

# User Bulletin

## ABI PRISM<sup>®</sup> 3100/3100-*Avant* Genetic Analyzers Using Data Collection Software v2.0

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December 2003

### **SUBJECT:   Protocols for Processing AmpF<sub>STR</sub> PCR Amplification Kit PCR Products**

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# Overview

This document describes:

- Protocols for processing AmpF $\ell$ STR<sup>®</sup> PCR Amplification Kit PCR products on the ABI PRISM<sup>®</sup> 3100/3100-*Avant* Genetic Analyzers
- Design changes and new features of ABI PRISM<sup>®</sup> 3100/3100-*Avant* Genetic Analyzer Data Collection Software v2.0
- Procedures and important information for running GeneMapper<sup>™</sup> ID Software v3.1 using Data Collection Software v2.0
- Verification testing for Data Collection Software v2.0
- Previous validation of the 3100/3100-*Avant* Genetic Analyzers

## Applicable AmpF $\ell$ STR Kits

PCR products generated from any of the AmpF $\ell$ STR<sup>®</sup> PCR Amplification Kits may be used with the 3100 and 3100-*Avant* protocols described in this user bulletin.

### Examples in This User Bulletin

Examples of results obtained using the AmpF $\ell$ STR PCR Amplification Kits listed below are provided in this user bulletin.

Kit	Dyes	Matrix Standard Set
AmpF $\ell$ STR <sup>®</sup> Identifiler <sup>®</sup> PCR Amplification Kit	<ul style="list-style-type: none"><li>• 6-FAM<sup>™</sup></li><li>• VIC<sup>®</sup></li><li>• NED<sup>™</sup></li><li>• PET<sup>™</sup></li><li>• LIZ<sup>®</sup></li></ul>	DS-33
AmpF $\ell$ STR <sup>®</sup> Profiler Plus <sup>®</sup> PCR Amplification Kit	<ul style="list-style-type: none"><li>• 5-FAM<sup>™</sup></li><li>• JOE<sup>™</sup></li><li>• NED<sup>™</sup></li><li>• ROX<sup>™</sup></li></ul>	DS-32
AmpF $\ell$ STR <sup>®</sup> SGM Plus <sup>®</sup> PCR Amplification Kit		

The examples are shown in:

- Appendix A: “Previous Validation of the 3100 Genetic Analyzer” on [page 52](#)
- Appendix B: “Previous Validation of the 3100-*Avant* Genetic Analyzer” on [page 58](#)

**Recommendations**

- When running the AmpF $\ell$ STR PCR Amplification Kits on the 3100 and 3100-*Avant* Genetic Analyzers, Applied Biosystems recommends and supports the use of:
  - 3100 POP-4<sup>TM</sup> Polymer
  - 36-cm capillary array
  - Hi-Di<sup>TM</sup> Formamide
- To successfully run the AmpF $\ell$ STR PCR Amplification Kits on the 3100 and 3100-*Avant* Genetic Analyzers, perform all of the procedures listed/referenced in the flowchart on [page 11](#).

## About the Software

**Data Collection Software v2.0**

Selected features of Data Collection Software v2.0 that pertain to the workflow, troubleshooting, and data analysis information included in this document are listed below.

Data Collection Software v2.0 includes:

- A new user interface
- New modules
  - Modules that support AmpF $\ell$ STR PCR Amplification Kits
  - An HID-specific module that includes recommended injection parameters
- G5 Variable Binning Module (G5vb)
  - Invoked through dye set selection
  - Spectral pattern has been re-mapped
- Calibration changes
  - Parameter files have been eliminated; settings are now edited through the Protocol Manager
  - Log files include more detailed descriptions of failure modes

**Note:** For a detailed description of Data Collection Software v2.0, refer to the *ABI PRISM<sup>®</sup> 3100/3100-*Avant* Genetic Analyzer User Guide* (PN 4347102).

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## GeneMapper ID Software v3.1

Selected features of GeneMapper ID Software v3.1 that pertain to the workflow and data handling information included in this document are listed below.

GeneMapper ID Software v3.1 includes:

- Predefined panels and bin sets for each AmpF $\mathbb{L}$ STR kit
- A Central Project window, which offers an interface between the sample, genotype, and raw data views
- Plot settings that you can customize, which aids data evaluation
- Simultaneous analysis of multiple samples from different AmpF $\mathbb{L}$ STR kits, which improves workflow
- Automated concordance checks of controls and overlapping loci from different AmpF $\mathbb{L}$ STR kits, which eliminates manual processing

**Note:** For a detailed description of GeneMapper ID Software v3.1, refer to the *GeneMapper™ ID Software v3.1 User Guide* (PN 4338775).

## Data Collection Software v2.0 and Fragment Analysis

When GeneMapper ID Software v3.1 is installed on a computer that has Data Collection Software v2.0, two applications are available through the Results Group Editor:

- GeneMapper-*<Computer Name>* (where *<Computer Name>* is the name of your computer)  
When you use the 3100/3100-*Avant* Genetic Analyzers with Data Collection Software v2.0 and the AmpF $\mathbb{L}$ STR kits, Applied Biosystems recommends that you use GeneMapper-*<Computer Name>*.
- GeneMapper-Generic

### GeneMapper-*<Computer Name>*

The GeneMapper-*<Computer Name>* application enables autoanalysis. The Size Standard, Analysis Method, and Panel columns in the Sample Sheet window read directly from the GeneMapper ID Software v3.1 database.

**IMPORTANT!** You must create entries in these columns in GeneMapper ID Software v3.1 prior to setting up the plate record for a run. Once you are inside the Plate Editor dialog box in Data Collection v2.0, you cannot create new entries in these columns. If you create a new size standard, analysis method, or panel in GeneMapper ID Software v3.1 while the Plate Editor dialog box is

open, the columns will not be updated. The plate record must be closed and reopened to update the entries. For information about setting up size standards, analysis methods, and panels in the GeneMapper *ID* Software v3.1 database, refer to the *GeneMapper™ ID Software v3.1: Human Identification Analysis Tutorial* (PN 4335523).

### GeneMapper-Generic

The GeneMapper-Generic application allows you to generate .fsa files, but does not enable autoanalysis. When completing the Sample Sheet, you need to fill in basic information for Data Collection Software v2.0 to complete the run; all other fields related to GeneMapper *ID* Software v3.1 are text entries.

The GeneMapper-Generic application is useful if you are:

- Using other software applications for analysis.
- Analyzing your samples in GeneMapper *ID* Software v3.1 on another computer, but do not have the same entries in the GeneMapper *ID* Software v3.1 database stored on the Data Collection v2.0 computer.

For example, if you have a customized size standard definition on the analysis computer, you can type that size standard name in the Size Standard text field to populate that column in your GeneMapper *ID* Software v3.1 project.

### Performing Autoanalysis

You may choose to perform autoanalysis of fragment analysis samples by using features of Data Collection Software v2.0 and GeneMapper *ID* Software v3.1.

You can only perform autoanalysis on the same instrument that collected the sample files. To analyze samples on another computer, you must transfer the files to that location.

If you perform autoanalysis on samples, but wish to edit/review results on another computer, you need to:

- Transfer the GeneMapper *ID* Software v3.1 project, analysis methods, size standards, panel, and bin set information to the other GeneMapper *ID* Software v3.1 database. All components need to be exported and imported individually.

**Note:** For import/export procedures, refer to the *GeneMapper™ ID Software v3.1: Human Identification Analysis Tutorial* (PN 4335523).

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- When completing the plate record, enter the Instrument Protocol information for Data Collection Software v2.0 to complete the run.
  - When creating a new Results Group for a set of samples to be autoanalyzed, check the Do Autoanalysis check box (and for remote analysis, define a default location for sample file storage).

## **Performing Manual Analysis**

For information on manual analysis, refer to the *GeneMapper ID Software v3.1 User Guide* (PN 4338775).

# Materials Required

The following tables list the materials required to run AmpF $\ell$ STR PCR Amplification Kit products on the ABI PRISM 3100 and 3100-*Avant* Genetic Analyzers.

## Dedicated Equipment and Supplies

Follow the guidelines for dedicated equipment and supplies to ensure that exogenous DNA and PCR products are confined to a designated area:

- Designate an amplified DNA work area for amplified DNA and for dedicated equipment and supplies used to handle amplified DNA.
- Applied Biosystems recommends that you do not remove amplified DNA, equipment, or supplies from the amplified DNA work area.
- Be careful not to let samples that are not amplified come into contact with supplies and equipment in the amplified DNA work area.

## Accessories

Accessories	Supplier	Part Number
3100 Capillary Array, 36 cm	Applied Biosystems	4315931
MicroAmp <sup>®</sup> Optical 96-Well Reaction Plate	Applied Biosystems	N801-0560
96-well plate septa	Applied Biosystems	4315933
Reservoir septa	Applied Biosystems	4315932
Array-fill syringe, 250- $\mu$ L glass syringe	Applied Biosystems	4304470
Polymer-reserve syringe, 5.0-mL glass syringe	Applied Biosystems	628-3731

## Chemicals

Chemicals	Supplier	Part Number
3100 POP-4™ Polymer	Applied Biosystems	4316355
Matrix Standard Set DS-32 for 3100 and 3100- <i>Avant</i> (containing the dyes 5-FAM™, JOE™, NED™, and ROX™)	Applied Biosystems	4323018
Matrix Standard Set DS-33 for 3100 and 3100- <i>Avant</i> (containing the dyes 6-FAM™, VIC®, NED™, PET™, and LIZ®)	Applied Biosystems	4323016
Hi-Di™ Formamide	Applied Biosystems	4311320
GeneScan™-500 ROX™ Size Standard	Applied Biosystems	401734
GeneScan™-500 LIZ® Size Standard	Applied Biosystems	4322682
10X Genetic Analyzer Buffer with EDTA	Applied Biosystems	402824
AmpFℓSTR® PCR Amplification Kit, one of the following:		
AmpFℓSTR® COfiler® PCR Amplification Kit	Applied Biosystems	4305246
AmpFℓSTR® Identifiler® PCR Amplification Kit	Applied Biosystems	4322288
AmpFℓSTR® Profiler® PCR Amplification Kit	Applied Biosystems	403038
AmpFℓSTR® Profiler Plus® PCR Amplification Kit	Applied Biosystems	4303326
AmpFℓSTR® Profiler Plus® <i>ID</i> PCR Amplification Kit	Applied Biosystems	4330284
AmpFℓSTR® SEfiler™ PCR Amplification Kit	Applied Biosystems	4335129
AmpFℓSTR® SGM Plus® PCR Amplification Kit	Applied Biosystems	4307133



**Software** GeneMapper *ID* Software v3.1 is required to analyze and genotype AmpF $\Lambda$ STR PCR Amplification Kit products on the 3100 and 3100-*Avant* Genetic Analyzers running Data Collection Software v2.0 or later.

**User Documentation** When processing AmpF $\Lambda$ STR PCR Amplification Kit products on the 3100 and 3100-*Avant* Genetic Analyzers, it may be helpful to refer to the Applied Biosystems instrument, software, and kit documentation listed below.

Document	Part Number
<i>ABI PRISM<sup>®</sup> 3100/3100-Avant Genetic Analyzer User Guide</i>	4347102
<i>GeneMapper<sup>™</sup> ID Software v3.1: Human Identification Analysis Tutorial</i>	4335523
<i>GeneMapper<sup>™</sup> ID Software v3.1 User Guide</i>	4338775
<i>AmpF<math>\Lambda</math>STR<sup>®</sup> COfiler<sup>®</sup> PCR Amplification Kit User Bulletin</i>	4305469
<i>AmpF<math>\Lambda</math>STR<sup>®</sup> Identifiler<sup>®</sup> PCR Amplification Kit User's Manual</i>	4323291
<i>AmpF<math>\Lambda</math>STR<sup>®</sup> Profiler<sup>®</sup> PCR Amplification Kit User's Manual</i>	402945
<i>AmpF<math>\Lambda</math>STR<sup>®</sup> Profiler Plus<sup>®</sup> PCR Amplification Kit User's Manual</i>	4303501
<i>AmpF<math>\Lambda</math>STR<sup>®</sup> Profiler Plus<sup>®</sup> ID PCR Amplification Kit User Bulletin</i>	4330429
<i>AmpF<math>\Lambda</math>STR<sup>®</sup> SEfiler<sup>™</sup> PCR Amplification Kit User's Manual</i>	4337410
<i>AmpF<math>\Lambda</math>STR<sup>®</sup> SGM Plus<sup>®</sup> PCR Amplification Kit User's Manual</i>	4309589

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## For More Information

The procedures in this user bulletin provide a broad overview of the steps required to perform a fragment analysis run and to perform data analysis. For detailed reference information, refer to the following documents.

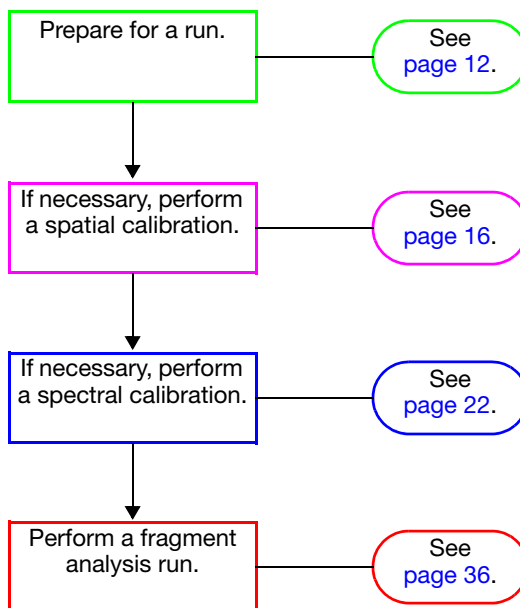
- Shadravan, F., 2001, Sizing Precision and Reproducibility Studies of AmpF $\mathcal{L}$ STR<sup>®</sup> Kits with ABI PRISM<sup>®</sup> 3100 Genetic Analyzer. *Proc. Am. Acad. Forensic Sci.* 7:26.
- Shadravan, F., Roby, R.K., Reeder, D.J., 2002, Characterization of AmpF $\mathcal{L}$ STR<sup>®</sup> Identifiler<sup>®</sup> PCR Amplification Kit for use with ABI PRISM<sup>®</sup> 3100 Genetic Analyzer. *Proc. Am. Acad. Forensic Sci.* 8:27.
- Daoudi, Y., Boland, C., Joe, L., Leibert, C., Wheaton, A., Roby, R.K., 2002. Validation of AmpF $\mathcal{L}$ STR<sup>®</sup> PCR Amplification Kits for Use with the ABI PRISM<sup>®</sup> 3100-*Avant* Genetic Analyzer. *Proceedings from the 13th International Symposium on Human Identification*, in press.

## Safety

Complete Safety information for the 3100/3100-*Avant* Genetic Analyzers may be found in the *ABI PRISM<sup>®</sup> 3100/3100-*Avant* Genetic Analyzer User Guide* (PN 4347102).

# Procedure Flowchart

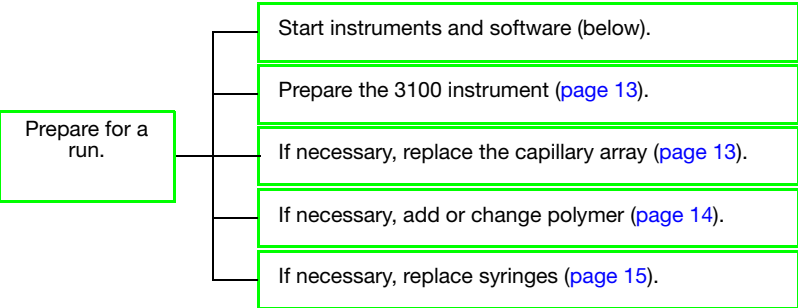
The flowchart below provides an overview of the procedures required to run the AmpF $\Phi$ STR PCR Amplification Kit products on the 3100 and 3100-*Avant* Genetic Analyzers. See the referenced section for detailed procedures.



# Prepare for the Run

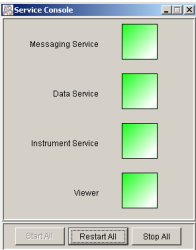
This section contains brief procedures to prepare for a run. For detailed information, refer to the *ABI PRISM® 3100/3100-Avant Genetic Analyzer User Guide* (PN 4347102).

## Procedure Flowchart





## Start the Instruments and Software

To start the instruments and software:

1.	Turn on the computer. The OrbixWeb™ Daemon software automatically launches.
2.	Turn on the 3100 or 3100- <i>Avant</i> Genetic Analyzer. Wait for the green light to appear.
3.	<div>Start Data Collection Software v2.0: Select <b>Start &gt; Programs &gt; Applied Biosystems &gt; Data Collection &gt; Run Data Collection 3100 v2.0</b> or <b>Run Data Collection 3100-<i>Avant</i> v2.0</b>. The Service Console displays; when all the applications are running, the Data Collection Viewer window displays.</div> <div><div>Service Console: When all squares are green, all the applications are running. This could take several minutes.</div></div>
4.	View and set preferences.

## Prepare the 3100 Instrument

To prepare the 3100/3100-*Avant* instrument:

1.	<p>Preset autosampler and place fresh deionized water and 1X running buffer in positions 1 to 4.</p> <p> <b>CAUTION</b> <b>CHEMICAL HAZARD.</b> 10X Genetic Analyzer Buffer with EDTA may cause eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.</p>
2.	<p>Place lower polymer block and anode buffer jar on the instrument.</p>
3.	<p>Clean the capillary array detection window with methanol, if necessary.</p> <p> <b>WARNING</b> <b>CHEMICAL HAZARD.</b> Methanol is a flammable liquid and vapor. Exposure causes eye and skin irritation, and may cause central nervous system depression and nerve damage. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.</p>

## Replace the Capillary Array

### When to Replace a Capillary Array

A capillary array should last approximately 100 runs. The following indications may suggest that a new capillary array is required:

- Poor sizing precision or allele calling
- Poor resolution and/or decreased signal intensity

**IMPORTANT!** A run will not start without the correct spectral calibration for a specific dye set and capillary array length. Refer to the *ABI PRISM® 3100/3100-Avant Genetic Analyzer User Guide* (PN 4347102) for procedures on setting the active spectral calibration.

### Replace the Capillary Array

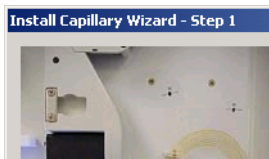
To replace a capillary array:

1.	Use a 36-cm capillary array for the AmpF $\mathcal{L}$ STR kits.
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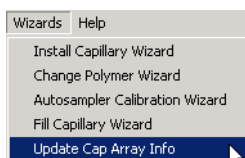
To replace a capillary array: *(continued)*

2. Install the capillary array:
  - Using the Install Capillary Array wizard



OR

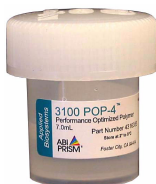
- Using the Update Capillary Array Info utility



## Add or Change Polymer

### To add or change polymer:

1. Use 3100 POP-4™ Polymer (PN 4316355) for the AmpFSTR kits.



**WARNING CHEMICAL HAZARD. POP-4 Polymer** causes eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

**To add or change polymer: (continued)**

2. Use the table below to determine whether to add or change the polymer before proceeding with instrument preparation.
- IMPORTANT!** Always replace polymer on the instrument that is older than 1 week.

If polymer on the instrument is...	Then ...
less than 1 week old, and sufficient in quantity to complete your runs <sup>a</sup>	ensure that there are no air bubbles, and then proceed with instrument preparation.
less than 1 week old, and insufficient in quantity to complete your runs	fill the syringes and the upper polymer block with polymer by following the Change Polymer wizard.
more than 1 week old	1. Remove and clean the polymer blocks and syringes. 2. Fill the syringes and the upper polymer block with polymer by following the Change Polymer wizard.
wrong type (changing between POP-6™ and POP-4™ polymers)	

a. A 3100 run (16 capillaries) uses 50 to 80  $\mu\text{L}$  of polymer and a 3100-*Avant* run (4 capillaries) uses approximately 20  $\mu\text{L}$  of polymer.

## Replace the Syringes

To maintain optimal performance, Applied Biosystems recommends that syringes be replaced approximately every 3 months.

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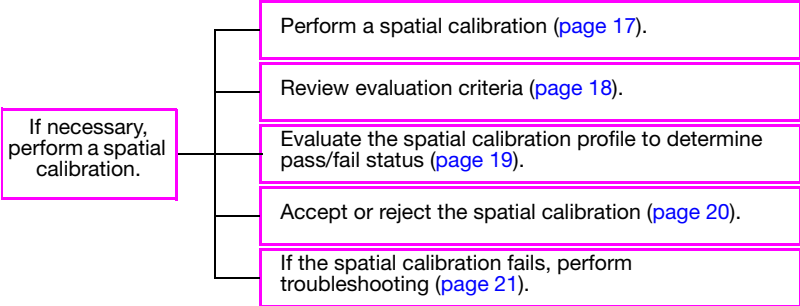
# Perform a Spatial Calibration

## When to Perform a Spatial Calibration

Perform a spatial calibration after:

- Installing or replacing a capillary array
- Temporarily removing the capillary array from the detection block
- Moving the instrument

## Procedure Flowchart





## Perform a Spatial Calibration

To perform a spatial calibration:

1. In the Tree pane of Data Collection Software v2.0, click **GA Instruments > ga3100 or ga3100-Avant > instrument name > Spatial Run Scheduler**.

Spatial profile

Positions (pixel) of each capillary

Capillary	Position (pixels)	Left spacing	Right spacing
1	9	15	15
2	24	15	15
3	39	15	15
4	55	16	15
5	70	15	15
6	85	15	15
7	100	15	16
8	115	16	15
9	131	15	15
10	145	15	15
11	161	15	16
12	177	16	15

Start and Accept or Reject Spatial profile here

2. In the Spatial Protocols section:
  - a. Select one of the following:
    - If the capillaries contain fresh polymer, select **Protocol > SpatialNoFill\_1**
    - Otherwise, select **Protocol > SpatialFill\_1**

Select one

- b. Click **Accept**.

**Note:** You do not need to fill the capillaries each time you perform a spatial calibration.

### To perform a spatial calibration: *(continued)*

3. Click .

The calibration run lasts approximately:

- 2 min without filling the capillaries
- 6 min with filling the capillaries

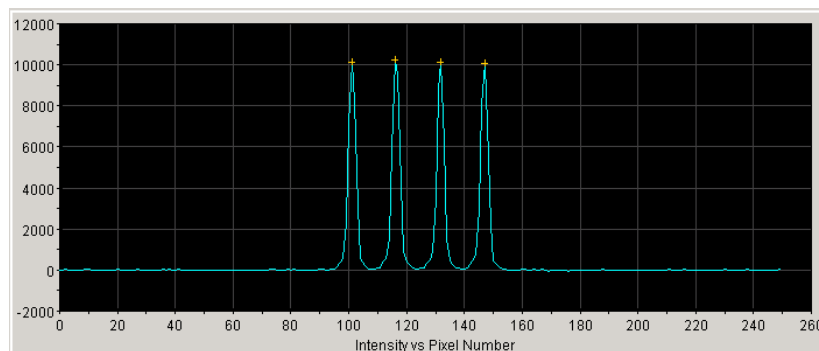
**Note:** The spatial profile window turns black when you start a spatial calibration.

### Review Evaluation Criteria

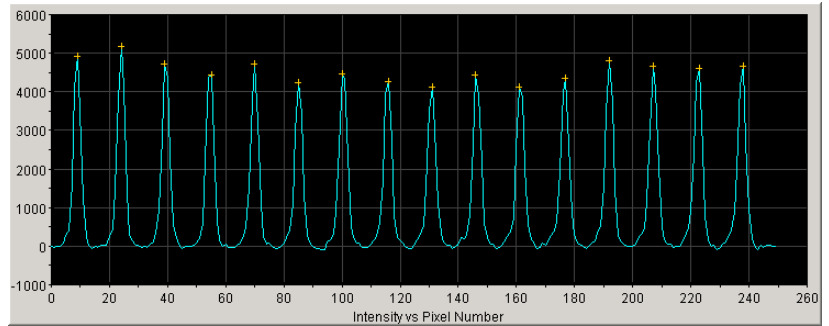
While viewing the calibration profile, use the following criteria to evaluate the data:

Peak Attribute	Criteria
Height	Similar heights for all peaks.
Shape	Single sharp peak for each capillary. Small shoulders are acceptable.
Spacing	Position values are 13–16 higher than the previous one for every capillary. Theoretical spacing between capillaries is 15.
Orange crosses	One orange cross marking the top of every peak. No misplaced crosses.

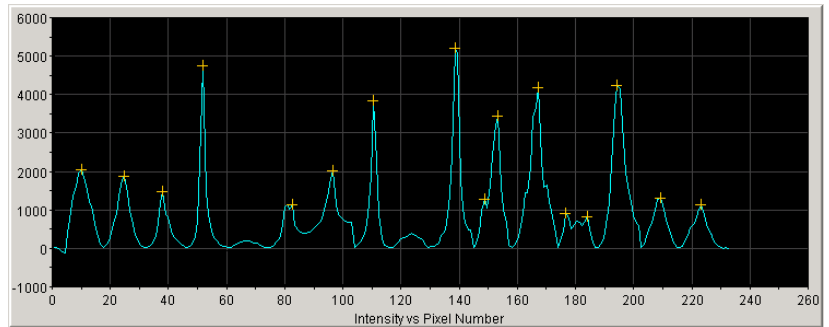
### Passing profile from a 3100-*Avant* system



### Passing profile from a 3100 system



### Failing profile from a 3100 system



## Evaluate the Spatial Calibration Profile

To evaluate the spatial calibration profile:

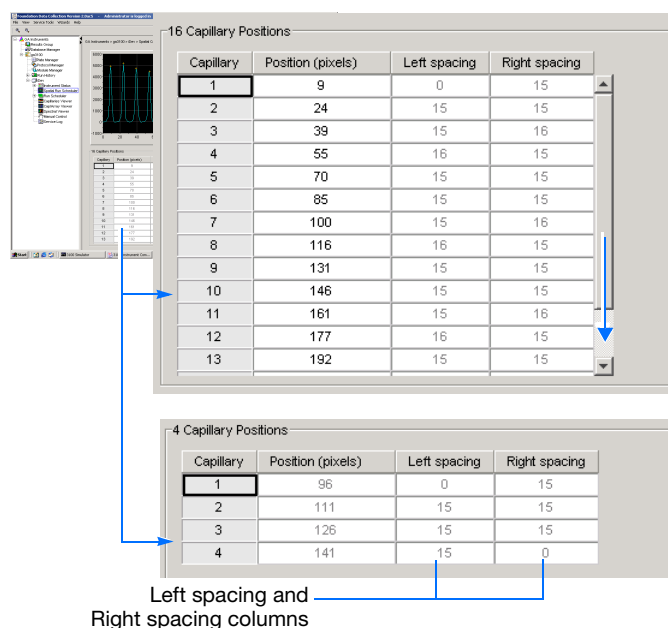
1. View the spatial calibration profile.
2. Examine each row in the Capillary Positions table.  
**Note:** There are 16 capillary positions for the 3100 instrument and 4 capillary positions for the 3100-*Avant* instrument.

### To evaluate the spatial calibration profile: *(continued)*

3. Verify that the values in the Left spacing and Right spacing columns are 13 to 16 pixels.

To move an orange cross:

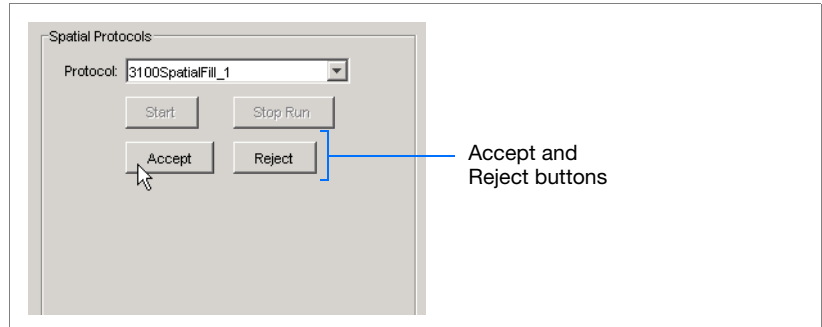
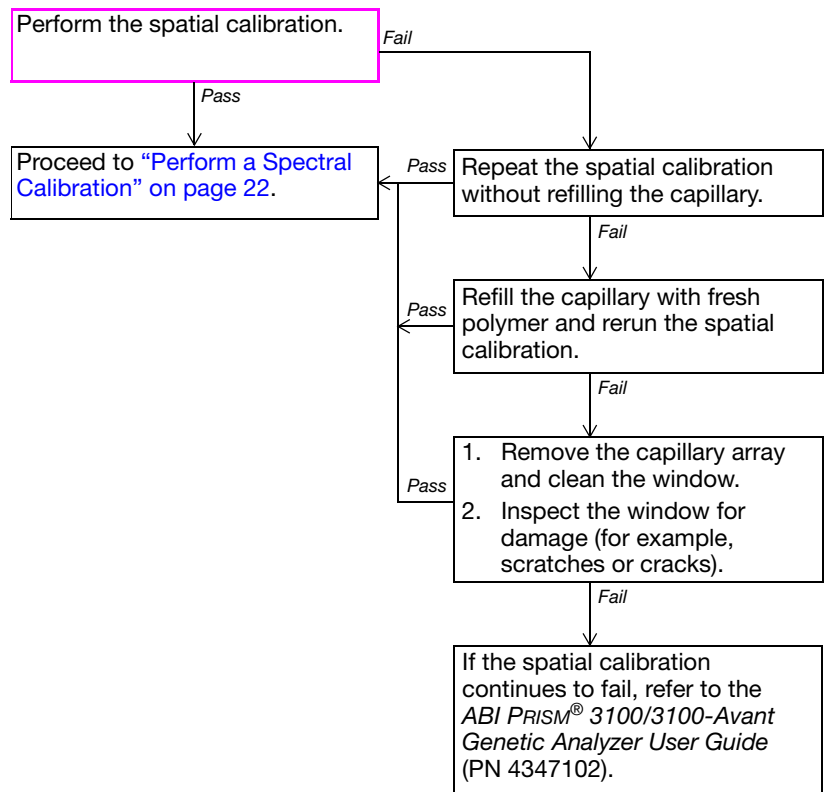
- a. Type a new value in the Positions (pixels) box for the capillary of interest.
- b. Click outside of that box or press **Enter**.



### Accept or Reject the Spatial Calibration

To accept or reject the spatial calibration:

1. If the calibration passed, click **Accept** to write the calibration data to the database and .ini file.
2. If the calibration failed, click **Reject**, refer to the [“Troubleshooting Flowchart”](#) below.

To accept or reject the spatial calibration: *(continued)*Troubleshooting  
Flowchart

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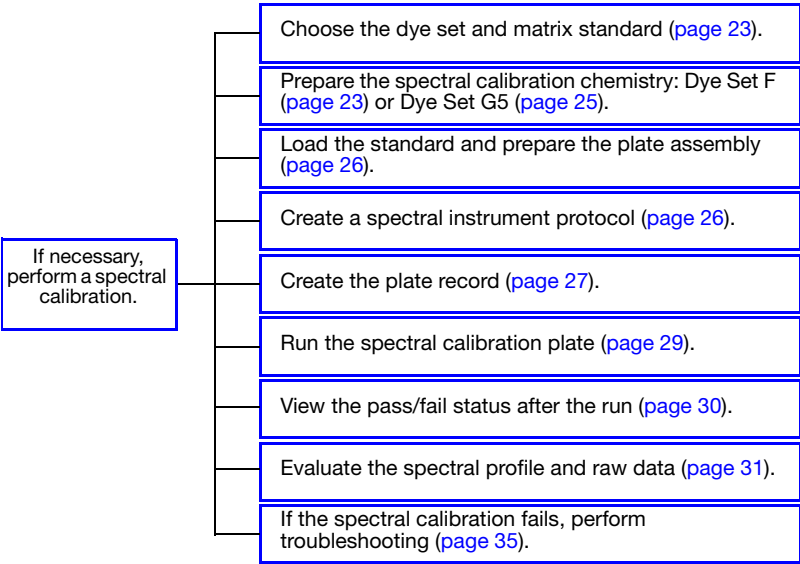
# Perform a Spectral Calibration

## When to Perform a Spectral Calibration

Perform a spectral calibration:

- When you use a new dye set on the instrument
- After a service engineer has realigned the laser or CCD camera
- If you begin to see an increase in spectral separation (“pull-up” and/or “pull-down” peaks)

## Procedure Flowchart



## Choose the Dye Set and Matrix Standard

Choose the appropriate dye set and matrix standard for the AmpF $\ell$ STR PCR Amplification Kit you are using as shown in the table below.

For AmpF $\ell$ STR PCR Amplification Kits that use a...	Use...	And use...	AmpF $\ell$ STR PCR Amplification Kit Examples
four-dye system, including the following dyes: <ul style="list-style-type: none"> <li>• 5-FAM<sup>™</sup></li> <li>• JOE<sup>™</sup></li> <li>• NED<sup>™</sup></li> <li>• ROX<sup>™</sup></li> </ul>	Dye Set F	Matrix Standard Set DS-32	<ul style="list-style-type: none"> <li>• AmpF<math>\ell</math>STR<sup>®</sup> COfiler<sup>®</sup> PCR Amplification Kit</li> <li>• AmpF<math>\ell</math>STR<sup>®</sup> Profiler<sup>®</sup> PCR Amplification Kit</li> <li>• AmpF<math>\ell</math>STR<sup>®</sup> Profiler Plus<sup>®</sup> PCR Amplification Kit</li> <li>• AmpF<math>\ell</math>STR<sup>®</sup> Profiler Plus<sup>®</sup> ID PCR Amplification Kit</li> <li>• AmpF<math>\ell</math>STR<sup>®</sup> SGM Plus<sup>®</sup> PCR Amplification Kit</li> </ul>
five-dye system, including the following dyes: <ul style="list-style-type: none"> <li>• 6-FAM<sup>™</sup></li> <li>• VIC<sup>®</sup></li> <li>• NED<sup>™</sup></li> <li>• PET<sup>™</sup></li> <li>• LIZ<sup>®</sup></li> </ul>	Dye Set G5	Matrix Standard Set DS-33	<ul style="list-style-type: none"> <li>• AmpF<math>\ell</math>STR<sup>®</sup> Identifier<sup>®</sup> PCR Amplification Kit</li> <li>• AmpF<math>\ell</math>STR<sup>®</sup> SEfiler<sup>™</sup> PCR Amplification Kit</li> </ul>

## Prepare the Matrix Standards for Dye Set F

Follow this procedure if you are setting up spectral (matrix) calibration standards for kits using a four-dye system, including the 5-FAM, JOE, NED, and ROX dyes.

### To set up the matrix standards for Dye Set F:

1.	Thoroughly vortex the four Matrix Standard Set DS-32 tubes for Dye Set F.
2.	Spin the tubes briefly in a microcentrifuge.

To set up the matrix standards for Dye Set F: *(continued)*

3. Prepare Matrix Standard Set DS-32 for Dye Set F by combining the following in a labeled 1.5-mL microcentrifuge tube:

Reagent	Volume (μL) 3100 System	Volume (μL) 3100-Avant System
5-FAM	2.5	1
JOE	2.5	1
NED	2.5	1
ROX	2.5	1
Hi-Di Formamide	190	76
<b>Final Volume</b>	<b>200</b>	<b>80</b>



**WARNING CHEMICAL HAZARD. Hi-Di**

**Formamide.** Exposure causes eye, skin, and respiratory tract irritation. It is a possible developmental and birth defect hazard. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

**Note:** For optimal performance of the new spectral calibration algorithm, Applied Biosystems recommends that you formulate the Matrix Standard Set DS-32 standards as indicated in this user bulletin.

**Note:** For the 3100 instrument, if necessary add additional matrix standards at increments of 2.5 μL to pass spectral calibration. This user bulletin recommends a higher concentration of the spectral standards than is suggested in the Matrix Standard Set DS-32 product insert.


4. Vortex thoroughly.
5. Spin the mixture briefly in a microcentrifuge.
6. Heat the tube at 95 °C for 3 min to denature the sample.
7. Immediately place the tube on ice for 3 min.



## Prepare the Matrix Standards for Dye Set G5

Follow this procedure if you are setting up spectral (matrix) calibration standards for kits using a five-dye system, including the 6-FAM, VIC, NED, PET, and LIZ dyes.

### To set up the matrix standards for Dye Set G5:

1.	Thoroughly vortex the Matrix Standard Set DS-33 tube for Dye Set G5.												
2.	Spin the tube briefly in a microcentrifuge.												
3.	Prepare Matrix Standard Set DS-33 for Dye Set G5 by combining the following in a labeled 1.5-mL microcentrifuge tube: <table><tr><th>Reagent</th><th>Volume (μL) 3100 System</th><th>Volume (μL) 3100-Avant System</th></tr><tr><td>Matrix Standard Set DS-33</td><td>5</td><td>2</td></tr><tr><td>Hi-Di Formamide</td><td>195</td><td>78</td></tr><tr><td><b>Final Volume</b></td><td><b>200</b></td><td><b>80</b></td></tr></table>	Reagent	Volume (μL) 3100 System	Volume (μL) 3100-Avant System	Matrix Standard Set DS-33	5	2	Hi-Di Formamide	195	78	<b>Final Volume</b>	<b>200</b>	<b>80</b>
Reagent	Volume (μL) 3100 System	Volume (μL) 3100-Avant System											
Matrix Standard Set DS-33	5	2											
Hi-Di Formamide	195	78											
<b>Final Volume</b>	<b>200</b>	<b>80</b>											
	<div><b>WARNING</b> <b>CHEMICAL HAZARD. Hi-Di Formamide.</b> Exposure causes eye, skin, and respiratory tract irritation. It is a possible developmental and birth defect hazard. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.</div>												
4.	Vortex thoroughly.												
5.	Spin the mixture briefly in a microcentrifuge.												
6.	Heat the tube at 95 °C for 3 min to denature the sample.												
7.	Immediately place the tube on ice for 3 min.												





## Load the Standard and Prepare the Plate Assembly

To load the standard and prepare the plate assembly:


1.	Place a 96-well reaction plate into the plate base provided with the instrument.
2.	Dispense 10 $\mu$ L of the denatured matrix standard into the 96-well reaction plate: <ul style="list-style-type: none"> <li>• Wells A1–H2 for the 3100 system</li> <li>• Wells A1–A4 for the 3100-<i>Avant</i> system</li> </ul>
3.	Align the septa strip on the reaction plate.
4.	Centrifuge the reaction plate so that each standard is collected at the bottom of its well.
5.	Snap the plate retainer onto the reaction plate and plate base.
6.	Verify that the holes of the plate retainer and the septa strip are aligned.
7.	Place the plate assembly on the autosampler.

## Create a Spectral Instrument Protocol

To create a spectral instrument protocol:





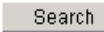

1.	In the Tree pane of Data Collection Software v2.0, click  <b>GA Instruments</b> >  <b>ga3100</b> or <b>ga3100-<i>Avant</i></b> >  <b>Protocol Manager</b> .
2.	In the Instrument Protocols pane, click  to open the Protocol Editor dialog box.

To create a spectral instrument protocol: *(continued)*

3.	<p>Complete the Protocol Editor dialog box:</p> <ol style="list-style-type: none"> <li>Enter a name for the protocol.</li> <li>Optional: Enter a description for the protocol.</li> <li>In the Type drop-down list, select <b>SPECTRAL</b>.</li> <li>In the Dye Set drop-down list, select <b>F</b> or <b>G5</b>.</li> <li>In the Polymer drop-down list, select <b>POP4</b>.</li> <li>In the Array Length drop-down list, select <b>36-cm</b>.</li> <li>In the Chemistry drop-down list, select <b>Matrix Standard</b>.</li> <li>In the Run Module drop-down list, select <b>Spect36_POP4_1</b>.</li> </ol>
4.	<p>Click .</p> <p>You have successfully created the spectral instrument protocol.</p>

## Create the Plate Record

## To create the plate record:

1.	<p>In the Tree pane of Data Collection Software v2.0, click  <b>GA Instruments</b> &gt;  <b>ga3100</b> or <b>3100-Avant</b> &gt;  <i>instrument name</i> &gt;  <b>Run Scheduler</b>.</p>
2.	<p>In the Run Scheduler view:</p> <ol style="list-style-type: none"> <li>In the Scan or Type Plate ID field enter a new plate name.</li> <li>Click .</li> </ol>
3.	<p>In the Create new plate dialog box, click .</p>


**To create the plate record: *(continued)***

4. Complete the New Plate dialog box:
  - a. Enter a name for the plate.
  - b. Optional: Enter a description for the plate record.
  - c. In the Application drop-down list, select **Spectral Calibration**.
  - d. In the Plate Type drop-down list, select **96-Well**.
  - e. Enter a name for the owner.
  - f. Enter a name for the operator.

5. Click **OK**.  
The Spectral Calibration Plate Editor opens.

The screenshot shows the 'Spectral Calibration Plate Editor' window. At the top, there are input fields for 'Plate Name' (containing 'jkh'), 'Operator' (containing 'jk'), 'Plate Sealing' (a dropdown menu showing 'Sealed'), and 'Owner' (containing 'jkh'). Below these fields is a table with the following columns: 'Well', 'Sample Name', 'Comment', 'Priority', and 'Instrument Protocol 1'. The table contains 96 rows, with the first row (A01) selected. At the bottom of the window, there is a 'Description' text area and two buttons: 'OK' and 'Cancel'.

**To create the plate record: (continued)**

6.	<p>In the Spectral Calibration Plate Editor dialog box, enter the following information:</p> <ul style="list-style-type: none"> <li>a. In the Sample Name column of row A, enter a sample name, then click the next cell. The value 100 is automatically displayed in the Priority column.</li> <li>b. In the Comments column of row A, enter any additional comments or notations for the sample at the corresponding position of the plate.</li> <li>c. In the Instrument Protocol 1 column of row A, select a protocol appropriate for the AmpF<math>\Lambda</math>STR kit you are using.</li> </ul>
7.	Highlight the entire row.
8.	<p>Select <b>Edit &gt; Fill Down Special</b>.</p> <p>Based on the plate type (96-well) and capillary array (16 or 4 capillaries) you are using, the software automatically fills in the appropriate well numbers for a single run.</p>
9.	<p>Click .</p> <p>You have successfully created the plate record for the spectral calibration plate.</p>

**Run the Spectral Calibration Plate**

Run the spectral calibration plate on the 3100/3100-*Avant* Genetic Analyzer.

For information on running the spectral calibration plate on the instrument, refer to the *ABI PRISM<sup>®</sup> 3100/3100-*Avant* Genetic Analyzer User Guide* (PN 4347102).

## View the Pass/Fail Status After the Run

After the instrument completes the spectral calibration run, the pass or fail status of each capillary is recorded in the Events Messages section of the Instrument Status window.

To view the pass/fail status:

1. In the Tree pane of Data Collection Software v2.0, click  **GA Instruments** >  **ga3100** or **ga3100-Avant** >  *instrument name* >  **Instrument Status** >  **Event Log**.

2. In the Events Messages section of the window, view the status of each capillary.

For a good-quality calibration, each capillary should have a:

- Q-value above 0.95
- Condition number within range of:

Dye Set	Condition Number Range
F	6 to 12
G5	7 to 12

Event Log		Cap #	Pass/Fail	Condition No.
				Q-value
Publisher	Description			
iDev	Finished saving spectral calibration data			
iDev	Saving spectral calibration data			
iDev	Capillary 16 successfully calibrated : q=0.988 c=9.12			
iDev	Capillary 15 successfully calibrated : q=0.986 c=9.15			
	Run completed			
iDev	Capillary 14 successfully calibrated : q=0.986 c=9.01			
iDev	Capillary 13 successfully calibrated : q=0.988 c=8.99			





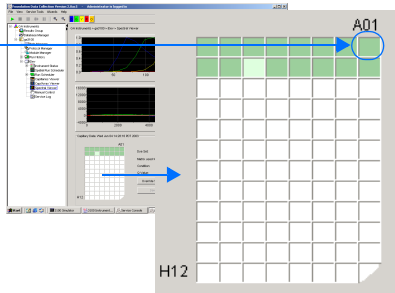
### Dye set G5 status results

3. If the entire spectral calibration failed, see the [“Troubleshooting Flowchart” on page 35](#).

## Evaluate the Spectral Profile and Raw Data

**IMPORTANT!** Review and evaluate the spectral calibration profile for each capillary, even if the Spectral Calibration Results box indicated that they all passed.

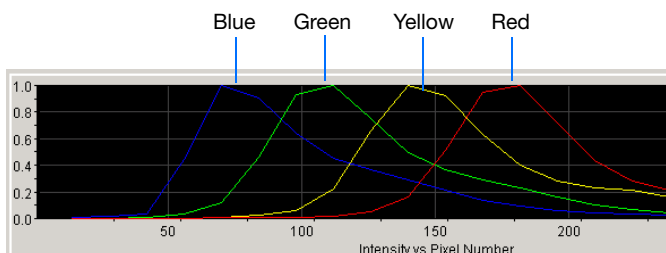
To evaluate the spectral profile and raw data:

1.	In the Tree pane of Data Collection Software v2.0, click  <b>GA Instruments</b> >  <b>ga3100</b> or <b>ga3100-Avant</b> >  <i>instrument name</i> >  <b>Spectral Viewer</b> .
2.	In the Dye Set drop-down list, select the dye set you just created.  <b>Note:</b> If the spectral calibration failed (no spectral profiles created), see the <a href="#">“Troubleshooting Flowchart”</a> on page 35.
3.	Select a well on the plate diagram to view the capillary spectral results.  <b>Note:</b> If a capillary fails, it is automatically assigned the spectral profile of its nearest passing capillary to the left. If there are no passing capillaries to the left, it is assigned the profile of the nearest passing capillary to the right.  <div style="display: flex; align-items: center;"> <div style="margin-right: 20px;"> <p>Well A01</p>  <p>Capillary status:</p> <div style="display: flex; align-items: center; margin-bottom: 5px;"> <div style="width: 20px; height: 15px; background-color: #006400; border: 1px solid black; margin-right: 5px;"></div> <span>Passed (dark green)</span> </div> <div style="display: flex; align-items: center; margin-bottom: 5px;"> <div style="width: 20px; height: 15px; background-color: #90EE90; border: 1px solid black; margin-right: 5px;"></div> <span>Selected (light green)</span> </div> <div style="display: flex; align-items: center;"> <div style="width: 20px; height: 15px; background-color: #D2B48C; border: 1px solid black; margin-right: 5px;"></div> <span>Failed (tan)</span> </div> </div> <div>  </div> </div>

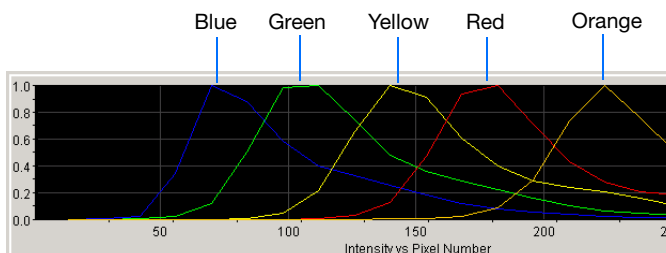
To evaluate the spectral profile and raw data: *(continued)*

4. For the selected capillary, verify that the order of the peaks in the spectral profile from left to right are:
  - Dye Set F: blue-green-yellow-red
  - Dye Set G5: blue-green-yellow-red-orange

If the peaks in the profile...	Then...
appear in the correct order	go to <a href="#">step 5</a> .
do not appear in the correct order	the spectral calibration run has failed. Go to the <a href="#">“Troubleshooting Flowchart”</a> on page 35.



**Example of a 4-dye spectral calibration profile**



**Example of a 5-dye spectral calibration profile**

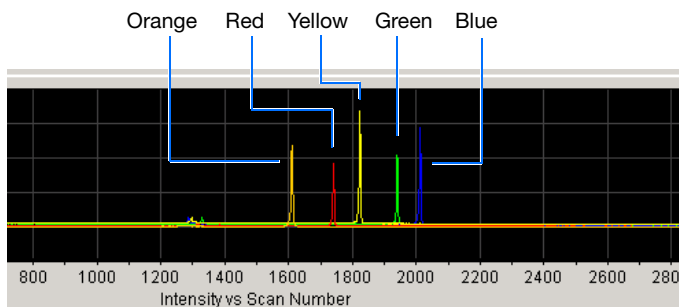


To evaluate the spectral profile and raw data: *(continued)*

5. Verify that the order of the peaks in the raw data profile from left to right are:

- Dye Set F: red-yellow-green-blue
- Dye Set G5: orange-red-yellow-green-blue

If...	Then...
the peaks are in the correct order and there are no extraneous peaks that adversely affect the spectral profile	go to <a href="#">step 6</a> .
the peaks are in the wrong order or there are any extraneous peaks that adversely affect the spectral profile	the spectral calibration run has failed. Go to the <a href="#">“Troubleshooting Flowchart”</a> on <a href="#">page 35</a> .



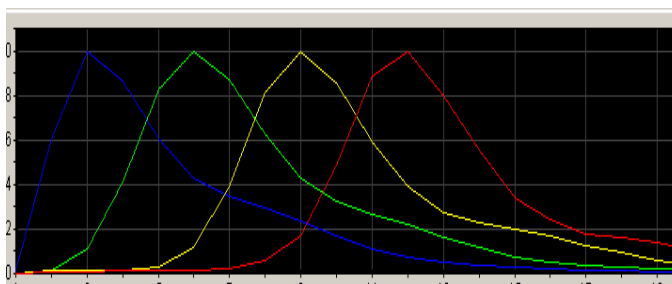
**Example of a 5-dye fragment analysis raw data profile**

To evaluate the spectral profile and raw data: *(continued)*

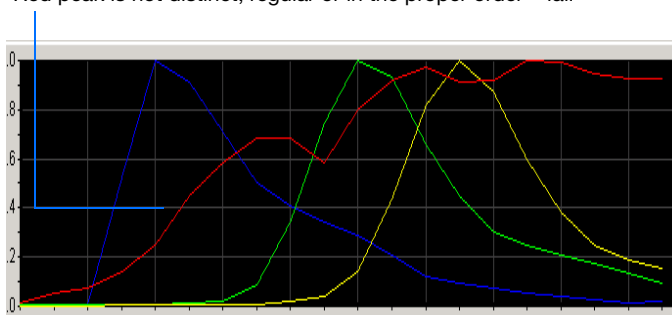
6. Verify that the peaks in the spectral profile do not contain gross overlaps, dips, or other irregularities.

If the peaks in the profile...	Then...
are separate and distinct	the capillary has passed. Go to <a href="#">step 7</a> .
are not separate and distinct	the spectral calibration run has failed. Go to the <a href="#">“Troubleshooting Flowchart”</a> on page 35.

Peaks are distinct, regular and in the proper order – pass



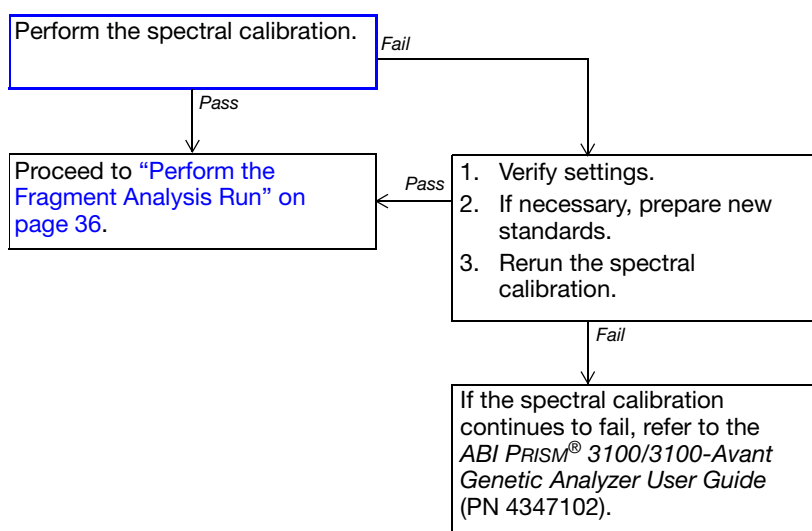
Red peak is not distinct, regular or in the proper order – fail



7. Repeat steps 3 through 6 for each capillary in the array.

To evaluate the spectral profile and raw data: *(continued)*

8. Rename the spectral run. The spectral file default name is the day, date and time of the run.
  - a. Click **Rename**.
  - b. In the Rename Calibration dialog box, enter a descriptive name for the spectral calibration including the dye set, array length and polymer type (optional).
  - c. Click **OK**.

Troubleshooting  
Flowchart

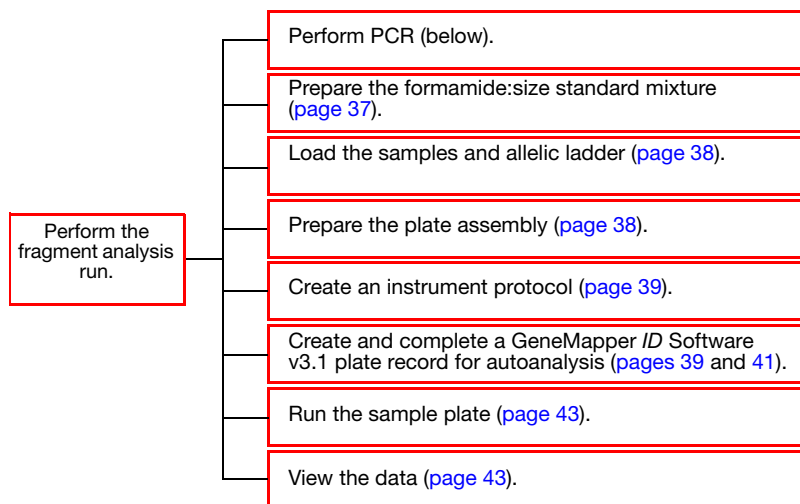
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# Perform the Fragment Analysis Run

**Run Definition** On the ABI PRISM 3100 Genetic Analyzer, a run corresponds to a defined set of 16 wells on a 96-well reaction plate.

On the ABI PRISM 3100-*Avant* Genetic Analyzer, a run corresponds to a defined set of 4 wells on a 96-well reaction plate.

## Procedure Flowchart



**Perform PCR** To prepare your DNA samples and perform PCR, follow the instructions in the appropriate AmpF $\mathcal{L}$ STR PCR Amplification Kit user manual. See "[User Documentation](#)" on [page 9](#) for names and part numbers.

## Prepare the Formamide:Size Standard Mixture

You can prepare the formamide:size standard mixture for each individual sample or prepare a bulk for all samples in the run.

To prepare the formamide:size standard mixture:

- For each sample, combine the following in a single microcentrifuge tube:

Reagent	Volume (µL)	
	Dye Set F	Dye Set G5
GeneScan™-500 ROX™ Size Standard	0.5	—
GeneScan™-500 LIZ® Size Standard	—	0.3
Hi-Di Formamide	8.5	8.7

Alternatively, for 16 samples, combine the following in a single microcentrifuge tube:

Reagent	Volume (µL)	
	Dye Set F	Dye Set G5
GeneScan-500 ROX Size Standard	10	—
GeneScan-500 LIZ Size Standard	—	6
Hi-Di Formamide	170	174

**Note:** Prepare the appropriate size standard formulation for your dye set.




**WARNING CHEMICAL HAZARD. Hi-Di Formamide.** Exposure causes eye, skin, and respiratory tract irritation. It is a possible developmental and birth defect hazard. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

- Vortex the tube to mix, then spin briefly in a microcentrifuge.

## Load the Samples and Allelic Ladder

To load the samples and allelic ladder:

1.	<p>In a 96-well reaction plate:</p> <ul style="list-style-type: none"> <li>• Dispense 9 <math>\mu</math>L of the formamide: size standard mixture into each well that will contain sample or allelic ladder.</li> <li>• Add 10 <math>\mu</math>L of the formamide to each blank well.</li> </ul> <div>  <b>WARNING</b> <b>CHEMICAL HAZARD. Formamide.</b> Exposure causes eye, skin, and respiratory tract irritation. It is a possible developmental and birth defect hazard. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.         </div>
2.	Load 1 $\mu$ L of the sample or allelic ladder into the wells.
3.	<p>Cover the reaction plate with an appropriate septa. Use:</p> <ul style="list-style-type: none"> <li>• Reservoir septa, or</li> <li>• 96-well plate septa if samples for more than one run were prepared</li> </ul>
4.	Briefly spin the reaction plate in a centrifuge to ensure that the contents of each well are mixed and collected at the bottom.
5.	To denature, heat the reaction plate in a thermal cycler at 95 °C for 3 min.
6.	Place the reaction plate immediately on ice for 3 min.

## Prepare the Plate Assembly






To prepare the plate assembly:

1.	Place the 96-well reaction plate into the plate base provided with the instrument.
2.	Align the septa strip on the reaction plate.
3.	Snap the plate retainer onto the reaction plate and plate base.
4.	Verify that the holes of the plate retainer and the septa strip are aligned.
5.	Place the plate assembly on the autosampler.

## Create an Instrument Protocol

You must create an instrument protocol prior to the first run for any AmpF $\Phi$ STR PCR Amplification Kit.

To create an instrument protocol:

1.	In the Tree pane of Data Collection Software v2.0, click  <b>GA Instruments</b> >  <b>ga3100</b> or <b>ga3100-Avant</b> >  <b>Protocol Manager</b> .
2.	In the Instrument Protocols pane, click  to open the Protocol Editor dialog box.
3.	Complete the Protocol Editor dialog box: <ol style="list-style-type: none"> <li>Enter a name for the protocol.</li> <li>Optional: Enter a description for the protocol.</li> <li>In the Type drop-down list, select <b>REGULAR</b>.</li> <li>In the Run Module drop-down list, select <b>HIDFragmentAnalysis36_POP4_1</b>.</li> <li>In the Dye Set drop-down list, select <b>F</b> or <b>G5</b>.</li> </ol>
4.	Click  . You have successfully created the spectral instrument protocol.

## Create a GeneMapper ID Software v3.1 Plate Record for Autoanalysis

To create a GeneMapper ID Software v3.1 plate record:

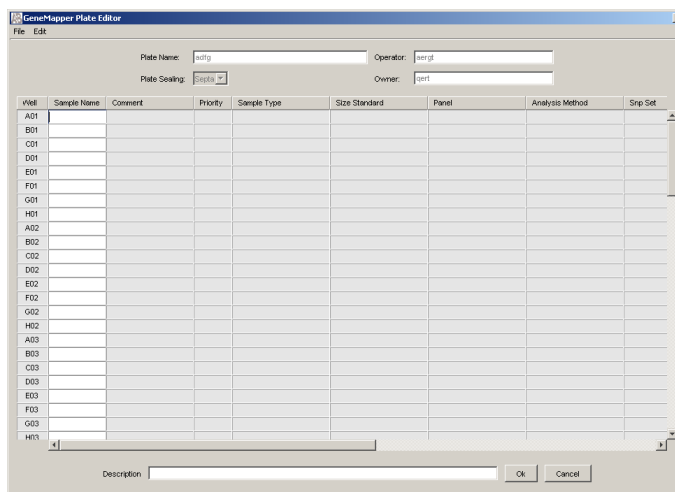
1.	In the Tree pane of Data Collection Software v2.0, click  <b>GA Instruments</b> >  <b>ga3100</b> or <b>ga3100-Avant</b> >  <b>Plate Manager</b> .
----	--

**To create a GeneMapper *ID* Software v3.1 plate record:**

2. Complete the New Plate dialog box:
  - a. Enter a name for the plate.
  - b. Optional: Enter a description for the plate record.
  - c. In the Application drop-down list, select:
    - **GeneMapper-Generic** or
    - **GeneMapper-<Computer Name>**

When you use the 3100/3100-*Avant* Genetic Analyzers with Data Collection Software v2.0 and the AmpF $\Phi$ STR kits, Applied Biosystems recommends that you select the **GeneMapper-<Computer Name>** application.
  - d. In the Plate Type drop-down list, select **96-Well**.
  - e. Enter a name for the owner.
  - f. Enter a name for the operator.

3. Click  .  
The GeneMapper Plate Editor opens.






**Complete the  
GeneMapper *ID*  
Software v3.1  
Plate Record for  
Autoanalysis**

To complete the GeneMapper *ID* Software v3.1 plate record:

1.	In the Sample Name column of a row, enter a sample name, then click the next cell. The value 100 is automatically displayed in the Priority column.
2.	In the Comment column, enter any additional comments or notations for the sample.
3.	In the Priority column, change the priority value, if desired.
4.	In the Sample Type column, select a sample type from the drop-down list that corresponds to the sample in that well.
5.	In the Size Standard column, select a size standard from the drop-down list: <ul style="list-style-type: none"> <li>• For 4-dye AmpF<math>\Lambda</math>STR kits, select <b>GS500 ROX</b>.</li> <li>• For 5-dye AmpF<math>\Lambda</math>STR kits, select <b>GS500 LIZ</b>.</li> </ul>
6.	In the Panel column, select the appropriate AmpF $\Lambda$ STR panel from the drop-down list.
7.	In the Analysis Method column, select a method from the drop-down list.  <b>Note:</b> For more information on analysis methods, refer to the <i>ABI PRISM<sup>®</sup> 3100/3100-Avant Genetic Analyzer User Guide</i> (PN 4347102).
8.	Leave the Snp Set column blank.
9.	Enter text for User-Defined columns 1 to 3.
10.	In the Results Group 1 column, select a group from the drop-down list.  <b>Note:</b> For more information on results groups, refer to the <i>ABI PRISM<sup>®</sup> 3100/3100-Avant Genetic Analyzer User Guide</i> (PN 4347102).

**To complete the GeneMapper *ID* Software v3.1 plate record:**

11.	<p>In the Instrument Protocol 1 column, select a module from the drop-down list.</p> <p>When you use the 3100/3100-<i>Avant</i> Genetic Analyzers with Data Collection Software v2.0 and the AmpF<math>\Lambda</math>STR kits, Applied Biosystems recommends that you select the <b>HIDFragmentAnalysis36_POP4_1</b> module.</p> <p><b>Note:</b> For more information on instrument protocols, refer to the <i>ABI PRISM<sup>®</sup> 3100/3100-Avant Genetic Analyzer User Guide</i> (PN 4347102).</p>						
12.	<p>To complete the rest of the plate record based on the samples loaded in your plate, do one of the following:</p> <table border="1"> <thead> <tr> <th>If the column contains...</th><th>Then...</th></tr> </thead> <tbody> <tr> <td>the same sample types and the same protocols</td><td> <p>highlight the entire column, then select <b>Edit &gt; Fill Down Special</b>.</p> <p>Based on the plate type (96-well) and capillary array (16 or 4 capillaries) you are using, the software automatically fills in the appropriate well numbers for a single run.</p> </td></tr> <tr> <td>different sample types and different protocols</td><td>complete the plate record manually.</td></tr> </tbody> </table>	If the column contains...	Then...	the same sample types and the same protocols	<p>highlight the entire column, then select <b>Edit &gt; Fill Down Special</b>.</p> <p>Based on the plate type (96-well) and capillary array (16 or 4 capillaries) you are using, the software automatically fills in the appropriate well numbers for a single run.</p>	different sample types and different protocols	complete the plate record manually.
If the column contains...	Then...						
the same sample types and the same protocols	<p>highlight the entire column, then select <b>Edit &gt; Fill Down Special</b>.</p> <p>Based on the plate type (96-well) and capillary array (16 or 4 capillaries) you are using, the software automatically fills in the appropriate well numbers for a single run.</p>						
different sample types and different protocols	complete the plate record manually.						
13.	<p>If you want to perform more than one run:</p> <ol style="list-style-type: none"> <li>Select <b>Edit &gt; Add Sample Run</b>. Additional Results Group, Instrument Protocol and Analysis Protocol columns are added to the right end of the plate record.</li> <li>Complete the columns for the additional runs.</li> </ol>						
14.	<p>Click  to save, then close the plate record.</p> <p><b>IMPORTANT!</b> After clicking <b>OK</b> within the Plate Editor, the completed plate record is stored in the Plate Manager database. Once in the Plate Manager database, the plate record can be searched for, edited, exported, or deleted.</p>						

## Run the Sample Plate

Run the fragment analysis sample plate on the 3100/3100-*Avant* Genetic Analyzer.

For information on running the sample plate on the instrument, refer to the *ABI PRISM® 3100/3100-*Avant* Genetic Analyzer User Guide* (PN 4347102).

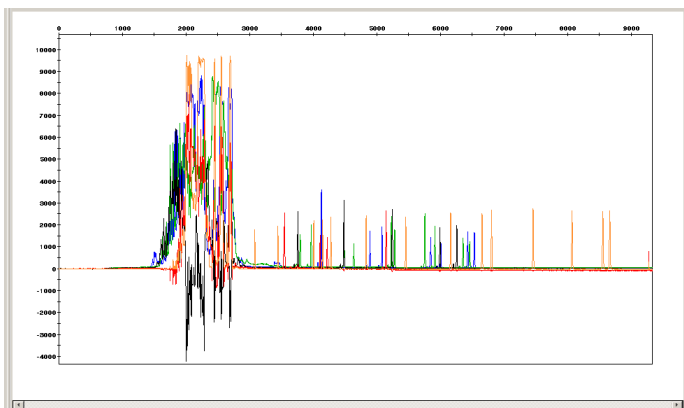
## View the Data After the Run

To view the data after the fragment analysis run:

1.	<p>When the run has completed, view the data:</p> <ul style="list-style-type: none"> <li>You can view the data as color data in the Array View page of Data Collection Software v2.0</li> </ul> <p><b>Note:</b> The electropherogram displayed in the Array View page is the raw, multicomponented data for a selected capillary.</p> <ul style="list-style-type: none"> <li>After autoanalysis, you can view the data as analyzed sample files in the following default location: <ul style="list-style-type: none"> <li><b>D:\AppliedBio\3100\DataExtractor\ExtractedRuns</b></li> </ul> </li> </ul> <p>or</p> <ul style="list-style-type: none"> <li><b>D:\AppliedBio\3100-Avant\DataExtractor\ExtractedRuns</b></li> </ul>
2.	<p>If you need to reanalyze the data, use GeneMapper <i>ID</i> Software v3.1.</p> <p><b>Note:</b> For details, refer to the <i>GeneMapper™ ID Software v3.1 User Guide</i> (PN 4338775).</p>

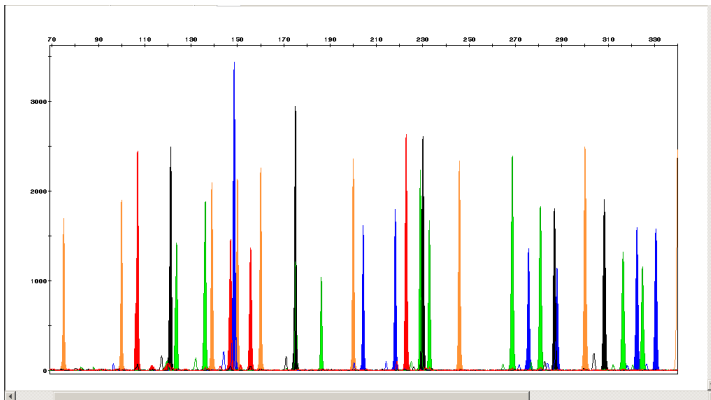
To view the data after the fragment analysis run: *(continued)*

3. The following is a representative display of raw data for a 1 ng sample amplified using the AmpF $\Phi$ STR Identifiler PCR Amplification Kit.



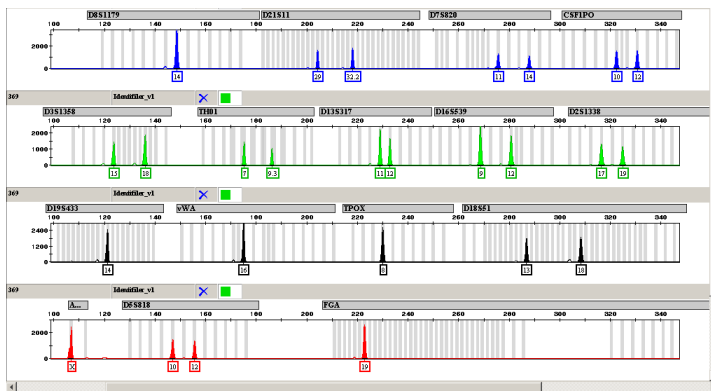
**Note:** When viewing raw data, peaks that fall below zero on the Y-axis will be observed in instances where the signal intensity exceeds the linear dynamic range of the instrument (that is, off-scale data).

4. The following is a representative sizing plot of the same sample shown in [step 3](#) above, viewed in GeneMapper *ID* Software v3.1.



To view the data after the fragment analysis run: *(continued)*

5. The following is a representative genotyping plot of the same sample shown in [step 3](#) on [page 44](#), viewed in GeneMapper ID Software v3.1.



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# Data Collection Software v2.0 Verification Testing

This section describes the verification testing performed to evaluate Data Collection Software v2.0 with samples processed on the 3100 and 3100-*Avant* Genetic Analyzers.

Data Collection Software v2.0 verification testing was performed to:

- Document and confirm the functionality of Data Collection Software v2.0
- Document and confirm that Data Collection Software v2.0 does not affect results obtained using the same analysis parameter settings as Data Collection Software v1.1 for the 3100 instrument or Data Collection Software v1.0 for the 3100-*Avant* instrument.

**Test Plan** The test plan, which Applied Biosystems has defined as verification of the software, was designed to develop a set of criteria to evaluate the performance of Data Collection Software v2.0 for the Human Identification laboratories.

GeneMapper *ID* Software v3.1 was used to analyze the data for this verification testing using the default settings as defined in the *GeneMapper™ ID Software v3.1 User Guide* (PN 4338775).

**Evaluation Categories** The verification was designed to evaluate the following categories:

- Genotype concordance
- Sensitivity
- Resolution
- Color balance
- Spectral pull-up

For each of these categories, the comparison results between Data Collection Software v1.1/v1.0 and Data Collection Software v2.0 are summarized in the following pages.

The data used was analyzed with GeneMapper *ID* Software v3.1.

## Genotype Concordance

Genotype concordance between Data Collection Software v1.1/v1.0 and Data Collection Software v2.0 was 100% across all runs. A total of 2582 loci were evaluated.

The table below shows the number of loci analyzed in the genotype concordance study for each system.

Instrument	AmpF $\Lambda$ STR Kit	Number of Loci Analyzed	Number of Samples
3100	Identifiler (5-dye)	960	15
3100- <i>Avant</i>	Identifiler (5-dye)	896	14
3100	SGM Plus (4-dye)	660	15
3100- <i>Avant</i>	SGM Plus (4-dye)	660	15

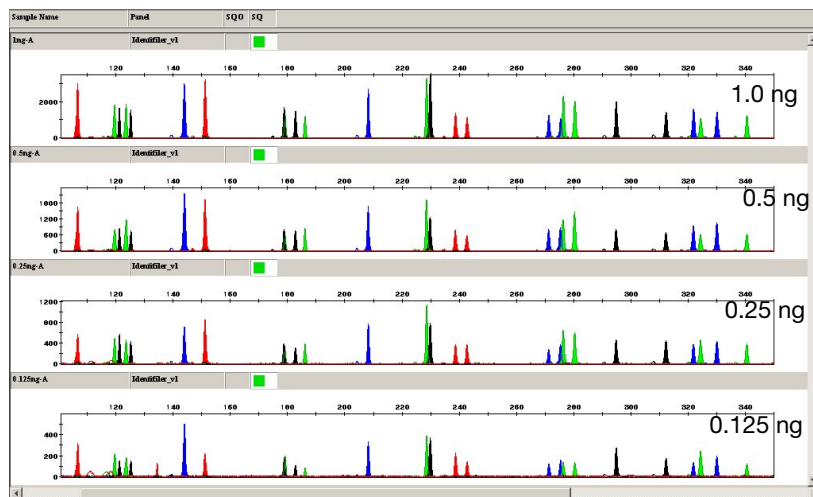
## Sensitivity

As has been previously demonstrated, samples amplified with the AmpF $\Lambda$ STR Identifiler PCR Amplification Kit produce full genotype profiles with 0.125 ng input DNA. Further, samples amplified with the AmpF $\Lambda$ STR SGM Plus PCR Amplification Kit produce full genotype profiles with 0.25 ng input DNA.

Data Collection Software v2.0 does not affect sensitivity as documented in previously published results for the 3100 or 3100-*Avant* instruments.

**Note:** Previously published results are in the *ABI PRISM<sup>®</sup> 3100 Genetic Analyzer User Bulletin, SUBJECT: Protocols for Processing AmpF $\Lambda$ STR PCR Amplification Kit PCR Products* (PN 4332345).

Figure 1 shows dilutions for a DNA sample amplified with the AmpF $\phi$ STR Identifiler PCR Amplification Kit: 1.0 ng, 0.5 ng, 0.25 ng, and 0.125 ng of input DNA. The Y-axis is magnified for lower input DNA amounts. A full profile is presented in each panel.



**Figure 1** DNA sample amplified with the AmpF $\phi$ STR Identifiler PCR Amplification Kit



Figure 2 shows dilutions for a DNA sample amplified with the AmpF $\Lambda$ STR SGM Plus PCR Amplification Kit: 2.0 ng, 1.0 ng, 0.5 ng, and 0.25 ng of input DNA. The Y-axis is magnified for lower input DNA amounts. A full profile is presented in each panel.

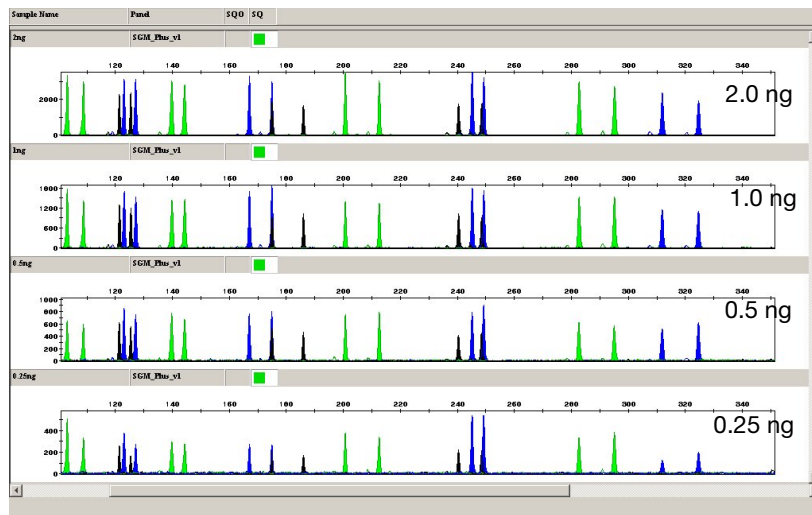
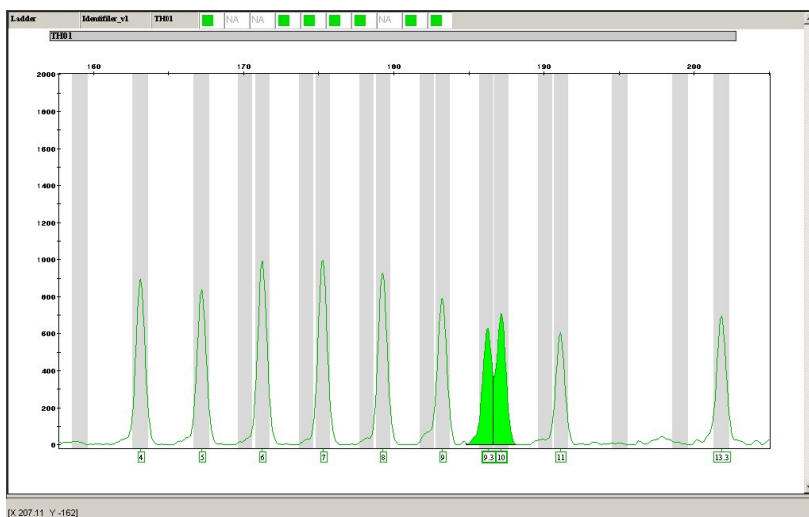


Figure 2 DNA sample amplified with the AmpF $\Lambda$ STR SGM Plus PCR Amplification Kit

**Resolution** Figure 3 shows single nucleotide resolution at the 9.3 and 10 alleles (highlighted) of the TH01 locus from the AmpF $\mathbb{L}$ STR Identifier Allelic Ladder. The 9.3 and 10 alleles were resolved and individually detected in all 5-dye AmpF $\mathbb{L}$ STR Identifier and 4-dye AmpF $\mathbb{L}$ STR SGM Plus runs.



**Figure 3** Single nucleotide resolution at the 9.3 and 10 alleles of the TH01 locus in the AmpF $\mathbb{L}$ STR Identifier Allelic Ladder

## Color Balance and Spectral Pull-Up

Color balance and spectral pull-up are consistent between Data Collection Software v1.1/v1.0 and Data Collection Software v2.0.

## Summary

Applied Biosystems findings demonstrate that the genotypes obtained between Data Collection Software v1.1/v1.0 (using GeneScan $\mathbb{R}$  Analysis Software and Genotyper $\mathbb{R}$  Software or GeneMapper $\mathbb{TM}$  ID Software) and Data Collection Software v2.0 (using GeneMapper ID Software v3.1) are concordant. Further, Data Collection Software v2.0 does not affect sensitivity, resolution, color balance, or spectral pull-up.

Applied Biosystems verification testing demonstrates that Human Identification laboratories can successfully adopt Data Collection Software v2.0.

## How to Obtain Services and Support

For the latest services and support information for all locations, go to **<http://www.appliedbiosystems.com>**, then click the link for **Services and Support**.

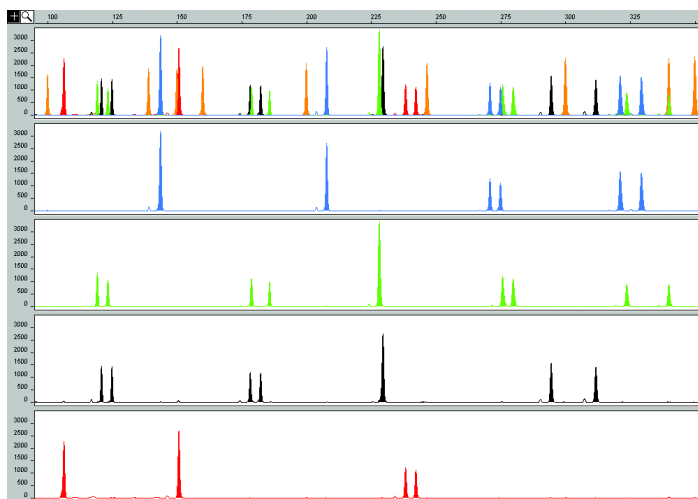
At the Services and Support page, you can:

- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support
- Order Applied Biosystems user documents, MSDSs, certificates of analysis, and other related documents
- Download PDF documents
- Obtain information about customer training
- Download software updates and patches

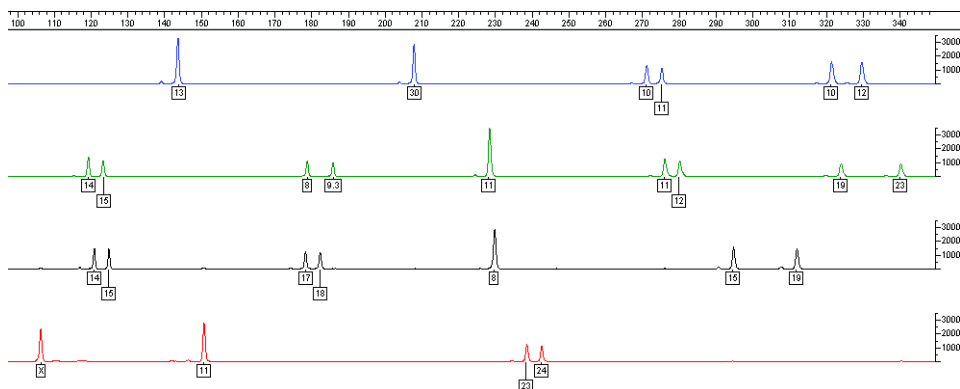
In addition, the Services and Support page provides access to worldwide telephone and fax numbers to contact Applied Biosystems Technical Support and Sales facilities.

## Appendix A: Previous Validation of the 3100 Genetic Analyzer

Figure 4 through Figure 10 are examples of DNA profiles obtained on the ABI PRISM 3100 Genetic Analyzer. The examples in Figure 4 (4a and 4b) show AmpF $\ell$ STR<sup>®</sup> Control DNA 9947A (1 ng) amplified with the AmpF $\ell$ STR Identifiler PCR Amplification Kit.



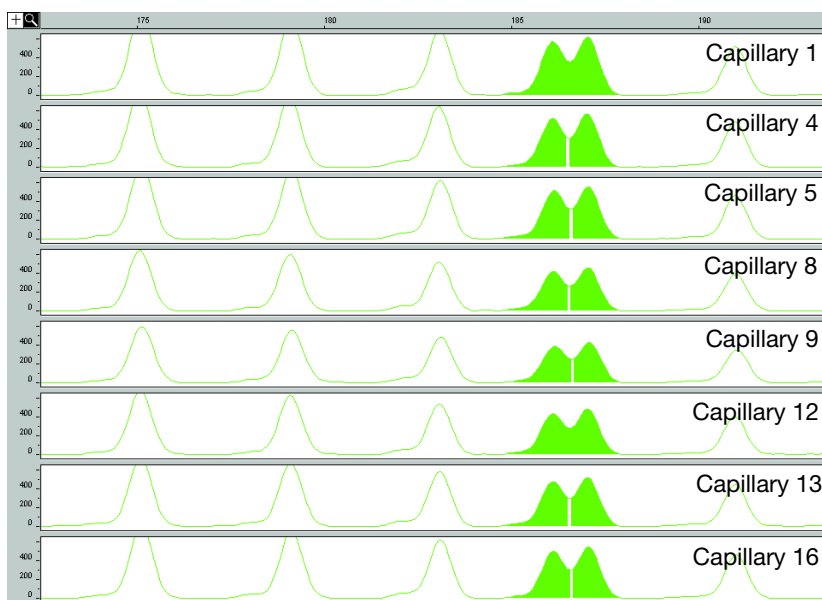
4a. Data analyzed with GeneScan<sup>®</sup> Analysis Software Version 3.7.1



4b. The same data (as 4a above) analyzed with Genotyper<sup>®</sup> Software v3.7

**Figure 4** AmpF $\ell$ STR DNA on the ABI PRISM 3100 Genetic Analyzer

Figure 5 shows eight (8) capillaries of a 16-capillary array displaying single nucleotide resolution at the 9.3 and 10 alleles (highlighted) of the TH01 locus from the AmpF $\Phi$ STR Identifiler Allelic Ladder. The 9.3 and 10 alleles were resolved and individually detected in all 16 capillaries.



**Figure 5** A 16-capillary array displaying single nucleotide resolution

Figure 6 shows a comparison of signal intensity between four (4) capillaries across a capillary array using the AmpF $\ell$ STR Control DNA 9947A (1 ng) amplified with the AmpF $\ell$ STR Identifier PCR Amplification Kit.

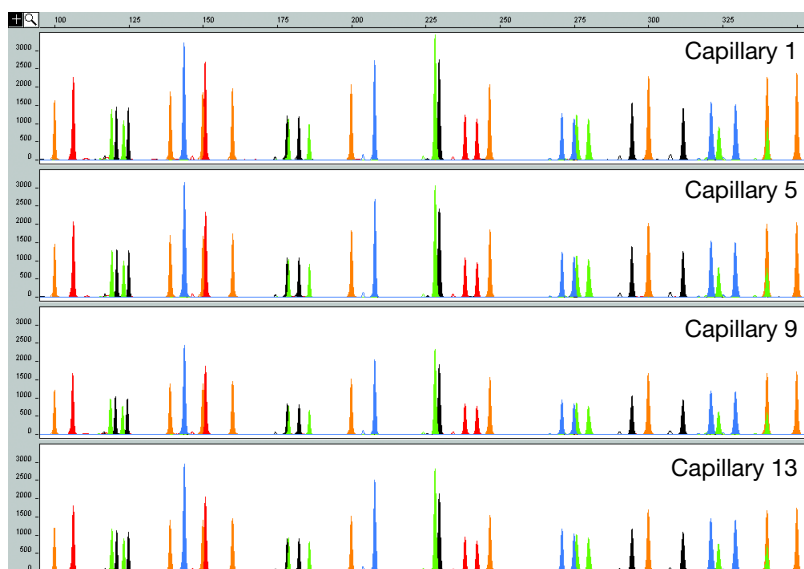
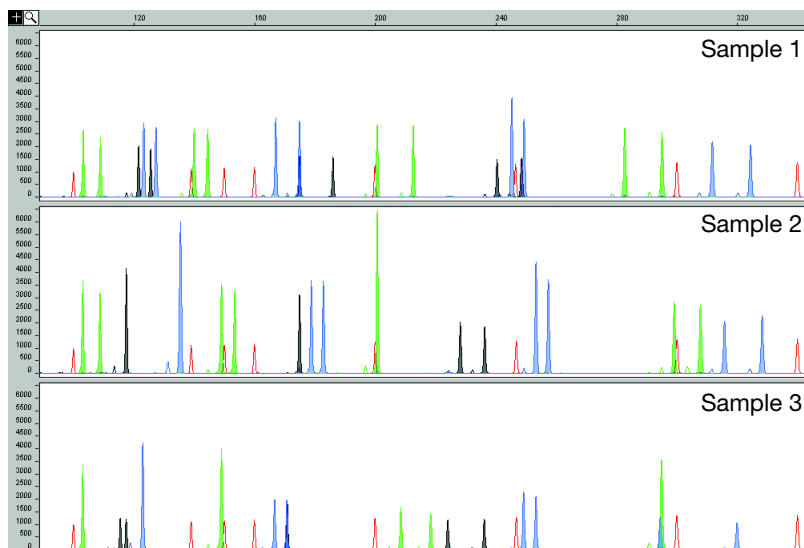
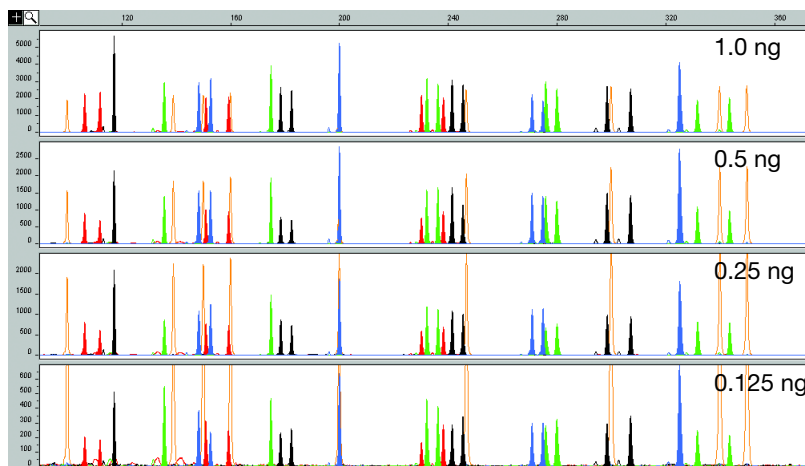


Figure 6 A comparison of signal intensity between capillaries



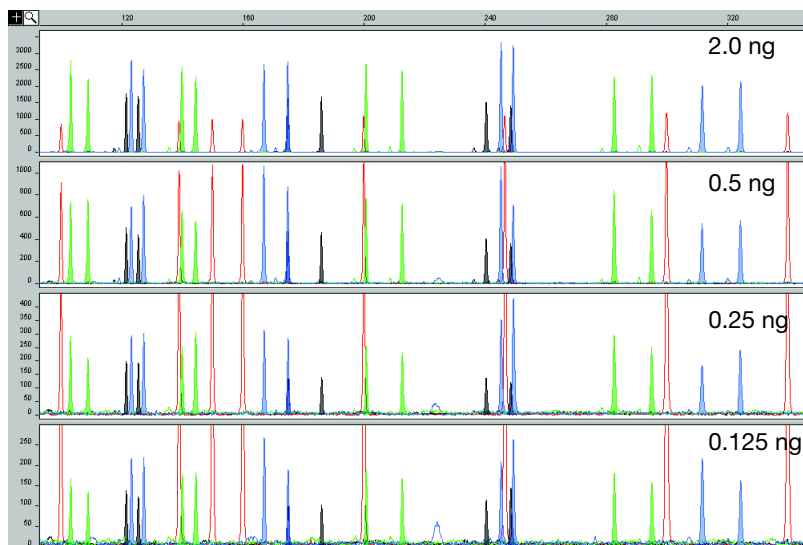
**Figure 7** Three (3) DNA samples (2 ng) amplified with the AmpF $\Lambda$ STR SGM Plus PCR Amplification Kit

**Figure 8** shows dilutions for a DNA sample amplified with the AmpF $\Lambda$ STR Identifier PCR Amplification Kit, 1.0 ng, 0.5 ng, 0.25 ng, and 0.125 ng input DNA. The Y-axis scale is magnified for lower input DNA amounts.



**Figure 8** DNA sample amplified with the AmpF $\Lambda$ STR Identifier PCR Amplification Kit

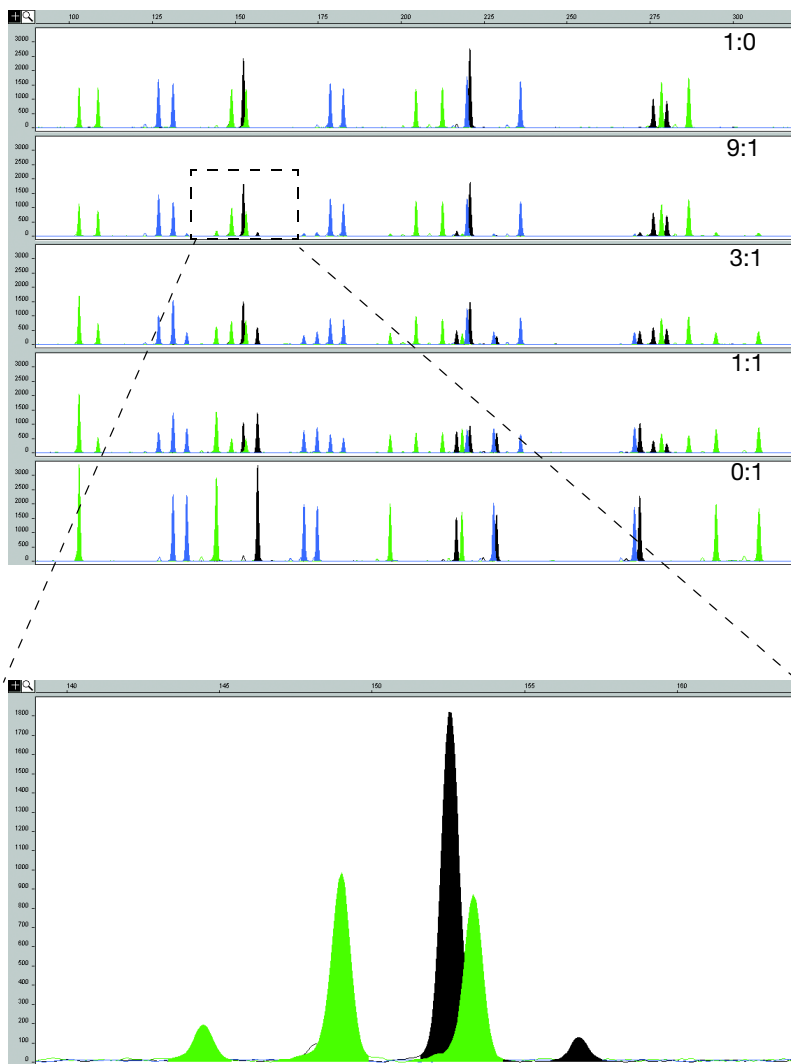
Figure 9 shows dilutions of a DNA sample amplified with the AmpF $\ell$ STR SGM Plus PCR Amplification Kit, 2.0 ng, 0.5 ng, 0.25 ng, and 0.125 ng input DNA. The Y-axis scale is magnified for lower input DNA amounts.



**Figure 9** DNA sample amplified with the AmpF $\ell$ STR SGM Plus PCR Amplification Kit



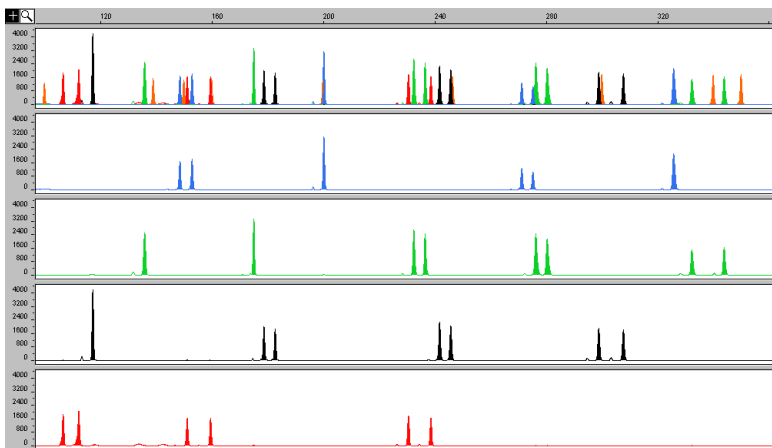
In [Figure 10](#), the first and last panels display the profiles of each DNA sample amplified individually with 2ng DNA using the AmpF $\Lambda$ STR Profiler Plus PCR Amplification Kit (the male sample is in the top panel and the female sample is in the bottom panel). The other three panels display the mixture of these DNA samples mixed at approximate ratios of 9:1, 3:1, and 1:1. The panel inset displays the expanded view of the DNA sample mixed at an approximate ratio of 9:1 at D8S1179 (green) and D5S818 (yellow).



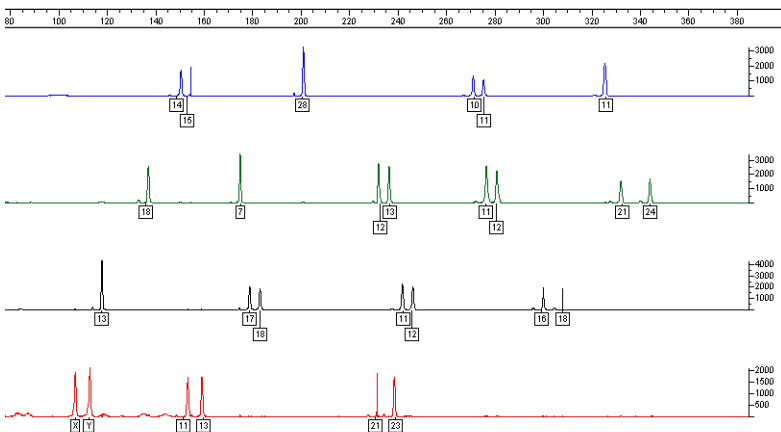
**Figure 10** Profiles of DNA samples amplified

## Appendix B: Previous Validation of the 3100-*Avant* Genetic Analyzer

Figure 11 through Figure 17 are examples of DNA profiles obtained on the ABI PRISM 3100-*Avant* Genetic Analyzer. The examples in Figure 11 (11a and 11b) show a 1 ng DNA sample amplified with the AmpF $\mathcal{L}$ STR Identifiler PCR Amplification Kit.



11a. Data analyzed with GeneScan<sup>®</sup> Analysis Software v3.7.1



11b. The same sample (as in 11a) analyzed with Genotyper<sup>®</sup> Software v3.7

**Figure 11** DNA profiles from the ABI PRISM 3100-*Avant* Genetic Analyzer

Figure 12 shows four capillaries from a single capillary array displaying single nucleotide resolution at the 9.3 and 10 alleles (highlighted). The 9.3 and 10 alleles are on the TH01 locus from the AmpF $\Phi$ STR Identifiler Allelic Ladder and were resolved and individually detected in all 4 capillaries.

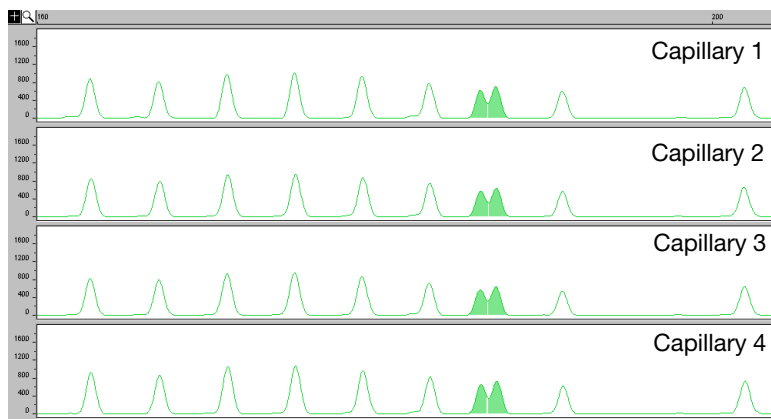


Figure 12 Single nucleotide resolution at the 9.3 and 10 alleles

Figure 13 shows a comparison of signal intensity between three capillary arrays using a 1 ng DNA sample amplified with the AmpF $\Phi$ STR Identifiler PCR Amplification Kit.

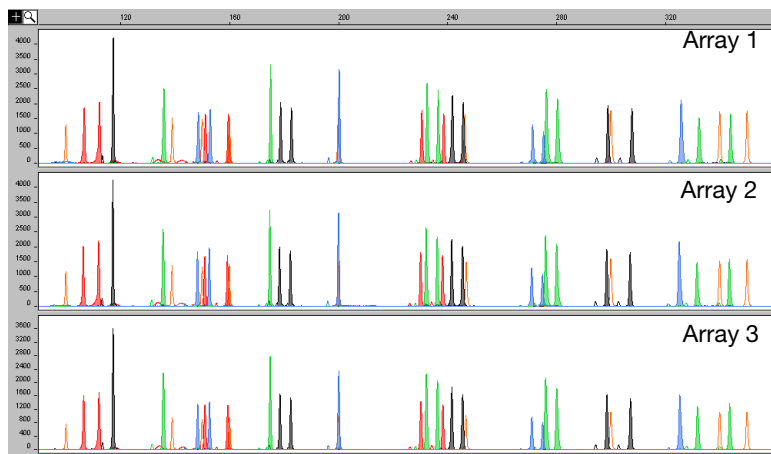
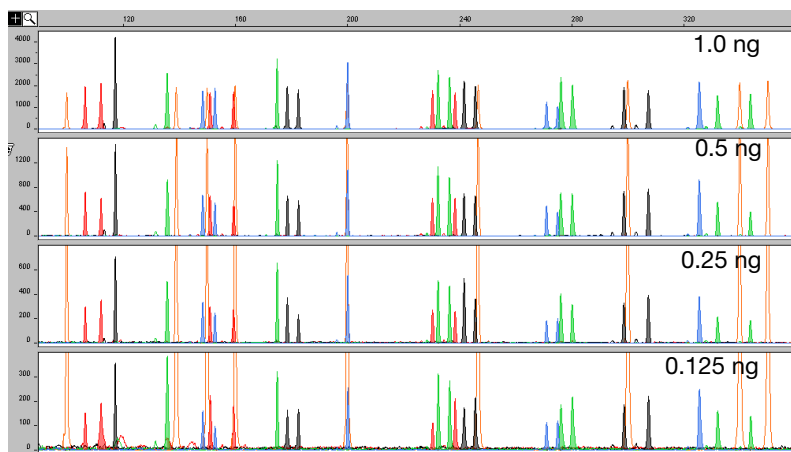
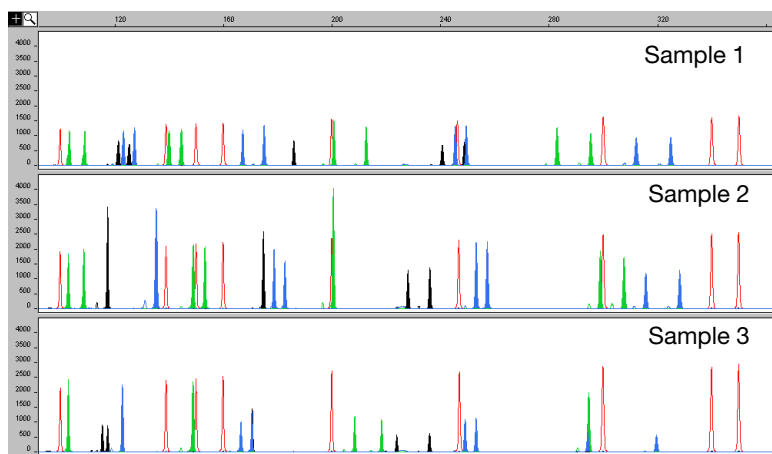


Figure 13 A comparison of signal intensity between three capillary arrays

Figure 14 shows dilutions of a DNA sample amplified with the AmpF $\ell$ STR Identifiler PCR Amplification Kit, 1.0 ng, 0.5 ng, 0.25 ng, and 0.125 ng input DNA. The Y-axis scale is magnified for lower input DNA amounts.

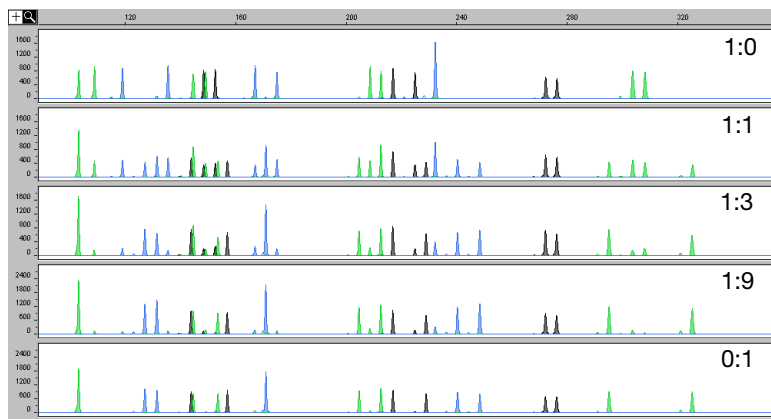


**Figure 14** DNA sample amplified with the AmpF $\ell$ STR Identifiler PCR Amplification Kit



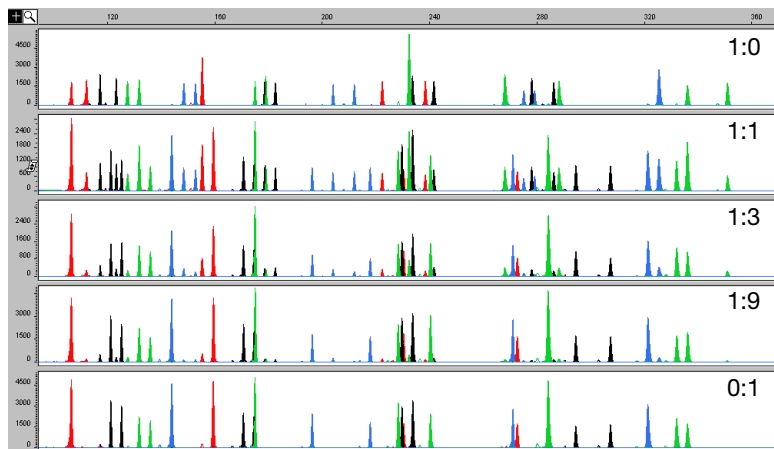
**Figure 15** Three (3) DNA samples (1 ng) amplified with the AmpF $\ell$ STR SGM Plus PCR Amplification Kit

In [Figure 16](#) the first and last panels display the profiles of each DNA sample amplified individually with 2 ng DNA using the AmpF $\ell$ STR Profiler Plus PCR Amplification Kit (the male sample is in the top panel and the female sample is in the bottom panel). The other three panels display the mixture of these DNA samples mixed at approximate ratios of 1:1, 1:3, and 1:9.



**Figure 16** DNA samples amplified using the AmpF $\ell$ STR Profiler Plus PCR Amplification Kit

In [Figure 17](#), the first and last panels display the profiles of each DNA sample amplified individually with 2 ng DNA using the AmpF $\ell$ STR Identifiler PCR Amplification Kit (the male sample is in the top panel and the female sample is in the bottom panel). The other three panels display the mixture of these DNA samples mixed at approximate ratios of 1:1, 1:3, and 1:9.



**Figure 17** DNA samples amplified using the AmpF $\ell$ STR Identifiler PCR Amplification Kit

# Notes

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