

Implementing Promega PowerPlex® STR Kits for Non-Forensic Applications

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Introduction

STR Analysis is a Powerful Genotyping Tool



- Promega PowerPlex® STR kits were originally developed for use in forensic and paternity laboratories under specific/validated conditions (36cm POP-4® polymer)
- STR technology produces highly discriminative multi-locus genotypes
- STR analysis is valuable tool for non-forensic/paternity applications requiring single-source and mixed sample genotyping/analyses
- Many labs using STRs for non-forensic applications are also performing DNA sequencing, which requires alternate array lengths and polymer types
- Promega STR kits are for human identification applications including forensic analysis, relationship testing and research use. Not for medical diagnostic use.

Objectives



1. Generate Promega STR kit data using common multi-capillary electrophoresis (CE) platforms under non-forensic configurations (36cm and 50cm arrays with POP-6TM or POP-7TM)
2. Set up data collection on the 3130/3130xL, 3500/3500xL and 3730 Genetic Analyzers
3. Highlight key differences between Promega PowerPlex[®] STR and Life Technologies AmpFℓSTR[®] kits and protocols
4. Explain the basic components of data analysis in GeneMapper[®] 4.1
5. Compare PowerPlex[®] 16 HS and AmpFℓSTR[®] Identifiler Plus data

NOTE This is a technical presentation geared towards current users of STR kits and capillary electrophoresis instruments.

Organization of the Webinar



- I. Brief introduction to STRs
 - Use of STR analysis in forensic and non-forensic applications
- II. Emphasize high level goals of STR analyses
- III. Describe capillary electrophoresis components and configurations
- IV. Basics of setting up the Genetic Analyzers
 - 3130/3130xL
 - 3500/3500xL
 - 3730/3730xL
- V. Artifacts
- VI. Comment on GeneMapper[®] 4.1 analysis of STR Kit data
- VII. Comparison of PowerPlex[®] 16 HS and Identifiler Plus[®] data

STR Overview

Short Tandem Repeats



Short Tandem Repeats (STR) are repetitive sequences:

- Pentanucleotide: AAAGA AAAGA AAAGA AAAGA
- Tetranucleotide: AAAG AAAG AAAG AAAG
- Trinucleotide: CTT CTT CTT CTT CTT
- Dinucleotide: AG AG AG AG AG AG

Tetranucleotides - favored in human identity

- Good balance of “ease of interpretation” and “variation found in nature”

Pentanucleotides – highly discriminating, low stutter percentages

- Included in Promega STR kits

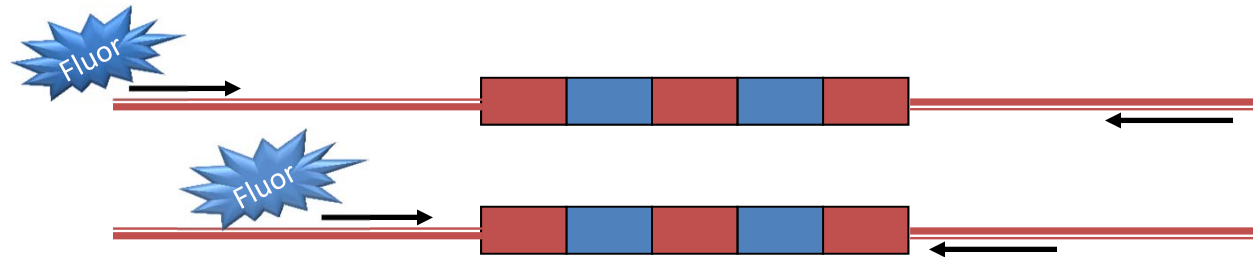
STR Overview

Multiplexing: Both Fragment Size & Color are Used

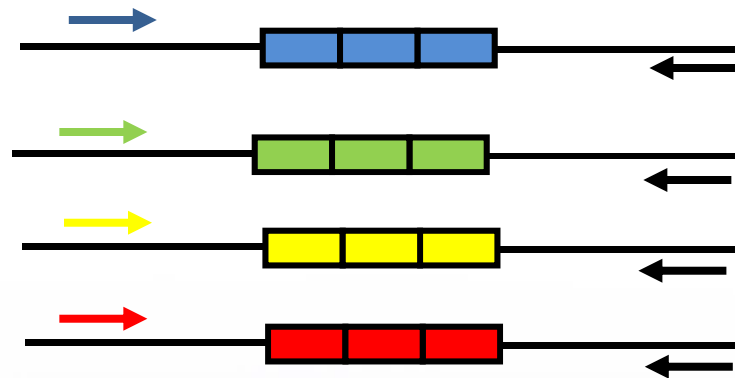


Size of the PCR product can be changed by moving primers closer or further from repeat region

SIZE



COLOR



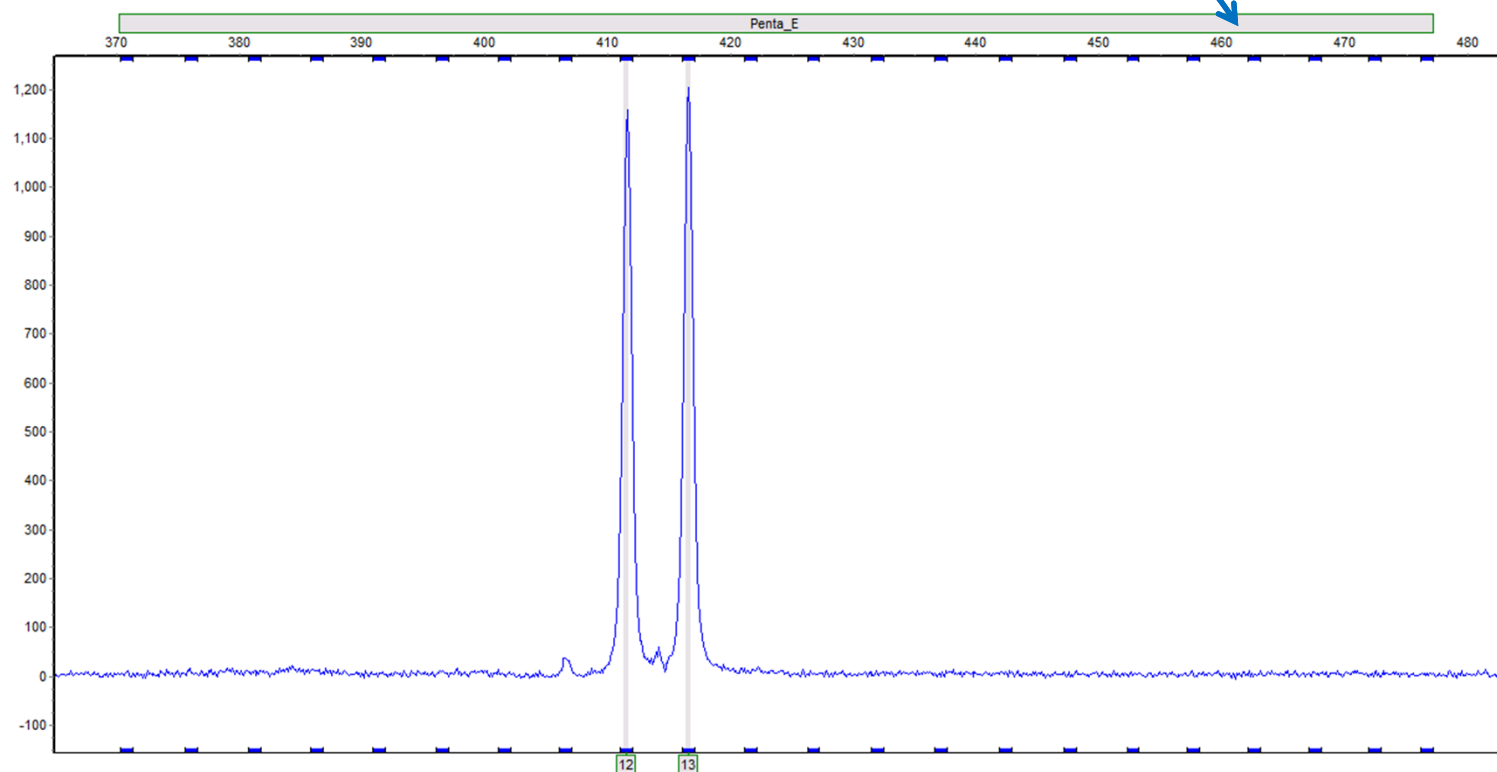
Different primers can be labeled with different fluorescent dyes

STR Overview

Example Electropherogram of One Locus/Genotype



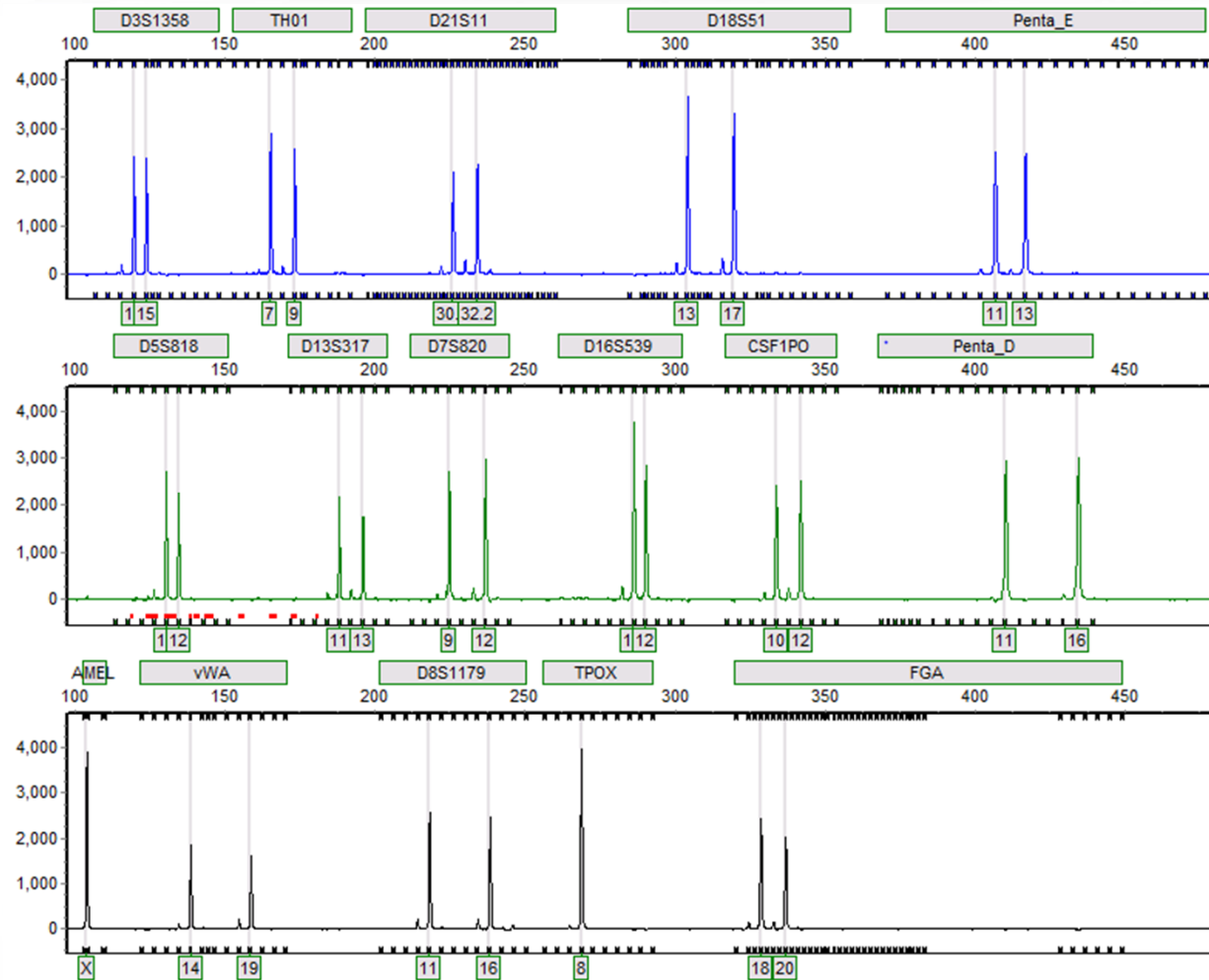
Penta E locus (size range of expected STR alleles)



Heterozygote – 12/13 genotype
(SoftGenetics GeneMarkerHID® v. 2.4.0)

STR Overview

Full PowerPlex® 16 HS Single-Source Profile



$\sim 3 \times 10^{-19}$ power of discrimination with PowerPlex® 16 HS

STR Analysis in Genetic Identity

Widespread and Still Increasing Use



Forensic casework laboratories

- Processing evidence from crime scenes
- Missing persons

Forensic database laboratories

- Creating criminal offender databases

Paternity testing laboratories

Other (less common) uses

- Genetic identification in plants, animals or humans

Emerging segments

- Police stations
- Immigration
- Department of Defense field testing



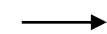
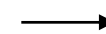
Crime
Scene



Lab

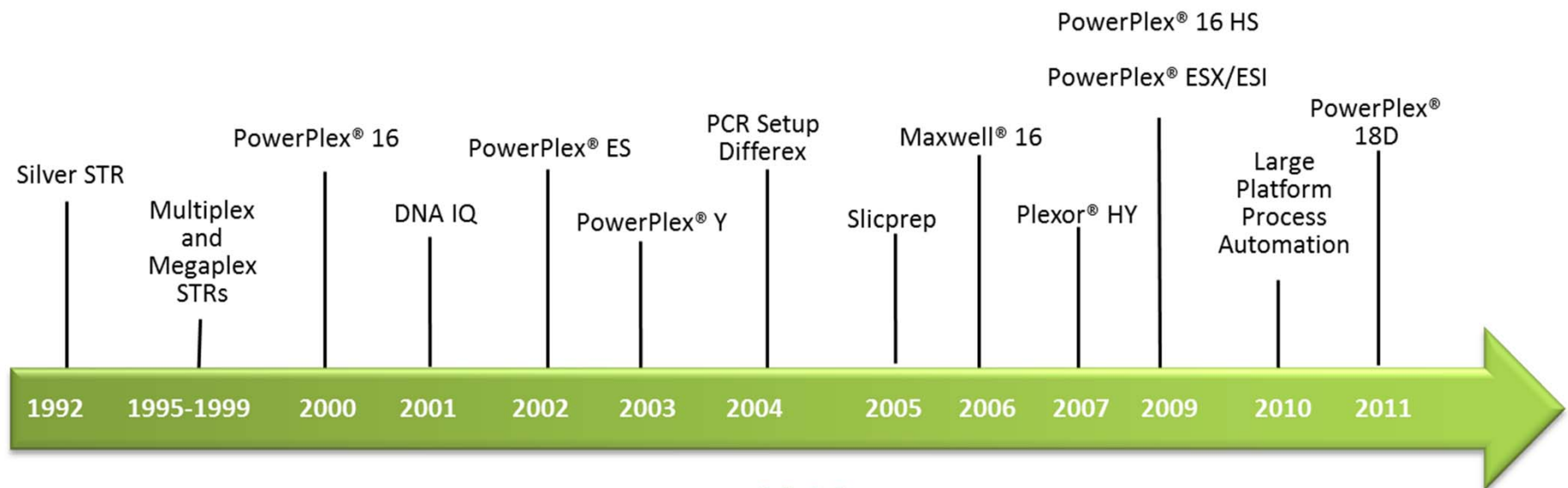


Court



Product Development and Innovation

Long History of Promega Products for STR Analysis



2012

PowerPlex® 21

PowerPlex® Y23

STR Overview

Comparison of Promega and Life Technologies STR Kits



Locus	PROMEGA PowerPlex® Systems				LIFE TECHNOLOGIES* AmpF&STR® PCR Amplification Kits		
	21	18D	16 & 16 HS	Cell ID™	Identifiler®/ Plus/ Direct	Profiler®	Cofiler®
Amel	Fluorescein	TMR-ET	TMR	TMR	PET®	JOE™	JOE™
CSF1PO	JOE™	JOE™	JOE™	JOE™	6-FAM™	JOE™	JOE™
D12S391	CXR-ET						
D13S317	Fluorescein	JOE™	JOE™	JOE™	VIC®	NED™	
D16S539	JOE™	JOE™	JOE™	JOE™	VIC®		5-FAM™
D18S51	JOE™	Fluorescein	Fluorescein		NED™		
D19S433	CXR-ET	CXR-ET			NED™		
D1S1656	Fluorescein						
D21S11	TMR-ET	Fluorescein	Fluorescein	Fluorescein	6-FAM™		
D2S1338	JOE™	CXR-ET			VIC®		
D3S1358	Fluorescein	Fluorescein	Fluorescein		VIC®	5-FAM™	5-FAM™
D5S818	TMR-ET	JOE™	JOE™	JOE™	PET®	NED™	
D6S1043	Fluorescein						
D7S820	TMR-ET	JOE™	JOE™	JOE™	6-FAM™	NED™	NED™
D8S1179	CXR-ET	TMR-ET	TMR		6-FAM™		
FGA	CXR-ET	TMR-ET	TMR		PET®	5-FAM™	
Penta D	JOE™	JOE™	JOE™				
Penta E	Fluorescein	Fluorescein	Fluorescein				
TH01	TMR-ET	Fluorescein	Fluorescein	Fluorescein	VIC®	JOE™	JOE™
TPOX	TMR-ET	TMR-ET	TMR	TMR	NED™	JOE™	JOE™
vWA	TMR-ET	TMR-ET	TMR	TMR	NED™	5-FAM™	

Non-Forensic Applications of STRs

A Wide-array of Powerful Applications



Sample Identification and Characterization

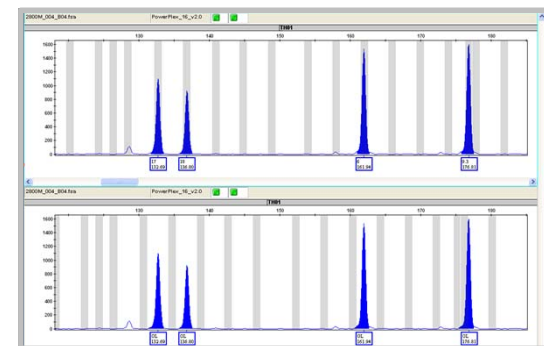
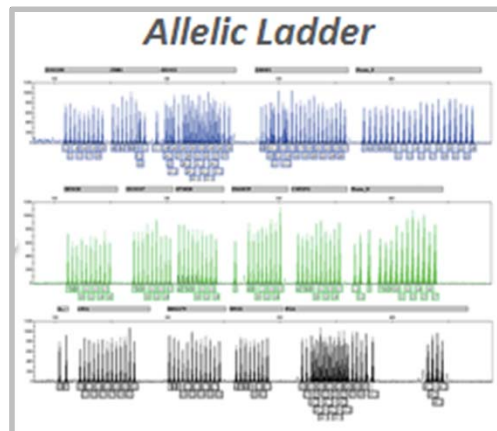
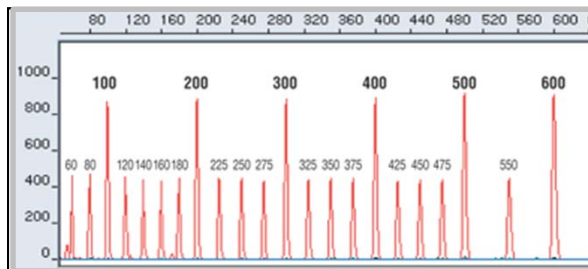
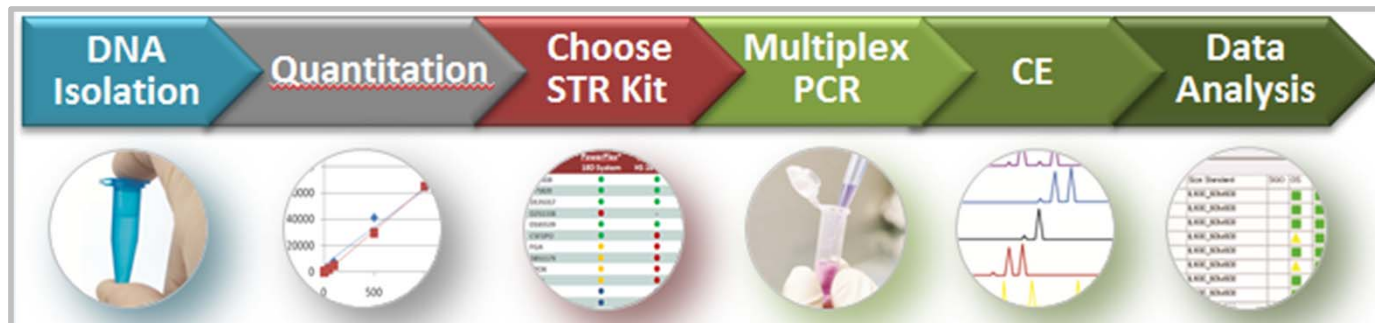
- Ancestry studies
- Cell line authentication
- Cancer research
- Twin zygosity testing
- Biobanking
- Research on genetic diseases
- Sample ID/Relationship confirmation
- Tissue provenance testing
- Slide floater analysis

Mixed Sample Identification

- Maternal cell contamination
- Biobanking (detect contamination)
- Cell line authentication (detect contamination)
- Semi-quantitative mixed sample analysis

High Level Goals of STR Analysis

STR Workflow Optimized for High Quality Data

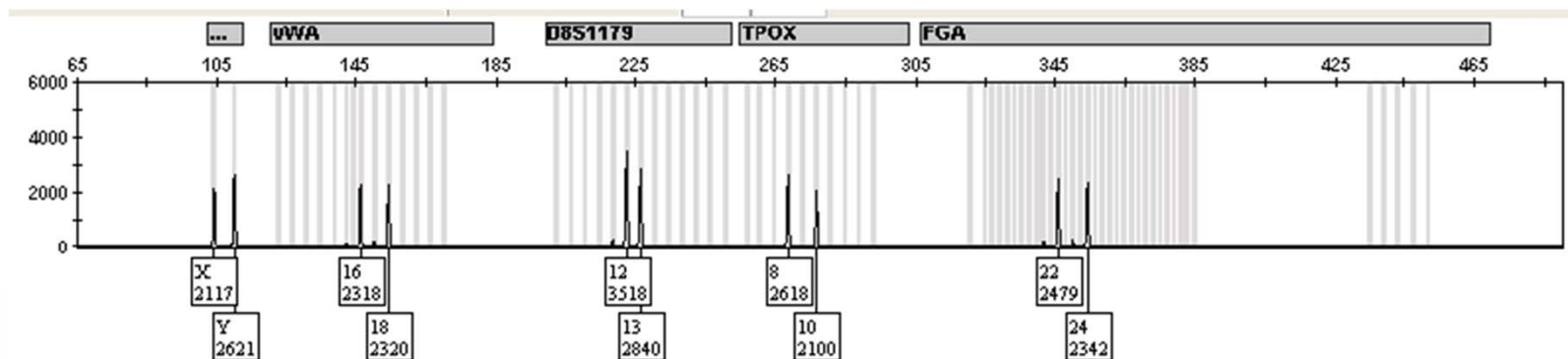


High Level Goals of STR Analysis

Generating Full, Balanced Profiles



- Generate a full, well-balanced DNA fingerprint/genetic profile
- Semi-quantitative mixture analysis
- Understand the capabilities and limitations of the instrument and chemistry
 - Dynamic range
 - Sensitivity



High Level Goals of STR Analysis

Optimize All Steps to Produce High Quality Data



PowerPlex® 16 HS Example

Quantitation and DNA Input
(500pg)

Reaction Volume
(25uL)

Thermal Cycling
(32cycles)

Injection Voltage and Time
(3 kV, 5 secs)
(default)

*All of these major factors,
working together, affect peak
height and balance.*



CE Components and Configurations

Arrays, Polymers, Spectrals and Run Modules



1. Array length (36cm or 50cm)
2. Polymer type (POP-4[®], POP-6[™] and POP-7[™])
3. Spectral calibration/dye set
 - a) Promega currently has two dye sets – one 4-dye set and one 5-dye set
 - b) Promega dye sets are not the same as the Life Technologies' dye sets
4. Spectral and Run module parameters
 - Run temp, run voltage, run time, polymer fill volume
 - injection voltage, injection time

CE Components and Configurations

Not All Combinations Are Possible for All Instruments



Instrument	POP-4 [®]		POP-6 [™]		POP-7 [™]	
	36cm	50cm	36cm	50cm	36cm	50cm
3100	✓		✓	✓		
3100 – Avant	✓		✓	✓		
3130-4	✓		✓	✓	✓	✓
3130xL	✓		✓	✓	✓	✓
3500 – 8	✓			✓		✓
3500 – 24	✓			✓		✓
3730 – 48					✓	✓
3730xL					✓	✓

 = Genetic Identity validated configurations

✓ = Instrument/POP/capillary length combination with run module available

CE Components and Configurations

3130/3130xL Run Modules



Run Module	POP-6™		POP-7™		Approx. Runtime (mins)
	36cm	50cm	36cm	50cm	
Fragment Analysis 36_POP7			✓		35
Fragment Analysis 50_POP7				✓	50
Fragment Analysis 50_POP6		✓			90
RapidSeq36_POP6	✓				60

Source: Life Technologies Capillary Electrophoresis Products Catalogue, p 14

CE Components and Configurations

3130 Run Module, FragmentAnalysis36_POP7



Run Module Editor

Run Module Description

Name:

Type: REGULAR

Template: FragmentAnalysis36_POP7

Description:

Run Module Settings

Name	Value	Range
Oven_Temperature	60	18...65 Deg. C
Poly_Fill_Vol	4840	4840...38000 steps
Current_Stability	5.0	0...2000 uAmps
PreRun_Voltage	15.0	0...15 kVolts
Pre_Run_Time	180	1...1000 sec.
Injection_Voltage	1.2	1...15 kVolts
Injection_Time	16	1...600 sec.
Voltage_Number_Of_Steps	20	1...100 nk
Voltage_Step_Interval	15	1...60 sec
Data_Delay_Time	60	1...3600 sec.
Run_Voltage	15.0	0...15 kVolts
Run_Time	1200	300...14000 sec.

Ok Cancel

Run Module Settings

Name	Value	Range
Oven_Temperature	60	18...65 Deg. C
Poly_Fill_Vol	4840	4840...38000 steps
Current_Stability	5.0	0...2000 uAmps
PreRun_Voltage	15.0	0...15 kVolts
Pre_Run_Time	180	1...1000 sec.
Injection_Voltage	1.2	1...15 kVolts
Injection_Time	16	1...600 sec.
Voltage_Number_Of_Steps	20	1...100 nk
Voltage_Step_Interval	15	1...60 sec
Data_Delay_Time	60	1...3600 sec.
Run_Voltage	15.0	0...15 kVolts
Run_Time	1200	300...14000 sec.

$$1.2\text{kV} \times 16\text{sec} = 19.2\text{kVsec}$$

CE Components and Configurations

3130 Run Module Comparison



36cm POP-6™

36cm POP-7™

50cm POP-6™

Run Module Editor

Run Module Description

Name:

Type: REGULAR

Template: RapidSeq36_POP6

Description:

Run Module Settings

Name	Value	Range
Oven_Temperature	55	18...65 Deg. C
Poly_Fill_Vol	4840	4840...38000 steps
Current_Stability	5.0	0...2000 uAmps
PreRun_Voltage	15.0	0...15 kVolts
Pre_Run_Time	180	1...1000 sec.
Injection_Voltage	1.2	1...15 kVolts
Injection_Time	12	1...600 sec.
Voltage_Number_Of_Steps	40	1...100 nk
Voltage_Step_Interval	15	1...60 sec
Data_Delay_Time	240	1...3600 sec.
Run_Voltage	15.0	0...15 kVolts
Run_Time	2400	300...14000 sec.

Ok Cancel

Run Module Editor

Run Module Description

Name:

Type: REGULAR

Template: FragmentAnalysis36_POP7

Description:

Run Module Settings

Name	Value	Range
Oven_Temperature	60	18...65 Deg. C
Poly_Fill_Vol	4840	4840...38000 steps
Current_Stability	5.0	0...2000 uAmps
PreRun_Voltage	15.0	0...15 kVolts
Pre_Run_Time	180	1...1000 sec.
Injection_Voltage	1.2	1...15 kVolts
Injection_Time	16	1...600 sec.
Voltage_Number_Of_Steps	20	1...100 nk
Voltage_Step_Interval	15	1...60 sec
Data_Delay_Time	60	1...3600 sec.
Run_Voltage	15.0	0...15 kVolts
Run_Time	1200	300...14000 sec.

Ok Cancel

Run Module Editor

Run Module Description

Name:

Type: REGULAR

Template: FragmentAnalysis50_POP6

Description:

Run Module Settings

Name	Value	Range
Oven_Temperature	60	18...65 Deg. C
Poly_Fill_Vol	5020	5020...38000 steps
Current_Stability	5.0	0...2000 uAmps
PreRun_Voltage	15.0	0...15 kVolts
Pre_Run_Time	180	1...1000 sec.
Injection_Voltage	1.6	1...15 kVolts
Injection_Time	15	1...600 sec.
Voltage_Number_Of_Steps	40	1...100 nk
Voltage_Step_Interval	15	1...60 sec
Data_Delay_Time	680	1...3600 sec.
Run_Voltage	15.0	0...15 kVolts
Run_Time	4000	300...14000 sec.

Ok Cancel

*NOTE 16-cap instruments have longer injection times

Setting up a Promega 4-dye Spectral/Matrix



1. *Make 1:10 dilution of each dye in water*
2. *Mix diluted dyes in fresh HiDi formamide*
3. *Denature and snap cool on ice*

NOTE: With Promega chemistry , vortex well and do not centrifuge

NOTE: Store at -20°C after each thaw

Setting up Data Collection for the 3130/3130xL

3130xL: Promega 4-dye Spectral Run Module – 36cm POP-6™



Run Module Editor

Run Module Description

Name: Spect36_POP6_Promega

Type: SPECTRAL

Template: Spect36_POP6

Description:

Run Module Settings

Name	Value	Range
Oven_Temperature	60	18...65 Deg. C
Poly_Fill_Vol	4840	4840...38000 steps
Current_Stability	5.0	0...2000 uAmps
PreRun_Voltage	15.0	0...15 kVolts
Pre_Run_Time	180	1...1000 sec.
Injection_Voltage	1.2	1...15 kVolts
Injection_Time	12	1...600 sec.
Voltage_Number_Of_Steps	40	1...100 nk
Voltage_Step_Interval	15	1...60 sec
Data_Delay_Time	100	1...3600 sec.
Run_Voltage	15.0	0...15 kVolts
Run_Time	1900	300...14000 sec.

Ok Cancel

36cm POP-6™ configuration uses a sequencing template

Change from default 55°C

Setting up Data Collection for the 3130/3130xL

3130xL: 4-dye Spectral Protocol – 36cm POP-6™



Protocol Editor

Name: Spect_36_POP6_Promega

Description:

Type: SPECTRAL

Dye Set: F

Polymer: POP6

Array Length: 36

Chemistry: Matrix Standard

Run Module: Spect36_POP6_Promega

Edit Param... OK Cancel

Edit Spectral Params

Matrix Condition Number Bounds Lower 4.0 Upper 12.0

Locate Start Point After Scan 300 Before Scan 5000

Limit Analysis (scans) 20000

Sensitivity 0.4

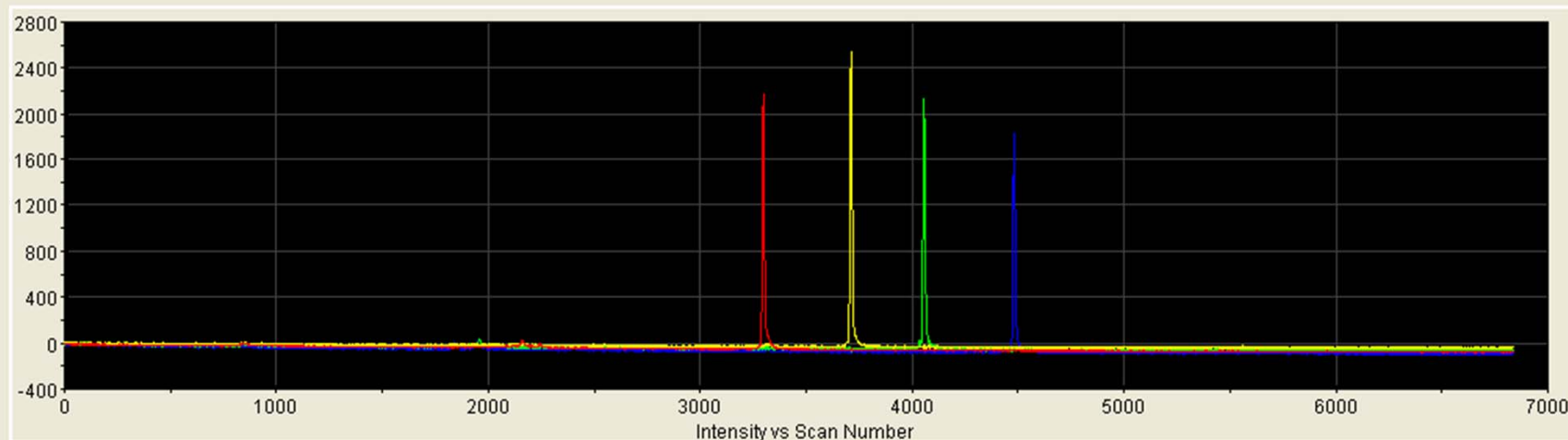
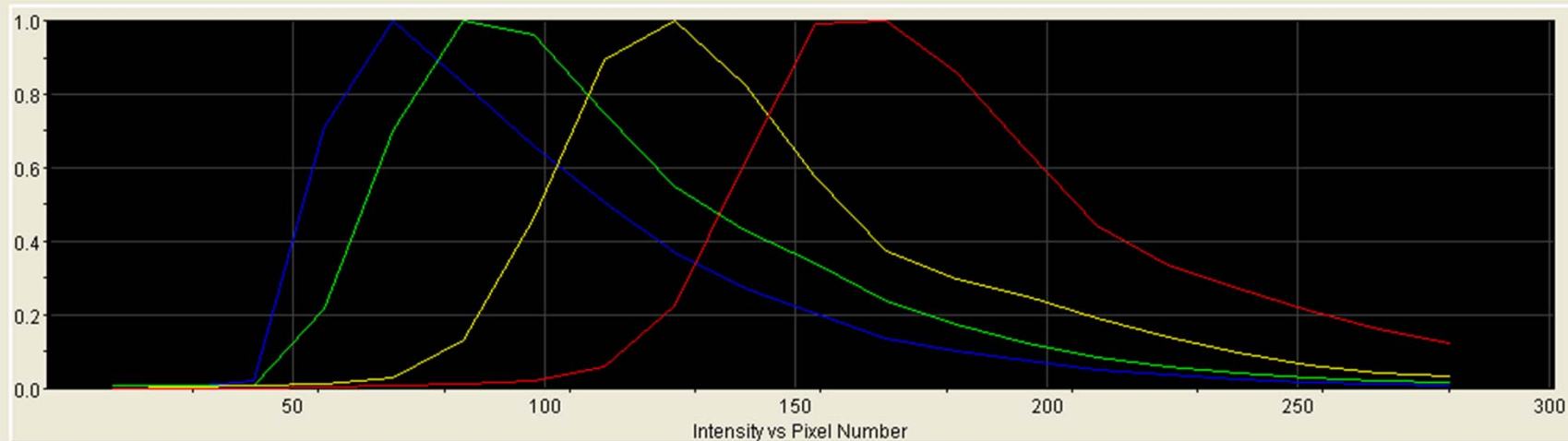
Minimum Quality Score 0.95

OK Cancel

For 4-dye spectral, drop lower condition bounds to 4.0 and leave upper at 12.

Setting Up Data Collection for the 3130/3130xL

Promega 4-Dye Spectral Calibration Result



PowerPlex® Chemistry



Hot Start STR Kits contain:

- Pre-amp and Post-amp boxes
- Enzyme in master mix
- Nuclease-free water
- Separately packaged allelic ladder
- Internal lane standard
- Control DNA (requires dilution)

16HS - 25 uL Reaction:

Primer Mix 2.5 uL

Master Mix 5.0 uL

Sample: up to 17.5 uL

Setting Up Data Collection for the 3130/3130xL 3130, RapidSeq36_POP6 Run Module



Run Module Editor

Run Module Description

Name:

Type: REGULAR

Template: RapidSeq36_POP6

Description:

Run Module Settings

Name	Value	Range
Oven_Temperature	55	18...65 Deg. C
Poly_Fill_Vol	4840	4840...38000 steps
Current_Stability	5.0	0...2000 uAmps
PreRun_Voltage	15.0	0...15 kVolts
Pre_Run_Time	180	1...1000 sec.
Injection_Voltage	1.2	1...15 kVolts
Injection_Time	12	1...600 sec.
Voltage_Number_Of_Steps	40	1...100 nk
Voltage_Step_Interval	15	1...60 sec
Data_Delay_Time	240	1...3600 sec.
Run_Voltage	15.0	0...15 kVolts
Run_Time	2400	300...14000 sec.

Ok Cancel

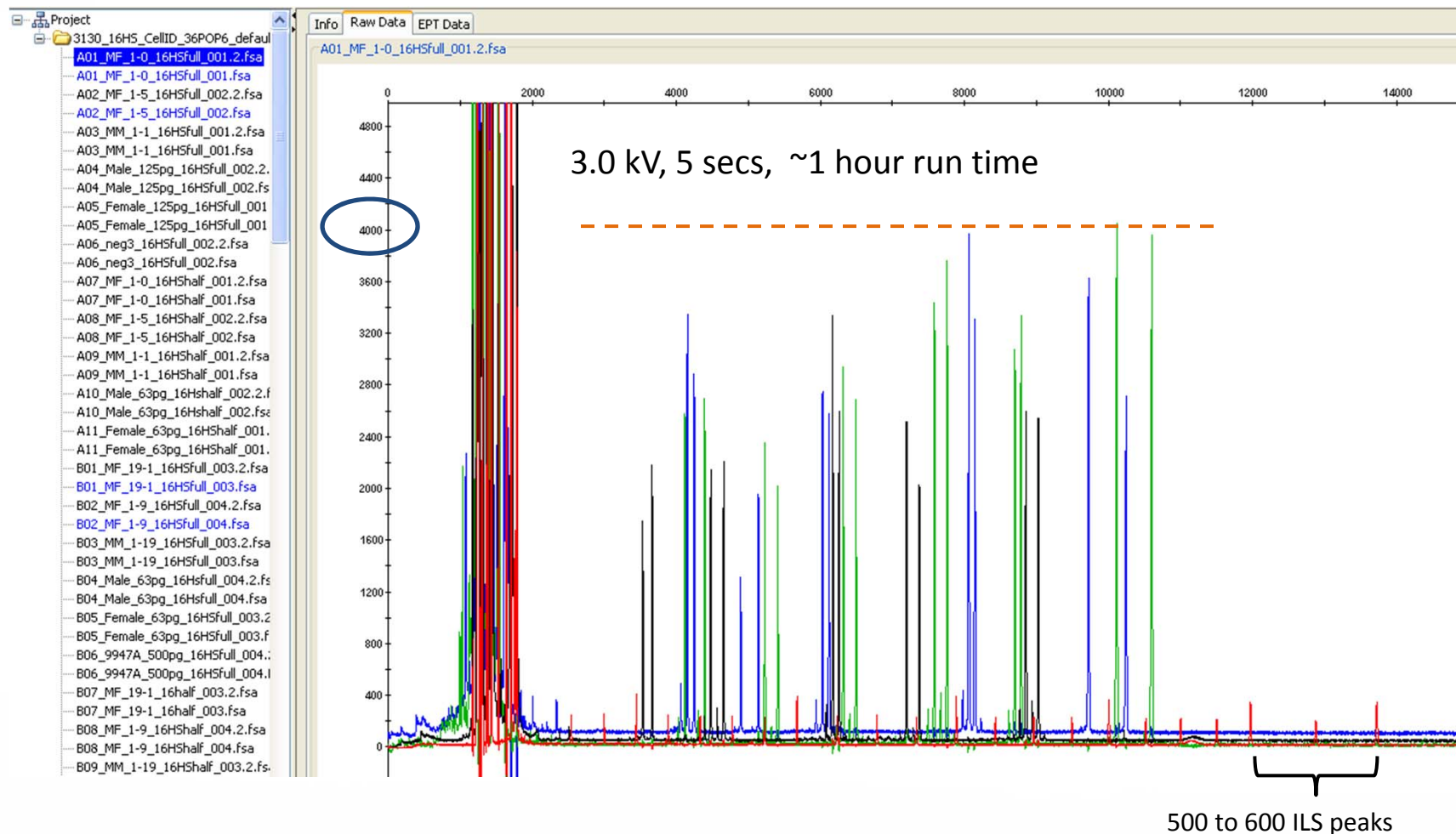
Change to 60°C

NOTE: Run time may need to be increased

**NOTE: On the 16 cap instrument,
1.2 kV for 18 seconds**

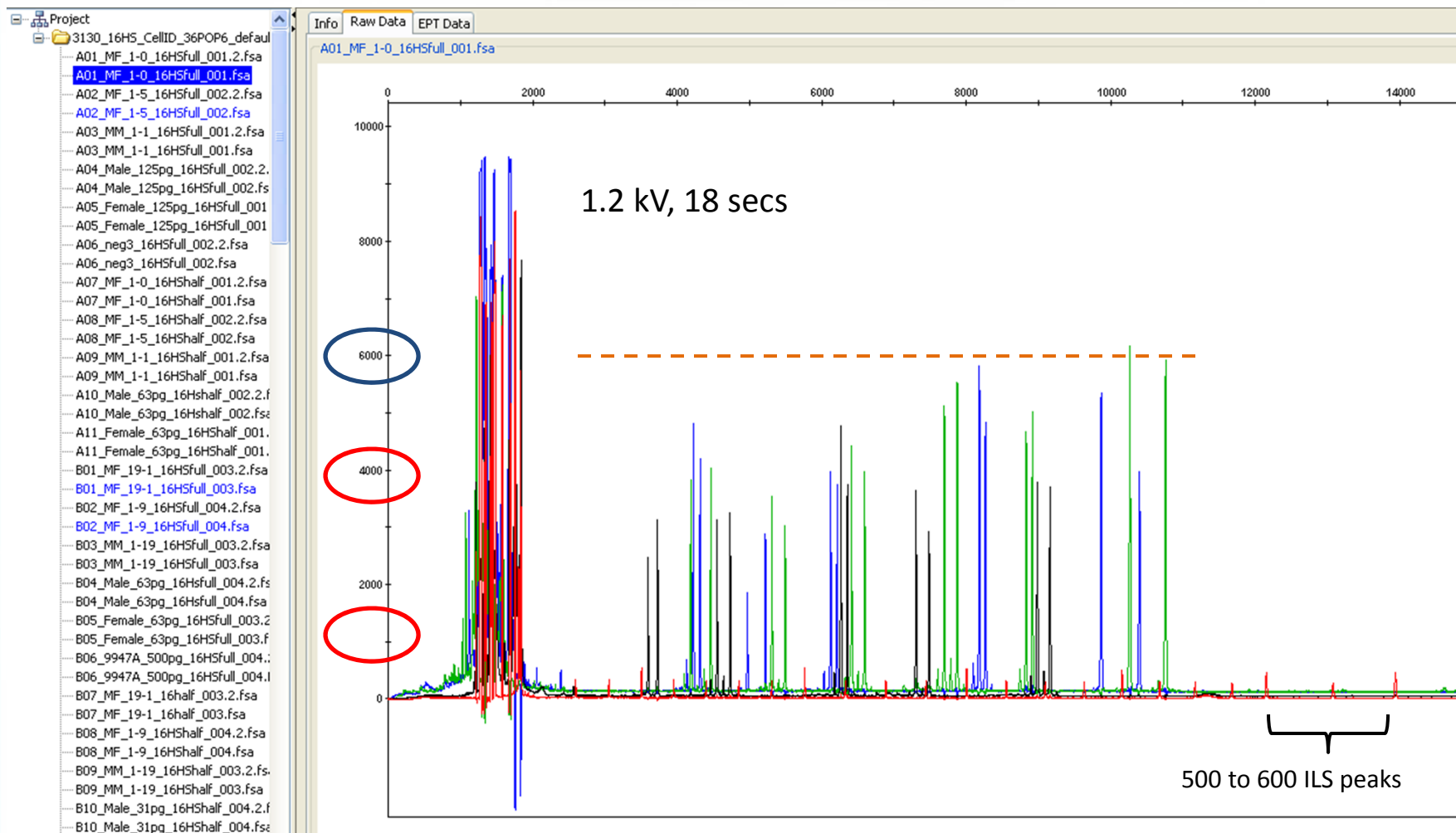
3130xL/ PowerPlex® 16 HS Example Data

36cm POP-6™: 3 kV, 5 sec Injection



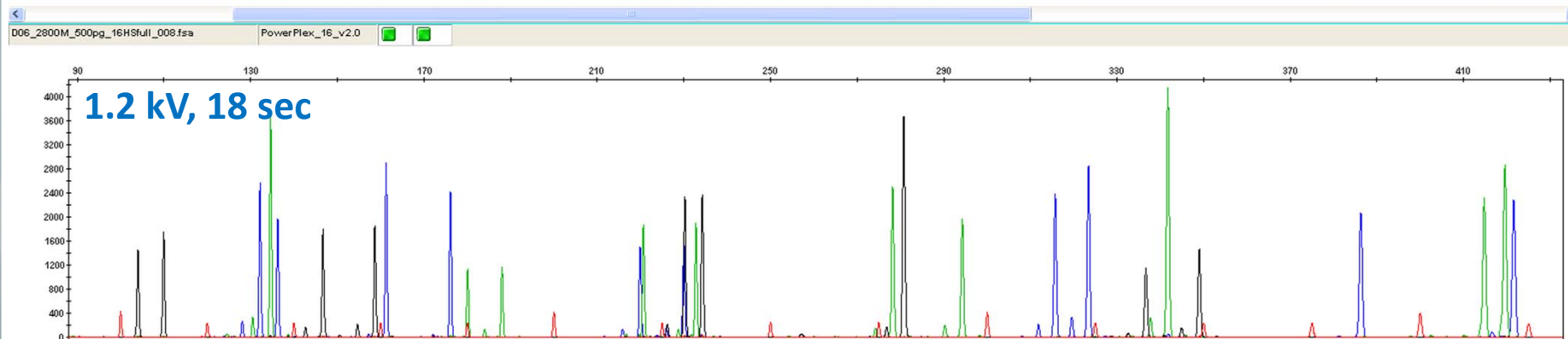
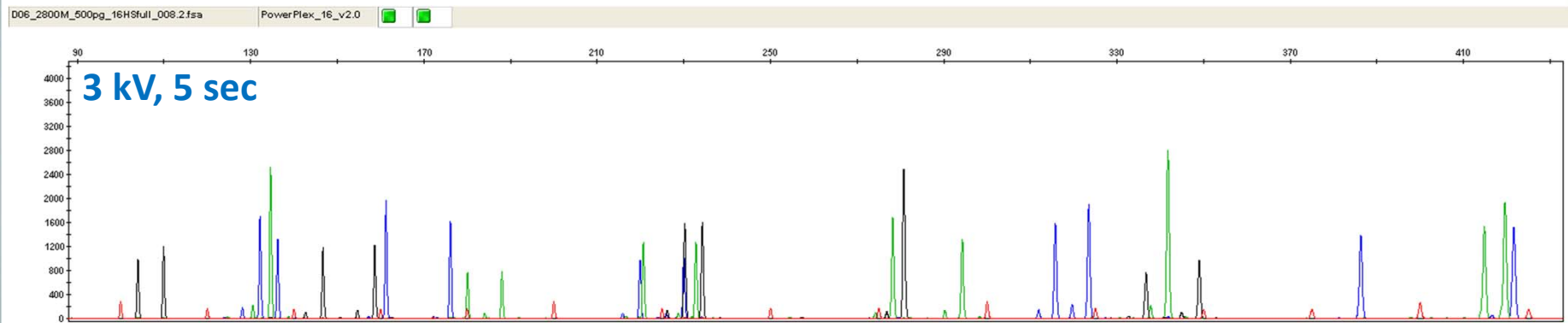
3130xL PowerPlex® 16 HS Example Data

36cm POP-6™: Default Injection Parameters



3130xL/ PowerPlex® 16 HS Example Data

36cm POP-6™: 2800M Control DNA Comparison

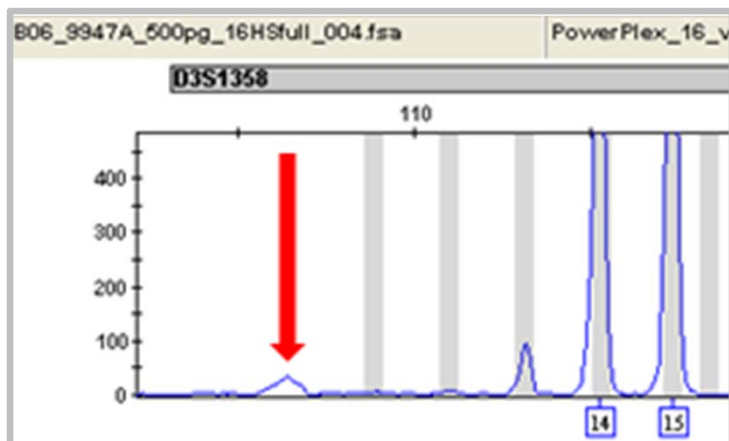


3130xL/ PowerPlex® 16 HS Example Data

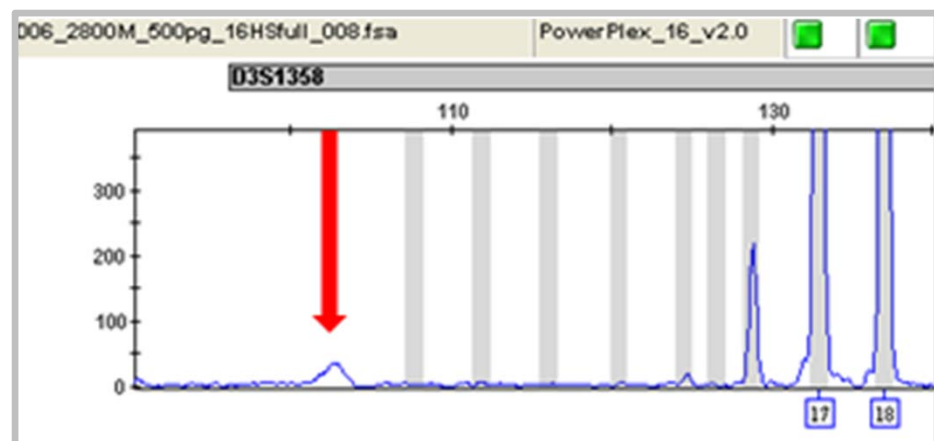
Known 16 HS Artifact in D3S1358 with POP-7™



36cm POP-7™



50cm POP-7™

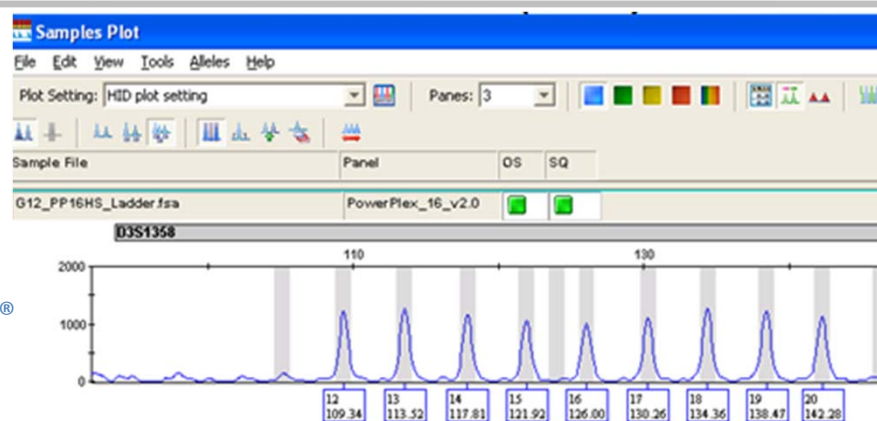


3130xL/ PowerPlex® 16 HS Example Data

Allelic Ladder Comparison with Three Polymer Types



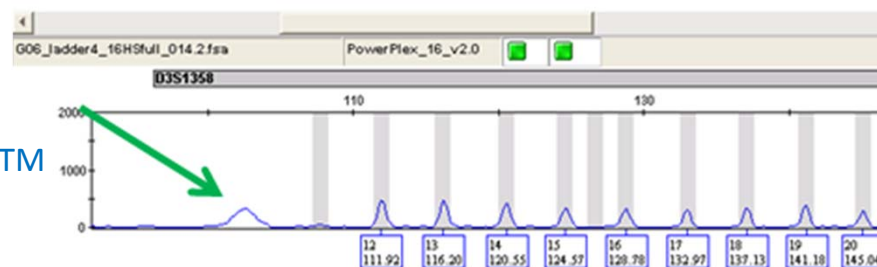
POP-4®



POP-6™



POP-7™



3130/3130xL Summary

Careful Implementation Produces High Quality Data



- ✓ Calibrate the CE with the Promega 4-dye spectral calibration
- ✓ Use the default run module for the configuration of choice
- ✓ Look for average peak heights, balance, artifacts
- ✓ Optimize by examining workflow to see where in the process it would be best to make changes (input DNA, cycle number, injection parameters)
- ✓ Contact genetic@promega.com with questions

CE Components and Configurations

3500/3500xL Genetic Analyzers



- Y-axis scale is 4x that of 3130/3130xL (~32,000 rfus)
- 3500 documentations cites 175 rfus for peak detection threshold
- “Research” and “HID” instruments are nearly identical
 - both can run all applications
- To create .fsa files:
 - 50cm array (not 36cm)
 - POP-7™ or POP-6™
- 36cm POP-4® = .hid files (Cannot be read by GeneMapper 4.1)
- 3500 .fsa files not backwards compatible to earlier versions of GeneMapper



CE Components and Configurations

Not All Combinations Are Possible for All Instruments



Instrument	POP-4 [®]		POP-6 [™]		POP-7 [™]	
	36cm	50cm	36cm	50cm	36cm	50cm
3100	✓		✓	✓		
3100 – Avant	✓		✓	✓		
3130-4	✓		✓	✓	✓	✓
3130xL	✓		✓	✓	✓	✓
3500 – 8	✓			✓		✓
3500 – 24	✓			✓		✓
3730 – 48					✓	✓
3730xL					✓	✓

✓ = Instrument/POP/capillary length combination with run module available

CE Components and Configurations

3130/3130xL Run Modules



Run Module	POP-6™		POP-7™		Approx. Runtime
	36cm	50cm	36cm	50cm	
FragAnalysis50_ POP7				✓	40
FragAnalysis50_ POP6		✓			100

Source: Life Technologies Capillary Electrophoresis Products Catalogue, p 14

Setting Up Data Collection for the 3500/3500xL

Create a New Dye Set



Dashboard Edit ▾ Library Maintenance Tools ▾ Manage ▾ Preferences Help ▾ Log Out

Library Resources

AS Applied Biosystems

Manage

- Plates
- Assays
- File Name Conventions
- Results Group

Analyze

- Instrument Protocols
- Dye Sets**
- Size Standards
- Basecalling Protocols
- Sizescalling Protocols
- QC Protocols
- Sequencing Analysis Protocols
- MicroSeqID Protocols
- Fragment Analysis Protocols
- HID Analysis Protocols

Main Workflow

Create New Dye Set

Create Edit Duplicate Delete Import Export E-Signature View Audit History View E-Signature History

Filter: All Search: All Go Clear ?

Dye Set	Chemistry Standard	Calibration Date	Capillary Array Serial Number	Is Signed
Create New Dye Set				
Setup a Dye Set				
* Dye Set Name Promega 4Dye ☐ Locked				
* Chemistry Matrix Standard				
* Dye Set Template F Template				
Arrange Dyes				
Dye Selection	☒	☒	☒	☒
Reduced Selection	☒	☒	☒	☒
Calibration Peak Order	4	3	2	1
Parameters				
The parameters will be used for instruments configured with 36cm capillary array and polymer POP4				
Matrix Condition Number Upper Limit 8.5				
Locate Start Point * After Scan 750 * Before Scan 5000				
* Limit Scans To 2500				
Sensitivity 0.4				
* Minimum Quality Score 0.95				
Notes				
Close Save				

Setting Up Data Collection for the 3500/3500xL Promega 4Dye Spectral Calibration



3500 Data Collection Software

Dashboard Edit Library Maintenance Tools Manage Preferences Help Log Out

Maintenance

AB Applied Biosystems

Calibrate

Spatial

Spectral

Performance Check

Sequencing Install Standard

Fragment Install Standard

HD Install Standard

Maintenance Wizards

Planned Maintenance

Notifications Log

Service Log

Schedule

Main Workflow

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

Calibration Run History View

Calibration Settings

Number of Wells: ☒ 96 ☐ 96-FastTube ☐ 384 Chemistry Standard: Matrix Standard

Plate Position: ☒ A ☐ B Dye Set: Promega 4Dye

☒ Allow Borrowing

Current Instrument Consumables

Polymer Type: POP4 Capillary Length: 36cm

Start Run

Status: Ready

Capillary Run Data

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																								
Run 2																								
Run 3																								
Overall																								

☒ Passed ☐ Failed ☐ Borrowed ☐ Not Calibrated

Quality Value: Condition #: Status: Message:

Intensity vs Scan Number

Raw Data

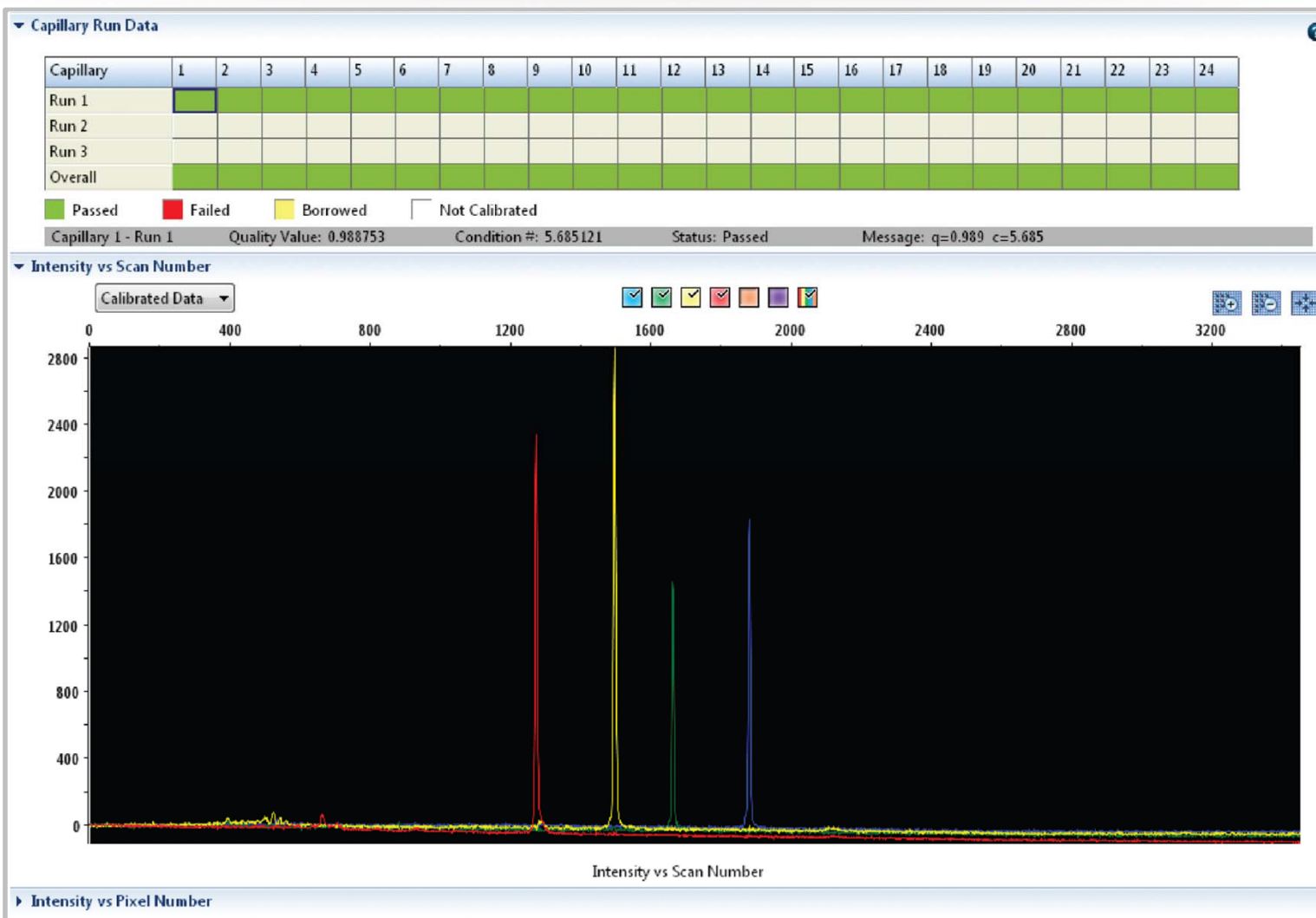
Intensity vs Scan Number

Intensity vs Pixel Number

Accept Reject

Setting Up Data Collection for the 3500/3500xL

Promega 4Dye Spectral Calibration Result



Setting Up Data Collection for the 3500/3500xL Instrument Protocol



3500 Data Collection Software

Dashboard Edit Library Maintenance To

Library Resources

AB Applied Biosystems

Manage

Plates

Assays

File Name Conventions

Results Group

Analyze

Instrument Protocols

Dye Sets

Size Standards

Basecalling Protocols

Sizercalling Protocols

QC Protocols

Sequencing Analysis Protocols

MicroSeqID Protocols

Fragment Analysis Protocols

HID Analysis Protocols

Main Workflow

Create Edit Duplicate Delete Import Export E-Signature View Audit History View E-Signature Hist

Filter: Fragment Search:

	Instrument Protocol Name	Type	Dye Set Name	Description
1	AB FragmentAnalysis50_POP6xL_1	Fragment	G5	
2	AB FragmentAnalysis50_POP6_1	Fragment	G5	
3	AB F			
4	AB F			
5	AB L			
6	AB L			
7	AB S			
8	AB S			
9	Copy			

Edit Instrument Protocol Copy Of FragmentAnalysis50_POP7_1

Setup an Instrument Protocol

Application Type: Fragment Capillary Length: 50 cm Polymer: POP7

Dye Set: Promega 4Dye

Instrument Protocol Properties

* Run Module: FragmentAnalysis50_POP7

* Protocol Name: 16HS 50cm POP7 Locked

Description:

Oven Temperature (°C): 60 Run Voltage (kVolts): 19.5 PreRun Voltage (kVolts): 15 Injection Voltage (kVolts): 1.6

Run Time (sec.): 1330 PreRun Time (sec.): 180 Injection Time (sec.): 8 Data Delay (sec.): 1

Advanced Options

Following values are not recommended to be changed.

Voltage Tolerance (kVolts): 0.7 Voltage # of Steps (nk): 30 Voltage Step Interval (sec.): 15

First Read Out Time (ms): 200 Second Read Out Time (ms): 200

Normalization Target: 4500.0 Normalization Factor Threshold Min: 0.3 Normalization Factor Threshold Max: 3.0

Close Save

Setting Up Data Collection for the 3500/3500xL

Verify Instrument Protocol



3500 Data Collection Software

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Sequencing Analysis Protocols

Create Edit Duplicate Delete Import Export E-Signature View Audit History View E-Signature History

Filter: Fragment Search: All Go Clear ?

	Instrument Protocol Name	Type	Dye Set Name	Description	Date Modified	Is Signed
1	16HS_ILS600	Fragment	Promega F		21-Jun-2012 07:50:40 AM	No
2	Copy Of FragmentAnalysis50_POP...	Fragment	G5		15-May-2009 05:17:15 PM	No
3	AB FragmentAnalysis50_POP6x1	Fragment	G5		15-May-2009 05:17:01 PM	No
4	AB FragmentAnalysis50_POP6_1	Fragment	G5		15-May-2009 05:17:01 PM	No
5	AB FragmentAnalysis50_POP7x1	Fragment	G5		15-May-2009 05:17:15 PM	No
6	AB FragmentAnalysis50_POP7_1	Fragment	G5		15-May-2009 05:17:15 PM	No
7	AB LongFragmentAnalysis50_PO...	Fragment	G5		15-May-2009 05:17:16 PM	No
8	AB LongFragmentAnalysis50_PO...	Fragment	G5		15-May-2009 05:17:15 PM	No
9	Promega 4Dye FragAnalysis50_PO...	Fragment	Promega 4Dye		21-Jun-2012 01:06:38 PM	No
10	AB SNaPshot50_POP7x1_1	Fragment	E5		15-May-2009 05:17:18 PM	No
11	AB SNaPshot50_POP7_1	Fragment	E5		15-May-2009 05:17:18 PM	No
12	16HS50cmPOP7	Fragment	Promega 4Dye		09-Aug-2012 12:13:36 PM	No

Setting Up Data Collection for the 3500/3500xL

Define the Size Standard/ILS



Edit Size Standard Promega CXR 600ROX 60 - 600

Setup a Size Standard

* Size Standard: ☐ Locked

Description:

* Dye Color:

Enter sizes in the field below separated by a comma, space, or return then click the "Add Size(s) >>" button to add them to the current size standard definition.

Enter new Size Standard definition: (e.g. 11.0, 34.2, 55)

Add Size(s) >>

* Current Size Standard definition:

60.0
80.0
100.0
120.0
140.0
160.0
180.0
200.0
225.0
250.0
275.0
300.0
325.0
350.0
375.0
400.0
425.0
450.0
475.0
500.0
550.0
600.0

Close Save

Setting Up Data Collection for the 3500/3500xL

Create a Sizecalling Protocol



Dashboard Edit Library Maintenance Tools

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QC Protocols

Sequencing Analysis Protocols

MicroSeqID Protocols

Fragment Analysis Protocols

HID Analysis Protocols

Main Workflow

Create Edit Duplicate Delete Import Export E-Signature View Audit History View E-Signature History

Edit Sizecalling Protocol MSI 50 IL S600

Setup a Sizecalling Protocol

* Protocol Name: 16HS IL S600 Locked

Description:

Size Standard: Promega IL S600

Sizecaller: 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: 60 Primer Peak: Present

Analysis Stop Point: 1000000 Sizing Stop Size: 600

	Blue	Green	Yellow	Red	Purple	Orange
Minimum Peak Height	175	175	175	175	175	175

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

NOTE: Verify that Sizecalling Protocol matches Instrument Protocol

Setting Up Data Collection for the 3500/3500xL

Set Up an Assay



3500 Data Collection Software

Dashboard Edit Library Maintenance Tools Manage Preferences Help Log Out

Create Edit Duplicate Delete Import Export E-Signature View Audit History View E-Signature History

Filter: Fragment Search: All Go Clear

Assay Name	Type	Instrument Protocol	Primary Analysis Protocol	Secondary Analysis Protocol	Color	Date Modified	Is Signed
1 AB Fragment_Analysis_...	Fragment	FragmentAnalysis50_PO...	Fragment_Analysis_PA_P...			19-May-2009 06:32:31 PM	No
2						19-May-2009 06:32:31 PM	No
3						19-May-2009 06:32:31 PM	No
4						19-May-2009 06:32:31 PM	No
5						03-Jul-2012 01:26:54 PM	No
6						19-May-2009 06:32:31 PM	No
7						19-May-2009 06:32:31 PM	No
8						19-May-2009 06:32:31 PM	No
9						19-May-2009 06:32:31 PM	No

Edit Assay PP16HS_50cm_POP7

Setup an Assay

* Assay Name: PP16HS_50cm_POP7 ☐ Locked Color: Magenta

Application Type: Fragment

Protocols

Do you wish to assign multiple instrument protocols to this assay? ☒ No ☐ Yes

* Instrument Protocol: PP16HS_50cm_POP7 Edit Create New

* Sizecalling Protocol: 16HS_ILS600 Edit Create New

GeneMapper Protocol: Edit Create New

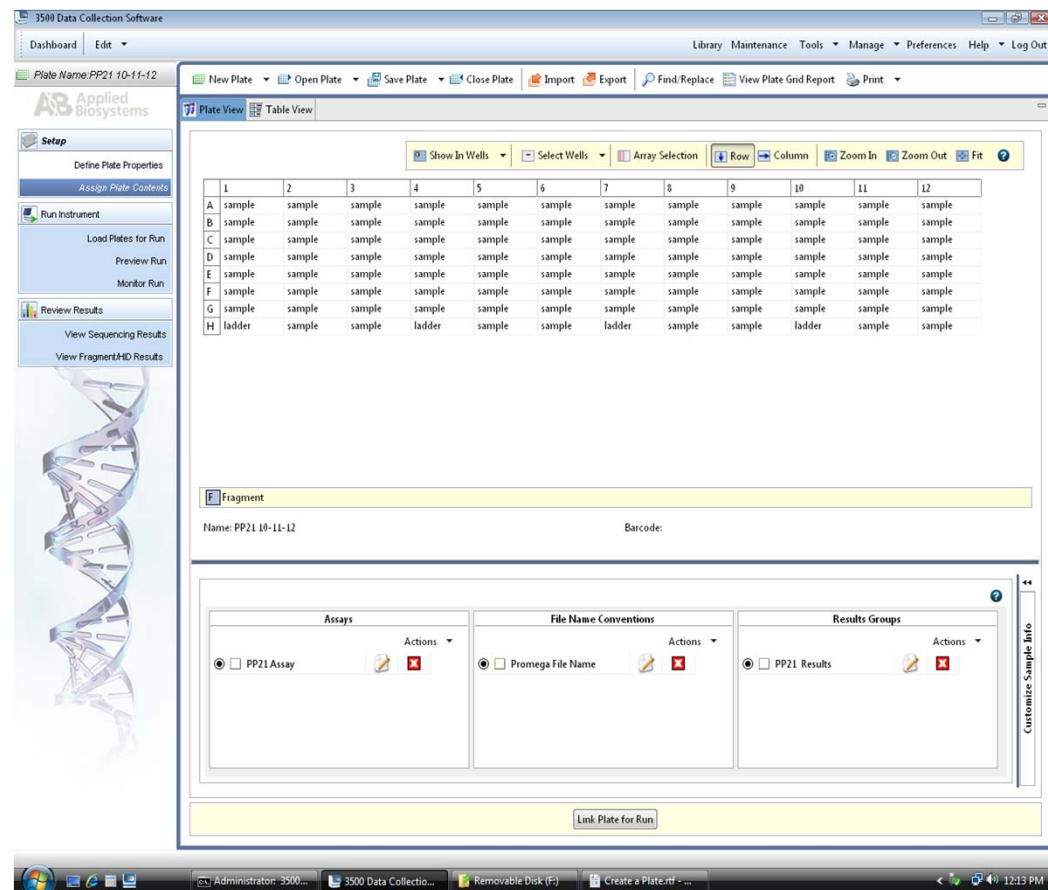
Close Save

Setting up Data Collection for the 3500/3500xL

Complete Data Collection Info/ Link Plate/Run



- Set up File Name Convention
- Set up Results Group
- Link Plate and Run



3500/3500xL Summary



- ✓ Create a new Promega4Dye Dye Set
- ✓ Run the Promega 4-Dye Spectral Calibration
- ✓ Create Data Collection Components needed to run the samples
- ✓ Optimize by examining workflow to see where in the process it would be best to make changes
 - Input DNA
 - Cycle number
 - Injection parameters

CE Components and Configurations

Only POP-7™ Configurations Available for 3730/3730xL



Instrument	POP-4®		POP-6™		POP-7™	
	36cm	50cm	36cm	50cm	36cm	50cm
3100	✓		✓	✓		
3100 – Avant	✓		✓	✓		
3130-4	✓		✓	✓	✓	✓
3130xL	✓		✓	✓	✓	✓
3500 – 8	✓			✓		✓
3500 – 24	✓			✓		✓
3730 – 48					✓	✓
3730xL					✓	✓

✓ = Instrument/POP/capillary length combination with run module available

CE Components and Configurations

3730/3730xL DNA Analyzers



CE Components and Configurations

3730/3730xL



- Y-axis scale is 4x that of the 3130/3130xL – saturates at ~32,000 rfus
- Most instruments use POP-7™
- For Promega 4-Dye chemistry use Any4Dye spectral calibration
- For Promega 5-Dye chemistry use the G5-RCT option
- Follow the same guidelines as discussed above for the 3130/3130xL instruments
- Some cross-talk to be expected on this instrument, so try to avoid over-loaded data
- Analyze with a peak window size of 11 (GeneMapper)

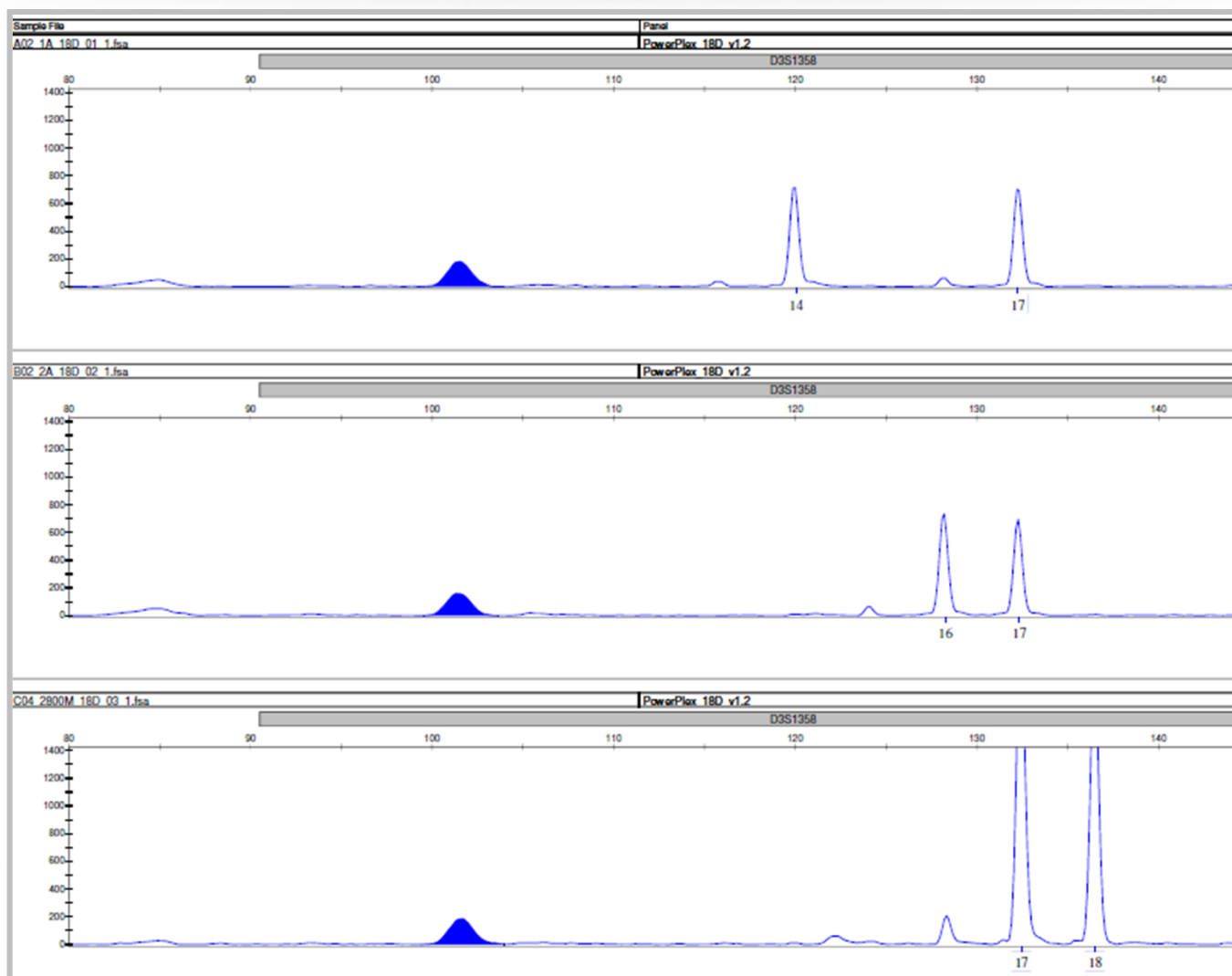
Important Overall Considerations



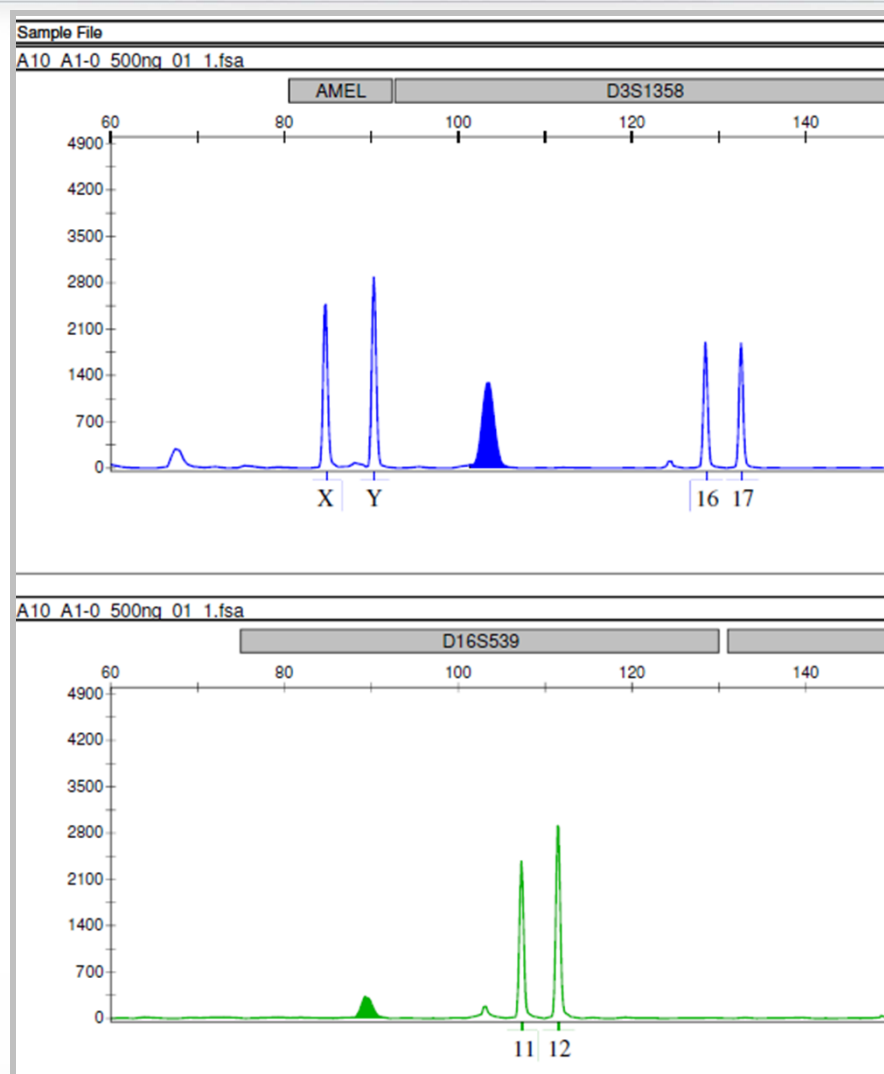
1. POP-7™ and POP-6™ polymer results in artifacts in some kits and configurations (in 16HS there is a known artifact that runs before the bins in the D3 locus)
2. Modified panels and binsets are needed with 18D and PP21 with POP-7™
3. Promega modified panels and binsets have an “NF” nomenclature for non-forensic.

Contact genetic@promega.com.

Known Artifact with PowerPlex® 18D in D3S1358 3500, 50cm POP-7™



Known Artifacts with PowerPlex® 21 in D3S1358 and D16D539 - 3500, 50cm POP-7 and 50cm POP-6



Data Analysis

GeneMapper® 4.1



- GeneMapper® ID or GeneMapper® ID-X was developed for STR kit data analysis
- GeneMapper® 4.x was developed for sizing and genotyping for custom fragment applications and other fragment analyses
 - can perform bin offsetting for genotyping
- Will need panel and binset files, analysis method file and table and plot settings files
- Obtain analysis files and instructions from genetic@promega.com

Data Analysis ***SoftGenetics Software***



SoftGenetics can provide help with software choices based on desired application (www.softgenetics.com)



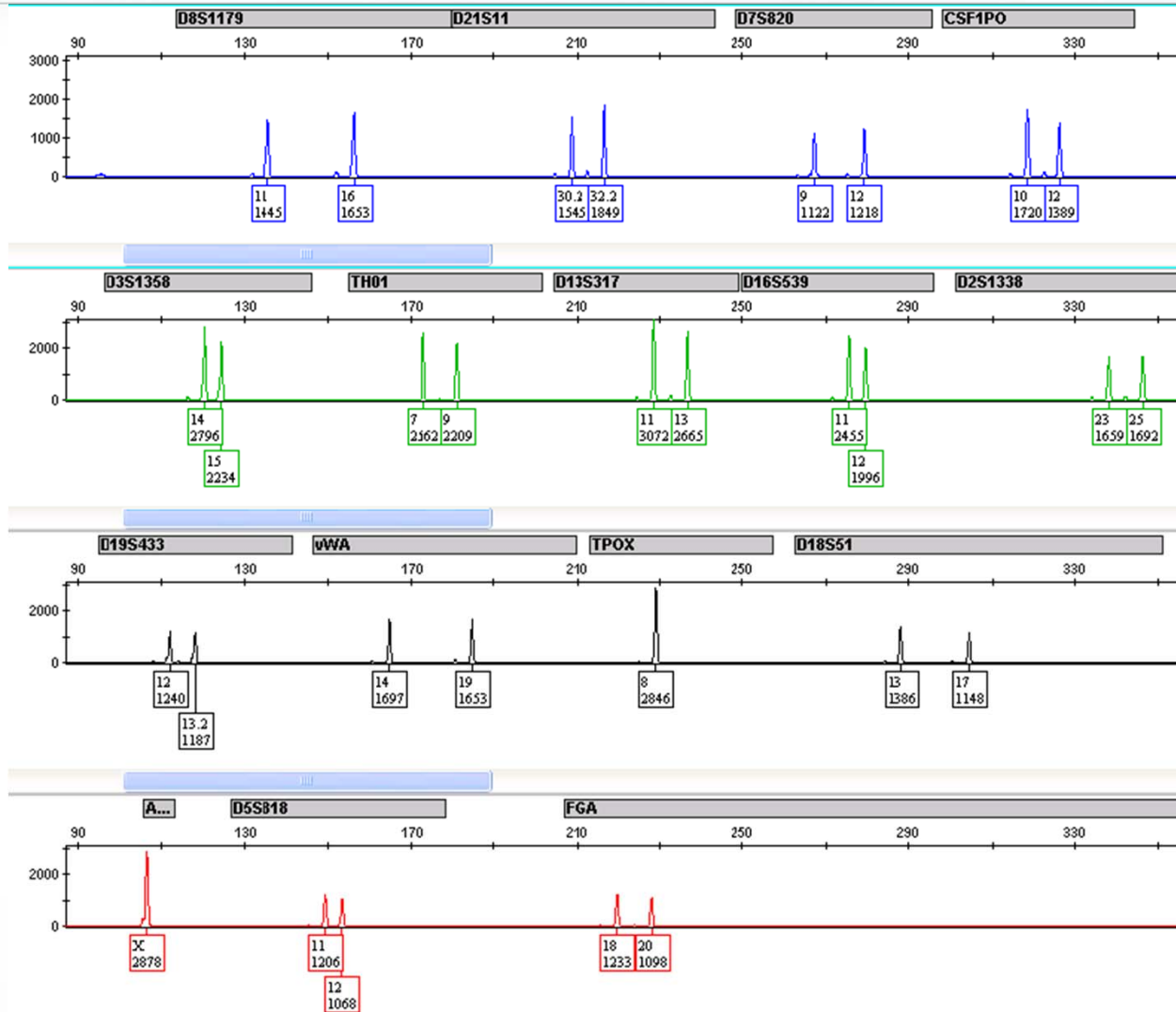
Comparison of 16 HS and Identifiler Plus® Data



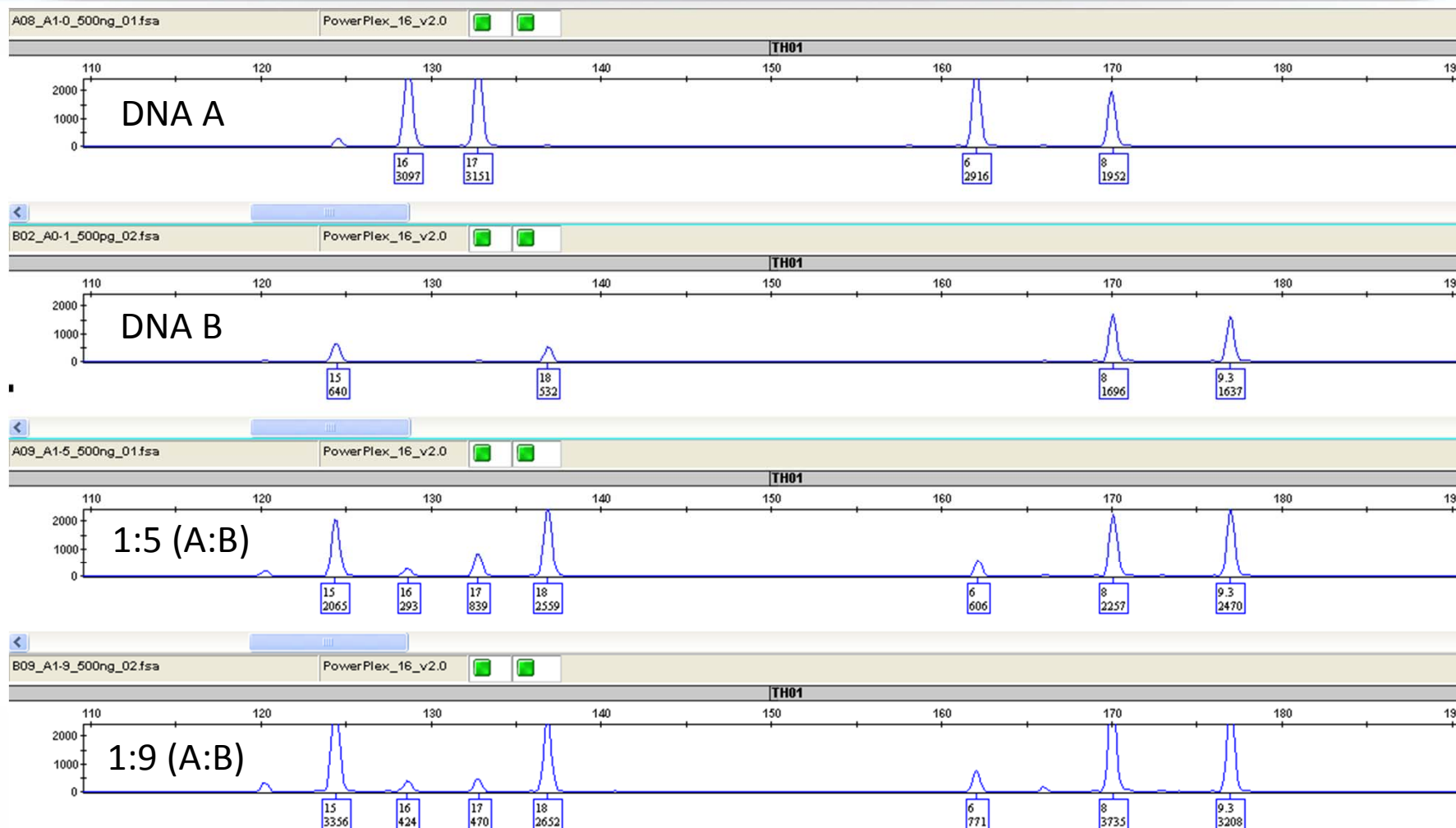
3130xL PowerPlex® 16HS 36 POP-7™



3130xL Identifiler Plus® 36 POP-7™



3500 PowerPlex® 16HS Mixtures



Mixture Analysis with 16 HS & Identifiler Plus®

Preliminary Comparison



		Promega 16 HS		Identifiler Plus®	
Input DNA Mixture					
A:B	Actual % A DNA	Estimated % A DNA	# Loci Used	Estimated % A DNA	# Loci Used
1:19	5%	3.58%	7	1.85%	8
1:9	10%	9.48%	8	7.96%	11
1:5	16.67%	17.11%	8	13.06%	9
1:2	33.33%	26.88%	10	failed injection	
1:1	50%	48.60%	11	35.30%	9
2:1	66.67%	61.32%	12	60.50%	9
5:1	83.33%	78.86%	7	81.12%	8
9:1	90%	90.05%	6	87.64%	4
19:1	95%	94.16%	3	95.62%	11

Summary of PowerPlex® 16 HS and Identifiler Plus® Comparison



- ✓ There are protocol differences between kits
 - ✓ E.g. Recommended input DNA amount and number of cycles
- ✓ Overall, there were no significant differences between the kits with respect to balance and ability to generate full profiles with recommended amounts of input DNA

Grand Summary



- It is possible to use the Promega PowerPlex® STR kits for non-forensic applications using configurations that were not validated specifically for forensic and paternity laboratories
- These conditions were not validated by Promega R&D scientists. Promega is providing this information as guidelines to help those wishing to use STR kits under alternate configurations
- Optimization will likely be required
- In some cases STR kits run with POP-7™ and POP-6™ may contain artifacts in the read region of the data.

Get Help

Promega Technical Service is Ready To Help



By phone: 800-356-9526

- Available 7am-6pm Central M-F

By email: genetic@promega.com

- Guaranteed response within 24hr
- Most responses within 2hrs



North American Branch Technical Support Team

Thank you.

Questions?

