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3500-Series instrument Overview

Considerations for efficient implementation of the 3500 series genetic analyzer for Human Identification

HID University Seminar Series 2011

3500 System Workflow and Feature Highlights

Data Collection

Optional signal normalization

New oven design
Shorter run times

QC Analysis

Data analysis QC tools and flags

Simplified reinjection scheme

Run Set-Up

Predefined protocols and HID templates

Intuitive Run Set-Up and system monitoring

GeneMapper *ID-X* v1.2

Upgrade to GeneMapper *ID-X* 1.1.1 supports Windows XP and Vista

Analyzes .fsa & new .hid file format

Instrument

Pre-packaged consumables with RFID Tracking

Redesigned laser and polymer pump block

Instrument → **Run Set-Up** → **Data Collection** → **QC Analysis** → **GMID-X v1.2**

Dashboard

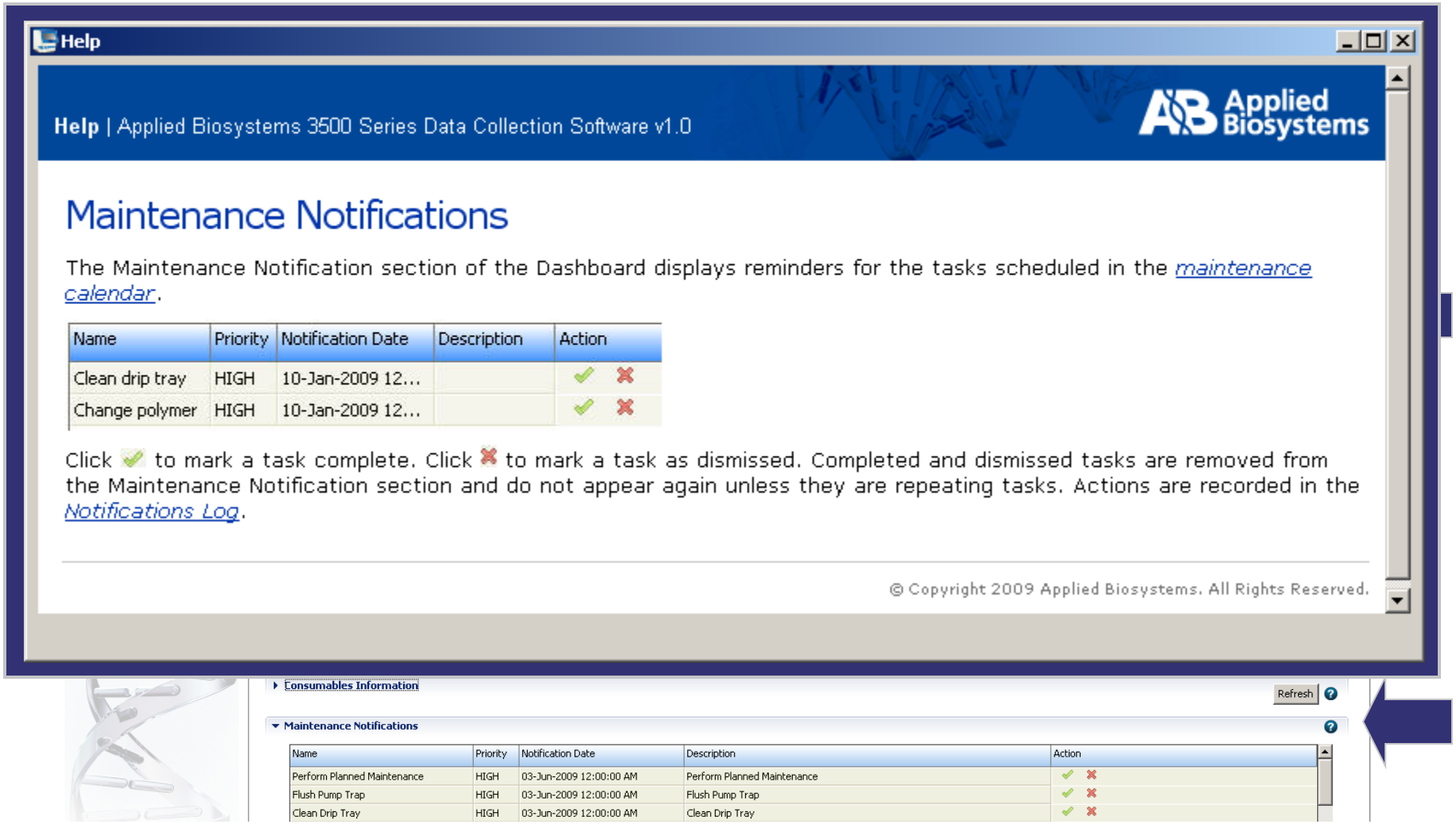
Key Operations

Consumable Gauges

Instrument Status

Maintenance Prompts

On-Screen Help



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Redesigned Consumable Packaging

- Pre-filled, quality-controlled reagents
- Radio Frequency Identification Tags
  - Fixed, passive tags
  - Limited read range
  - Memory only, cannot be read by the computer and cannot execute code
- Comparable per sample running cost to 31xx instruments

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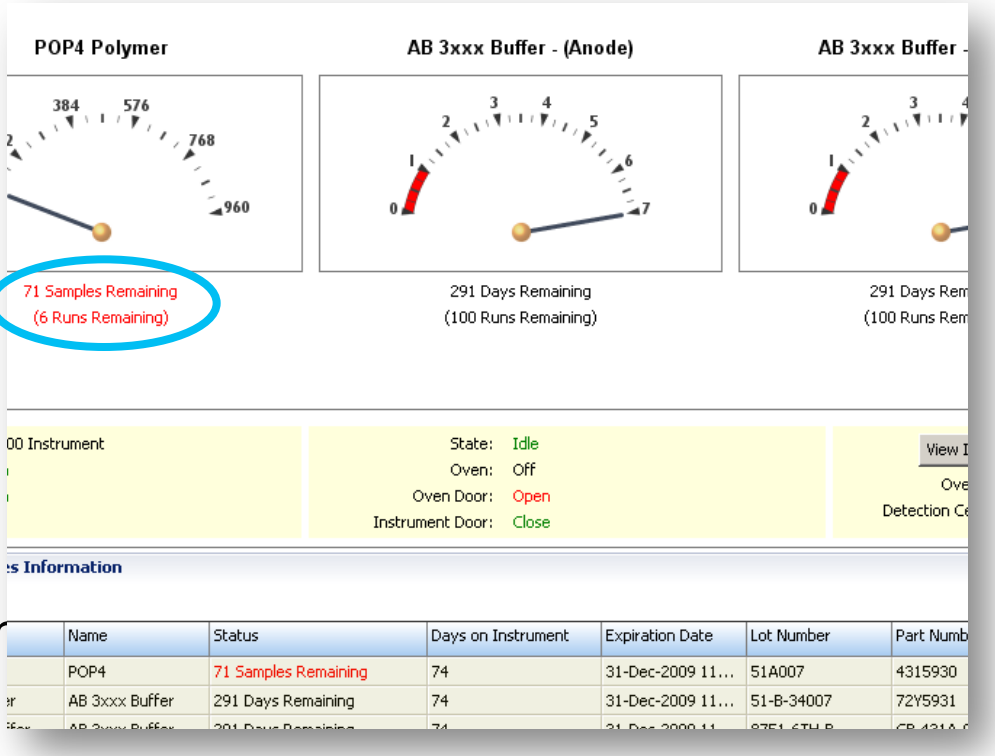
Radio Frequency Identification (RFID)

- Three independent RFID readers track and record information on four primary consumables (with RFID labels)
- Consumables tracked
  - Polymer
  - Cathode Buffer
  - Anode Buffer
  - Array
- Information tracked
  - Lot numbers
  - Part numbers
  - Serial numbers
  - Dates (expiration and installation
  - Capacity/Usage



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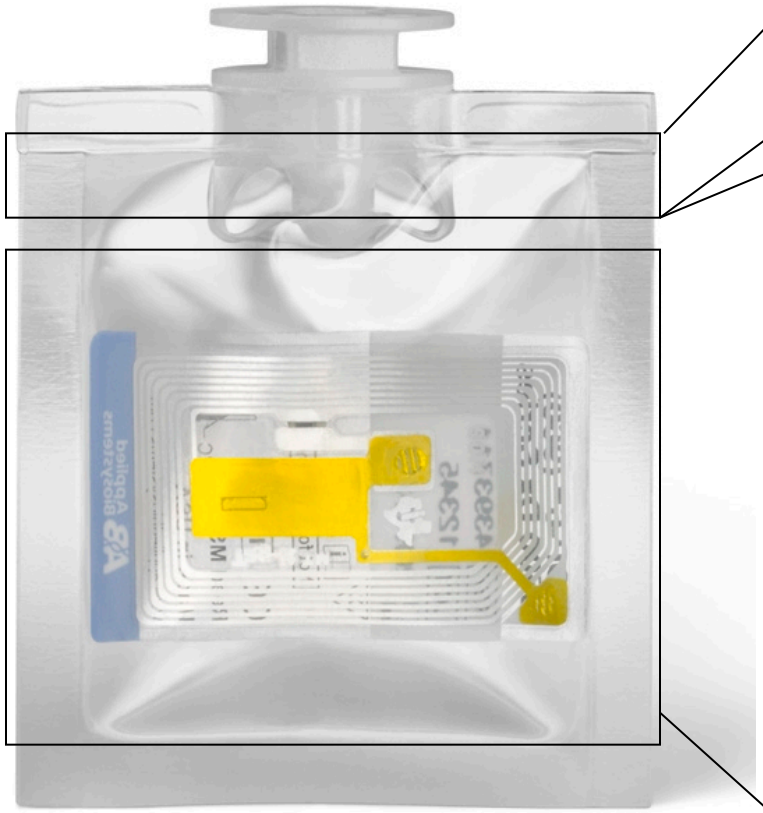
Consumables: Polymer Pouch Volume



Excess volume for installation, wizards and proper pouch functionality

Purchased volume

- 960, 384 or 96 samples

Provides:

- Predictable cost per sample
- Ease of use
- Data tracking (via RFID)

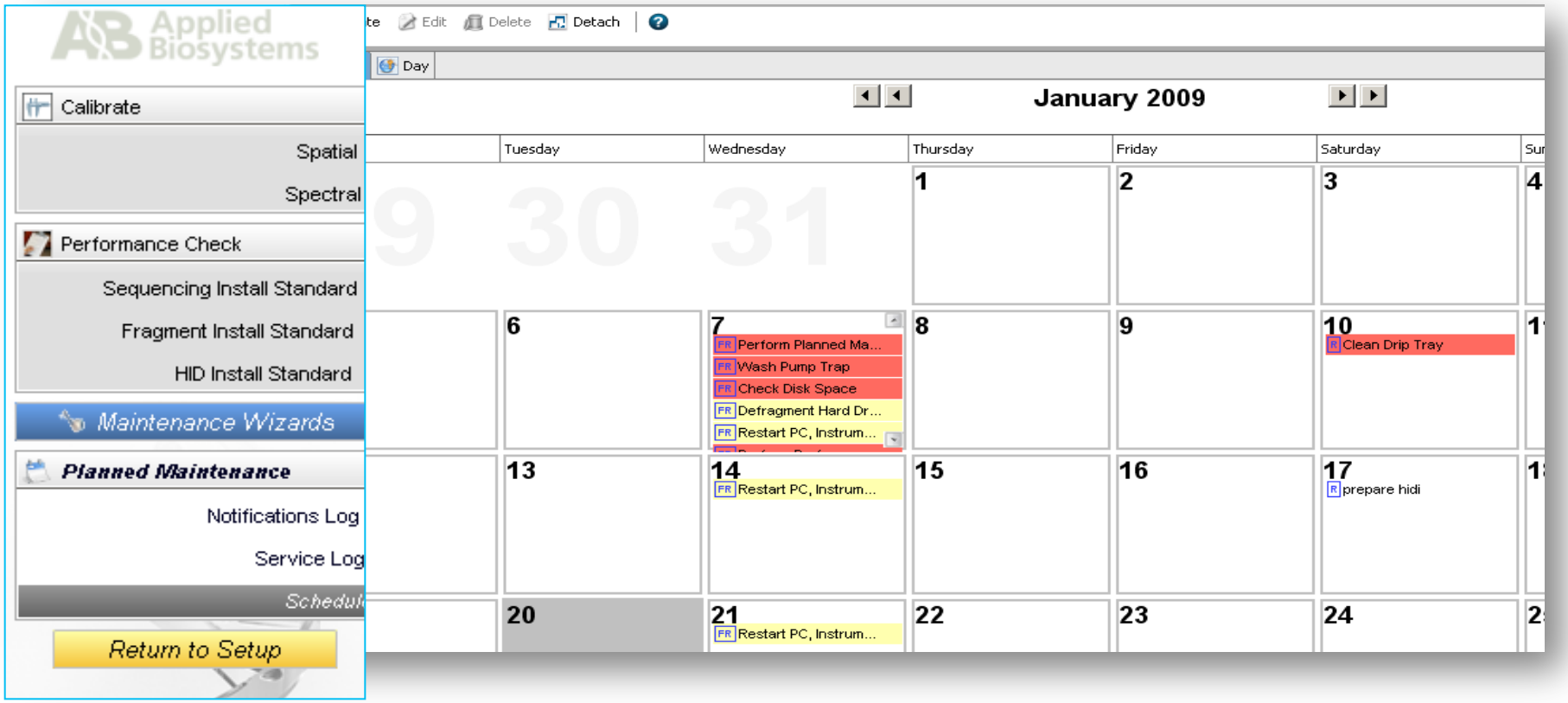
RFID Tracking Limits for Consumables

Consumable		Shelf Life	On Instrument		RFID Tracking Limits	
			Lifetime[1]	Usage[2]	Warning	Hard Stop
Polymer Pouch	**960 Samples**	Expiry Date	7 Days	960 Samples 50 Injections (24 cap) 120 Injections (8 cap)	7 Days	Expiry Date # Samples, Injections
	384 Samples	Expiry Date	7 Days	384 Samples 20 Injections (24 cap) 60 Injections (8 cap)	7 Days	Expiry Date # Samples, Injections
Array		Expiry Date	160 Injections	160 Injections	Expiry Date 160 Injections	None
Buffer		Expiry Date	7 Days	50 Injections (24 cap) 120 Injections (8 cap)	None	Expiry Date On Instrument Lifetime
Conditioning Reagent		Expiry Date	24 Hours	Single Use	24 Hours	Expiry Date

[2]On-instrument life/sample usage limit is based on whichever limit is reached first
[1]Polymer validated for 7 days on instrument and array guaranteed for 160 injections

Reagent and consumable lifetime guarantees/ recommendations are designed to promote the production of high quality data, for all applications, in whichever laboratory environment the instrument is located

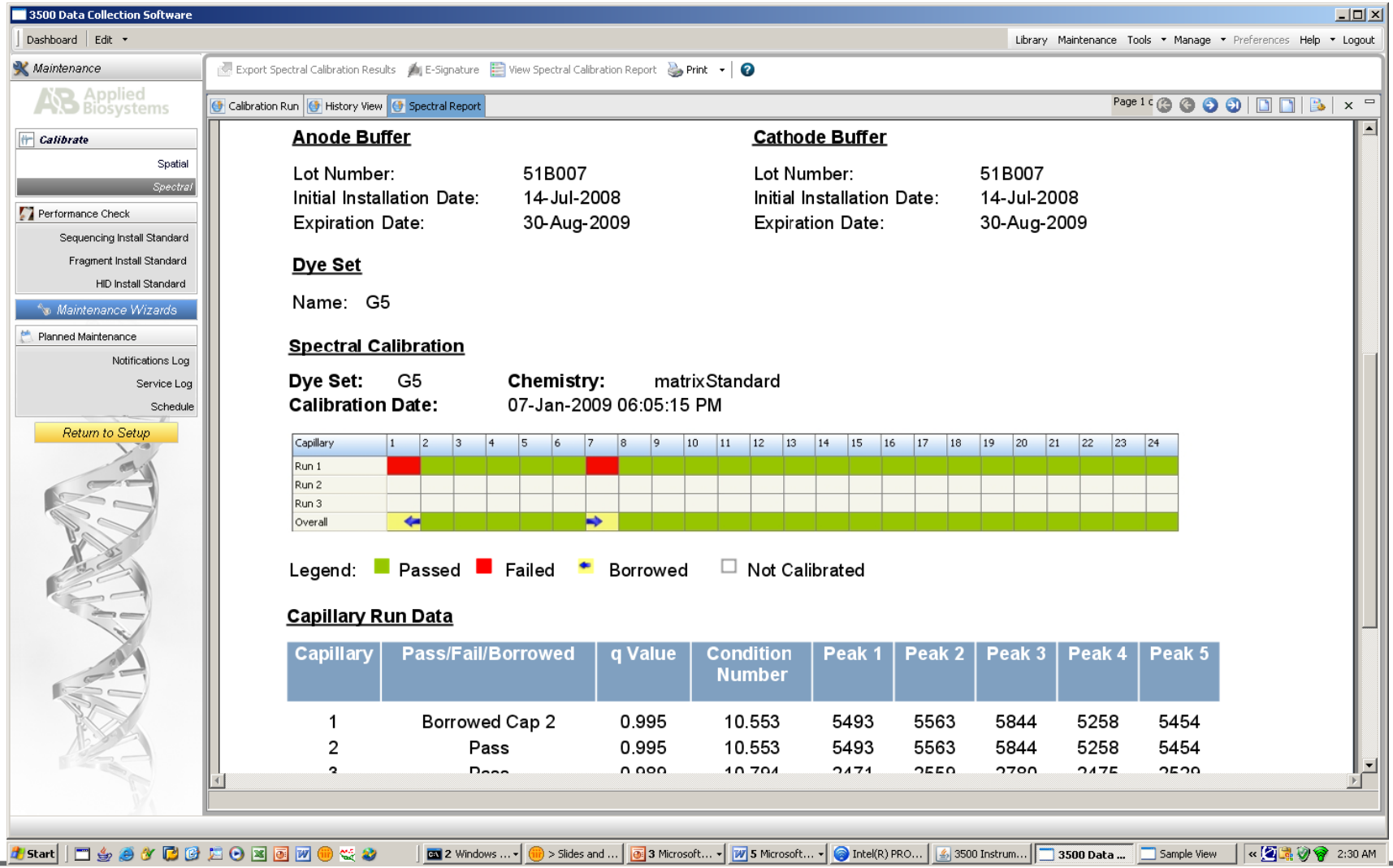
Calibration and Maintenance Calendar



FR or F = Applied Biosystems recommended tasks, user editable - specify dates, priority

R = User-specified repeating tasks

Calibration and Maintenance Reports and Export Files



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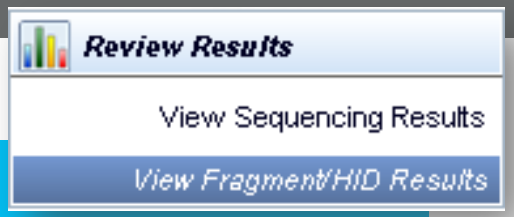
QC Analysis: QC Protocol

- QC protocol is the required primary analysis protocol for HID applications
- It defines peak detection, sizing, and quality values
- Factory-provided QC protocols are available in the software or can be created by the user

G5_LS(80-400)+Normalization

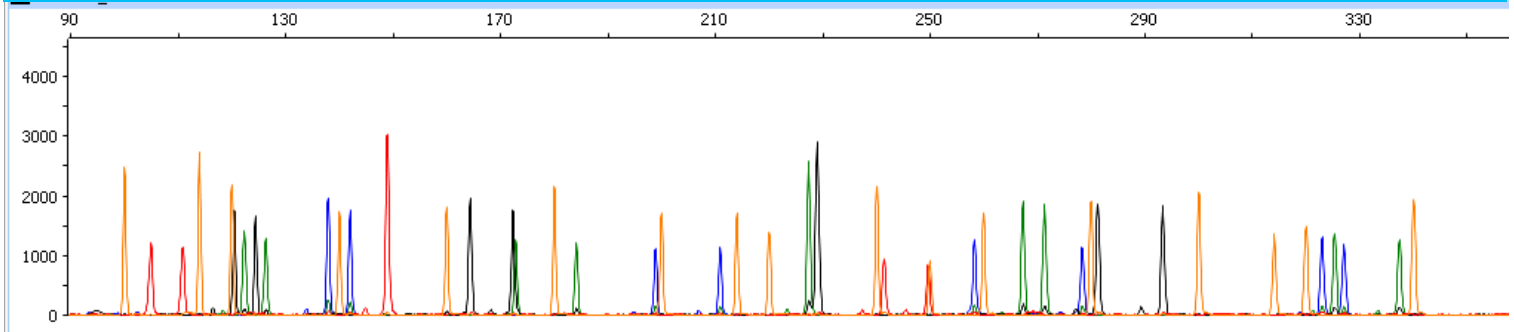
QC Analysis: Result Review



Review QC Flag Details – Set Up Reinjections

	Sample Name	Sample Type	Size Standard	Assay Name	PA Protocol	SA Protocol	Offscale	Broad Peak	Normalization Limit	Sizing Quality	Sample
1	1	Sample	HID_GS600_LIZ...	IF_POP4	G5_LS(80-400)		☐	☐		☑	
2	9	Sample	HID_GS600_LIZ...	IF_POP4	G5_LS(80-400)		☐	☐		☑	
3	17	Sample	HID_GS600_LIZ...	IF_POP4	G5_LS(80-400)		☐	☐		☑	
4	2	Sample	HID_GS600_LIZ...	IF_POP4	G5_LS(80-400)		☐	☐		☑	
5	10	Sample	HID_GS600_LIZ...	IF_POP4	G5_LS(80-400)		☐	☐		☑	
6	18	Sample	HID_GS600_LIZ...	IF_POP4	G5_LS(80-400)		☐	☐		☑	

Plot view with plot options: by dye, overlay, off scale



Sizing Table

	Dye Color	Dye/Sample Peak	Sample File Name	Size	Height	Area in Point	Area in BP	Data Point	Begin Point	Begin BP
1		O, 1	1_A01.hid		1098	4817	0.0	1857	1849	-1.0
2		O, 2	1_A01.hid		872	5016	0.0	1898	1890	-1.0

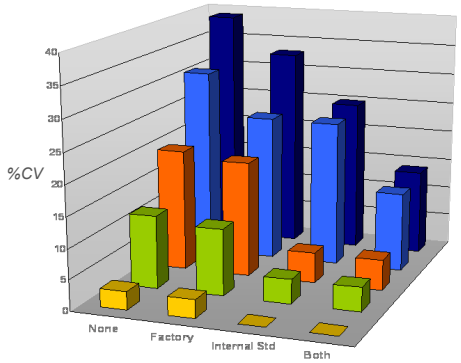
Improved Signal Consistency: Normalization Methods

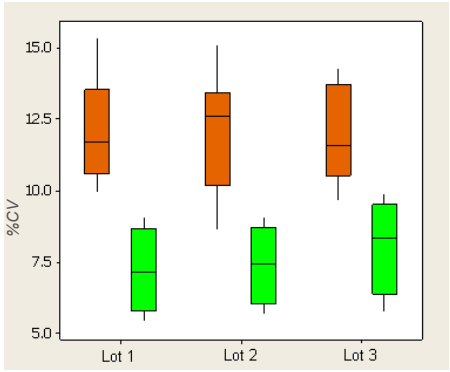
- **Factory Standardization:** Hardware-based calibration for more consistent instrument to instrument performance

- Internal Standard (IS) Normalization: Chemistry and Software based method for more consistent signal across capillaries, injections and instruments
  - User enabled
  - Utilizes re-designed GeneScan™600 LIZ Size Standard
  - Sample level peak heights are scaled relative to the intensity of the co-injected size standard compared to an optimized average size standard peak height (Normalization Target)

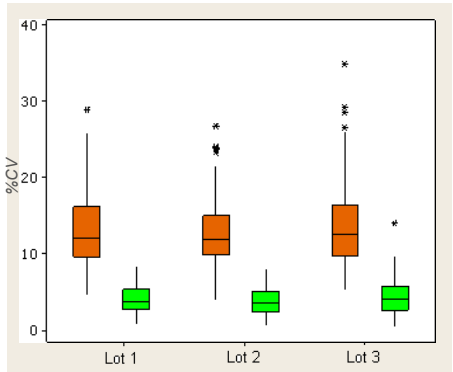
Signal Normalization Tools

- Forensic laboratories with multiple instruments have reported that signal variation between instruments can impact data interpretation
- Improved factory standardization and user-selectable internal standard normalization minimize signal variation between instruments and from injection to injection



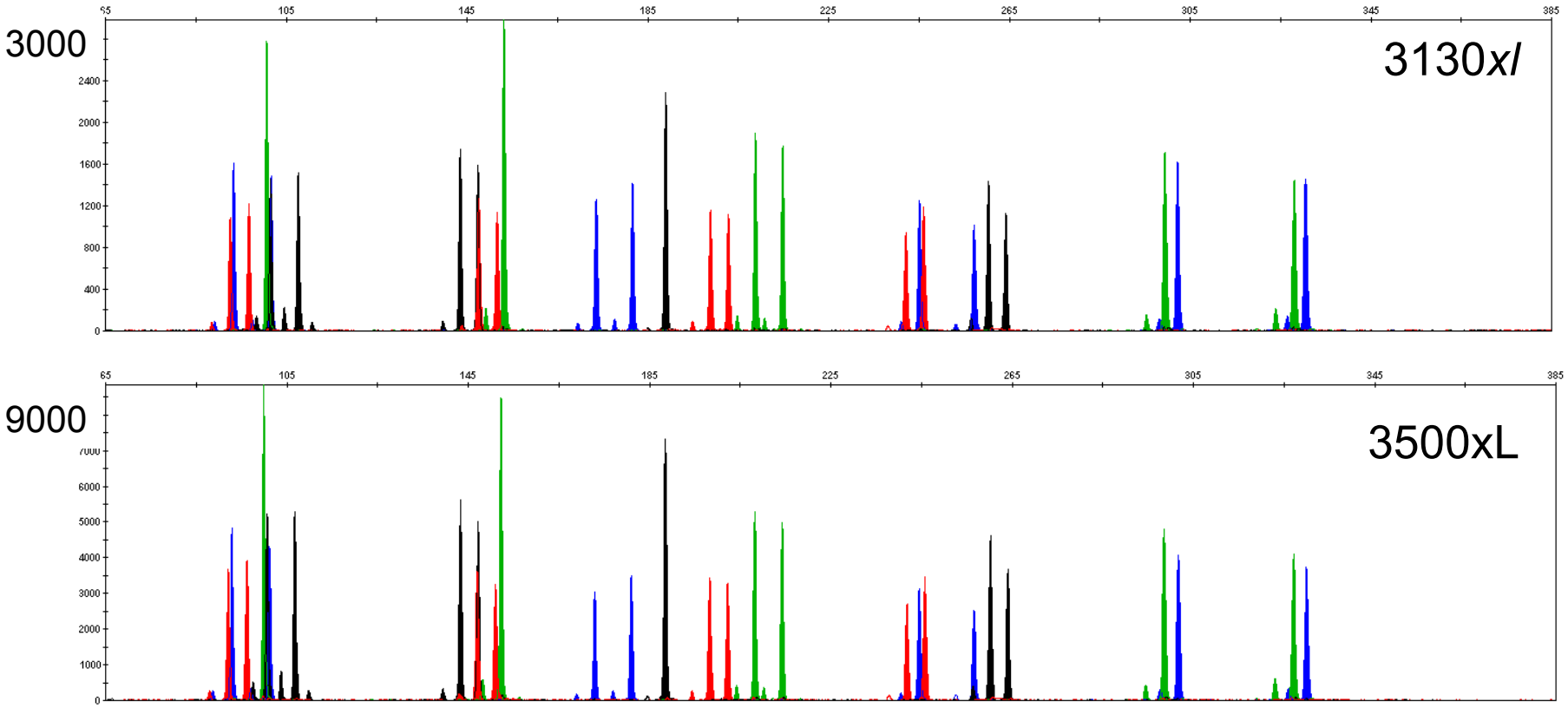


Instrument to Instrument Peak Height Consistency



Injection to Injection Peak Height Consistency

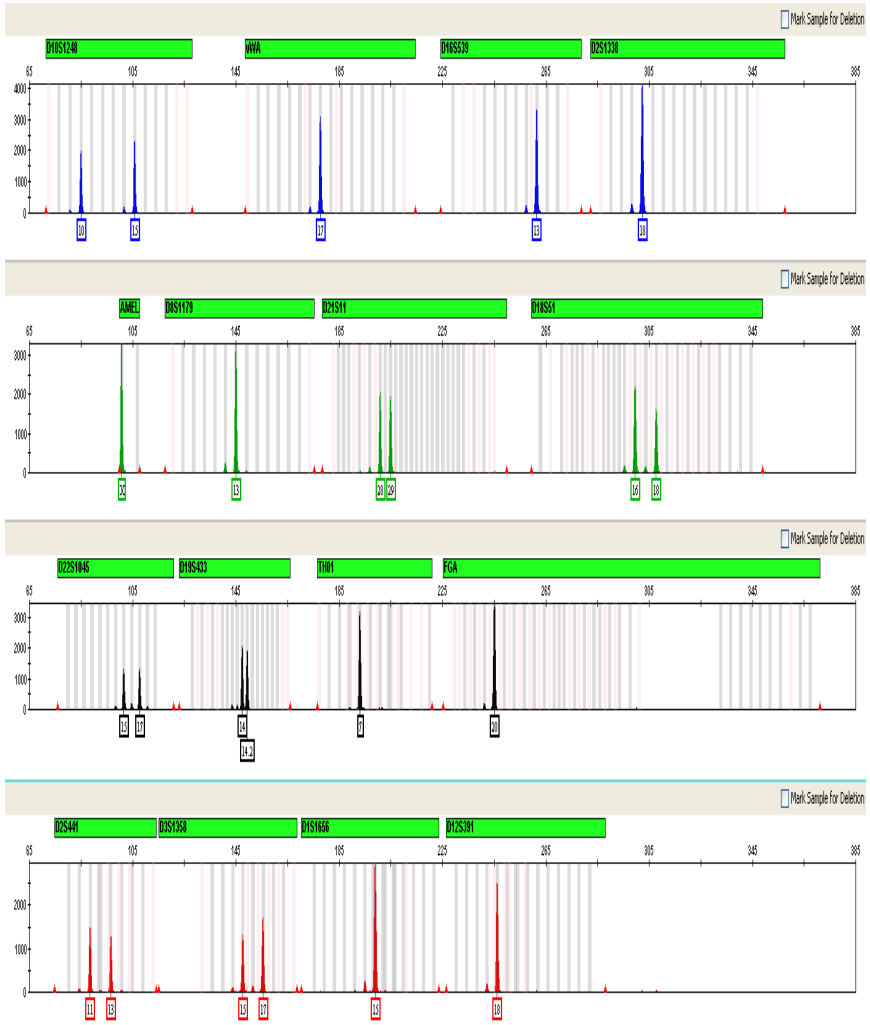
Data Scaling Considerations



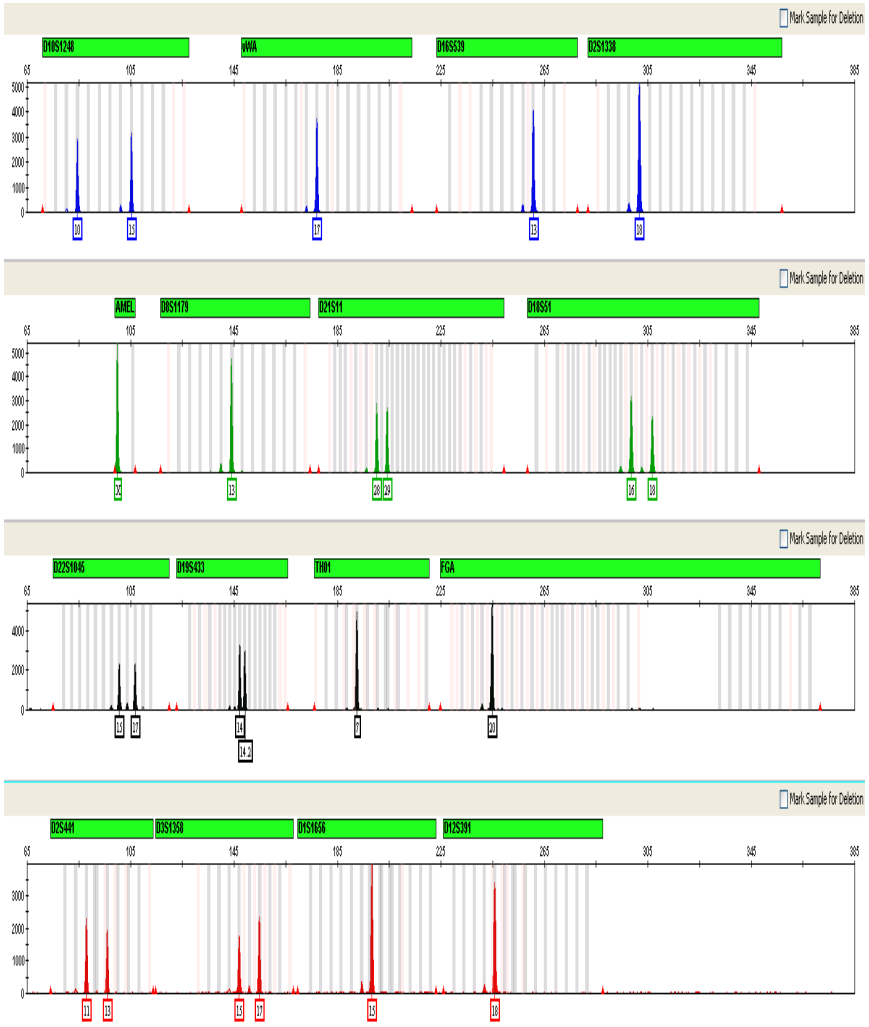
- Peak heights on the 3500 ~3-4 x greater than 31XX platforms due to differences in data scaling
- Higher peak amplitude threshold required for 3500 analysis if using a static threshold
  - Use of the Global Cut-off function in the analysis method may allow for fine-tuning of analysis thresholds

Comparison of 3130*xl* and 3500xL Data

3130*xl* PAT = 50 RFU



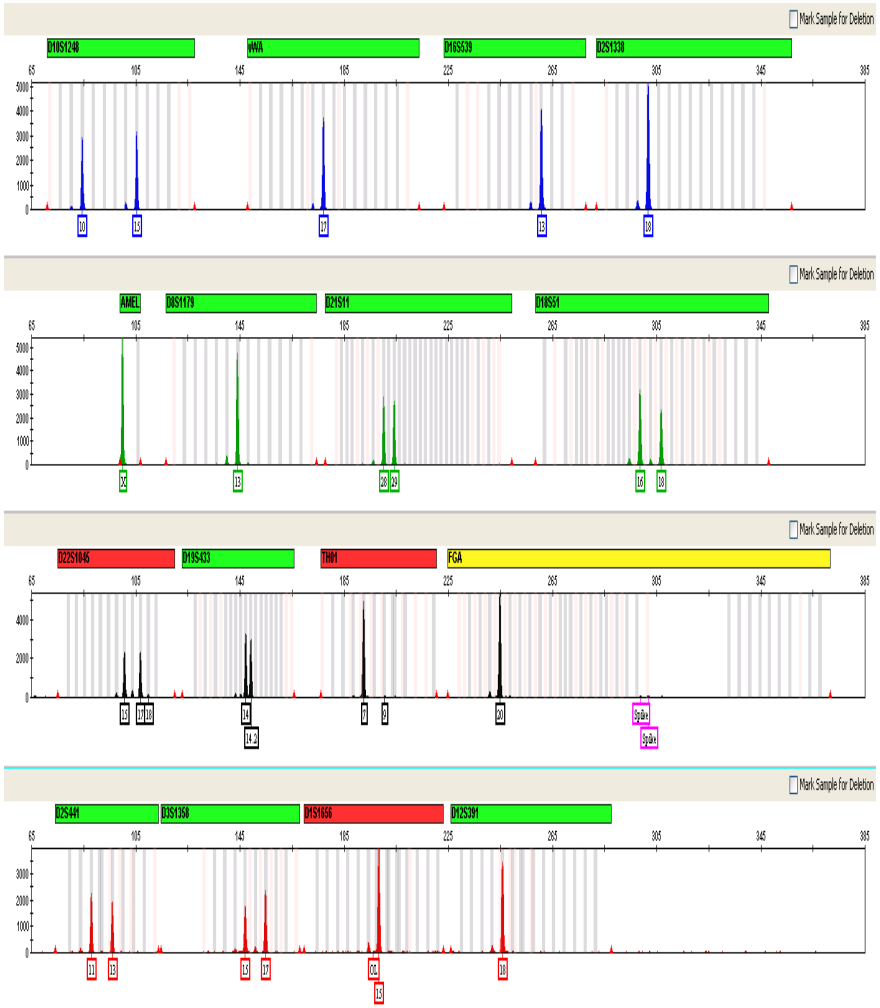
3500xL PAT = 175 RFU



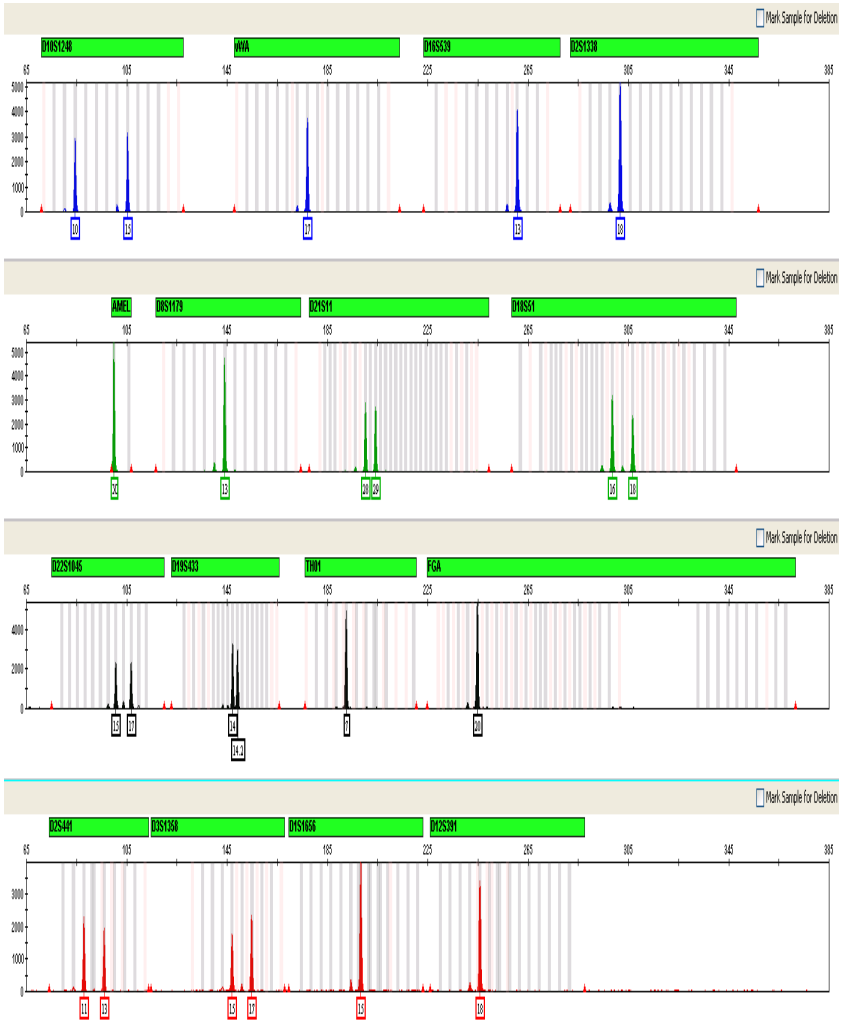
Identification of appropriate analysis thresholds promotes consistency of analysis outcome

PAT Definition for 3500 Data

PAT: 50rfu



PAT: 175rfu

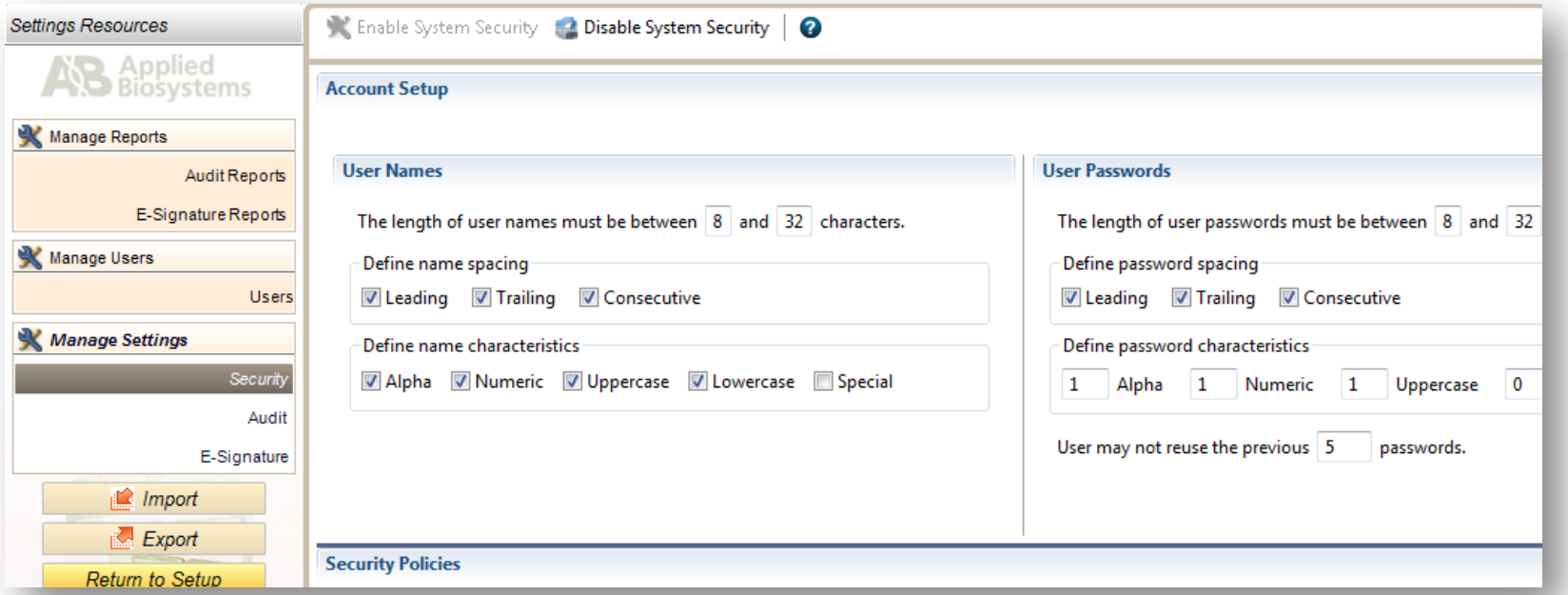


PAT Definition for 3500 Data

- Peak Amplitude Threshold
  - Minimum suggestion of [Mean Baseline Noise] + [3-10 x SD]
    - Default Data Collection value based on G5 Dye Set noise evaluation is ~175 RFU (independent tests recommended on individual instruments and workflows)
      - Dependent upon multiple factors including choice of chemistry, injection conditions, injection recipe, instrument platform
  - Other considerations
    - Artifacts
    - Spectral pull-up
    - Stutter
- Stochastic Threshold
  - Determine level of confidence for homozygote calls

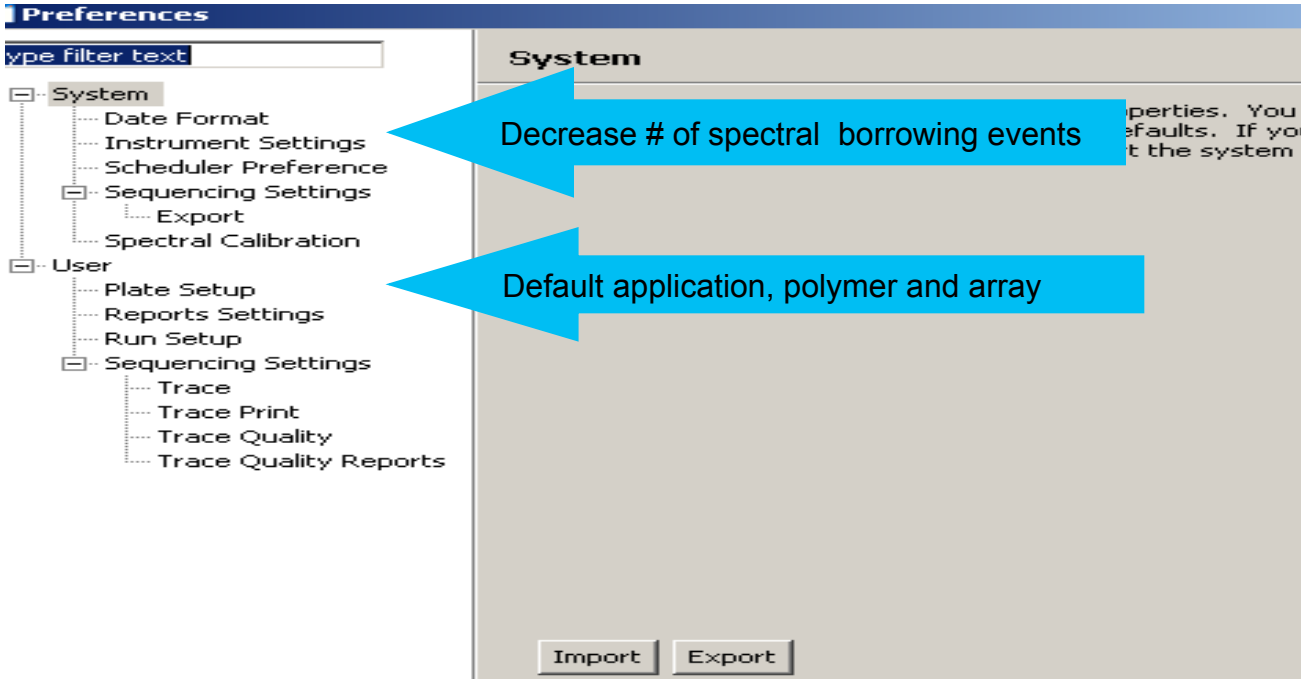
Consider differential thresholds for casework and database applications

Security, Audit and E-Sig



- User Configurable according to laboratory's needs
- **Security** controls user access to system functions and data
- **Auditing** tracks changes and provides history reports
- **E-signature** requires user-authentication before changes are saved

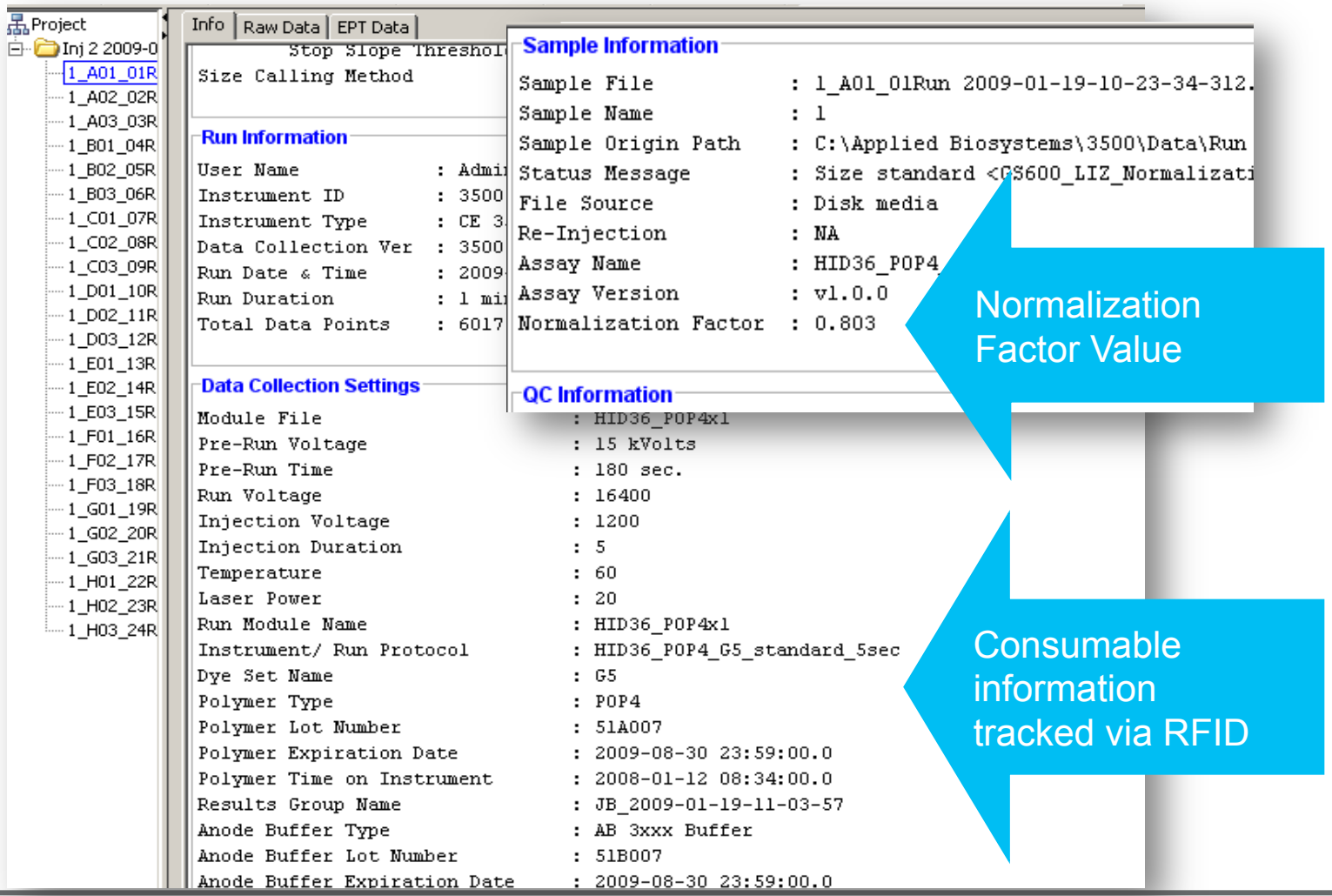
System Preferences



GeneMapper™ *ID-X* Software v1.2 and higher

- Provides comprehensive support for files generated by the 3500 Series Genetic Analyzers
  - Analyzes both .fsa and .hid 3500 files
    - Both file types cannot be analyzed with earlier versions of GeneMapper *ID-X* Software
  - Records RFID information regarding consumable lot numbers, usage, and expiration dates
  - Records instrument status and electrophoresis and analysis parameters
  - Supports signal normalization
  - Compatible with Windows® XP and Windows® Vista operating systems
- Includes printing patch update for increased speed
  - 30-180% reduction of printing time vs. version 1.1
- Can be updated to v1.2.1 using the CODIS Export Patch
  - Enables the user to configure the list of markers that can be included in CODIS Export tables
- Continues to support all existing sample QC and data chain of custody features to facilitate compliance with ISO17025

GeneMapper™ *ID-X* Software
Sample Info Tab



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Consumable Cost Comparison

Consumable	Instrument Platform					
	310	3130	3500	3130*xl*	3500xL	3730
Capillary/Array	$0.78	$1.26	$0.96	$0.59	$0.43	$0.25
Polymer	$0.52	$0.50	$0.48	$0.50	$0.48	$0.44
GA/Anode Buffer	$0.07	$0.07	$0.03	$0.07	$0.02	$0.01
Cathode Buffer			$0.04		$0.03	
Plates/Tubes	$0.10	$0.06	$0.06	$0.06	$0.06	$0.06
Septa	$0.27	$0.18	$0.18	$0.18	$0.18	$0.18
Total Cost/ Sample	$1.74	$2.07	$1.74	$1.40	$1.20	$0.95

- The cost/sample of 3500 consumables are comparable to the 3130 series
- Quality control of reagents coupled with pre-packaging saves operator time, eliminates sources of human error and promotes data optimum data quality
- The RFID tags automatically record lot numbers, part numbers, instrument serial numbers, reagent expiration and installation dates, and Capacity/Usage

Summary

- The 3500 Series Genetic Analyzers incorporate new hardware and software features designed specifically to support and enhance the Human Identification workflow
- Pre-packaged, quality controlled reagents deliver the same or improved on-instrument lifetime compared to previous platforms and help to maximize data quality
- RFID labelling of key consumables allow the user to monitor consumable status and all part and lot numbers are automatically recorded and transferred to the .fsa/.hid files
- GeneMapper™ *ID-X* Software v1.2 and higher supports analysis of the .fsa/.hid files including signal normalization
- The 3500 Genetic Analyzers represent a strong collaboration between Thermo Fisher Scientific and forensic laboratories worldwide

THANK YOU!!

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