

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

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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 a new 8 or 24 capillary genetic analyzer that builds on our legacy capillary electrophoresis instruments.

An onboard instrument software with an interactive touch screen removes the need for a desktop computer and simplifies plate setup and instrument operation. This intuitive software offers autocalibration and minimizes the burden of routine manual spectral calibrations. Integrated pull-up reduction and off-scale recovery algorithms streamline data analysis with Applied Biosystems™ GeneMapper™ ID-X Software v1.7.

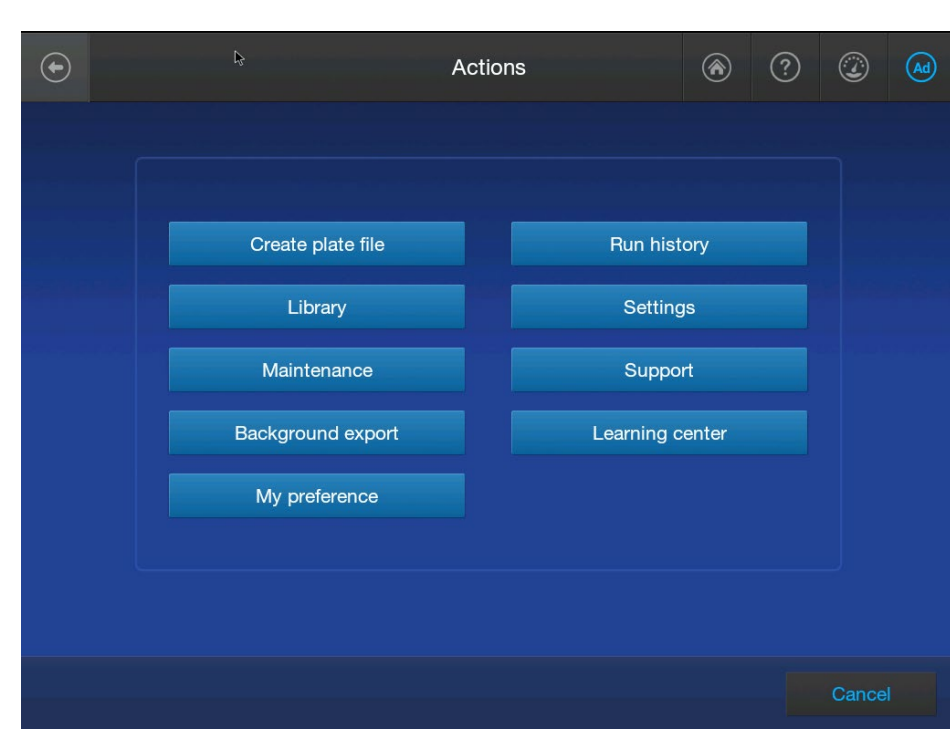
The system uses the same consumables as the Applied Biosystems 3500 Genetic Analyzer with the benefit of an easier-to-install capillary array. Four plate positions allow for increased walk-away capacity, continuous plate loading, and sample prioritization.

The work presented here provides details on key features and summarizes highlights from feasibility testing performed at Thermo Fisher Scientific for the human identification and forensic community.

KEY FEATURES

1. Increased Ease of Use

- Desktop & cloud-based plate manager software
- On-board computer with integrated touchscreen computer and intuitive Applied Biosystems software



- Easier to use capillary arrays: Retractable array allows for easier installation and removal



2. Increased Flexibility

- Continuous plate loading allows the user to load multiple plates without interrupting the current run
- Four plate positions accommodating 8-tube strips and 96-well plates



- Automated plate linking: ability to maintain traceability from sample to result when using barcoded plates
- Urgent sample reprioritization enables injection re-ordering without pausing an in-progress run

3. Connectivity

- Thermo Fisher Connect for user remote monitoring and data sharing
- Remotely setup, link/unlink and edit of runs of existing plates on instruments
- Wi-Fi capability

4. Enhanced Service & Support

- Smart Help with on-board Tech support ticket submission, and sharing of log and run files
- On board instrument help videos

HUMAN IDENTIFICATION FEASIBILITY TESTING

Feasibility testing was done on the initial release of the SeqStudio Flex instrument with Data Collection v1.0 to test readiness for the HID community. This baseline testing helped determine if additional software development was required prior to developmental validation of the HID workflow.

Materials and Methods

Instruments

- Two SeqStudio 24 Flex Genetic Analyzers and Data Collection software v1.0
- One SeqStudio 8 Flex Genetic Analyzer with Internal Developmental Version of Data Collection software
- One 3500xl Genetic Analyzer with Data Collection Software v4.0.1

Chemistry

- Casework Sample Preparation:
 - Extraction
 - 10 genomic DNA samples
 - Prepfil™ Express Forensic DNA Purification Kit
 - Automate Express™ Forensic DNA Extraction System



Parts of the instrument

- 1) Pump compartment
- 2) Power Button
- 3) Speakers
- 4) Drawer compartment
- 5) USB ports
- 6) Touchscreen
- 7) Instrument Status
- 8) Capillary array and detection cell compartment

Materials and Method (continued)

- Amplification
 - GlobalFiler™ IQC PCR Amplification Kit
 - GlobalFiler™ PCR Amplification Kit
 - NGM Detect™ PCR Amplification Kit
 - NGM SElect™ PCR Amplification Kit
 - Applied Biosystems ProFlex™ PCR System
 - Applied Biosystems 9700 PCR System
- Database Sample Preparation
 - Amplification
 - 10 blood samples on treated paper
 - 10 blood samples on untreated paper
 - 10 buccal swabs (VFE only)
 - Verifier™ Express(VFE) PCR Amplification Kit
 - Yfiler™ Platinum PCR Amplification Kit
 - ProFlex PCR System
 - 9700 PCR System

CE Plate Setup and Runs

Run modules for the two SeqStudio Flex instruments were created based on the 3500(xL) instrument run modules. Off-scale recovery(OSR) and pull-up reduction algorithms were applied in the default run modules on the SeqStudio Flex instrument. For this study, the pull-up algorithm was applied across all instrument runs, while off-scale recovery was selectively chosen across different runs.

One µL of amplicon/ladder and 9 or 10µL of a GeneScan™ 600 LIZ™ Size Standard v2.0 - Hi-Di™ Formamide mixture was used to run on the CE systems.

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

		Casework Kits			Database Kits	
		GlobalFiler IQC	NGM Detect	NGM Select	Verifier Express	Yfiler Platinum
Genotype Concordance	100% concordance of the genotypes across a profile	Pass	Pass	Pass	Pass	Pass
	Allelic Ladders	Pass	Pass	Pass	Pass	Pass
	Positive Control	Pass	Pass	Pass	Pass	Pass
	Samples	Pass	Pass	Pass	Pass	Pass
Dynamic Range and Off-scale Recovery	On scale profile with at least 2x recommended input	Pass	Pass	Pass	Pass	Pass
Pull-up Artifacts	Average Percent pull-up across sample < 3% of the parent peak, for 95% of samples	Pass	Pass	Pass	Pass	Pass
		Pass	Pass	Pass	Pass	Pass

Representative Electropherograms

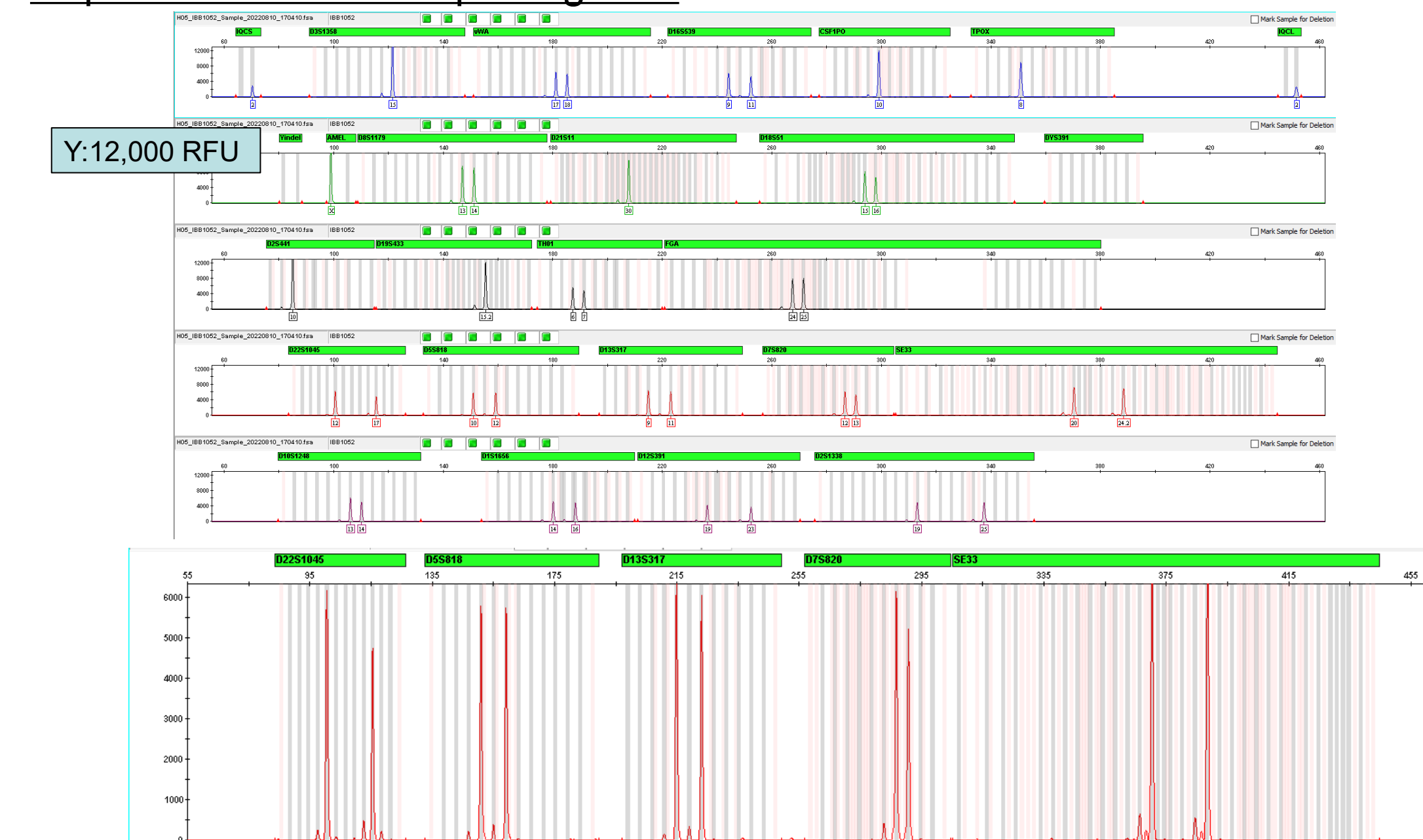


Figure 1: GlobalFiler IQC data - 1ng extracted sample analyzed at 100 RFU and no filters; zoomed into red dye channel

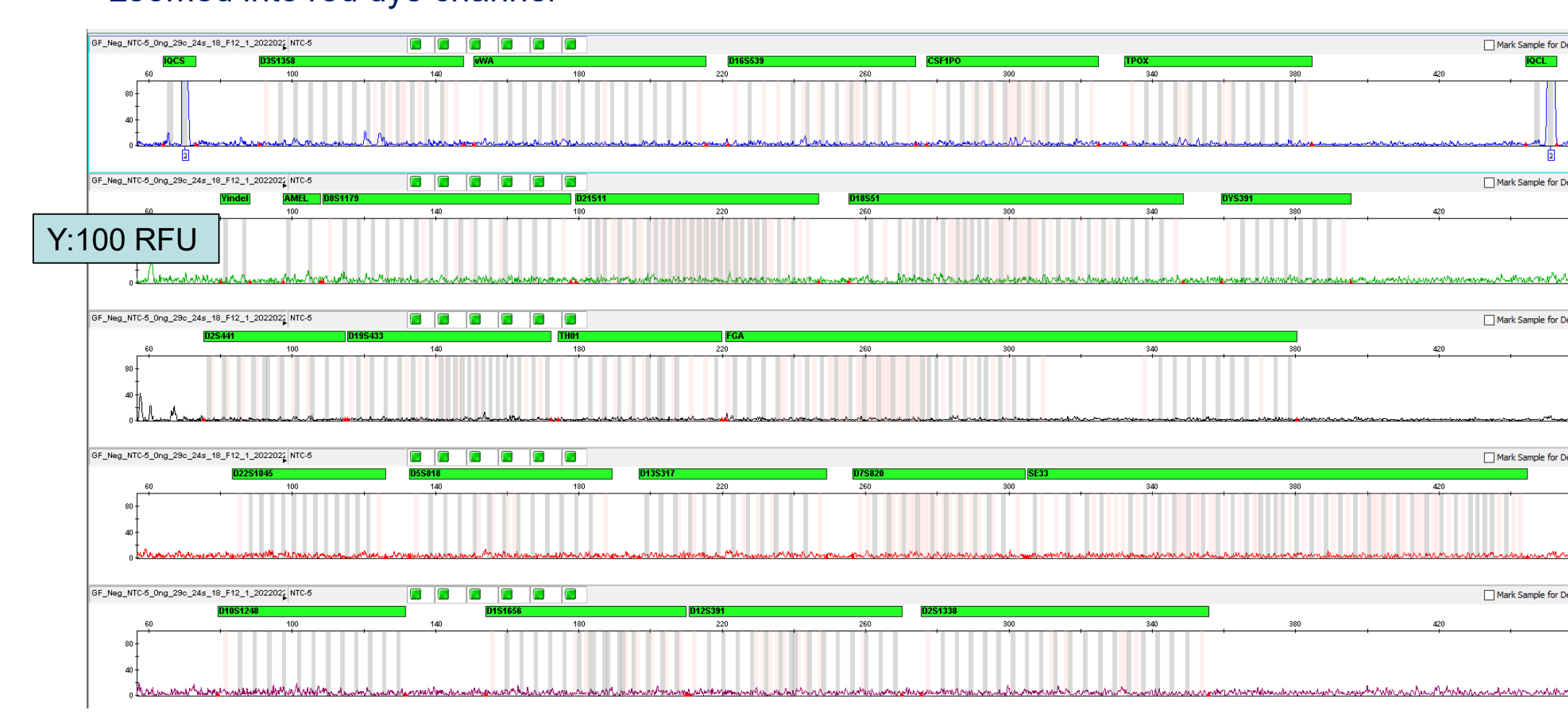


Figure 2: GlobalFiler IQC negative amplification control, analyzed at 25 RFU with no filters

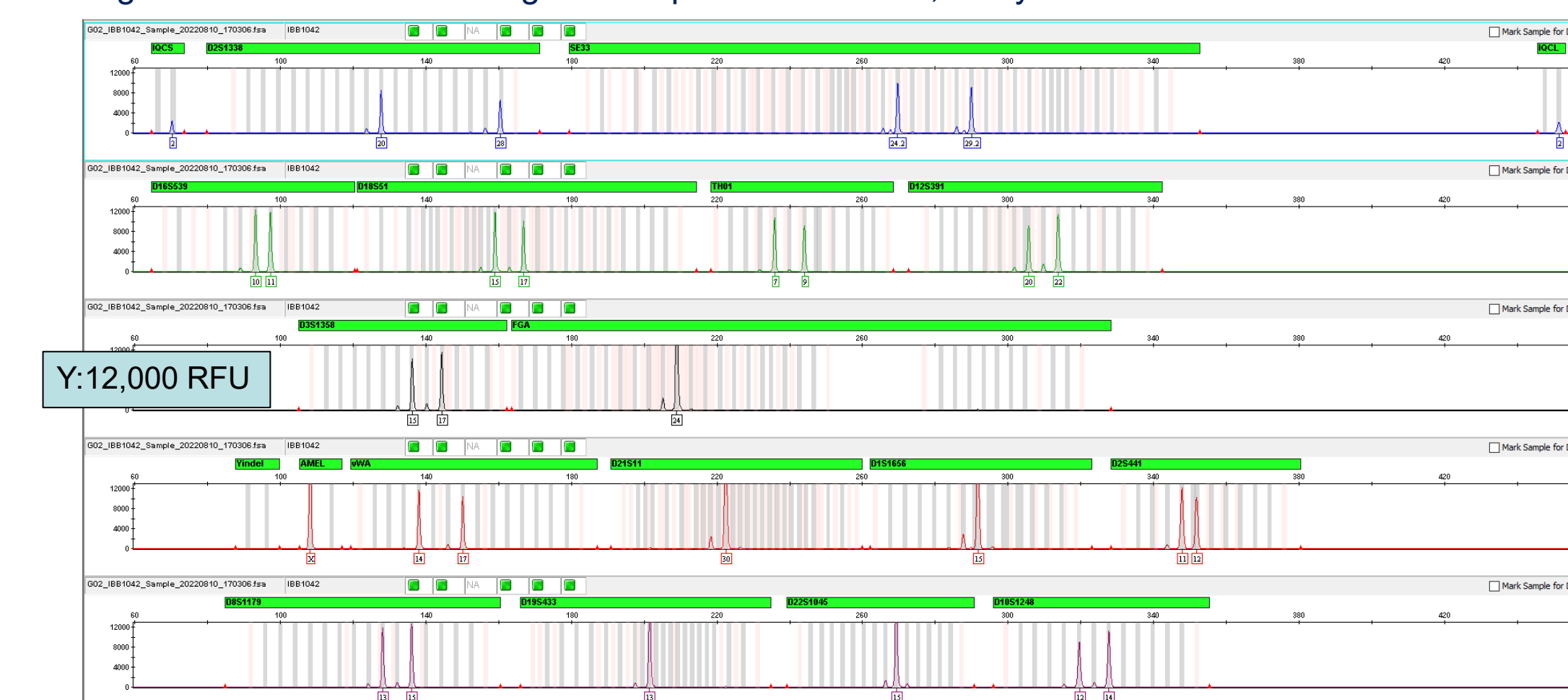


Figure 3: NGM Detect data - 500 pg, 30 cycles, analyzed at 100 RFU with a 10% global filter

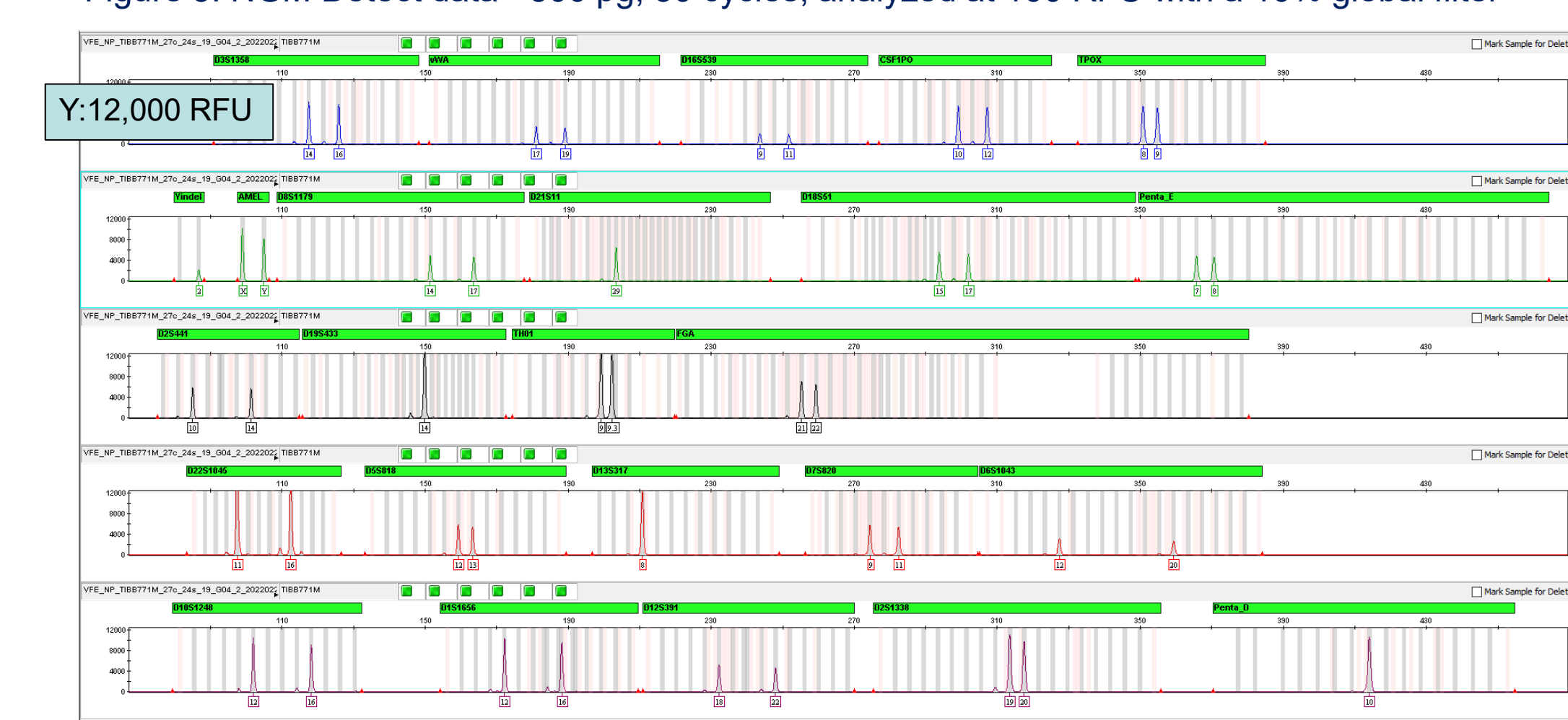


Figure 4: Verifier Express data from 1mm punch, direct amplification, analyzed at 100 RFU with a 20% global filter. No associated pull-up peaks were called.

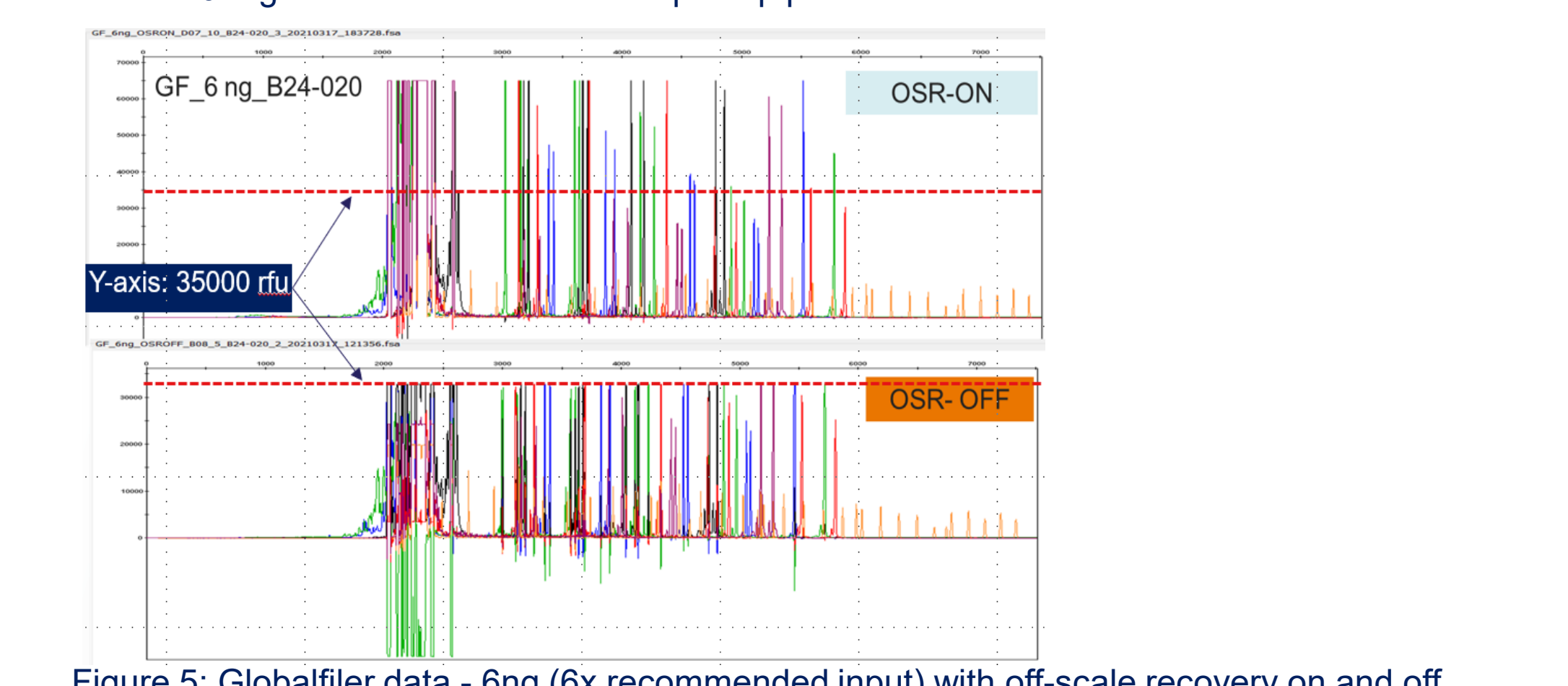


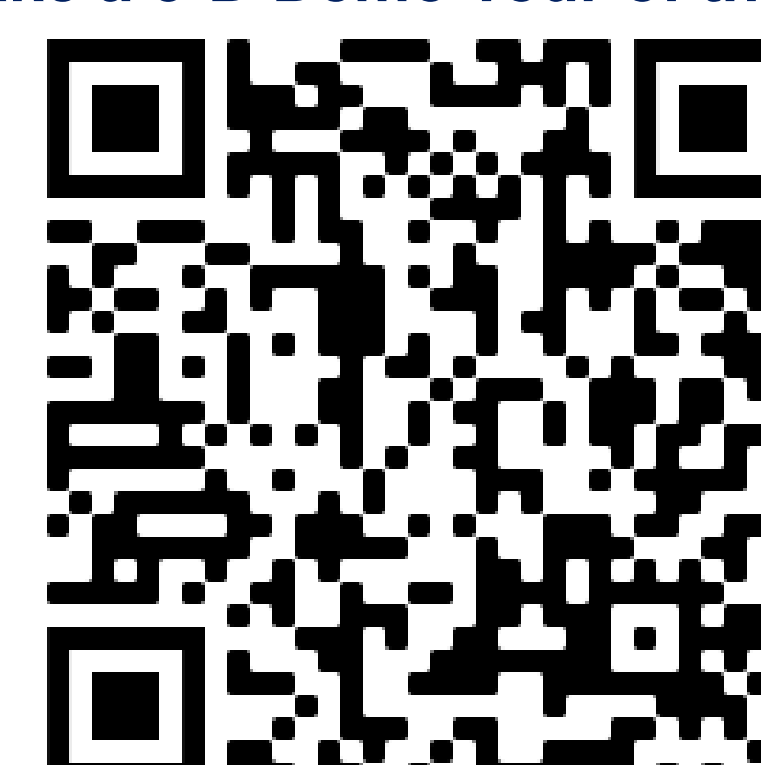
Figure 5: Globalfiler data - 6ng (6x recommended input) with off-scale recovery on and off

CONCLUSION

Feasibility testing shows that the SeqStudio Flex Genetic Analyzer meets the needs of the HID and forensic communities. Innovative features were integrated to allow for easier use and streamline sample processing. Further work is commencing on the developmental validation of Applied Biosystems STR kits on the system with GeneMapper ID-X Software v1.7.

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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