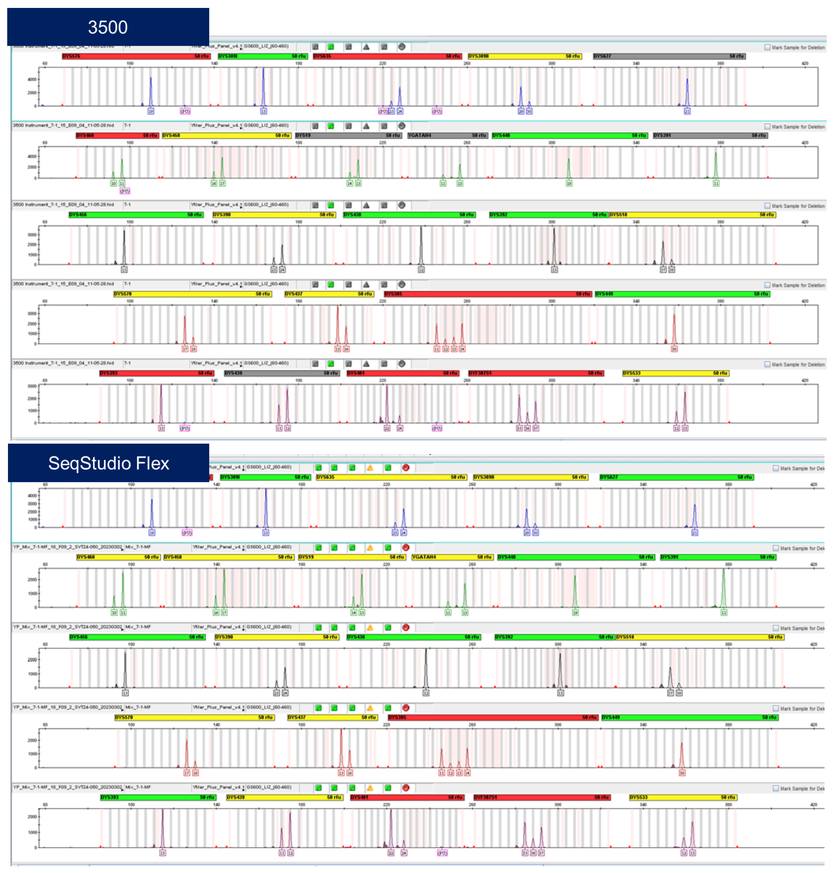

Figure 5: Comparison of a Yfiler Plus Male:Male mixture on the 3500 and SeqStudio Flex shows equivalency between the two systems.

CONCLUSION

The SeqStudio Flex Series Genetic Analyzers generate high-quality data with Applied Biosystems PCR amplification kits analyzed with GeneMapper ID-X Software v1.7. The studies demonstrate that this instrument meets the same specifications for concordance, reproducibility, precision, accuracy, sensitivity, resolution, crosstalk and carryover as the 3500xL.

For more information and instrument details refer to thermofisher.com/seqstudioflex.

Take a 3-D Demo Tour of the instrument here:

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