

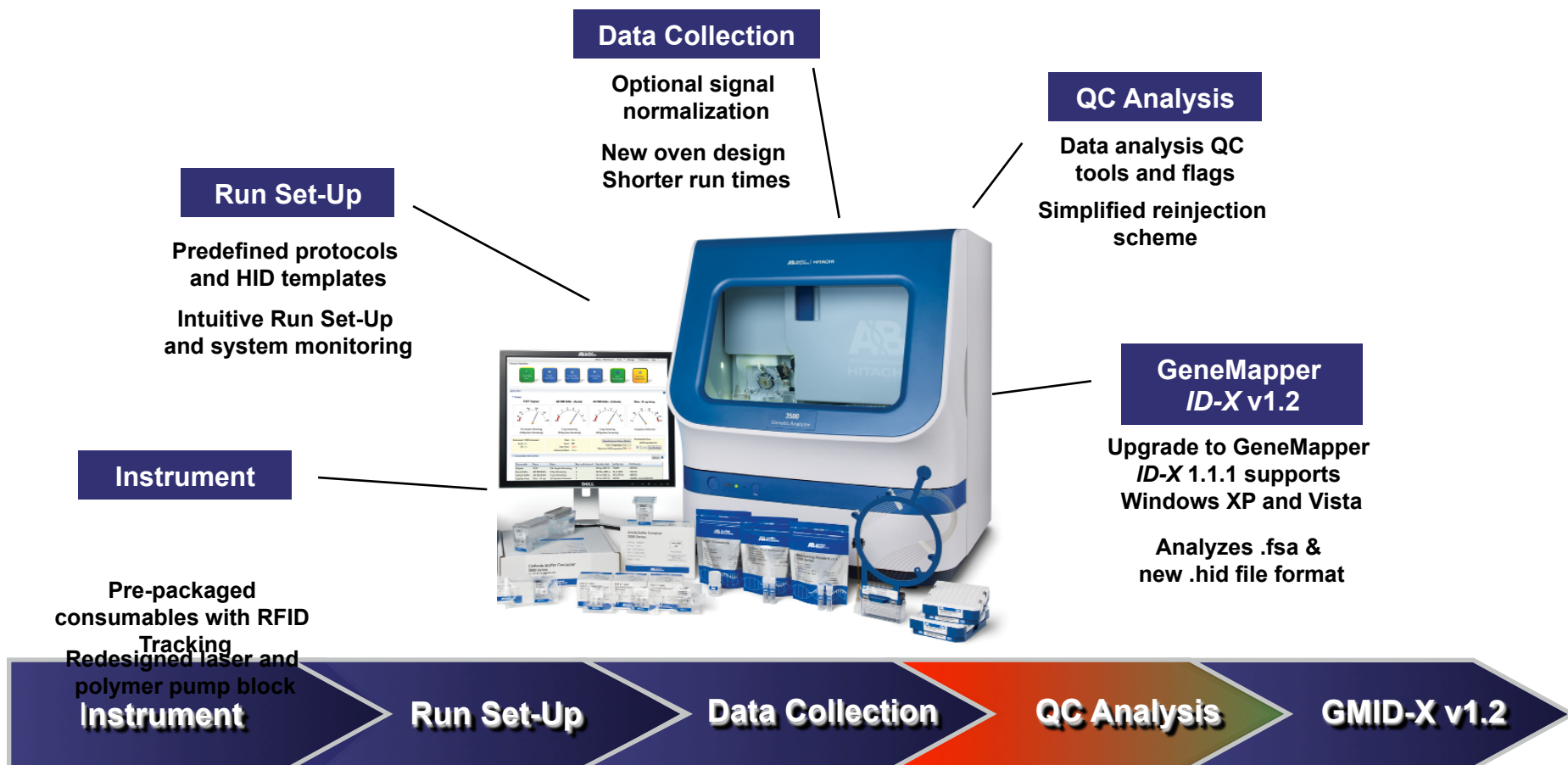


## **3500-Series instrument Overview**

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

HID University Seminar Series 2011

# 3500 System Workflow and Feature Highlights



# Dashboard

3500 Data Collection Software

Library Maintenance Tools Manage Preferences Help Log Out

Common Operations



Key Operations

Quick View

Gauges

POP4 Polymer



509 Samples Remaining  
(40 Injections Remaining)

AB 3500 Buffer - (Anode)



3 Days Remaining  
(45 Injections Remaining)

AB 3500 Buffer - (Cathode)



3 Days Remaining  
(45 Injections Remaining)

36cm - 24 cap Array



72 Injections Performed

Consumable Gauges

Instrument: 3500 Instrument

Laser: On  
EP: On

State: Idle

Oven: Off  
Oven Door: Open  
Instrument Door: Close

View Instrument Sensor Details

Oven Temperature (°C): 53.5  
Detection Cell Temperature (°C): 23.5

Pre-Heat the Oven

Set Temperature to:

60 (°C) Start Pre-Heat

Instrument Status

Consumables Information

Refresh

Maintenance Notifications

Name	Priority	Notification Date	Description	Action
Perform Planned Maintenance	HIGH	03-Jun-2009 12:00:00 AM	Perform Planned Maintenance	✓ ✗
Flush Pump Trap	HIGH	03-Jun-2009 12:00:00 AM	Flush Pump Trap	✓ ✗

Maintenance Prompts

**Help** | Applied Biosystems 3500 Series Data Collection Software v1.0

## Maintenance Notifications

The Maintenance Notification section of the Dashboard displays reminders for the tasks scheduled in the [maintenance calendar](#).

Name	Priority	Notification Date	Description	Action
Clean drip tray	HIGH	10-Jan-2009 12...		✓ ✗
Change polymer	HIGH	10-Jan-2009 12...		✓ ✗

Click ✓ to mark a task complete. Click ✗ to mark a task as dismissed. Completed and dismissed tasks are removed from the Maintenance Notification section and do not appear again unless they are repeating tasks. Actions are recorded in the [Notifications Log](#).

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▶ **Consumables Information**

▼ **Maintenance Notifications**

Name	Priority	Notification Date	Description	Action
Perform Planned Maintenance	HIGH	03-Jun-2009 12:00:00 AM	Perform Planned Maintenance	✓ ✗
Flush Pump Trap	HIGH	03-Jun-2009 12:00:00 AM	Flush Pump Trap	✓ ✗
Clean Drip Tray	HIGH	03-Jun-2009 12:00:00 AM	Clean Drip Tray	✓ ✗

Refresh ?



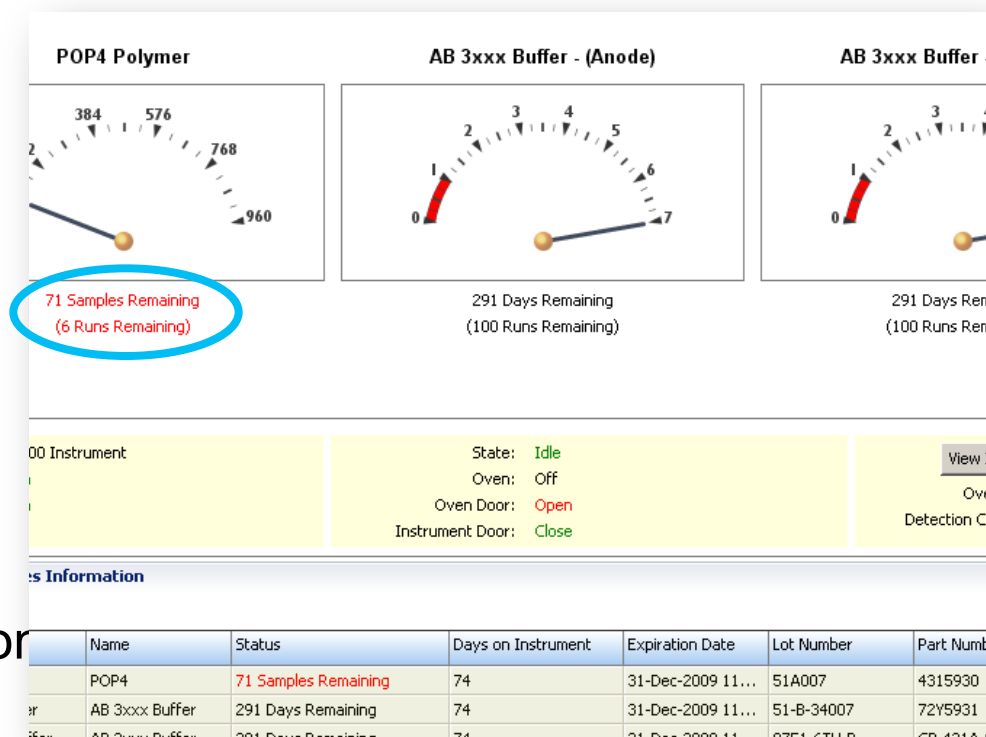
# Redesigned Consumable Packaging

- 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

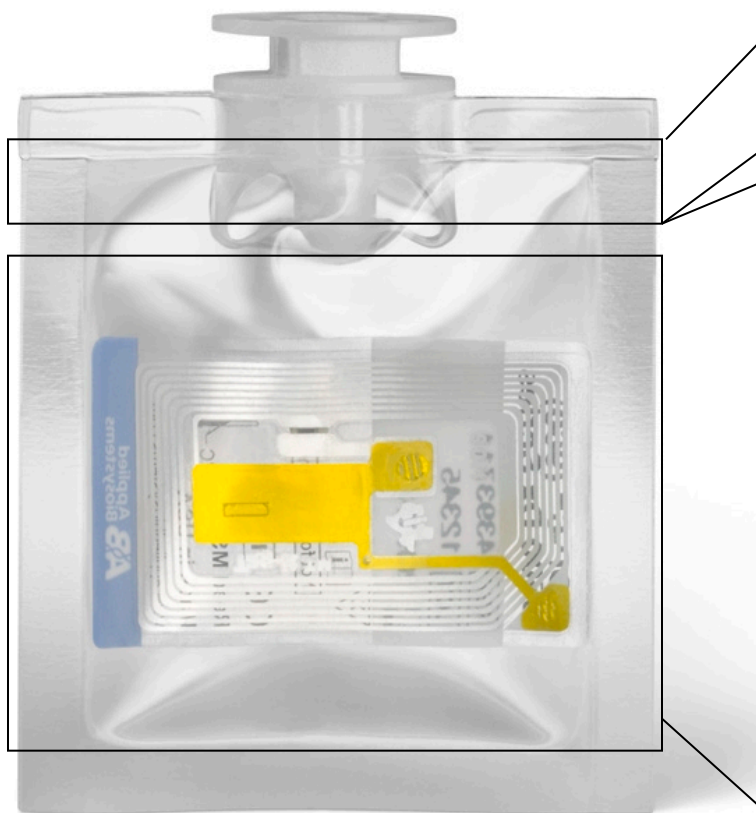


# Radio Frequency Identification (RFID)

- 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



# 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 <sup>1</sup>	Usage <sup>2</sup>	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

<sup>2</sup>On-instrument life/sample usage limit is based on whichever limit is reached first

<sup>1</sup>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

The screenshot shows the Applied Biosystems Calibration and Maintenance Calendar for January 2009. The interface includes a sidebar with navigation options and a main calendar grid. The sidebar contains the following sections:

- Calibrate:** Spatial, Spectral
- Performance Check:** Sequencing Install Standard, Fragment Install Standard, HID Install Standard
- Maintenance Wizards**
- Planned Maintenance:** Notifications Log, Service Log, Scheduling
- Return to Setup** (button)

The main calendar grid shows the following tasks:

Day	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
9	30	31	1	2	3	4
5	6	7	8	9	10	11
12	13	14	15	16	17	18
19	20	21	22	23	24	25

Tasks listed in the calendar include:

- Perform Planned Ma... (FR)
- Wash Pump Trap (FR)
- Check Disk Space (FR)
- Defragment Hard Dr... (FR)
- Restart PC, Instrum... (FR)
- Clean Drip Tray (R)
- Restart PC, Instrum... (FR)
- Restart PC, Instrum... (FR)
- Restart PC, Instrum... (FR)
- prepare hidi (R)

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

R = User-specified repeating tasks

# Calibration and Maintenance Reports and Export Files

3500 Data Collection Software

Dashboard Edit Library Maintenance Tools Manage Preferences Help Logout

Maintenance

ASB Applied Biosystems

Calibrate

Spatial

Spectral

Performance Check

Sequencing Install Standard

Fragment Install Standard

HID Install Standard

Maintenance Wizards

Planned Maintenance

Notifications Log

Service Log

Schedule

Return to Setup

Export Spectral Calibration Results E-Signature View Spectral Calibration Report Print

Calibration Run History View Spectral Report Page 1 of 1

**Anode Buffer**

Lot Number: 51B007  
Initial Installation Date: 14-Jul-2008  
Expiration Date: 30-Aug-2009

**Dye Set**

Name: G5

**Spectral Calibration**

Dye Set: G5 Chemistry: matrixStandard  
Calibration Date: 07-Jan-2009 06:05:15 PM

Capillary	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Run 1	Failed	Passed	Passed	Passed	Passed	Passed	Failed	Passed	Passed	Passed	Passed	Passed	Passed	Passed	Passed	Passed	Passed	Passed	Passed	Passed	Passed	Passed	Passed	Passed
Run 2	Passed	Passed	Passed	Passed	Passed	Passed	Passed	Passed	Passed	Passed	Passed	Passed	Passed	Passed	Passed	Passed	Passed	Passed	Passed	Passed	Passed	Passed	Passed	Passed
Run 3	Passed	Passed	Passed	Passed	Passed	Passed	Passed	Passed	Passed	Passed	Passed	Passed	Passed	Passed	Passed	Passed	Passed	Passed	Passed	Passed	Passed	Passed	Passed	Passed
Overall	Borrowed	Passed	Passed	Passed	Passed	Passed	Passed	Passed	Passed	Passed	Passed	Passed	Passed	Passed	Passed	Passed	Passed	Passed	Passed	Passed	Passed	Passed	Passed	Passed

Legend: ■ Passed ■ Failed ■ Borrowed  Not Calibrated

**Capillary Run Data**

Capillary	Pass/Fail/Borrowed	q Value	Condition Number	Peak 1	Peak 2	Peak 3	Peak 4	Peak 5
1	Borrowed Cap 2	0.995	10.553	5493	5563	5844	5258	5454
2	Pass	0.995	10.553	5493	5563	5844	5258	5454
3	Pass	0.999	10.704	5474	5550	5790	5475	5520

**Cathode Buffer**

Lot Number: 51B007  
Initial Installation Date: 14-Jul-2008  
Expiration Date: 30-Aug-2009

Start | 2 Windows ... | Slides and ... | 3 Microsoft ... | 5 Microsoft ... | Intel(R) PRO... | 3500 Instrum... | 3500 Data ... | Sample View | 2:30 AM

# QC Analysis: QC Protocol

Setup a QC Protocol

\* Protocol Name: G5\_LS(80-400)+Normalization

Description: G5600LIZ, Default analysis Range set to Full

Size Standard: G5600\_LIZ+Normalization\_(80-400)

Size Caller: SizeCaller v1.1.0

Analysis Settings | **QC Settings**

Analysis Range: Full | Sizing Range: Partial | Size Calling Method: Local Southern

Analysis Start Point: 0 | Sizing Start Size: 80

Analysis Stop Point: 1000000 | Sizing Stop Size: 400

Peak Amplitude Threshold

<input checked="" type="checkbox"/> Blue	<input checked="" type="checkbox"/> Green	<input checked="" type="checkbox"/> Yellow	<input checked="" type="checkbox"/> Red	<input type="checkbox"/> Purple	<input checked="" type="checkbox"/> Orange
50	50	50	50	175	50

Common Settings

Use Smoothing: Light

Use Baseline (Baseline Window (Pts)):  51

Minimum Peak Half Width: 2

Peak Window Size: 15

Polynomial Degree: 3

Slope Threshold Peak Start: 0.0


Slope Threshold Peak End: 0.0

Close Save

- 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

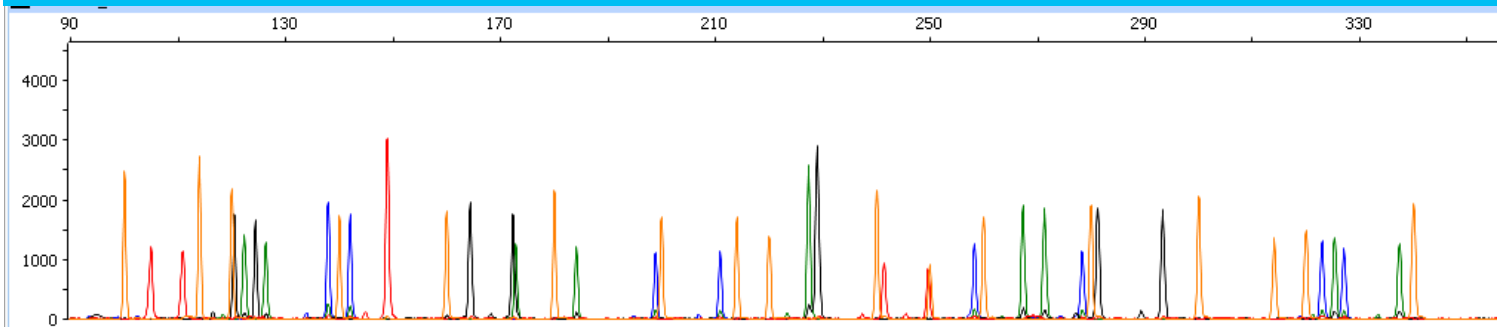
# QC Analysis: Result Review


Review Results
  
  
[View Sequencing Results](#)
  
  
[View Fragment/HID Results](#)

## 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 1	Sample
1	Sample	HID_GS600_LIZ...	IF_POP4	G5_L5(80-400)		☑	☑		☑	
2	Sample	HID_GS600_LIZ...	IF_POP4	G5_L5(80-400)		☑	☑		☑	
3	Sample	HID_GS600_LIZ...	IF_POP4	G5_L5(80-400)		☑	☑		☑	
4	Sample	HID_GS600_LIZ...	IF_POP4	G5_L5(80-400)		☑	☑		☑	
5	Sample	HID_GS600_LIZ...	IF_POP4	G5_L5(80-400)		☑	☑		☑	
6	Sample	HID_GS600_LIZ...	IF_POP4	G5_L5(80-400)		☑	☑		☑	

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



## Sizing Table

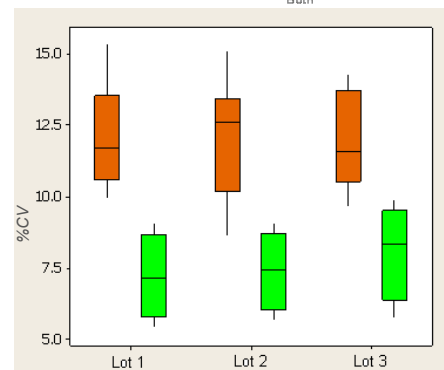
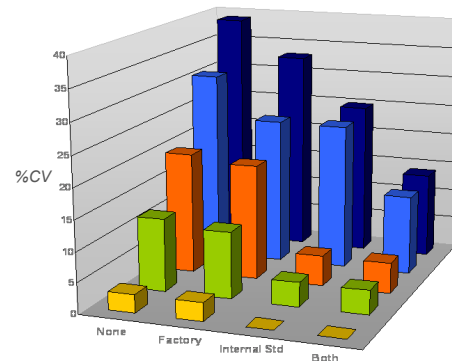
Dye Color	Dye/Sample Peak	Sample File Name	Size	Height	Area in Point	Area in BP	Data Point	Begin Point	Begin BP
Orange	O, 1	1_A01.hid		1098	4817	0.0	1857	1849	-1.0
Orange	O, 2	1_A01.hid		872	5016	0.0	1898	1890	-1.0

# Improved Signal Consistency: Normalization Methods

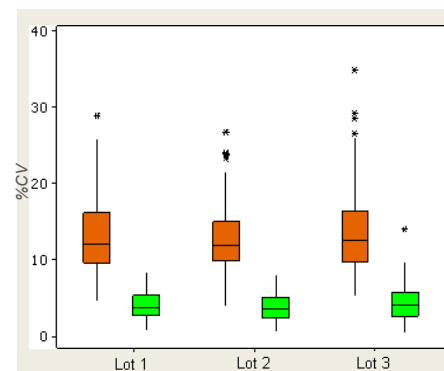
- **Factory Standardization:** Hardware-based calibration for more consistent instrument to instrument performance
- **Internal Standard (IS) Normalization:** Chemistry and Software based method for more consistent signal across capillaries, injections and instruments
  - User enabled
  - Utilizes re-designed 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

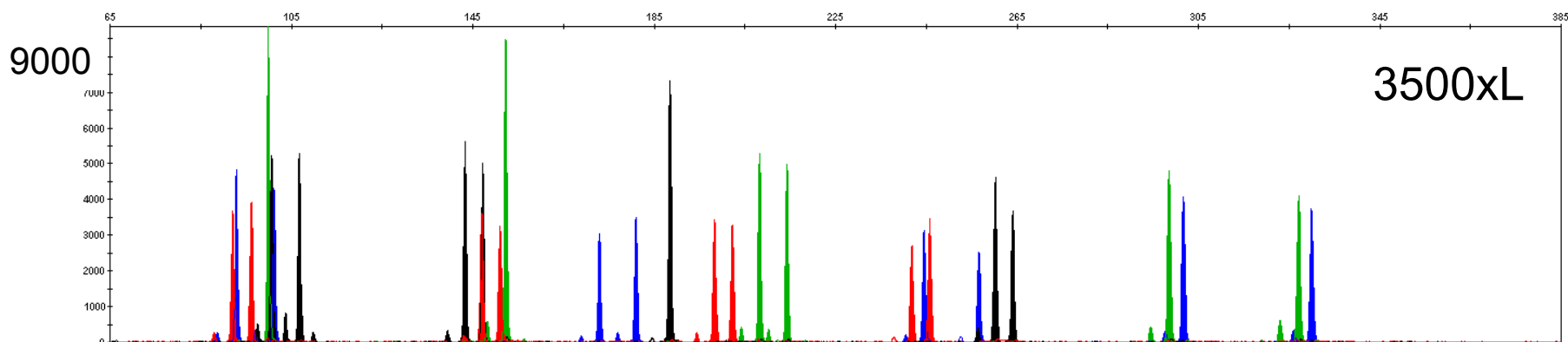
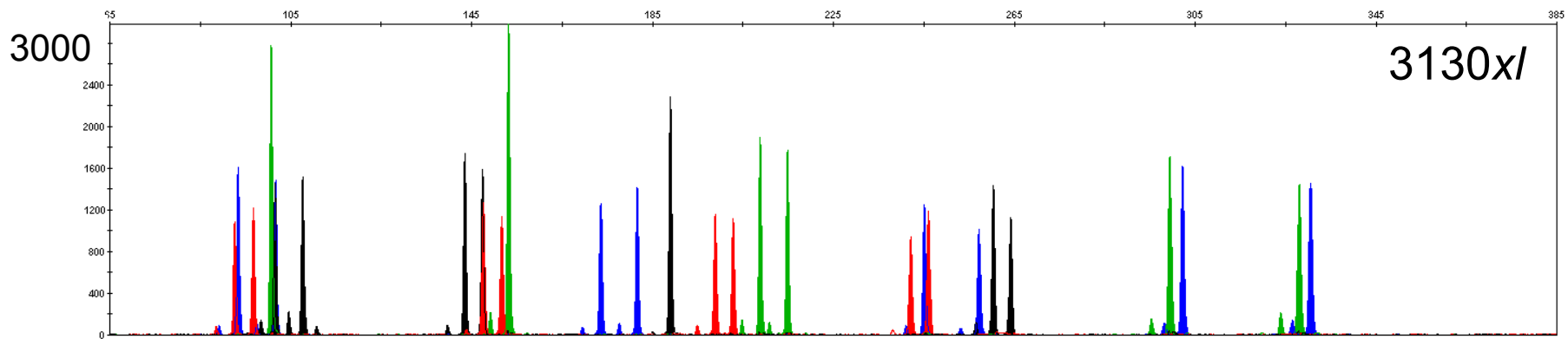


Instrument to  
Instrument Peak  
Height  
Consistency



Injection to  
Injection Peak  
Height  
Consistency

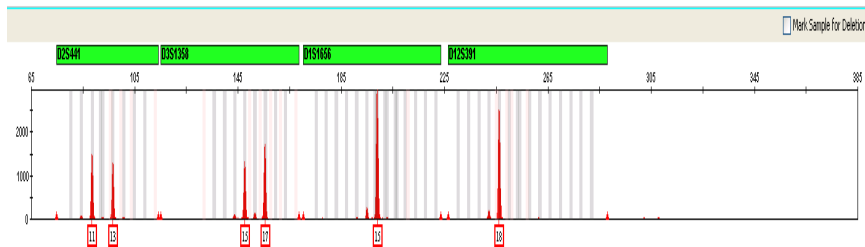
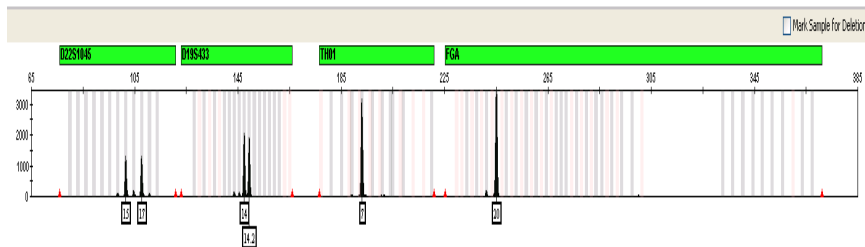
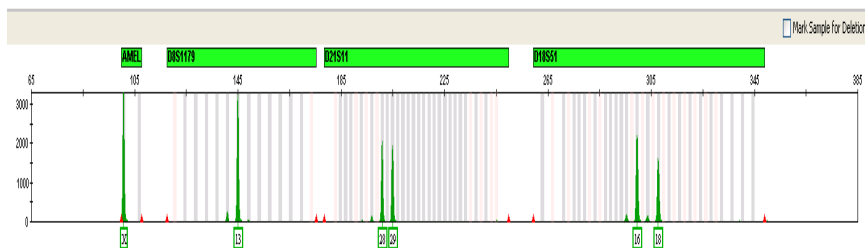
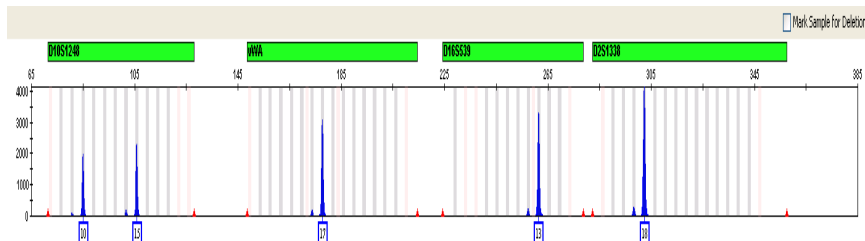
# Data Scaling Considerations



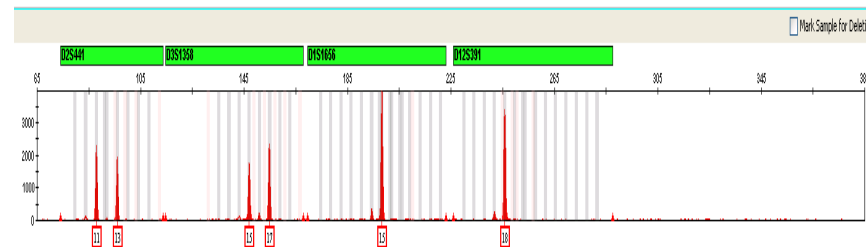
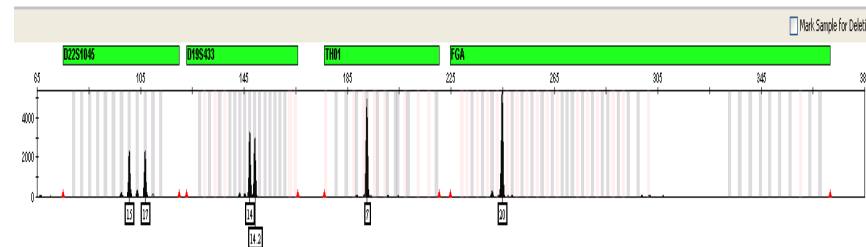
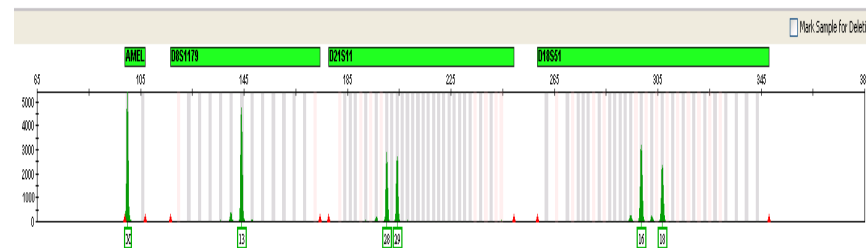
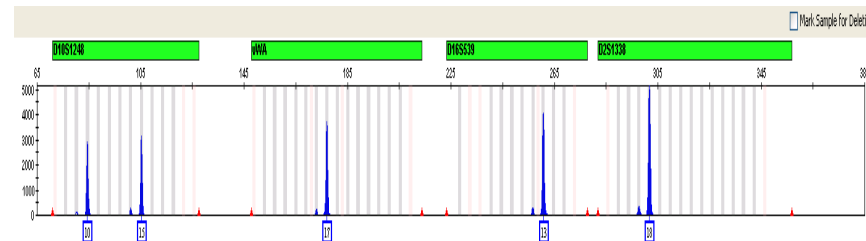
- Peak heights on the 3500 ~3-4 x greater than 31XX platforms due to differences in data scaling
- Higher peak amplitude threshold required for 3500 analysis if using a static threshold
  - Use of the Global Cut-off function in the analysis method may allow for fine-tuning of analysis thresholds

# Comparison of 3130xI and 3500xL Data

3130xI PAT = 50 RFU



3500xL PAT = 175 RFU

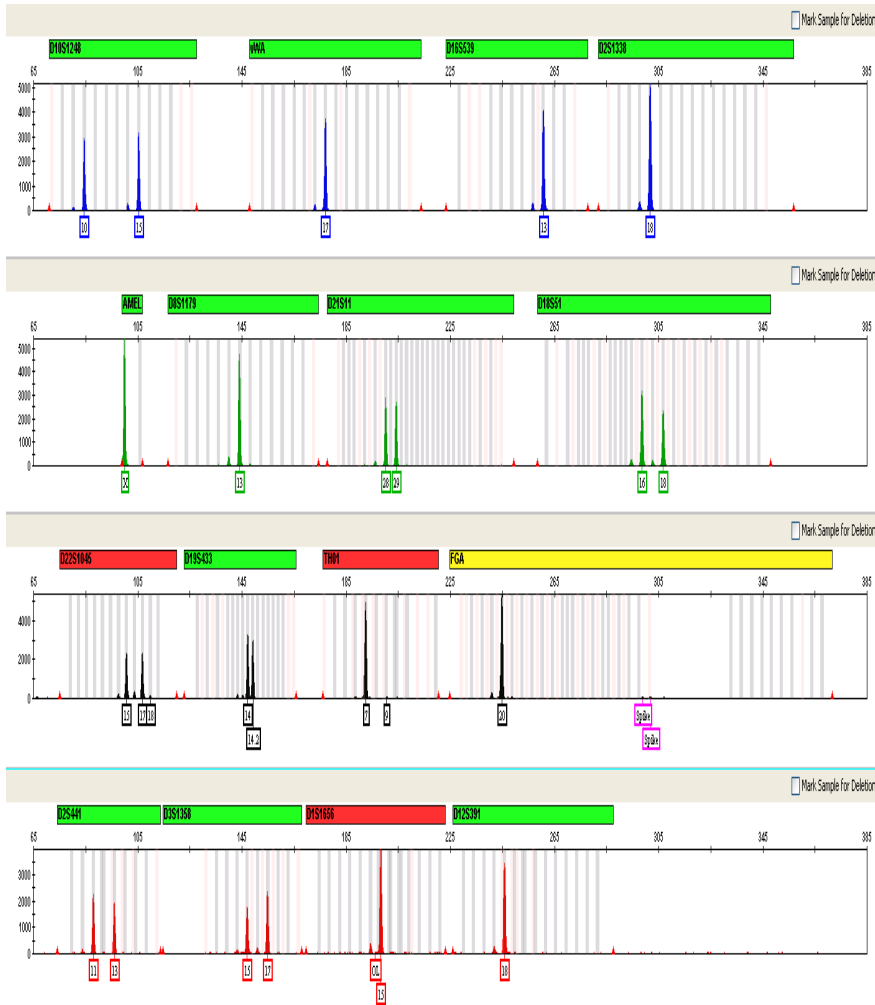


*Identification of appropriate analysis thresholds promotes consistency of analysis outcome*

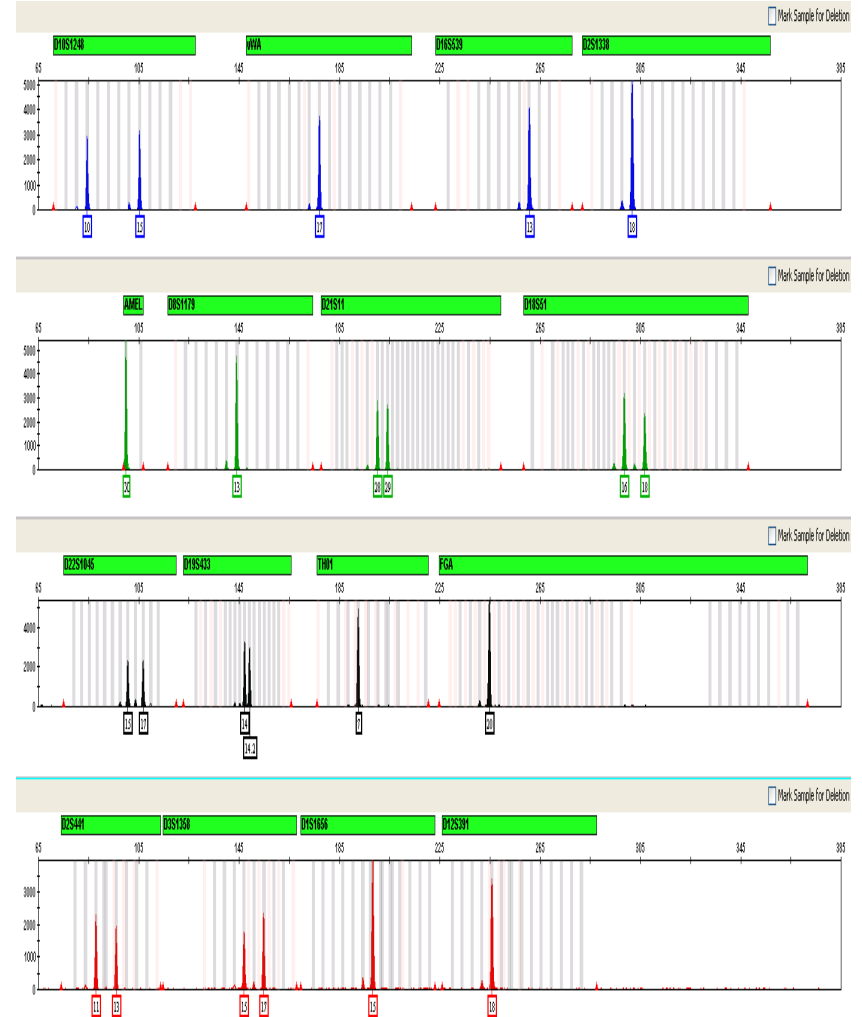


# PAT Definition for 3500 Data

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

Settings Resources

Applied Biosystems

Manage Reports  
Audit Reports  
E-Signature Reports

Manage Users  
Users

Manage Settings  
Security  
Audit  
E-Signature

Import  
Export  
Return to Setup

Enable System Security Disable System Security ?

### Account Setup

#### User Names

The length of user names must be between  and  characters.

Define name spacing  
 Leading  Trailing  Consecutive

Define name characteristics  
 Alpha  Numeric  Uppercase  Lowercase  Special

#### User Passwords

The length of user passwords must be between  and  characters.

Define password spacing  
 Leading  Trailing  Consecutive

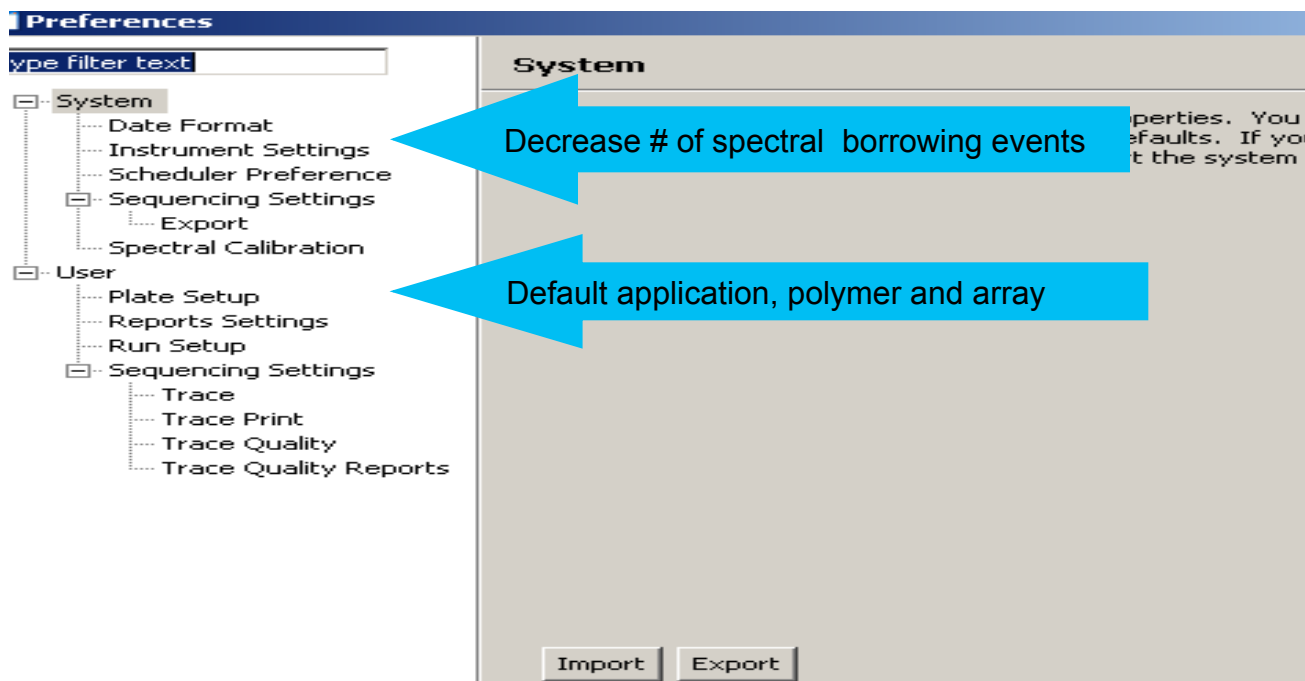
Define password characteristics  
 Alpha  Numeric  Uppercase  Lowercase

User may not reuse the previous  passwords.

#### Security Policies

- 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

The screenshot displays the GeneMapper ID-X software interface. On the left is a project tree showing a folder 'Inj 2 2009-0' with sub-items 1\_A01\_01R through 1\_H03\_24R. The main window is divided into three panes: 'Info', 'Raw Data', and 'EPT Data'. The 'Info' pane is active and shows 'Sample Information', 'Run Information', 'Data Collection Settings', and 'QC Information' sections. A blue arrow points to the 'Normalization Factor' value of 0.803 in the 'Sample Information' section. Another blue arrow points to the 'Consumable information tracked via RFID' section, which includes details like 'Run Module Name', 'Instrument/ Run Protocol', 'Dye Set Name', 'Polymer Type', 'Polymer Lot Number', 'Polymer Expiration Date', 'Polymer Time on Instrument', 'Results Group Name', 'Anode Buffer Type', 'Anode Buffer Lot Number', and 'Anode Buffer Expiration Date'.

Section	Field	Value
Sample Information	Sample File	1_A01_01Run 2009-01-19-10-23-34-312.
	Sample Name	1
	Sample Origin Path	C:\Applied Biosystems\3500\Data\Run
	Status Message	Size standard <GS600_LIZ_Normalizati
Run Information	User Name	Admi
	Instrument ID	3500
	Instrument Type	CE 3
	Data Collection Ver	3500
	Run Date & Time	2009
	Run Duration	1 mi
Data Collection Settings	Module File	HID36_POP4x1
	Pre-Run Voltage	15 kVolts
	Pre-Run Time	180 sec.
	Run Voltage	16400
	Injection Voltage	1200
	Injection Duration	5
	Temperature	60
	Laser Power	20
	Run Module Name	HID36_POP4x1
	Instrument/ Run Protocol	HID36_POP4_G5_standard_5sec
	Dye Set Name	G5
	Polymer Type	POP4
	Polymer Lot Number	51A007
	Polymer Expiration Date	2009-08-30 23:59:00.0
	Polymer Time on Instrument	2008-01-12 08:34:00.0
	Results Group Name	JB_2009-01-19-11-03-57
	Anode Buffer Type	AB 3xxx Buffer
Anode Buffer Lot Number	51B007	
Anode Buffer Expiration Date	2009-08-30 23:59:00.0	

# Consumable Cost Comparison

Consumable	Instrument Platform					
	310	3130	3500	3130xI	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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