



“Future trends in forensic DNA technology” seminar series

ThermoFisher
S C I E N T I F I C

A System Approach to Product Development: How your “asks” during expanded multiplex kit adoption drive our “answers”

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Insight into product development

You ask, we answer: Data Analysis Solutions

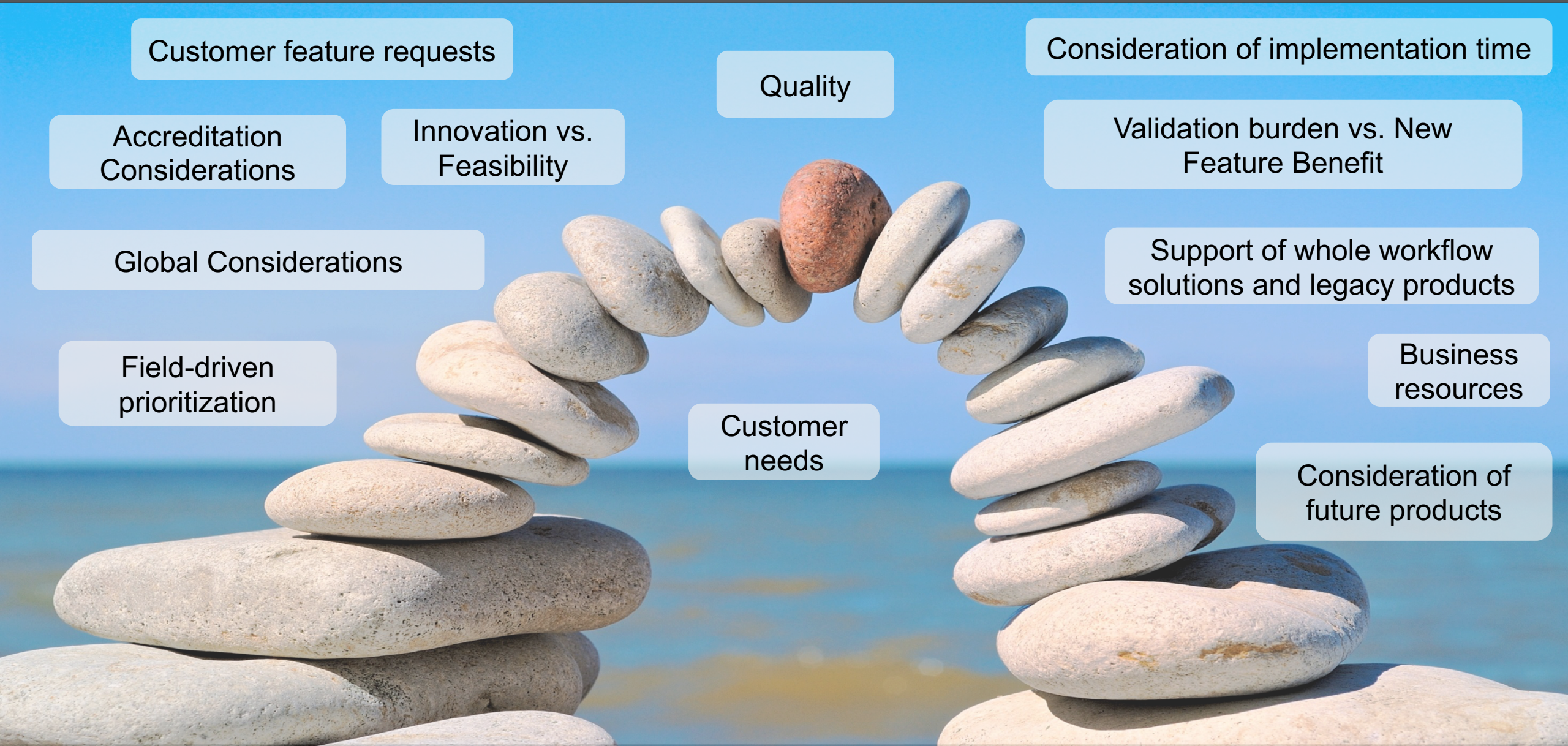
Forensic customer needs continue to evolve

- Higher number of samples submitted
- Difficult and compromised samples
- Analysis of more complex data sets
- Flat or decreasing funding
- Request for faster turnaround time
- Pressure to solve cases & clear backlogs

1. Voice of Customer Survey, Thermo Fisher Scientific

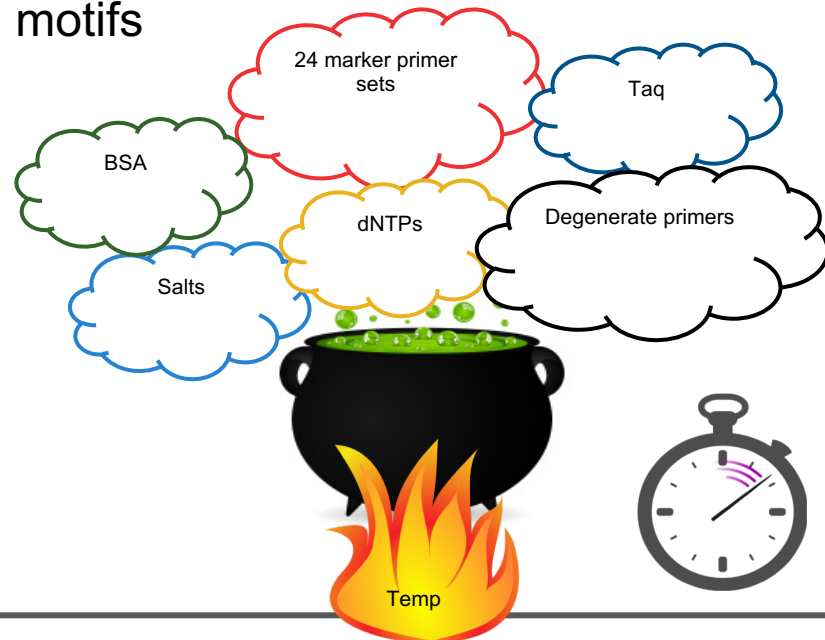


Development involves a thoughtful balance of needs, risks, and challenges

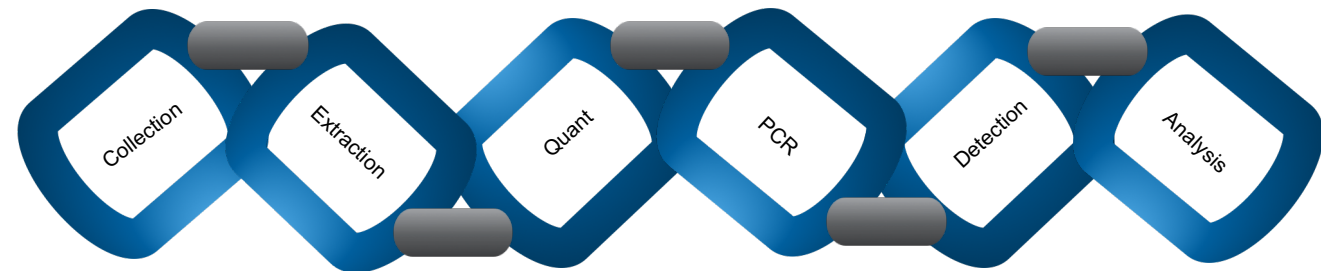


Workflow considerations

- The GlobalFiler kit is very complex
 - More Markers:
 - Identifiler marker set + 8 additional markers
 - Includes Y-indel and YSTR marker
 - Reduced amplification time
 - Some new markers have very complex repeat motifs



- The workflow is very complex
 - Complex sample and substrates
 - The GlobalFiler profile may look poor when in fact there is an underlying workflow issue or complication
 - 3500 series instrument sensitivity
 - Advanced mixture deconvolution and statistical calculations





You ask, we answer: Data Analysis Solutions

Data Collection v4 & GeneMapper™ *ID-X* v1.6

Your Ask: Applied Biosystems Workflow Improvements

Improve Data Interpretation

Reduce pull-up edits

Improve 1st pass success rate for database labs

More efficient data transfer to probabilistic genotyping

Improve the user experience

Added flexibility for the 3500 series instruments

Streamlined workflow

Support languages

6-dye installation standard

IT Compliance

Windows 10 Support

CODIS compatibility

Internationalization

Our Answer: Data Collection v4 and GeneMapper *ID-X* v1.6

Your Ask: Improved Data Interpretation

Current Landscape: 3500 series instrument

- Enhanced dynamic range
- Statistical methods that require identification of number of contributors
- Minimal baseline noise (for example, the GlobalFiler™ and Yfiler™ Plus kits)
- Multiple pull-up edits per 1ng sample

Current Accreditation Guidance

SWGDM 3.1.1.2:

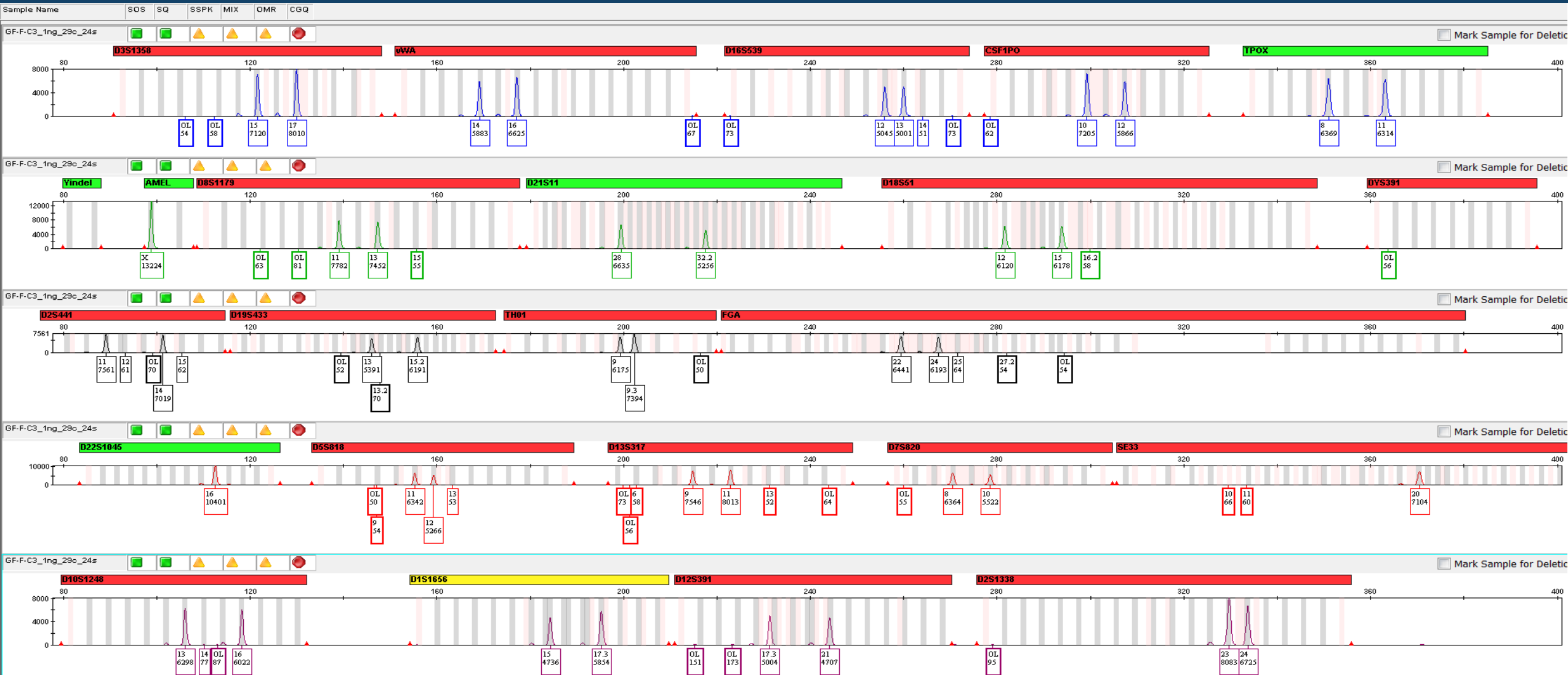
The analytical threshold should not be established for purposes of avoiding artifact labeling as such may result in the potential loss of allelic data.

Theoretical Pull-up Example

DNA Input (ng)	Heterozygous Peak Height (RFU)	RFU Product of 1.0 % Pull -up	RFU Product of 3.0 % Pull -up
0.5	1969	20	59
1	4958	50	149
2	6961	70	209

DNA Input (ng)	Homozygous Peak Height (RFU)	RFU Product of 1.0 % Pull -up	RFU Product of 3.0 % Pull -up
0.5	3938	39	118
1	9916	99	297
2	13922	139	418

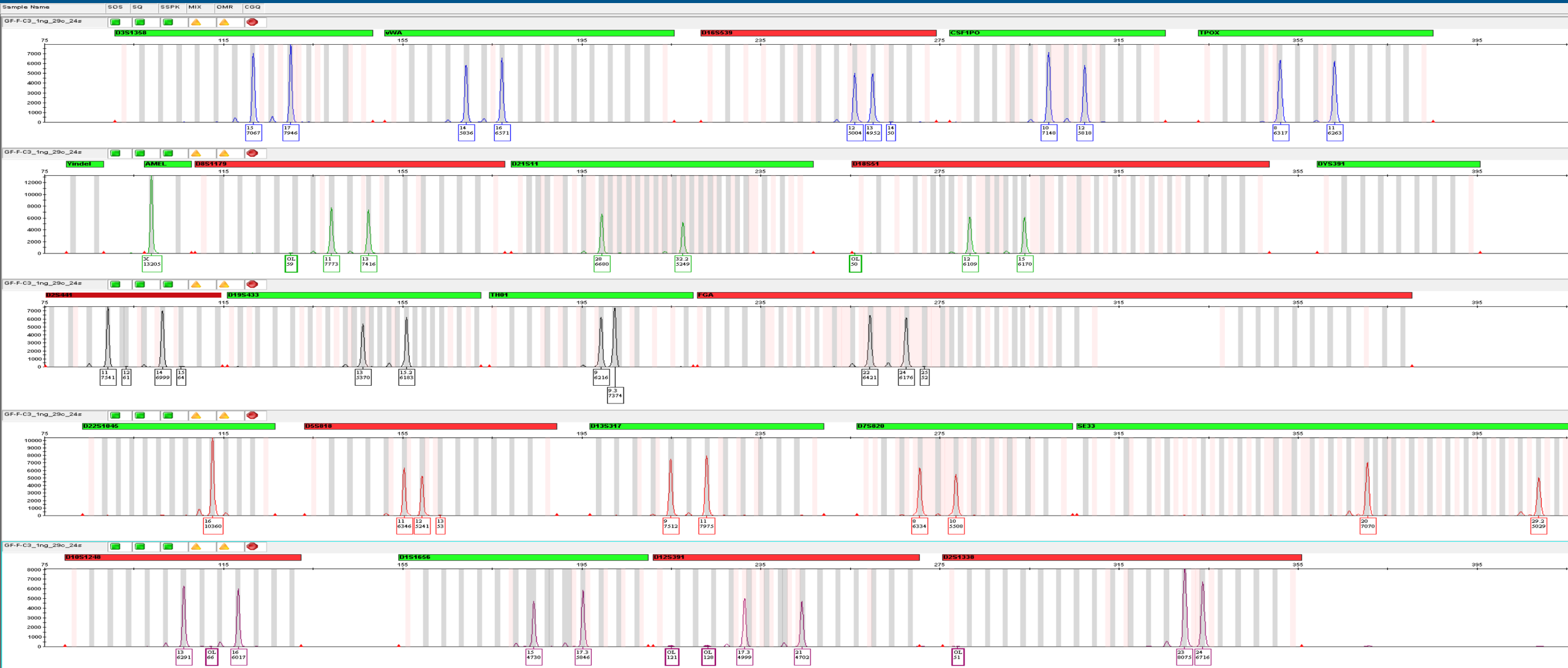
Your Ask: Improved Data Interpretation – Pull-up



Single-source 1ng sample amplified with the GlobalFiler kit analyzed at 50 RFU

31 pull-up edits

We Answer: Improved Data Interpretation – Pull-up Reduction



The same GlobalFiler profile analyzed at 50 RFU after pull-up reduction is applied.

6 pull-up edits

We Answer: Improved Data Interpretation – Pull-up Reduction

- Field testing was performed to assess the pull-up reduction feature

- Samples:

- (10) Single Source 1ng GF Samples
- (4*) 2-person 1:7 GF Mixtures (1ng input)
- (1) GF Positive Control

*Test Site 1: only 3 mixtures included in analysis

- Amplified with the GlobalFiler kit
- The samples were injected twice:
 - Pull-up reduction disabled
 - Pull-up reduction enabled
- The number of edits were compared

Field Testing Results

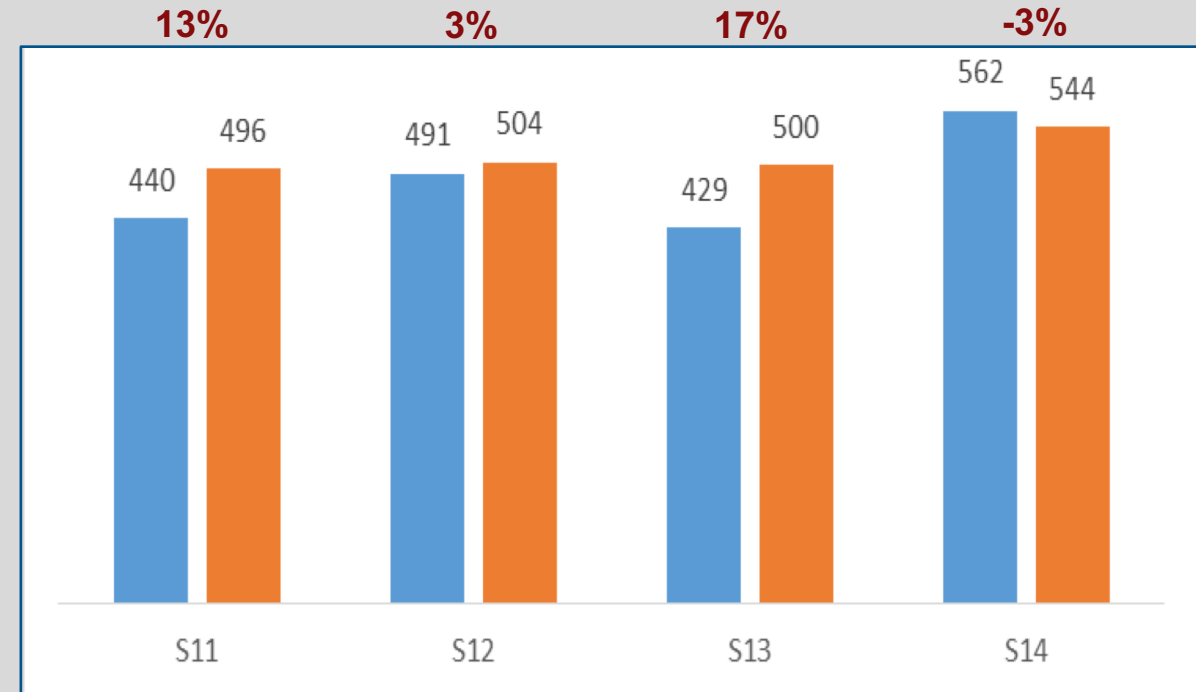
	Pull-Reduction Disabled	Pull-Up Reduction Enabled	Percent edits reduced
Test Site 1	268	86	68%
Test Site 2	59	9	85%
Test Site 3	64	17	73%

We Answer: Improved Data Interpretation – Pull-up Reduction

- Minor allele peak height was evaluated in the data from **one** of the test sites
 - (4) 1:7 GF Mixtures
 - 1ng total DNA input
 - Both with and without pull-up reduction enabled
 - Minor profiles concordant
 - No significant difference in peak height

Field Testing Results:

Minor Allele Peak Height Comparison



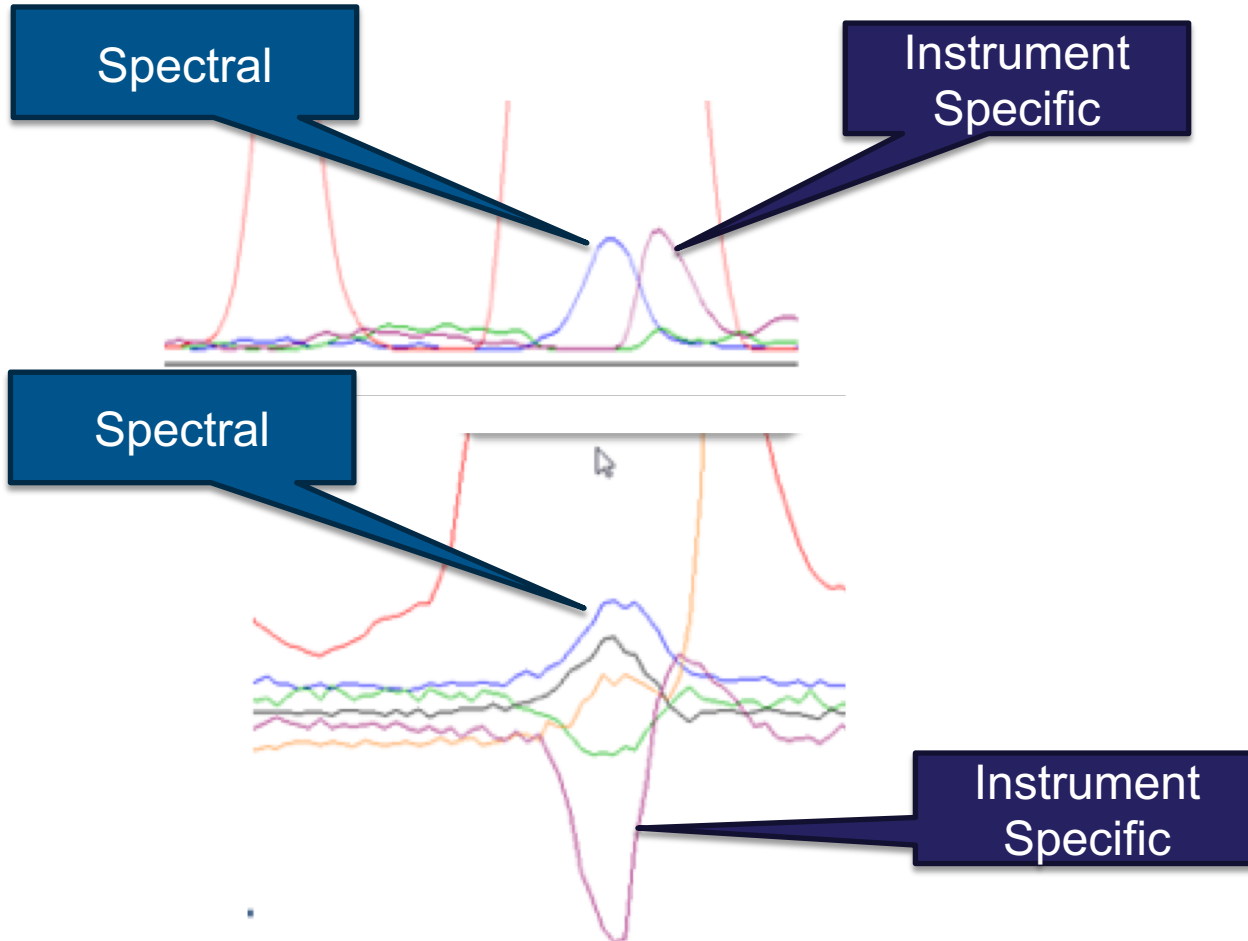
Pull up reduction ON

Pull up reduction OFF

% Difference in Peak Height

Myth or Fact?

- There is only one type of pull-up observed on the current Capillary Electrophoresis systems?



- **Myth**

- There are multiple types of pull-up that can be observed on capillary electrophoresis systems
 - Spectral pull-up and/or off-scale pull-up
 - Smaller peak centered under larger peak
 - Raw data shows similar parent and pull-up peak shape
 - Caused by variability between pure dyes and amplified product
 - Instrument specific pull-up
 - Smaller peak off-centered a bit from larger peak
 - Raw data shows sinusoidal curve
 - Caused by migration of dyes

You Ask: Higher First Pass Success Rate, Less Editing for Database Samples

Significant amount of time is spent editing database samples even after

- “High” threshold
- Implementation of 20% global filter

Main cause of editing is associated with off-scale samples

- Pull-up
- Elevated stutter

Off-scale data is useable for database samples, but it takes additional, manual review time.

Off-scale data is one of the main reasons a sample requires manual review.

10-15% of samples processed are off-scale and require additional attention.

We Answer: Off-Scale Data Recovery (OSR) for Database Samples

Capture “off-scale”

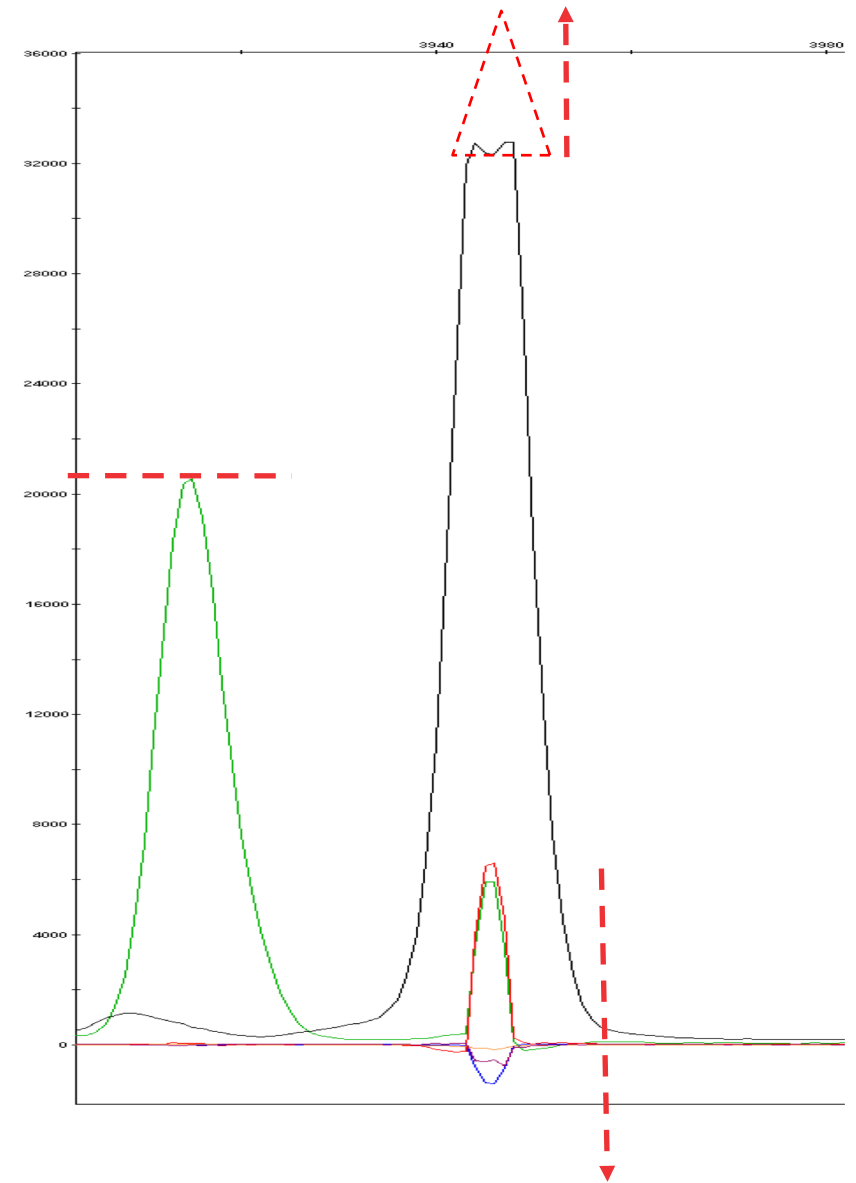
- Truer peak height
- Decrease manual interpretation
 - Off-scale data now approaching ~65,000 RFU
 - Global filters more effective
 - Stutter filters more applicable

Decrease pull-up associated with off-scale peak

On-scale data not impacted

Higher 1st pass success rate

Database kits: Identifiler™ Direct, Yfiler™ Direct, GlobalFiler™ Express, VeriFiler™ Express, Yfiler™ Plus kits
Casework kit in direct mode: GlobalFiler kit

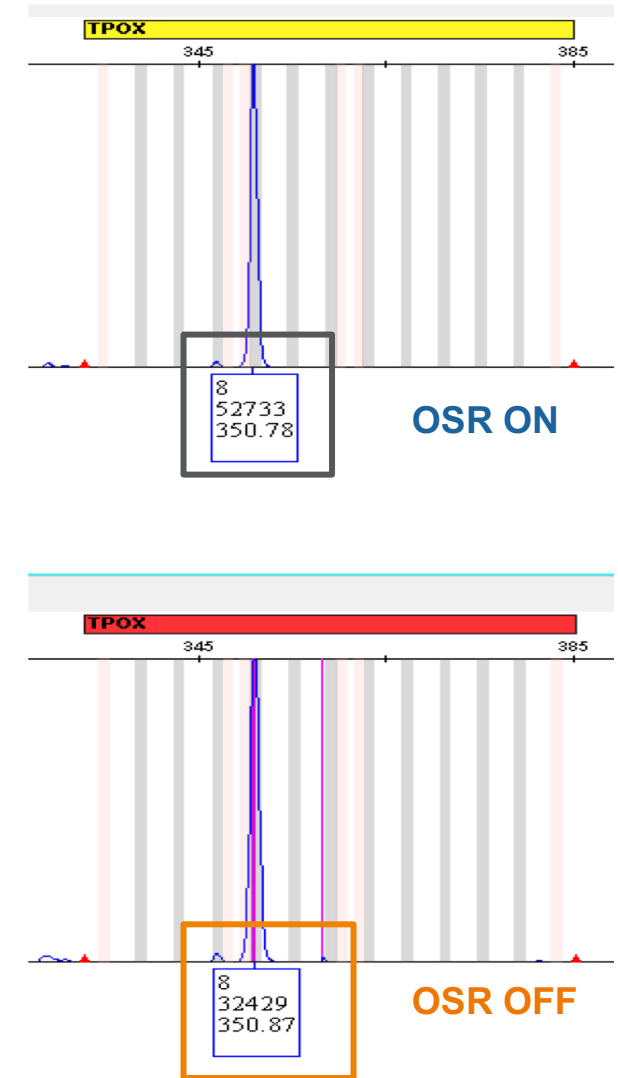


We Answer: Off-Scale Data Recovery (OSR) for Database Samples

During field testing, OSR increased first-pass success rate for all laboratories

- (10) reference samples run with the GlobalFiler Express Kit
- (3) 007 Positive Control DNA samples run in high concentration

	OSR Disabled	OSR Enabled
Test Site 1	5 markers in 2 samples	0 markers in 0 samples
Test Site 3	99 markers in 12 samples	4 markers in 1 sample
Test Site 2	5 markers in 2 samples	0 markers in 0 samples



Myth or Fact?

- The off-scale data recovery feature is perfect for those samples where I have a low-level minor in a mixture and would like to input more total DNA (such as 4 or 6 ng) to increase the peak heights of the minor contributor

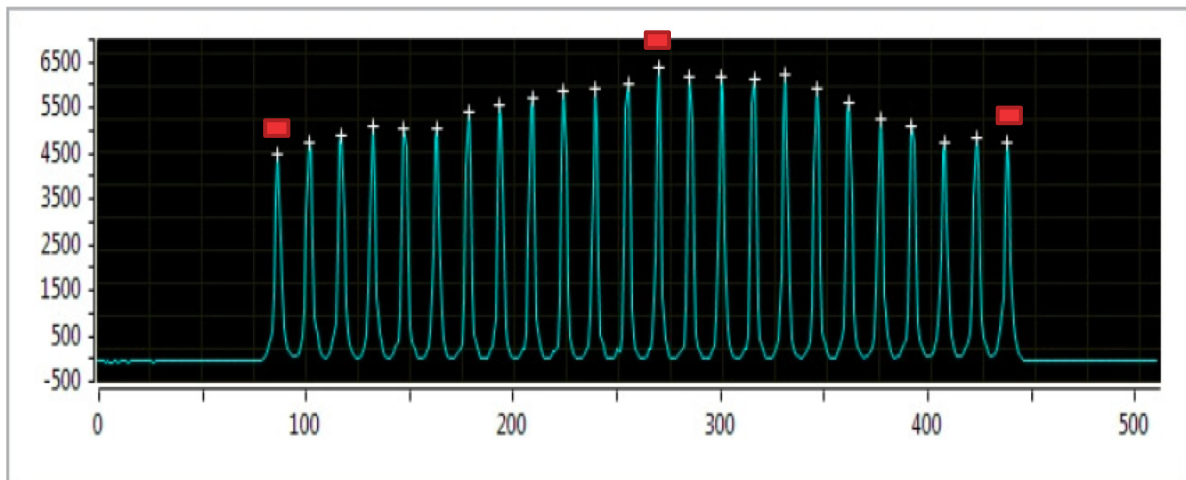


- **Myth**

- This feature was developed for and intended to be used in systems where robust, expected profiles are tested allowing for high thresholds (such as 175 RFU) and the implementation of a global filter
- As you increase the amount of DNA into the system, it is more likely to generate low-level peaks caused by cross-talk and/or carry-over
- Amplification kits are designed for an optimal amount of input DNA and there are reagent limitations as you increase the amount (i.e. increased stutter, minus A, peak height plateau)
- This feature is not recommended for the processing of casework samples

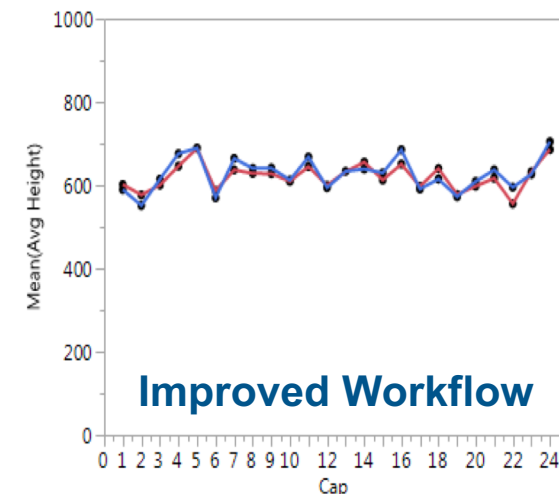
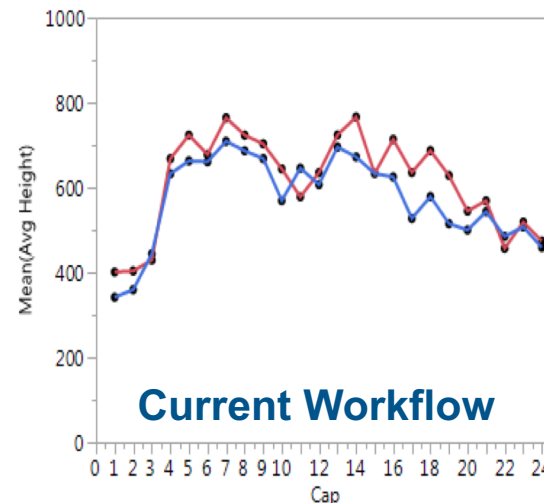
Continuous Instrument Improvement: 3500 Series Instruments

You Ask: Minimize signal variation



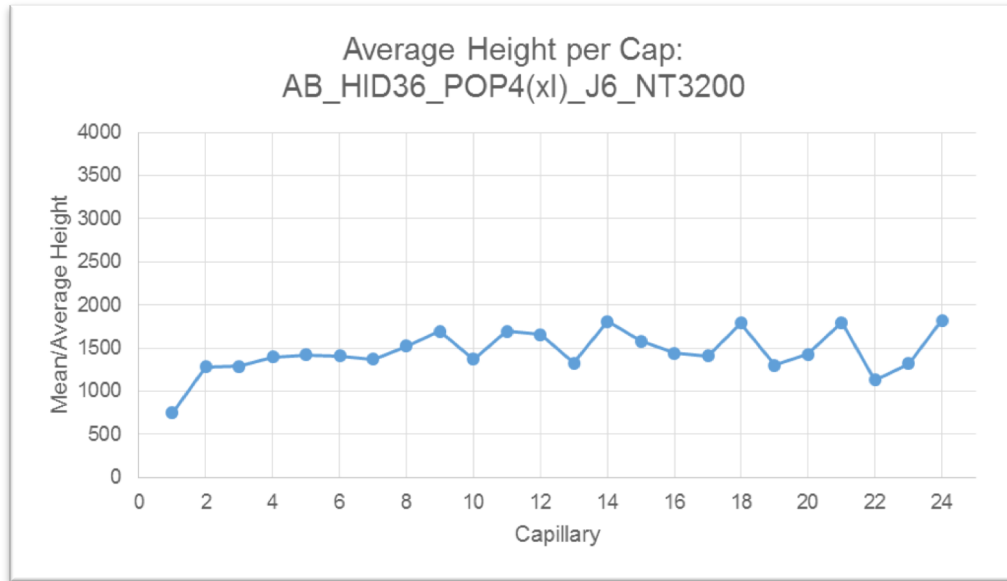
- Peak heights for samples in the edge capillaries may be lower than the middle capillaries
 - Adds to variation in the workflow, where higher peak heights may be observed for middle vs. edge capillaries
- Customer letter released on October 21, 2014, “Degree of signal variation observed in 3500xL multi-capillary instruments”

We Answer: Improve across-capillary consistency



- Signal Optimization: Two-step method to decrease variation across the 24 capillaries in the array
 - A **signal optimization factor** is determined based upon the spatial calibration and applied to the data
- Optional **signal optimization run** module that further aligns edge and middle capillaries

Spatial & Signal Optimization: Test Site 1 Data

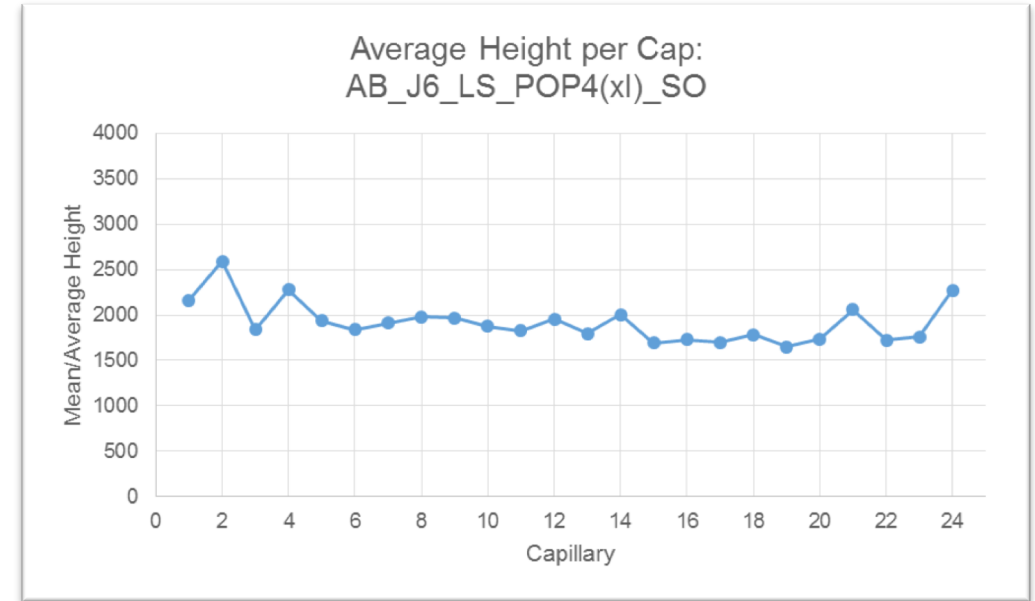


- Average of (3) 3500xL injections of GlobalFiler Allelic Ladder

- **Spatial Optimization Only (Factor)**

- CV% = 22%

- $\frac{\text{Maximum Capillary RFU}}{\text{Minimum Capillary RFU}} = 2.4$



- Average of (3) 3500xL injections of GlobalFiler Allelic Ladder

- **Spatial + Signal Optimization (Run Module)**

- CV% = 14%

- $\frac{\text{Maximum Capillary RFU}}{\text{Minimum Capillary RFU}} = 1.57$

Myth or Fact?

- Signal variation improvements were first introduced with the 3500 instrument

- **Myth**

- In the 3500 instrument, there is factory level optimization that attempts to address variations in signal intensity across the capillary introduced by the optics
- Service engineers also have always had the ability to adjust optics and hardware to help decrease variation and perform laser normalization during PM
- With the 3500, Size Standard Normalization was introduced as an additional, optional step
- With DC4.0 we have an added two step approach with the spatial based Signal Optimization and unique run modules

Efficient Data Transfer: Probabilistic Genotyping

You Ask: Streamline current workflow

Initial Data Analysis

- Apply stutter filters
- Edit artifacts
- Export sample allele table

Secondary Data Analysis

- Re-analyze with stutter filters turned off
- Mimic primary artifact editing
- Export allele table (including stutter peaks) for probabilistic genotyping software

Review: Primary Data Analysis

- Review of artifact calls from initial data analysis
- Confirm edits

Review: Secondary Data Analysis

- Review of artifact calls from secondary data analysis
- Confirm edits
- Verify concordance of initial and secondary analysis

We Answer: One-analysis, two export files

Data Analysis

- Apply stutter filters
- Edit artifacts
- Export allele tables:
 - Without stutter peaks
 - With stutter peaks

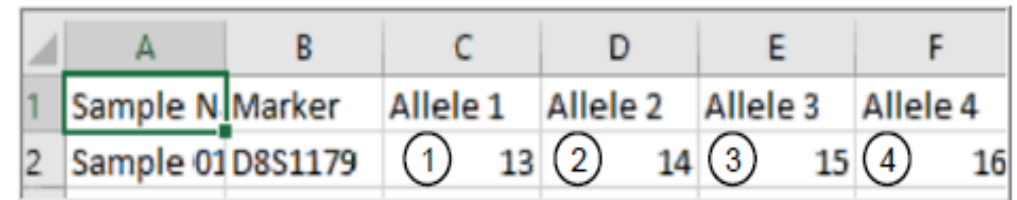
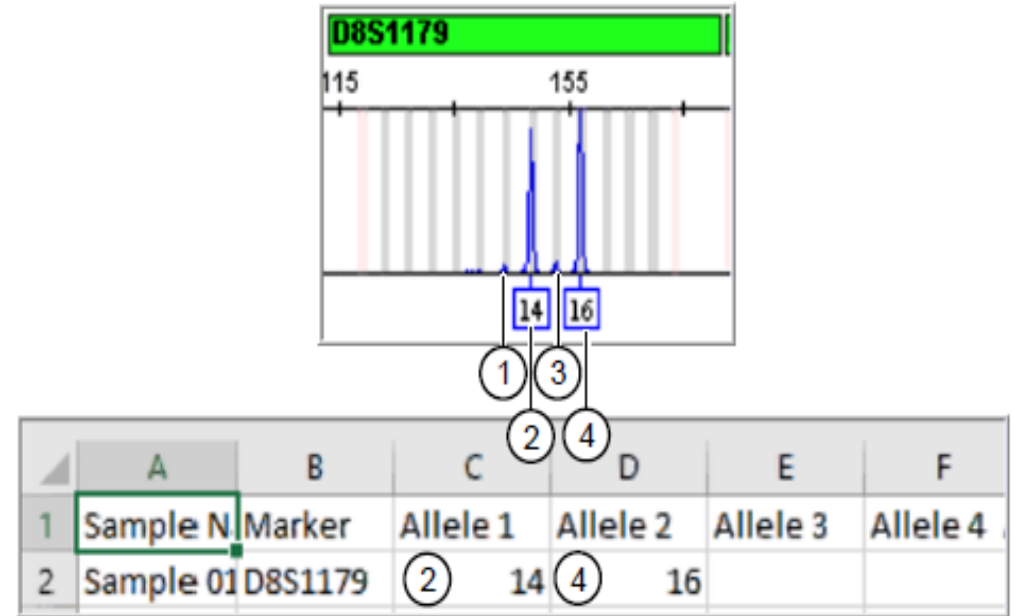
Review

- Single review of artifact edits

Save time and increase confidence in your data

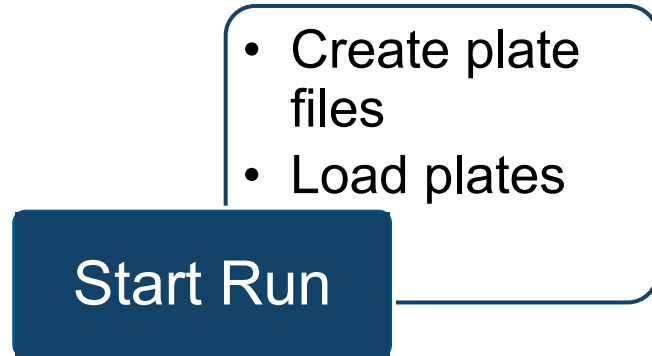
Probabilistic Genotyping – Data Export

- Two export file type options:
 - .txt or .csv
 - Select from the right-hand drop-down menu
- When you export without stutter:
 - Labeled allele peaks are identified as Allele 1 and Allele 2
- When you export with stutter:
 - Stutter peaks and labeled allele peaks are labeled sequentially

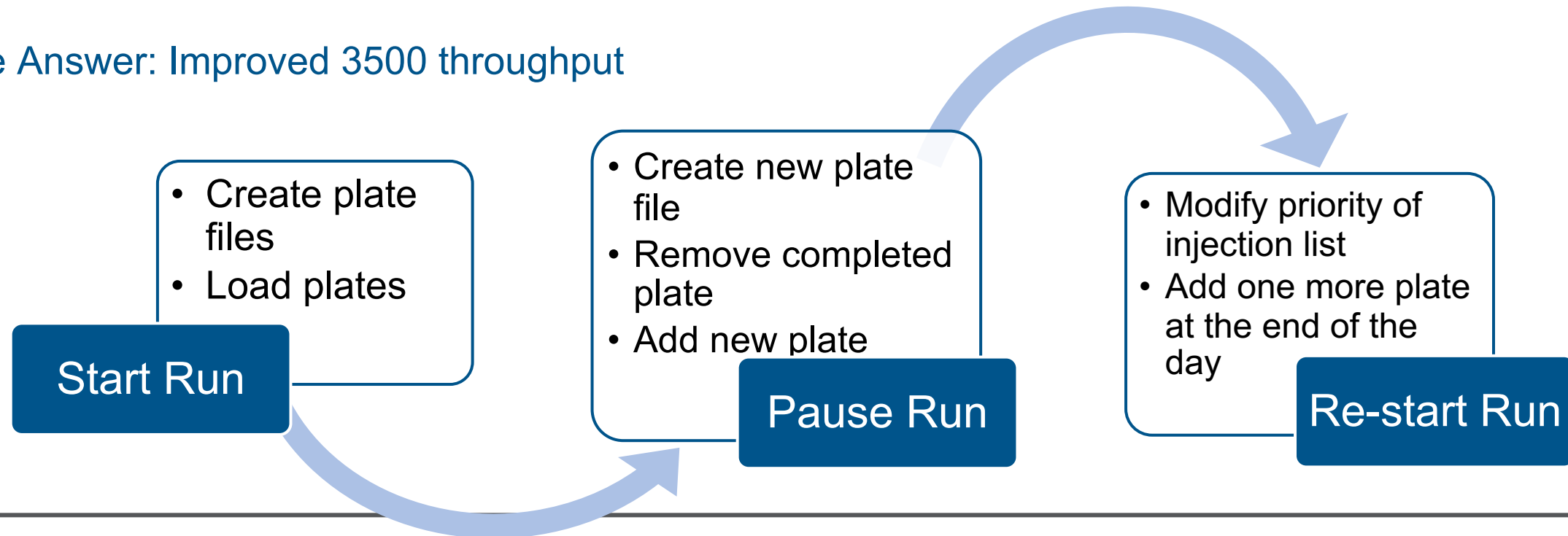


Enhanced Flexibility of the 3500 Series Instrument

You Ask: The ability to process more samples



We Answer: Improved 3500 throughput



Myth or Fact?

- Running of additional plates is great, but my laboratory does not need additional throughput therefore this feature is not going to impact me.



- **Myth**
 - In addition to having the flexibility to run more plates, this feature also allows you to quickly process priority cases
 - For example, you have a rush sample ready to run but there is a full 96 well plate in-progress. Now you do not have to wait for the plate to finish. You can pause the run, add your rush plate, prioritize the rush sample to inject next.



- Win 10 solution for GeneMapper ID-X and 3500 Data Collection Software
- CODIS 8 compatibility: 3.2R16 and 3.3 CMF export
- Internationalization
 - Multiple paper sizes
 - Two languages supported

GeneMapper *ID-X* v1.6 Key Features

Windows 10
64-bit
Compatibility

Windows 7
64-bit
Compatibility

Off-Scale Data
Recovery

Data Export for
Probabilistic
Genotyping
Software

Latest CODIS
support

Command Line
Interface (CLI)
Updates

Enhanced User
Interfaces

Printing
Enhancements

Sorting
Improvements

SeqStudio File
Support

Profile
Comparison Tool
Updates

Support for
Chinese
Language

Your Asks

What's next?

Our Answers

3500 Data Collection v4

3500 Data Collection v4 & GeneMapper ID-X v1.6

GeneMapper ID-X v1.6



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