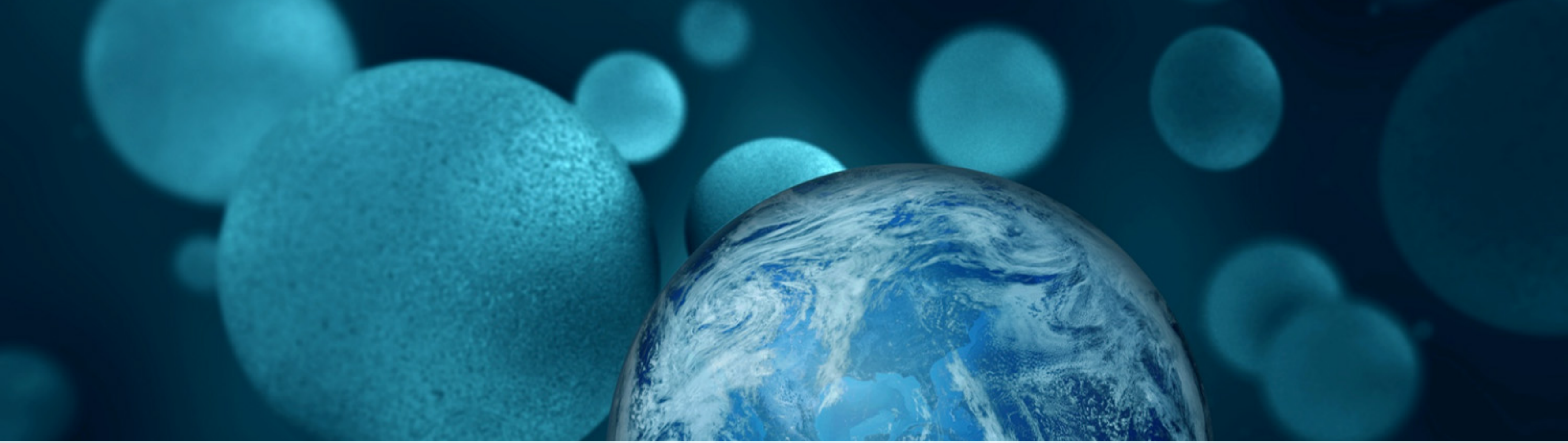


The Nuts and Bolts behind our Solutions to your Latest Laboratory Challenges

Presenter Name

Overview

You Ask, We Answer

3500/3500xL Data Collection v4.0 & GeneMapper^{TM} *ID-X* v1.6

Pull-up Reduction, Off-scale Data Recovery, Signal Optimization

We are on the Journey with You: You Ask, We Answer

- Customer needs continue to evolve
- Implementation of expanded multiplex kits opened a door to new challenges
- Your asks have helped shape the products we develop

Customer Feedback

Decrease Analysis Time

Improve Efficiency

Support Flexible Workflows

DC v4.0

GMIDX v1.6

The Exterior: Data Collection v4.0 and GeneMapper IDX v1.6

Decrease Analysis Time

Improve Efficiency

Support Flexible Workflows

Improve Data Interpretation

- Reduce pull-up edits
- Improve 1st pass success
- Signal Optimization
- 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
- Feature requests

IT Compliance

- Win 10 support
- CODIS compatibility
- Internationalization

Under the Hood: Pull-Up Reduction

Pull-up definition and expectations

- Software does not perfectly separate the dyes based on the calibration data
- Generally 1-3% of parent peaks
- Calibration cannot be accurately applied to off-scale data, may result in higher pull-up peaks

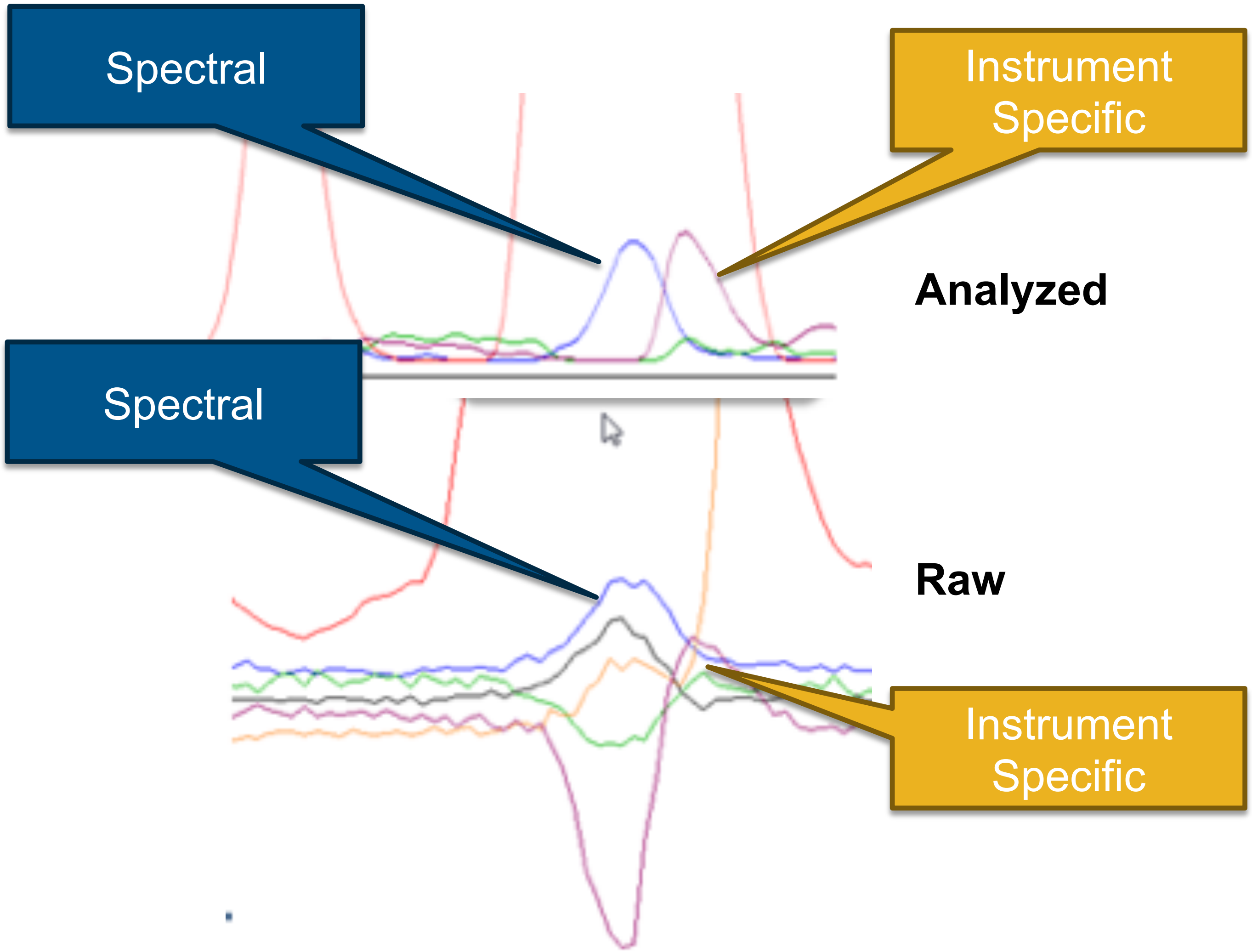
Spectral pull-up

- Calibration dye data does not perfectly match sample dye data
- Pure dyes are used for calibration
- In samples the dyes are attached to DNA fragments which changes the spectra from the pure dye spectra
- The difference in the spectra leads to the imperfect correction and spectral pull-up

Instrument-specific pull-up

- Movement of fluorescent molecules across the detection window causes the spectrum on the CCD to shift
- May be additive with spectral pull-up

Under the Hood: Pull-Up Reduction



Spectral

- Analyzed data: centered under parent peak
- Raw data: same shape as parent peak

Instrument-Specific

- Analyzed data: not centered under parent peak
- Raw data: sinusoidal curve

Under the Hood: Pull-Up Reduction

Pull-up reduction

Addresses spectral (including off-scale) and instrument-specific pull-up

Utilizes sample-specific spectral data

To enable pull-up reduction

Utilize an Applied Biosystems (AB) STR kit

Perform spectral calibration using an AB dye matrix and AB dye set

Choose applicable AB assay

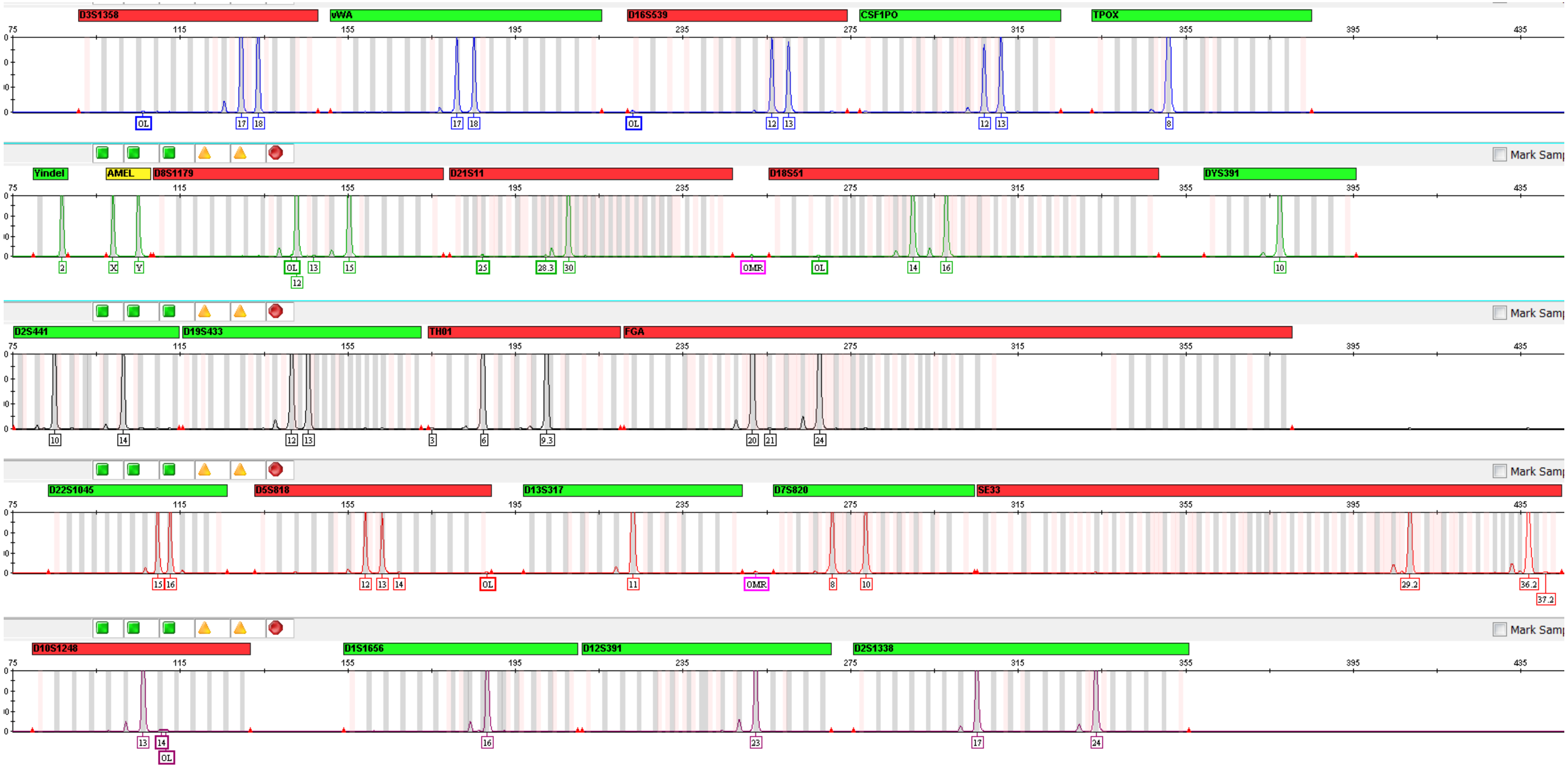
Spectral correction

Takes place prior to creation of .hid files

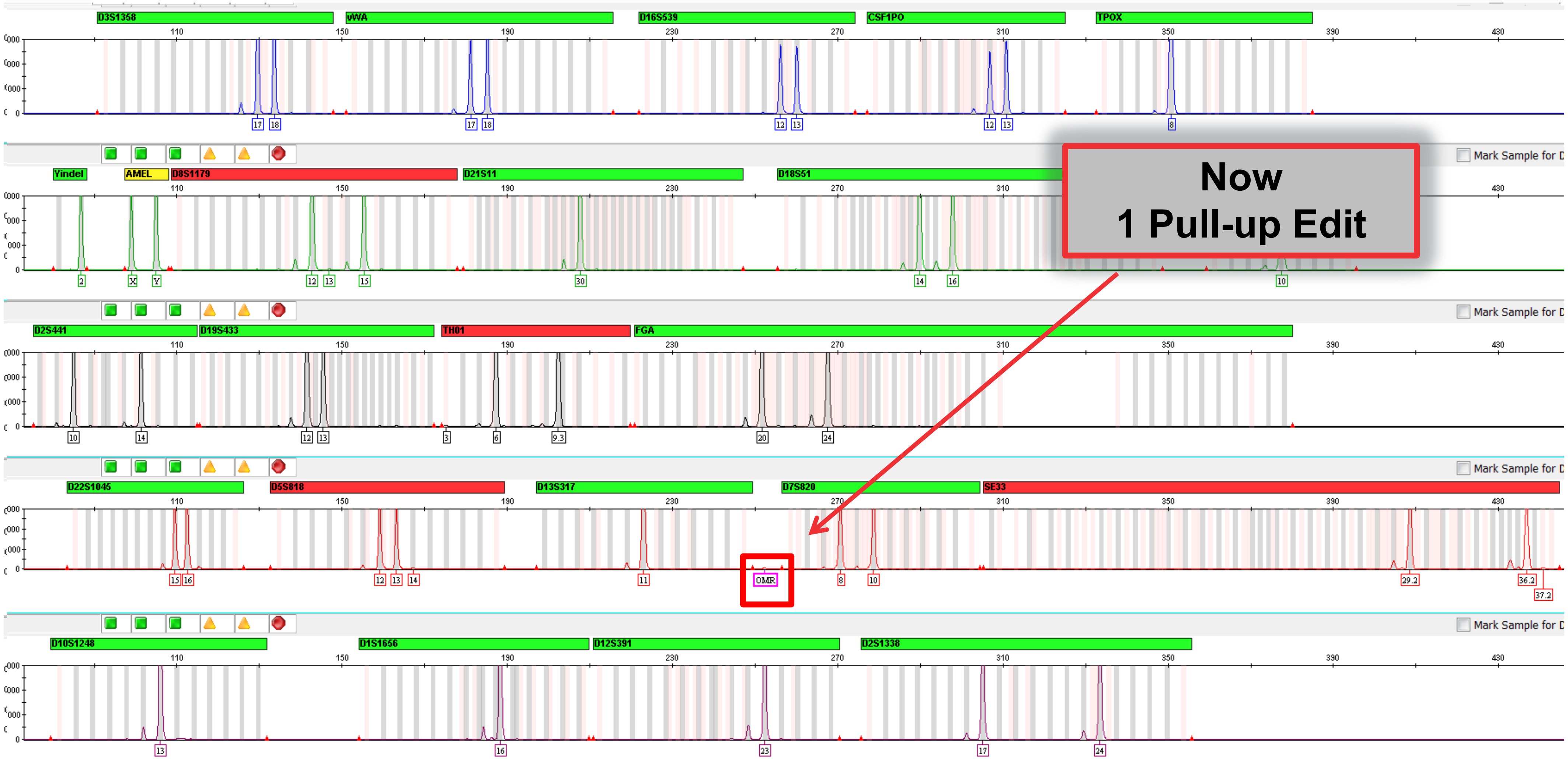
Raw Data viewed in GeneMapper IDX (all versions) will have the reduction applied

Under the Hood: Pull-Up Reduction

Single Source, 1ng, GF sample



Under the Hood: Pull-Up Reduction



Now
1 Pull-up Edit

Under the Hood: Pull-Up Reduction

Allelic ladders – pull-up reduction **DISABLED**

- Identified by labeling as allelic ladder sample type
- Identified by density of peaks in profile

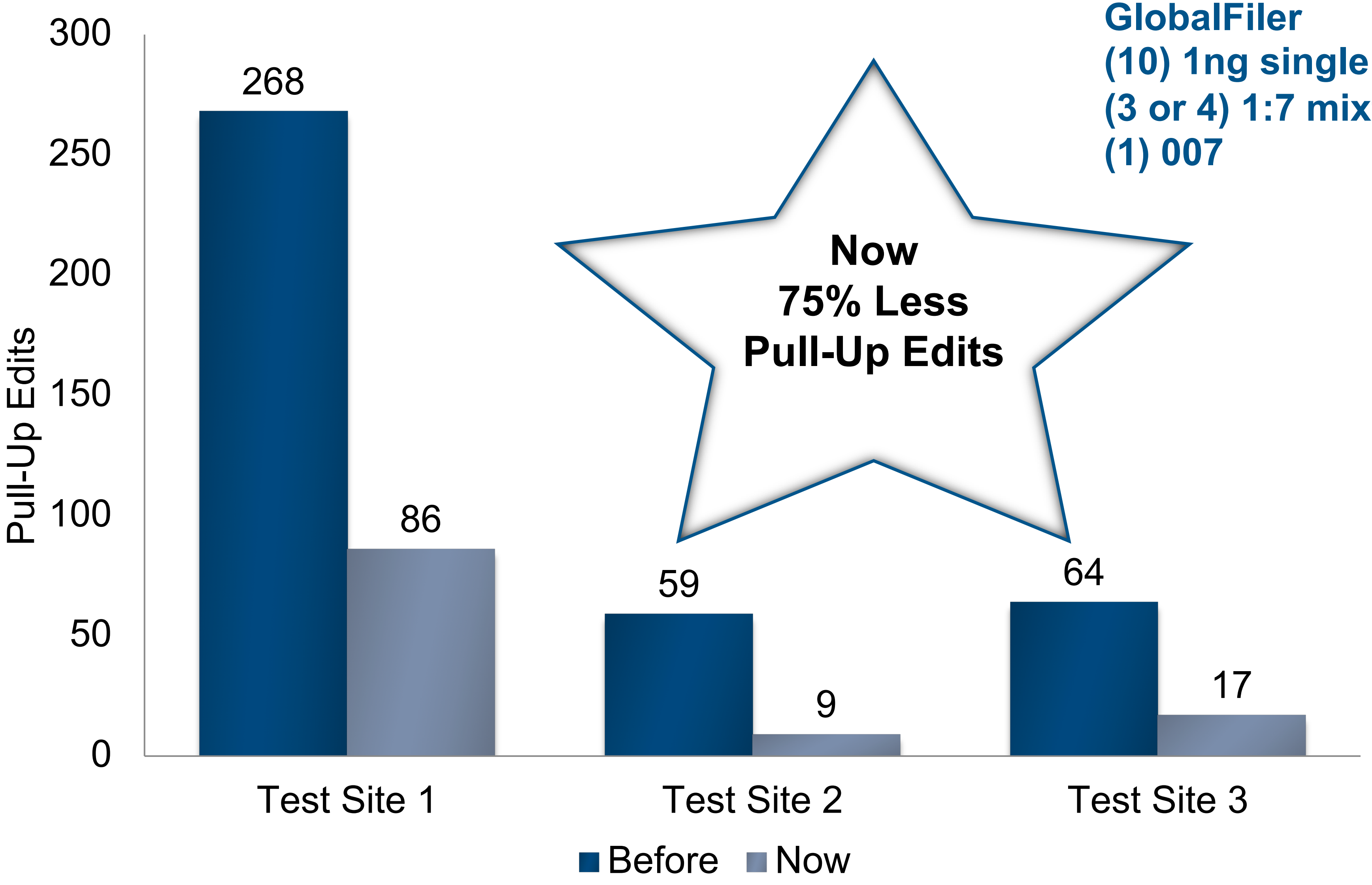
Poor quality samples – pull-up reduction **DISABLED**

- Signal-to-noise ratio
- Number of peaks and dyes detected
- Calibrated matrix will still be applied (ie sample-specific spectral data not utilized due to poor quality)

Important Notes

- Adds approximately 2-3 minutes per injection for processing (3500xL)
- Pull-up frequency is reduced
- Peak height percentage of remaining pull-up generally falls within 1-3%

Under the Hood: Pull-Up Reduction Field Testing



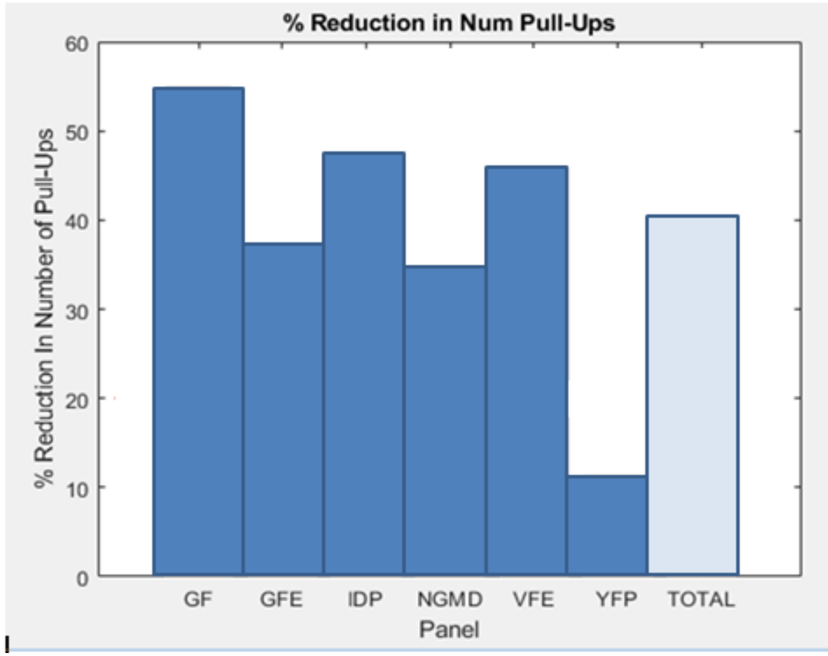
Now
75% Less
Pull-Up Edits

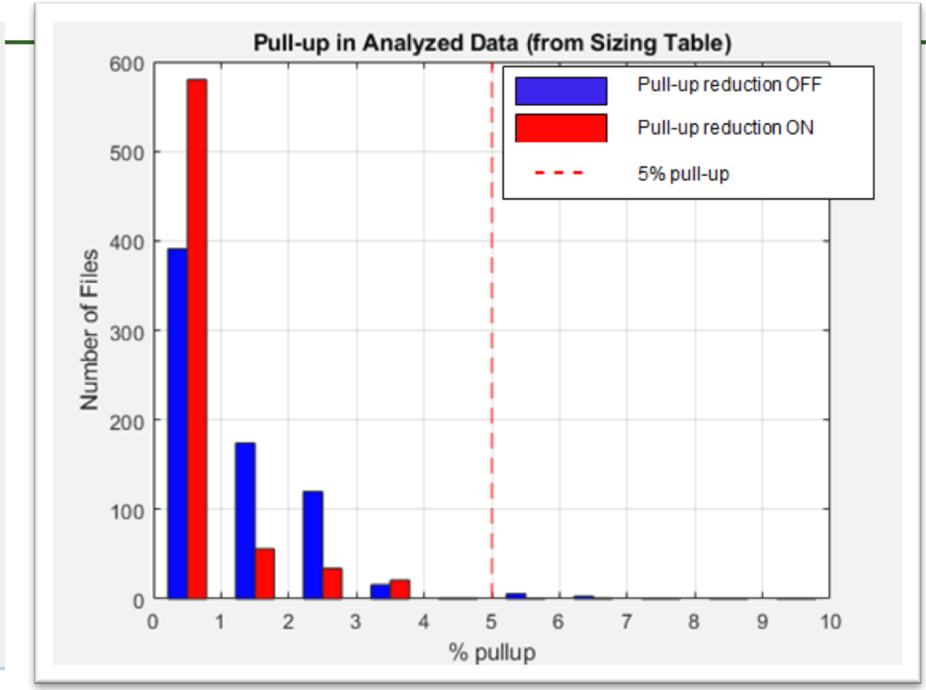
GlobalFiler
(10) 1ng single source samples
(3 or 4) 1:7 mixtures
(1) 007

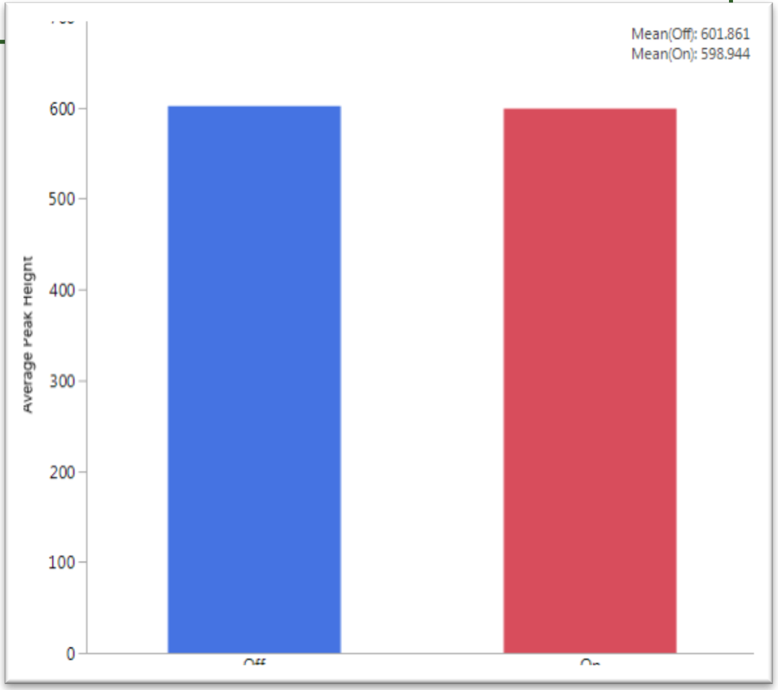
Under the Hood: Pull-Up Reduction Internal Validation

Results

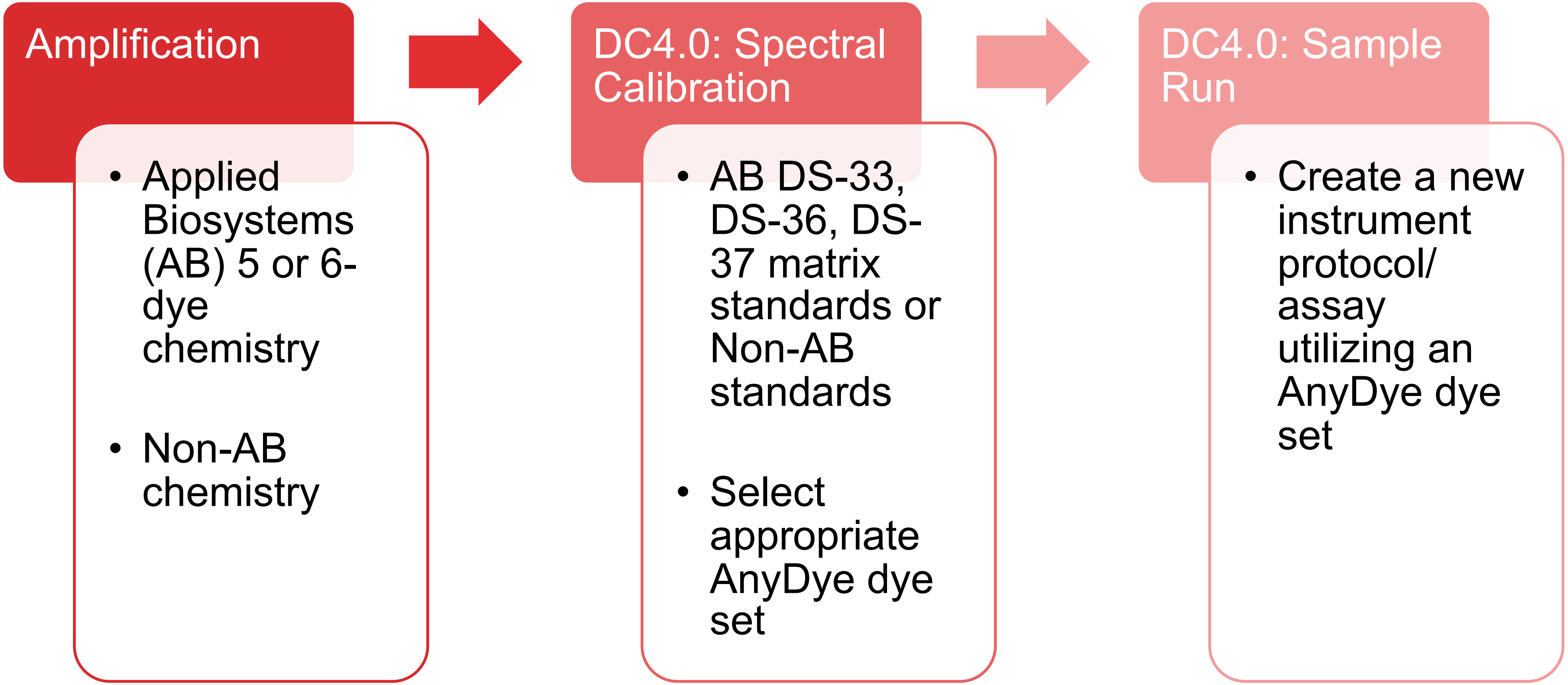
- Number of pull-up peaks above PAT reduced for all 6 kits tested
  - Average reduction across kits ~40%
- Decrease in the number of samples with pull-up >5% (Off: 1.5%; On 0.25%)
- No impact to minor genotype or significant difference to peak heights (Off: ~602 RFU; On: ~599 RFU)







Under the Hood: Pull-Up Reduction Disable



Amplification

- Applied Biosystems (AB) 5 or 6-dye chemistry
- Non-AB chemistry

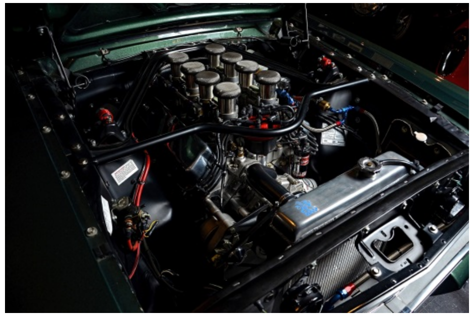
DC4.0: Spectral Calibration

- AB DS-33, DS-36, DS-37 matrix standards or Non-AB standards
- Select appropriate AnyDye dye set

DC4.0: Sample Run

- Create a new instrument protocol/ assay utilizing an AnyDye dye set

Under the Hood: Off-Scale Data Recovery (OSR)



Off-scale data requires manual review

- Editing of pull up and elevated stutter

Customers report:

- 10-15% of database samples are off-scale
- Even with a 20% global filter
- Even with a “high” 175 RFU threshold

Off-scale data: 3500 Series Characteristics

- Peak heights capped at ~32,000 RFU
  - Does not reflect true peak intensity
  - Global filter based on capped peak height value
- Pull-up peaks common under OS peaks
  - Pull-up percentage based on capped peak height value

Under the Hood: Off-Scale Data Recovery (OSR)

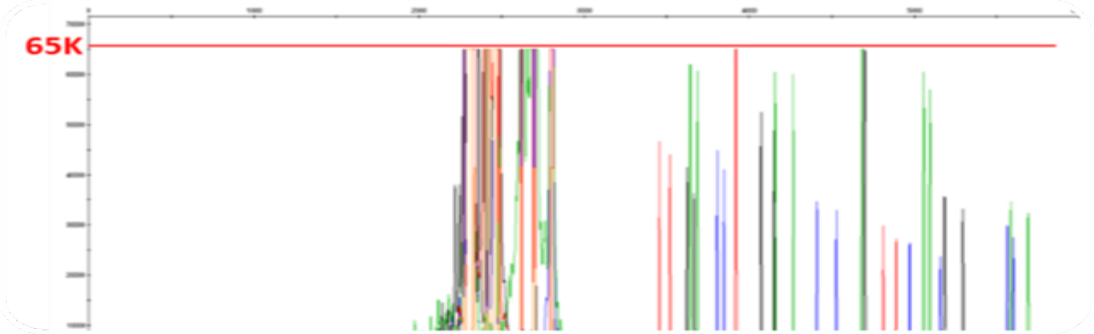
Saturation

- Bin data signals saturation
- Bin data converted to dye data
- Dye data capped at ~32K RFU values

Off-Scale Data Recovery

- CCD camera saturation limit is extended
- More bin data can be utilized
- Dye data capped at ~65K RFU values

GMIDX 1.6 is required to analyze/view OSR data above ~32K RFU.



Under the Hood: Off-Scale Data Recovery (OSR)

RFU upper limit extended for DC 4.0

- Upper limit: ~65,000 RFU
- **MUST** use GeneMapper™ IDX v 1.6

Better peak height estimate

- Global filters more effective
- Stutter filters more applicable
- Decreased pull up
- Higher 1st pass success rate
- On-scale data not impacted

Severely off-scale data (>65K RFU)

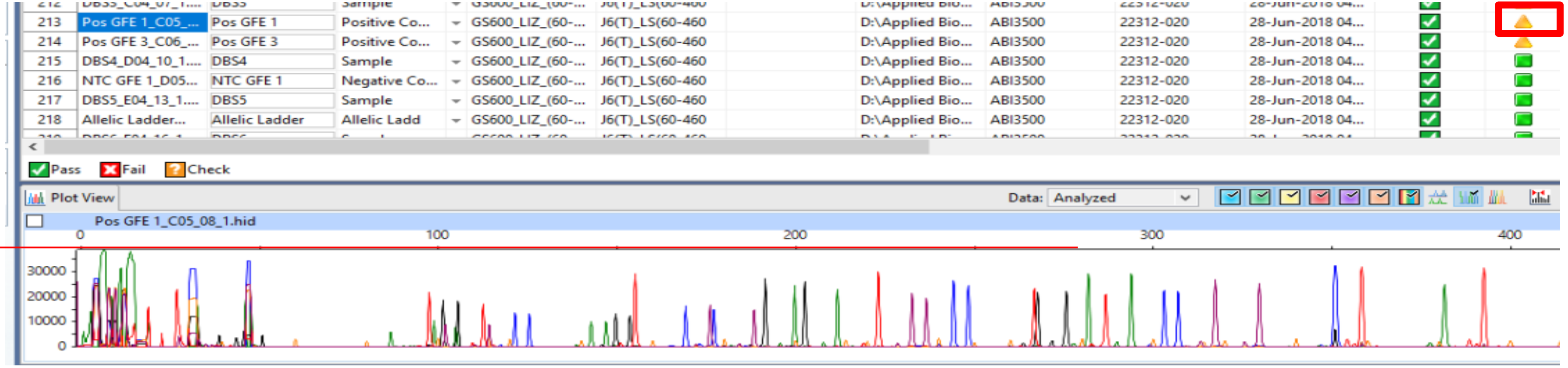
- Peaks will be capped
- Secondary dye colors may be pulled-up to 65K
- Analyze with caution!

Under the Hood: Off-Scale Data Recovery (OSR)

DC 4.0 results view collecting without and with OSR (reinjection of same sample)

No OSR

35K

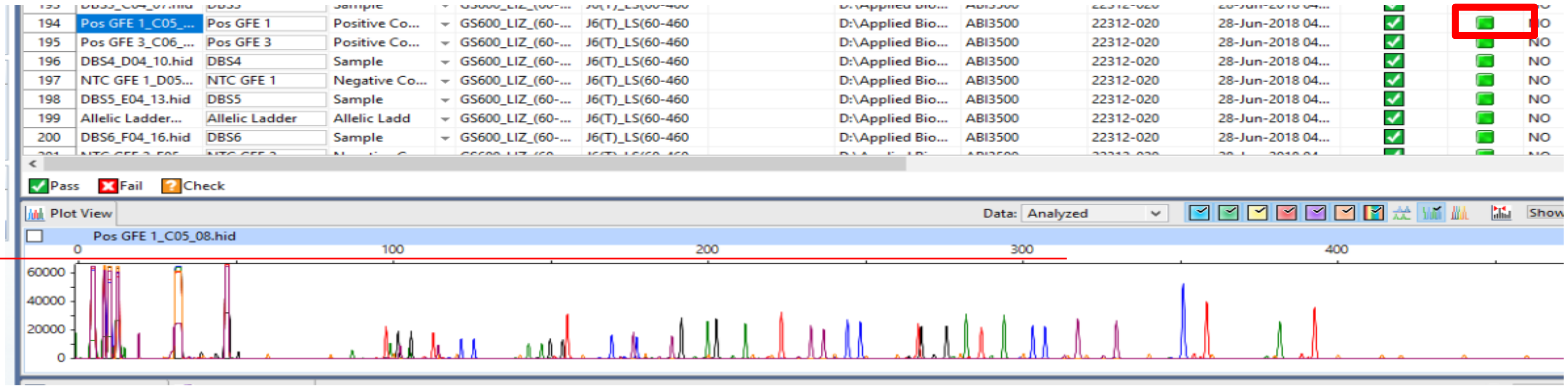


OS flag fired

213	Pos GFE 1_C05_...	Pos GFE 1	Positive Co...	GS600_LIZ_(60-...	J6(T)_LS(60-460	D:\Applied Bio...	ABI3500	22312-020	28-Jun-2018 04...	✓	▲
214	Pos GFE 3_C06_...	Pos GFE 3	Positive Co...	GS600_LIZ_(60-...	J6(T)_LS(60-460	D:\Applied Bio...	ABI3500	22312-020	28-Jun-2018 04...	✓	▲
215	DBS4_D04_10_1....	DBS4	Sample	GS600_LIZ_(60-...	J6(T)_LS(60-460	D:\Applied Bio...	ABI3500	22312-020	28-Jun-2018 04...	✓	🟩
216	NTC GFE 1_D05...	NTC GFE 1	Negative Co...	GS600_LIZ_(60-...	J6(T)_LS(60-460	D:\Applied Bio...	ABI3500	22312-020	28-Jun-2018 04...	✓	🟩
217	DBS5_E04_13_1....	DBS5	Sample	GS600_LIZ_(60-...	J6(T)_LS(60-460	D:\Applied Bio...	ABI3500	22312-020	28-Jun-2018 04...	✓	🟩
218	Allelic Ladder...	Allelic Ladder	Allelic Ladd	GS600_LIZ_(60-...	J6(T)_LS(60-460	D:\Applied Bio...	ABI3500	22312-020	28-Jun-2018 04...	✓	🟩

OSR

65K



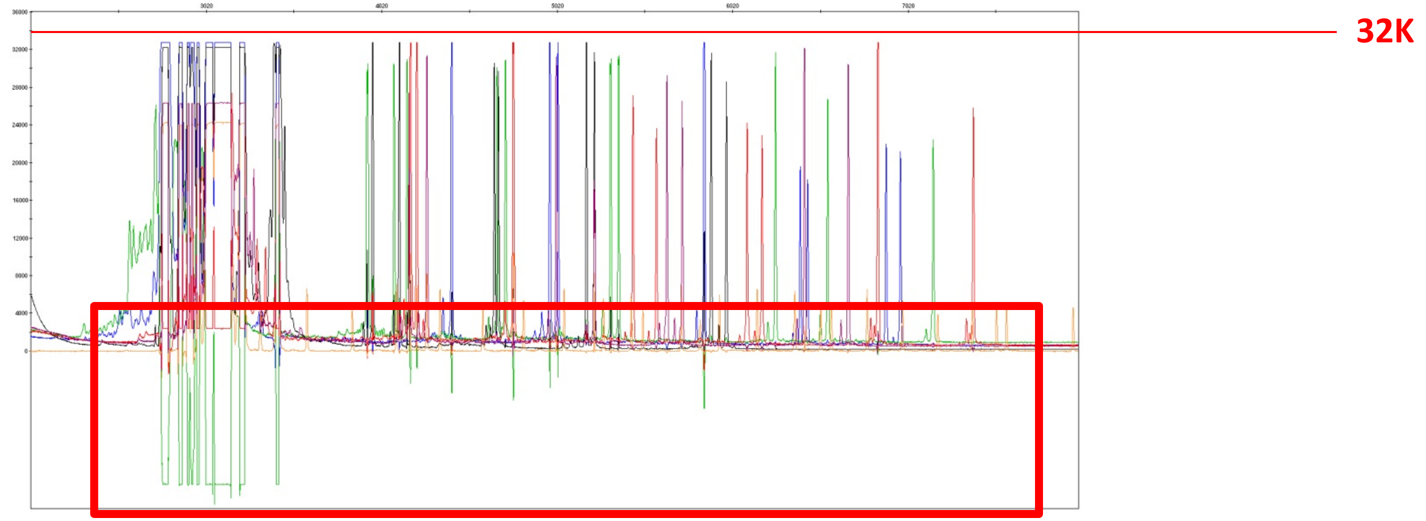
OS flag PASS

194	Pos GFE 1_C05_...	Pos GFE 1	Positive Co...	GS600_LIZ_(60-...	J6(T)_LS(60-460	D:\Applied Bio...	ABI3500	22312-020	28-Jun-2018 04...	✓	🟩	NO
195	Pos GFE 3_C06_...	Pos GFE 3	Positive Co...	GS600_LIZ_(60-...	J6(T)_LS(60-460	D:\Applied Bio...	ABI3500	22312-020	28-Jun-2018 04...	✓	🟩	NO
196	DBS4_D04_10.hid	DBS4	Sample	GS600_LIZ_(60-...	J6(T)_LS(60-460	D:\Applied Bio...	ABI3500	22312-020	28-Jun-2018 04...	✓	🟩	NO
197	NTC GFE 1_D05...	NTC GFE 1	Negative Co...	GS600_LIZ_(60-...	J6(T)_LS(60-460	D:\Applied Bio...	ABI3500	22312-020	28-Jun-2018 04...	✓	🟩	NO
198	DBS5_E04_13.hid	DBS5	Sample	GS600_LIZ_(60-...	J6(T)_LS(60-460	D:\Applied Bio...	ABI3500	22312-020	28-Jun-2018 04...	✓	🟩	NO
199	Allelic Ladder...	Allelic Ladder	Allelic Ladd	GS600_LIZ_(60-...	J6(T)_LS(60-460	D:\Applied Bio...	ABI3500	22312-020	28-Jun-2018 04...	✓	🟩	NO
200	DBS6_F04_16.hid	DBS6	Sample	GS600_LIZ_(60-...	J6(T)_LS(60-460	D:\Applied Bio...	ABI3500	22312-020	28-Jun-2018 04...	✓	🟩	NO

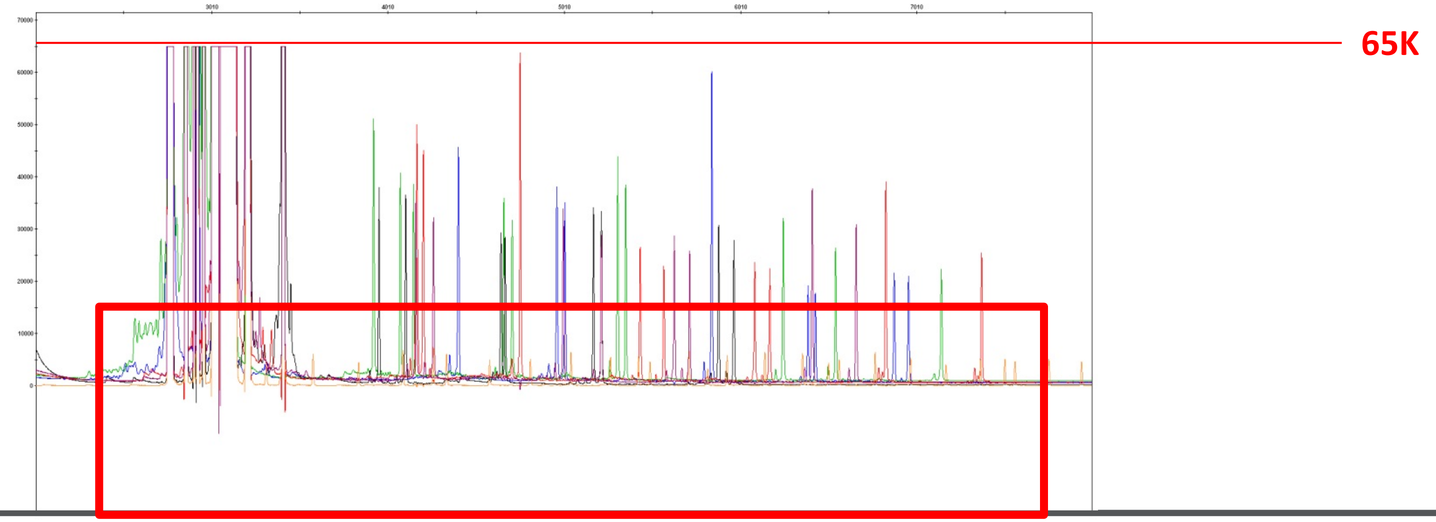
Under the Hood: Off-Scale Data Recovery (OSR)

GMIDXv1.6 raw data collecting without and with OSR (reinjection of same sample)

No OSR



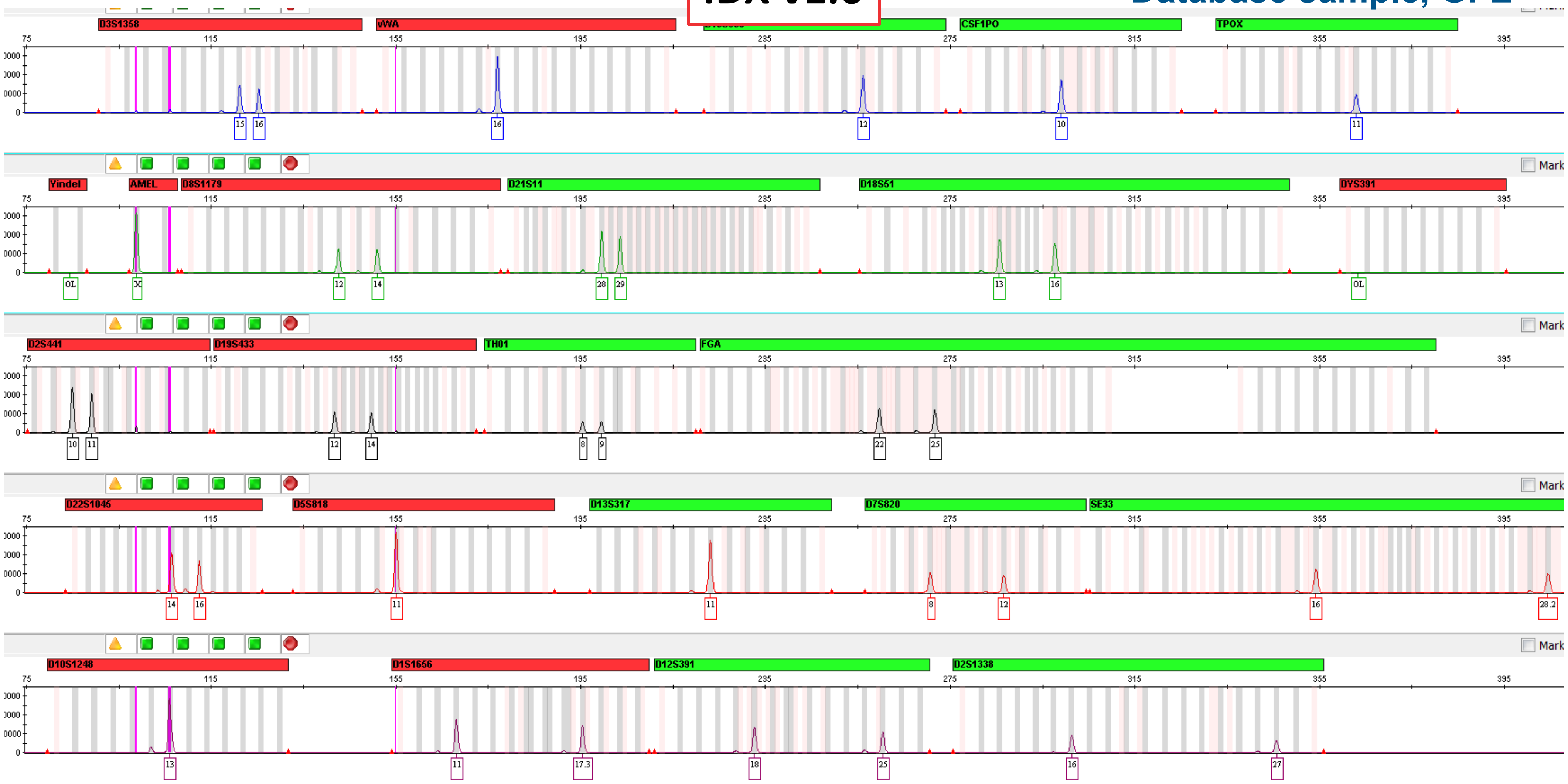
OSR



3500 Data Collection v4.0: Off-Scale Recovery

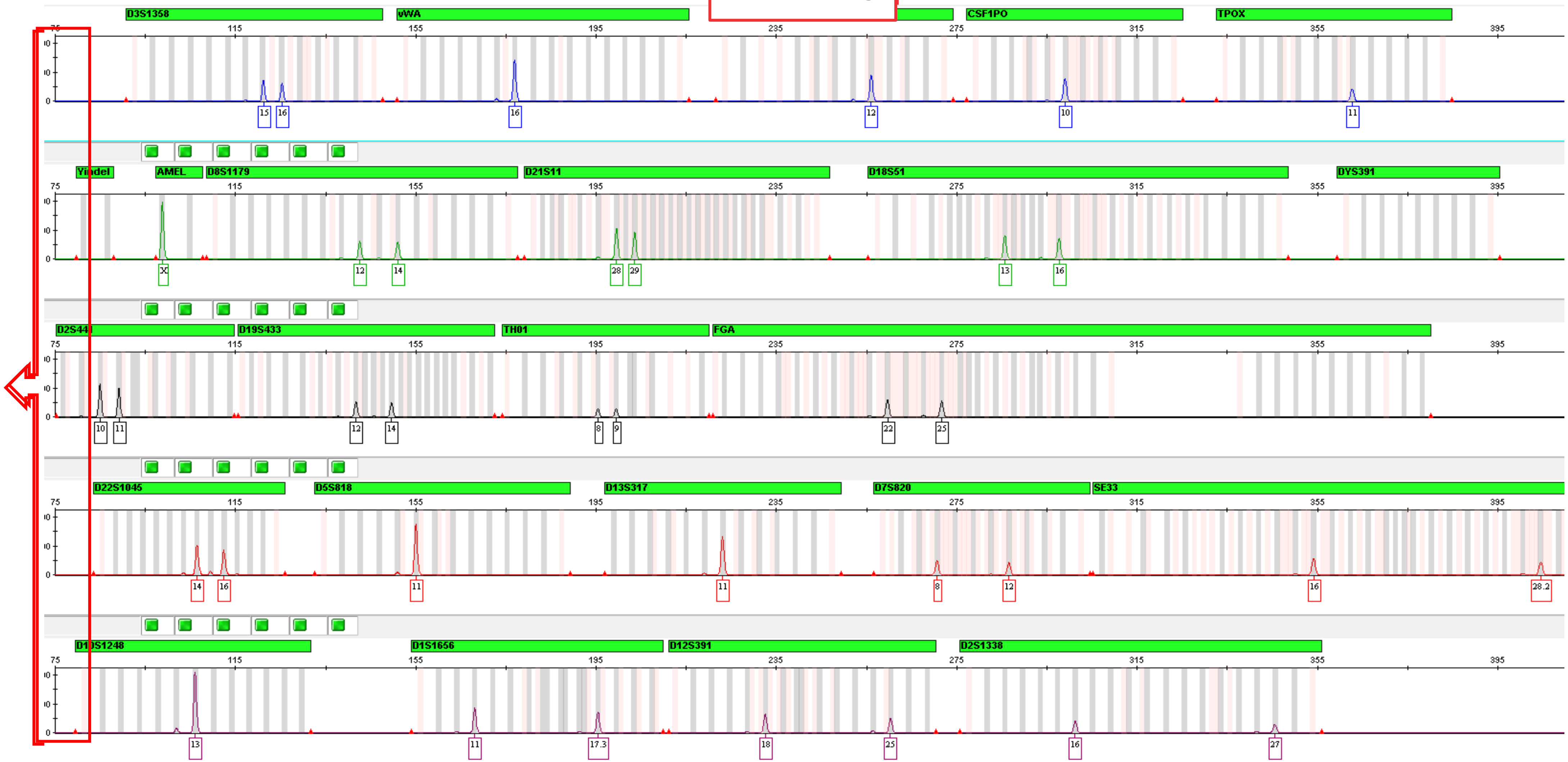
IDX v1.6

Database sample, GFE



3500 Data Collection v4.0: Off-Scale Recovery

IDX v1.6



45K

35K

BEFORE: 10 Off-scale markers, 16 Marker level flags

NEW: 10 Off-scale markers, 0 Marker level flags

Under the Hood: Off-Scale Data Recovery (OSR)

Developed for database samples

- Robust, single source profiles
- High thresholds
- Global filter

Increased DNA input causes

- Cross talk
- Carryover
- PCR-related artifacts
- Peak height plateau due to reagent consumption

Not intended for casework samples

Under the Hood: Off-Scale Data Recovery (OSR) Disable

Passing spectral

- Calibrate AnyDye dye set

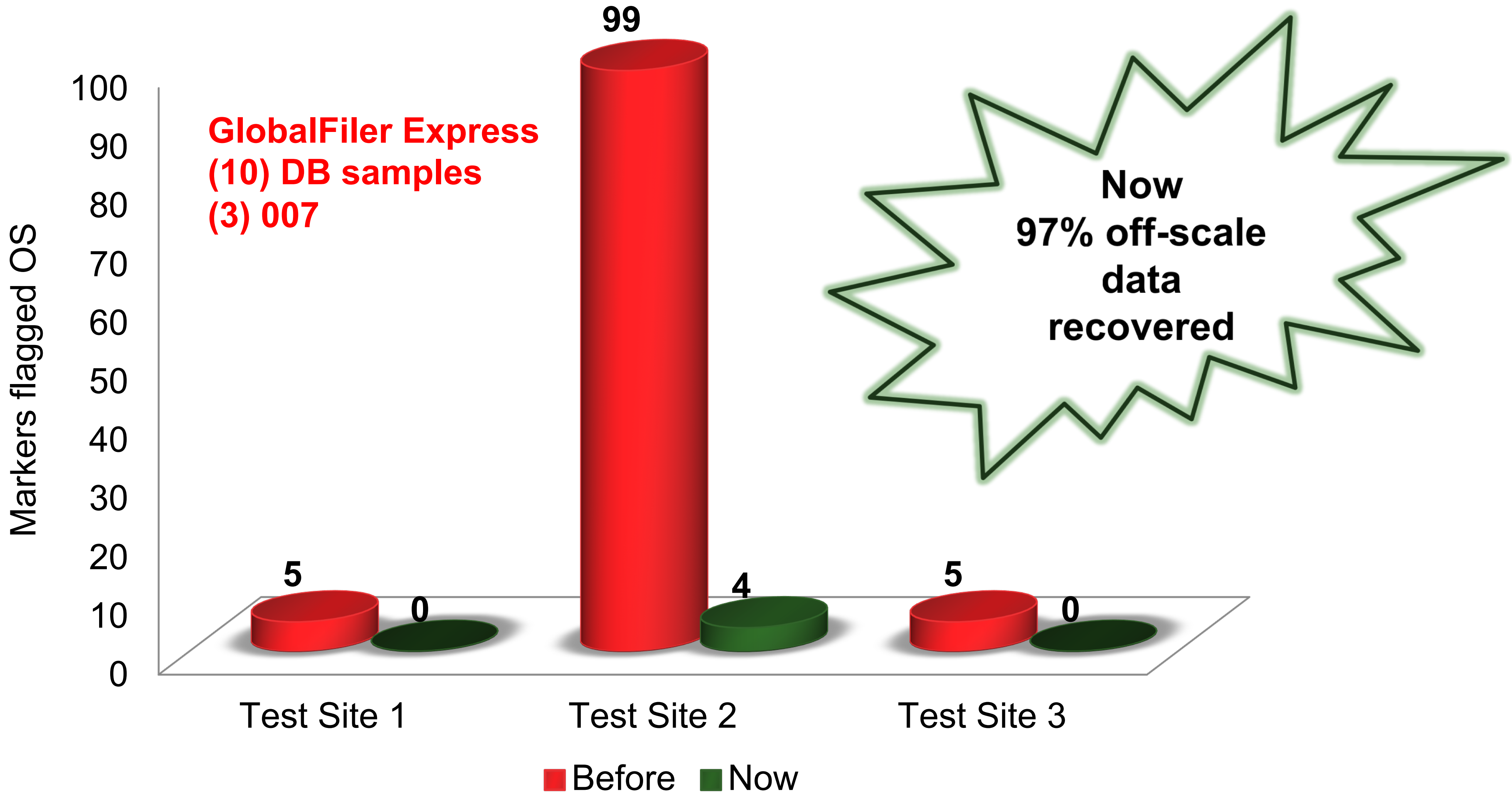
Set up plate

- Run with AnyDye dye set

Import Data into GMIDX

- All versions: OS ~32K

Under the Hood: Off-Scale Data Recovery (OSR) Field Testing



GlobalFiler Express
(10) DB samples
(3) 007

Now
97% off-scale
data
recovered

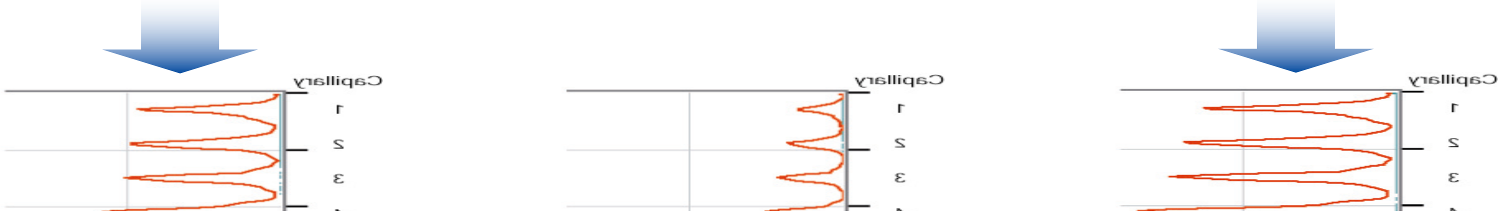
Thermo Fisher
SCIENTIFIC

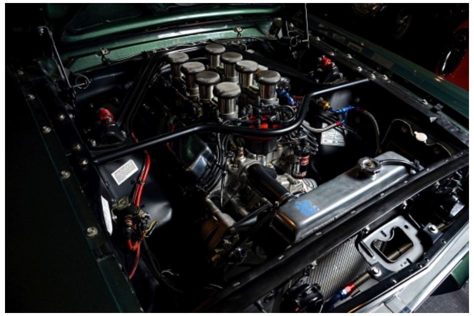
Under the Hood: Off-Scale Data Recovery (OSR) Internal Validation

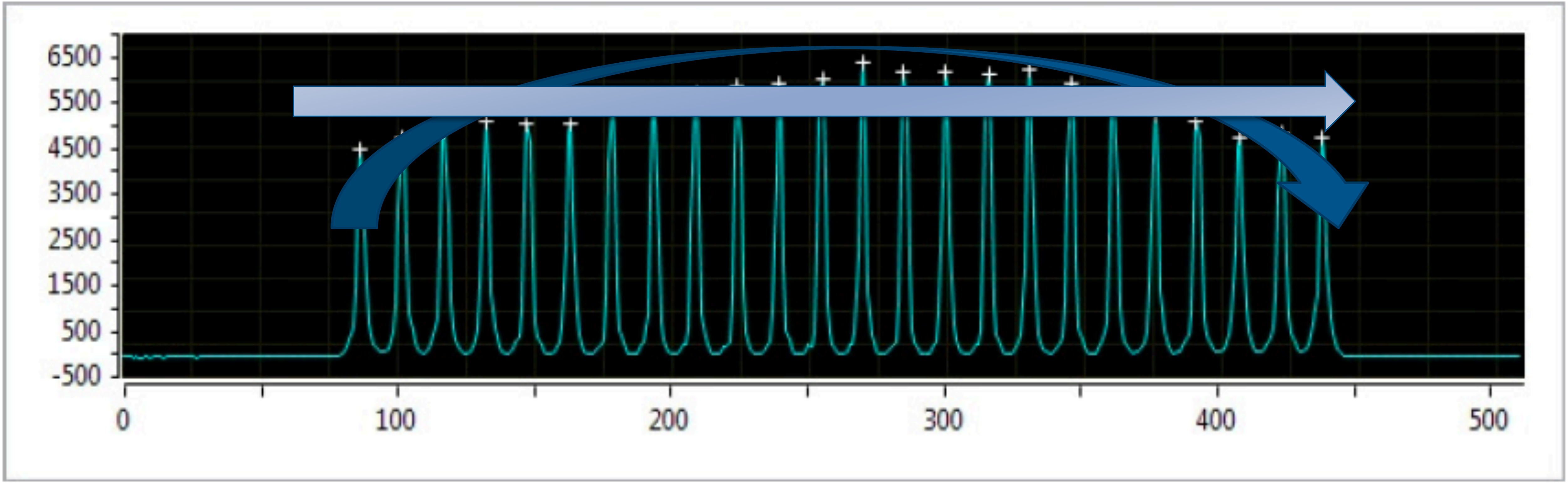
Results

Data Set (RFU)	<32K		32-65K		>65K	
IDX Version	1.5	1.6	1.5	1.6	1.5	1.6
PQV	On Scale		SOS	On Scale	SOS	SOS
Peak Morphology	Full Shape		Capped	Full Shape	Capped	Capped
	All alleles concordant for all ranges of peak heights Intra-locus balance was within 10% comparing <32K injection to 32-65K injection in v1.6 Pull-up related to OS data reduced in both 1.5 and 1.6					

Under the Hood: Signal Optimization







Under the Hood: Signal Optimization

Signal optimization

- Goal: reduce capillary-to-capillary variation within an injection
- Dual-focused approach developed

Optical approach: Reduce variation introduced by optics

- Utilize spatial calibration data
- 24-capillary instruments only
- Automatically applied

Injection approach: Reduce variation introduced by injection conditions

- Optimize injection conditions in the run module
- Both 8- and 24-capillary instruments
- Optional setting

Under the Hood: Signal Optimization Optical Approach

Use spatial calibration

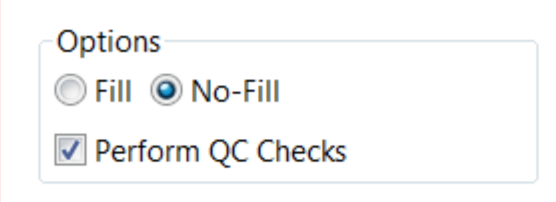
- Signal Optimization Factor calculated during spatial calibration
- Applied during data collection
- Applied to all injections

Calculations

- Fitted curve method
  - Used to adjust signal intensity
  - Minimizes background signal and reduces noise
- Signal Optimization Factor calculated from adjusted signal intensity

Calibration data unchanged

- QC check calculation based on displayed signal intensity
  - Avg PH
  - Uniformity
  - Capillary spacing


Options

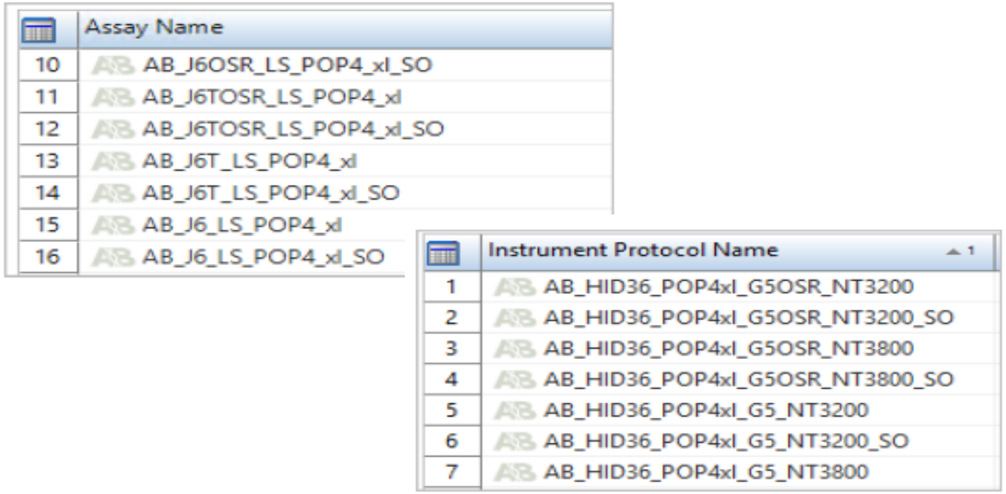
○ Fill ● No-Fill

☑ Perform QC Checks

Under the Hood: Signal Optimization Optical Approach

Signal optimization Factor	• 8 cap: Factor = 1 for all capillaries • Displayed in Spatial calibration Screen and exportable
Range	• Minimum: 0.5 • Maximum: 2 • Values outside this range rounded to maximum or minimum value
Size standard normalization	• Signal Optimization Factor is applied prior to size standard normalization factor calculation
Signal	• Signal optimization factor applies during collection of signal data

Under the Hood: Signal Optimization Injection Approach



	Assay Name
10	AB_J6OSR_LS_POP4_xl_SO
11	AB_J6TOSR_LS_POP4_xl
12	AB_J6TOSR_LS_POP4_xl_SO
13	AB_J6T_LS_POP4_xl
14	AB_J6T_LS_POP4_xl_SO
15	AB_J6_LS_POP4_xl
16	AB_J6_LS_POP4_xl_SO

	Instrument Protocol Name
1	AB_HID36_POP4xl_G5OSR_NT3200
2	AB_HID36_POP4xl_G5OSR_NT3200_SO
3	AB_HID36_POP4xl_G5OSR_NT3800
4	AB_HID36_POP4xl_G5OSR_NT3800_SO
5	AB_HID36_POP4xl_G5_NT3200
6	AB_HID36_POP4xl_G5_NT3200_SO
7	AB_HID36_POP4xl_G5_NT3800

New signal optimization (SO) run module

- Choose an assay: _SO suffix
  - Contains instrument protocol: _SO suffix
    - Contains run module: **HID36_POP4(xL)_SO**

Enhanced run module

- Injection conditions similar to sequencing applications
  - Position of capillary in sample optimized
  - A small amount of POP is introduced into the sample prior to injections
- These conditions have been demonstrated to improve uniformity across capillaries

Under the Hood: Signal Optimization Internal Validation

Objectives

- Determine most effective injection parameters
- Ensure no adverse effects of new parameters
- Test two-step approach

Materials

- 3500 and 3500xl instruments
- Default run module + 4 additional with different z-offsets and introduction of polymer
- GF and IDP
- GF carryover
- GF and GFE stability

Testing

- Peak height variability
- Sizing precision
- Genotype concordance
- Carryover
- Sample stability

Under the Hood: Signal Optimization Internal Validation

Results

Peak Height Variability

- 2 modules with a higher position in the well showed reduced variability
  - Position between high extreme and default selected
- Overall improvement seen with %CV and Max:Min ratio when comparing default to SO run module
- Still need to visually inspect spatial to check for outliers to ensure no overcorrection
- Spatial optimization showed little to no improvement in variation on the (2) 3500 instruments

Sizing and Concordance

- No significant different in sizing precision results with any of the 5 run modules
- All allele calls for each gDNA sample were 100% concordance

Sample Stability

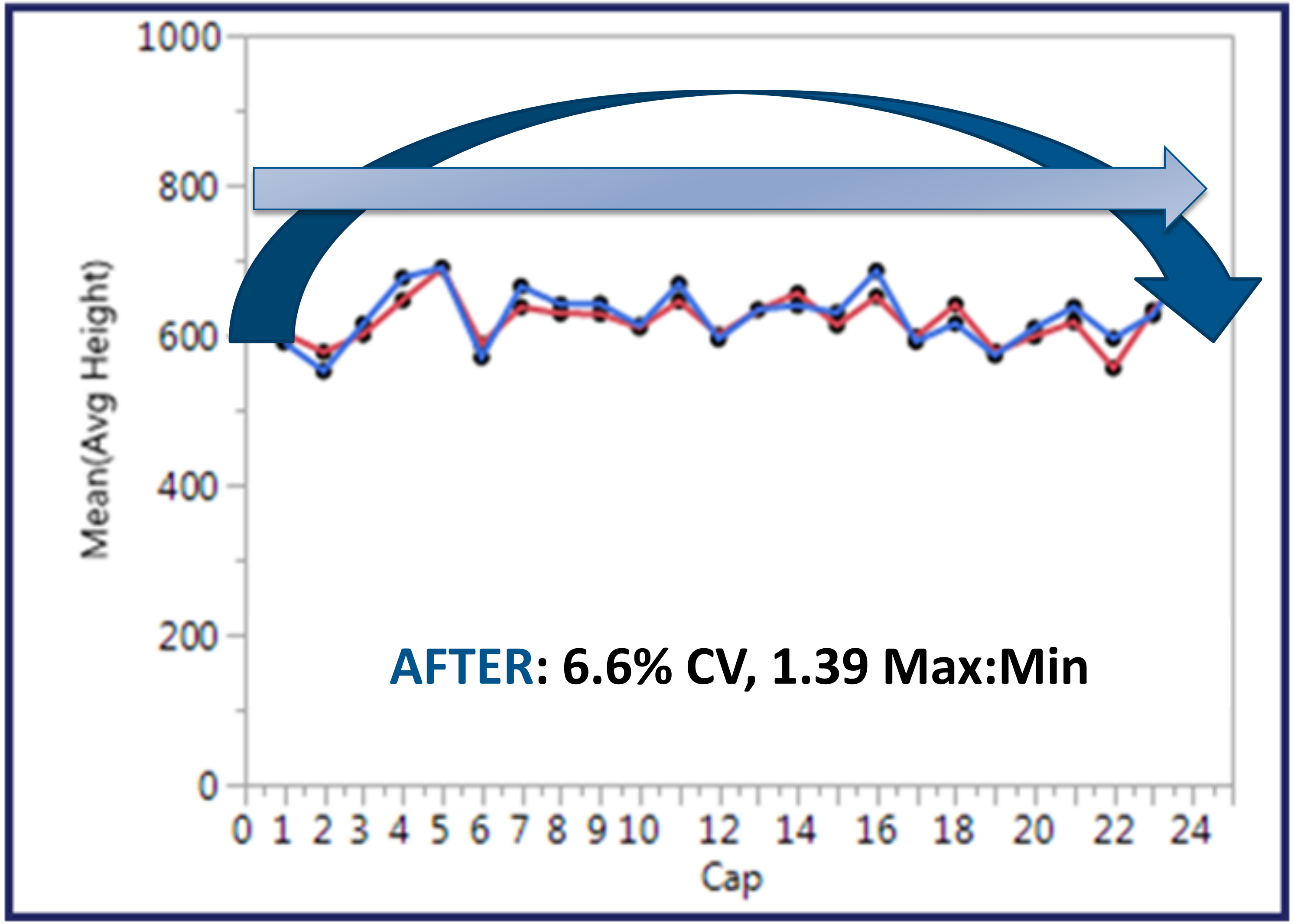
- Overall, no significant difference with introduction of polymer after 3d (4C, RT) and 8d (4C)
- No effect on resolution, sample peak heights, or intra-color balance at any time points with either run module
- Migration slower after 8d at 4C with SO run module
- Size standard peaks were lower after 3d RT and 8d 4C with SO run module

Carryover

- No additional carryover instances with SO compared to the default module
- %CV, Max:Min, and Average peak height metrics also evaluated for this data set: Improvement with variation and no significatnt different ot average peak height

Under the Hood: Signal Optimization Internal Validation

Combination of both methods shows greatest decrease in variation for 24 capillary instruments



AFTER: 6.6% CV, 1.39 Max:Min

Red and Blue lines depict two injections on same 3500xL instrument

In the Driver’s Seat with GMIDX v1.6

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

Feature Requests

- Have an idea to improve AB chemistry, instrument or software?
- Want to let us know about a pain point?
- Email your suggestions and comments to us at HID.TechSupport@thermofisher.com
- If there is already a workaround or solution, we'll let you know. If not, we'll make sure our developers hear what you have to say!

"Hi, I'm Jane Doe from ABC laboratories and I have a feature request. I would like to be able to XXXXX. Here's a screen shot of the current view and I circled the problem I'm having."