User Bulletin

Applied Biosystems 3130/3130*xl* Genetic Analyzers Using Data Collection Software v3.0

September 9, 2010

SUBJECT: Protocols for Processing AmpFℓSTR PCR Amplification Kit PCR Products

In This User Bulletin

This user bulletin includes the following topics:

Overview

This document describes:

- The design changes and new features that differentiate the Applied Biosystems 3130/3130*xl* Genetic Analyzers from the ABI PRISM® 3100/3100-*Avant* Genetic Analyzers.
- The tasks required to perform Human Identification (HID) analysis with the 3130/3130*xl* Genetic Analyzers, using 3130/3130*xl* Genetic Analyzer Data Collection Software v3.0 and GeneMapper® *ID* Software v3.2.
- Examples of DNA profiles generated on the 3130/3130*xl* Genetic Analyzers.
- Maintenance schedule and maintenance procedures for the 3130/3130*xl* Genetic Analyzers.

Audience

This user bulletin is intended for customers who will be using the 3130/3130*xl* Genetic Analyzers to perform HID analysis.

Applied Biosystems

Related Documentation

The following documents are referenced in this user bulletin. Be sure to have these on hand when you are performing experiments with the 3130/3130*xl* Genetic Analyzers.

- *Applied Biosystems 3130/3130xl Genetic Analyzers Getting Started Guide* (PN 4352715, Rev. B).
- *GeneMapper® ID Software v3.1 and v3.2: Human Identification Analysis Tutorial* (PN 4357520, Rev. A)
- *Applied Biosystems 3130/3130xl Genetic Analyzers Maintenance, Troubleshooting, and Reference Guide* (PN 4352716, Rev. B)

Safety

Complete Safety information for the 3130/3130*xl* Genetic Analyzers may be found in the *Applied Biosystems 3130/3130xl Genetic Analyzers Getting Started Guide*.

How to Obtain Support

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

At the 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 Support page provides access to worldwide telephone and fax numbers to contact Applied Biosystems Technical Support and Sales facilities.

New Features and Design Changes

Instrument Modifications

- Polymer delivery system (PDS)

  Applied Biosystems has taken a proven PDS technology from the Applied Biosystems 3730 DNA Analyzer and applied it to the
  4- and 16-capillary instruments. The PDS improves instrument performance and maintenance by reducing manual intervention.

  The primary benefit lies in the automation of the polymer delivery process. The automated PDS of the 3130/3130*xl* Genetic Analyzers does not use polymer syringes. Eliminating the manual steps for filling, priming, and loading polymer provides faster, easier, and more reliable instrument setup. You simply load the polymer bottle onto the instrument, then perform setup using the software wizards (see "Data Collection Software Modifications" on page 4).

  Note: The positions of the waste and rinse reservoirs are reversed on the 3130/3130*xl* Genetic Analyzers (as compared to the 3100/3100-*Avant* Genetic Analyzers). See step 7 on page 64.

- Oven door

  Applied Biosystems has changed the composition of the gasket material used to seal the oven door.

- CCD camera shutter

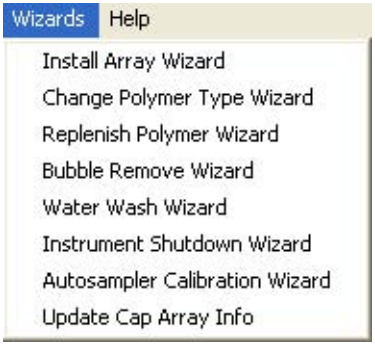
  This modification affects the CCD camera shutter only. It does not alter mechanically or introduce variation to the performance of the CCD camera or optical path components.

- Detection cell heater

  The detection cell heater is only activated when using run modules created for the POP-7™ polymer. Applied Biosystems does not recommend activation of the detection cell heater for HID applications.

Data Collection Software Modifications

Data Collection Software v3.0 represents Applied Biosystems latest controlling software for the capillary electrophoresis instruments. As in Data Collection Software v2.0, there are software wizards to guide you through maintenance procedures:



Several of these wizards are new in Data Collection Software v3.0 and support the new pump-based PDS of the 3130/3130xl Genetic Analyzers:

- Change Polymer Type Wizard (to change to a different polymer type)
- Replenish Polymer Wizard (to replenish with the same polymer, from the same or different lot)
- Bubble Remove Wizard
- Water Wash Wizard
- Instrument Shutdown Wizard

Reagents, Kits, and Run Module for HID Analysis

The reagents, AmpFℓSTR kits, and run module listed in this section are available for performing HID analysis using the 3130/3130*xl* Genetic Analyzers.

Reagents

Part Number	Name	3130 Genetic Analyzer	3130*xl* Genetic Analyzer
4345831	Matrix Standard Set DS-32 (Dye Set F) **Note:** Matrix Standard Set DS-32 is for use on multi-capillary instruments (that is, the 3100/3100-*Avant* Genetic Analyzers and the 3130/3130*xl* Genetic Analyzers).	√	√
4345833	Matrix Standard Set DS-33 (Dye Set G5) **Note:** Matrix Standard Set DS-33 is for use on multi-capillary instruments (that is, the 3100/3100-*Avant* Genetic Analyzers and the 3130/3130*xl* Genetic Analyzers).	√	√
4352755	3130 POP-4™ polymer	√	√
4311320	Hi-Di™ formamide	√	√
402824	10× Genetic Analyzer Buffer with EDTA **Note:** Use 10× Genetic Analyzer Buffer with EDTA to prepare 1× Genetic Analyzer Buffer with EDTA (1× running buffer).	√	√
4333464	3130 and 3100-*Avant* Capillary Array, 36 cm	√	—
4315931	3130*xl* and 3100 Capillary Array, 36 cm	—	√

AmpFℓSTR Kits

Kit	Dye Set	Matrix Standard Set
• AmpFℓSTR® Identifiler® PCR Amplification Kit • AmpFℓSTR® Yfiler™ PCR Amplification Kit • AmpFℓSTR® SEfiler™ PCR Amplification Kit	G5	DS-33
• AmpFℓSTR® Profiler Plus® PCR Amplification Kit • AmpFℓSTR® COfiler® PCR Amplification Kit • AmpFℓSTR® Profiler Plus® ID PCR Amplification Kit • AmpFℓSTR® SGM Plus® PCR Amplification Kit • Other four-dye AmpFℓSTR® kits	F	DS-32

Run Module

Run Module	Capillary Length (cm)	Polymer Type	Run Time (min)	24-hr Throughput (GT[a])		Resolution (bp)	Specification (SD)[b]
				3130 Analyzer	3130*xl* Analyzer		
HIDFragment Analysis36_POP4_1	36	POP-4	45	2,560	10,240	500	0.15

a. 20 GT (genotypes)/capillary/injection.
b. 1-bp resolution at 99.99% accuracy.

Section 1 Performing HID Analysis

This section covers the following topics:

How to Perform the Tasks in This Section

In this section, the tasks required to perform HID analysis on the 3130/130*xl* Genetic Analyzers are grouped under four major steps:

1. "Preparing the Instrument" on page 8
2. "Performing a Spatial Calibration" on page 11
3. "Performing a Spectral Calibration" on page 12
4. "Performing a Sample Run" on page 25

Many of the tasks listed under each of these major steps are described in detail in the *Applied Biosystems 3130/3130xl Genetic Analyzers Getting Started Guide* (*3130/3130xl Genetic Analyzers GSG*). Consequently, this user bulletin is meant to be used in conjunction with the *3130/3130xl Genetic Analyzers GSG*:

- When tasks required to perform HID analysis are the same as those described in the *3130/3130xl Genetic Analyzers GSG*, you are referred to the appropriate page number in the *3130/3130xl Genetic Analyzers GSG.*

  IMPORTANT! Be sure to use the correct revision of the *3130/3130xl Genetic Analyzers GSG:* Part Number 4352715, Revision B (PN 4352715, Rev. B). If you do not use PN 4352715, Rev. B, the referenced page numbers may be incorrect. If you do not have this revision, you can download it from Applied Biosystems website; see "How to Obtain Support" on page 2.

- When tasks required to perform HID analysis are different from those described in the *3130/3130xl Genetic Analyzers GSG*, you are referred to the appropriate page number in this user bulletin. The user bulletin references are highlighted in **blue text** (for *.pdf files) or **bold text** (for printed documents).

Preparing the Instrument

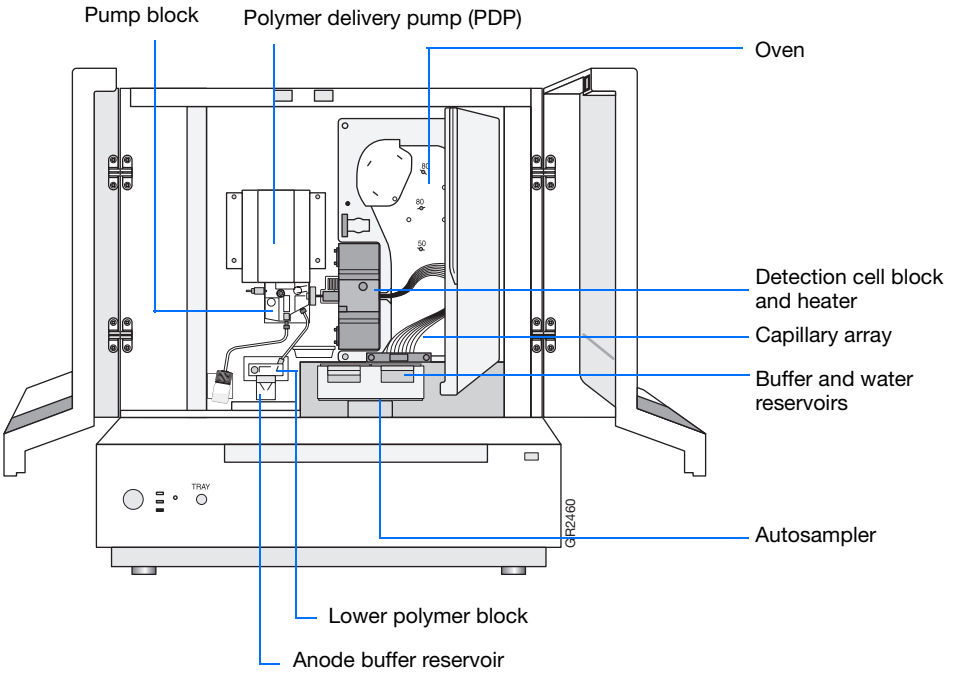
Workflow

To prepare the instrument for a run:

Step	Task	See...
1	Review the instrument parts and functions.	**page 10 of this user bulletin.**
2	Start the computer workstation, then start the 3130/3130*xl* Genetic Analyzer.	page 7 of the *3130/3130xl Genetic Analyzers GSG.*
3	Start the 3130/3130*xl* Genetic Analyzer Data Collection Software v3.0.	page 9 of the *3130/3130xl Genetic Analyzers GSG.*
4	Inspect the instrument, then perform any necessary maintenance tasks. **IMPORTANT!** As part of instrument maintenance, you must change or replenish polymer weekly. For HID analysis, be sure to use 3130 POP-4 polymer (PN 4352755). **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.	• pages 12 to15 of the *3130/3130xl Genetic Analyzers GSG.* • **Appendix A on page 55 of this user bulletin.**

Step	Task	See...
5	Use 10× Genetic Analyzer Buffer with EDTA (PN 402824) to prepare 1× Genetic Analyzer Buffer with EDTA (1× running buffer), then fill the instrument reservoirs. **CAUTION CHEMICAL HAZARD. 10× 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. **CAUTION CHEMICAL HAZARD. 1× 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.	page 16 of the *3130/3130xl Genetic Analyzers GSG.*

Instrument Layout



Part	Function
Anode buffer reservoir	Contains 16 mL of 1X running buffer.
Buffer and water reservoirs (four)	Each contain 16 mL of 1X running buffer or water.
Autosampler	Holds the sample plates and reservoirs and moves to align the samples, water, or buffer with the capillaries.
Capillary array	Enables the separation of the fluorescent-labeled DNA fragments by electrophoresis. It is a replaceable unit composed of 4 or 16 silica capillaries.
Detection cell block and heater	Holds the capillaries in place for laser detection.

Part	Function
Lower polymer block	Contains the buffer valve, anode electrode, and anode buffer reservoir.
Oven	Maintains uniform capillary array temperature.
Polymer delivery pump (PDP)	Pumps polymer into the array and performs maintenance procedures.
Pump block	Includes the displacement pump chamber, piston water seal, array attachment point (array port), and connection to the lower polymer block through the interconnect tube.

Performing a Spatial Calibration

The Data Collection software uses images collected during the spatial calibration to establish a relationship between the signal emitted by each capillary and the position where that signal falls on and is detected by the CCD camera.

When to Perform the Calibration

You are required to perform a spatial calibration when you:

- Install or replace a capillary array
- Temporarily remove the capillary array from the detection block
- Move the instrument

Workflow

To perform a spatial calibration:

Step	Task	See...
1	Create a spatial calibration file.	page 22 of the *3130/3130xl Genetic Analyzers GSG.*
2	Evaluate the spatial calibration file.	page 23 of the *3130/3130xl Genetic Analyzers GSG.*

Performing a Spectral Calibration

A spectral calibration creates a matrix that corrects for the overlapping fluorescence emission spectra of the dyes. Although each of these dyes emits its maximum fluorescence at a different wavelength, there is some overlap in the emission spectra between the dyes. The goal of multicomponent analysis is to effectively correct for spectral overlap. Performing a spectral calibration is similar to performing a sample run, except that calibration standards are run in place of samples and a spectral calibration module is used in place of a run module.

When to Perform the Calibration

Perform a spectral calibration:

- When you use a new dye set on the instrument
- When you change capillary array length or polymer type

  Note: For every fragment analysis dye set, you must create a separate spectral calibration for each capillary array length and polymer type combination you use.
- After the laser or CCD camera has been realigned/replaced by a service engineer
- If you begin to see a decrease in spectral separation (pull-up and/or pull-down peaks)

Workflow

To perform a spectral calibration:

Step	Task	See...
1	Choose the correct dye set and matrix standard set for the AmpF*ℓ*STR kit you are using. **Note:** New matrix standard sets are available for all validated multi-capillary instruments (that is, the 3100/3100-*Avant* Genetic Analyzers and the 3130/3130*xl* Genetic Analyzers).	**page 14 of this user bulletin.**

Step	Task	See...
2	Select a spectral calibration protocol for Dye Set G5 or Dye Set F and create a plate record. **IMPORTANT!** For HID applications, the default condition number boundaries for the Dye Set G5 spectral calibration protocol should be edited in Data Collection Software v3.0. You will only need to perform this edit once. See **"Edit the Dye Set G5 Spectral Calibration Protocol" on page 15 of this user bulletin.**	**page 15 of this user bulletin.**
3	Prepare the spectral calibration chemistry.	**page 21 of this user bulletin.**
4	Load the spectral calibration samples.	page 32 of the *3130/3130xl Genetic Analyzers GSG.*
5	Place the plate assembly into the instrument.	page 39 of the *3130/3130xl Genetic Analyzers GSG.*
6	Run the spectral calibration plate, which includes: • Linking the plate • Starting the run • Viewing the pass/fail status	pages 40 to 42 of the *3130/3130xl Genetic Analyzers GSG.*
7	Evaluate the spectral calibration data. **IMPORTANT!** If you obtain off-scale peaks in a 3130/3130*xl* Genetic Analyzer spectral calibration when using the new matrix standard sets, see **"Troubleshooting: Off-Scale Spectral Calibration Peak Height" on page 23 of this user bulletin**.	page 43 of the *3130/3130xl Genetic Analyzers GSG.*
8	Activate the spectral calibration.	page 47 of the *3130/3130xl Genetic Analyzers GSG.*

Choose the Dye Set and Matrix Standards

Choose the appropriate dye set and matrix standard for the AmpFℓSTR PCR Amplification Kit you are using, as shown in the table below.

For AmpFℓSTR kits that use a...	Use...	And use...	AmpFℓSTR Kit Examples
five-dye system, which includes the following dyes: • 6FAM™ • VIC® • NED™ • PET® • LIZ®	Dye Set G5	Matrix Standard Set DS-33	• AmpFℓSTR® Identifiler® PCR Amplification Kit • AmpFℓSTR® Yfiler™ PCR Amplification Kit • AmpFℓSTR® SEfiler™ PCR Amplification Kit
four-dye system, which includes the following dyes: • 5FAM™ • JOE™ • NED™ • ROX™	Dye Set F	Matrix Standard Set DS-32	• AmpFℓSTR® Profiler Plus® PCR Amplification Kit • AmpFℓSTR® COfiler® PCR Amplification Kit • AmpFℓSTR® Profiler Plus® ID PCR Amplification Kit • AmpFℓSTR® SGM Plus® PCR Amplification Kit • Other four-dye AmpFℓSTR kits

Select a Spectral Calibration Protocol and Create a Plate Record

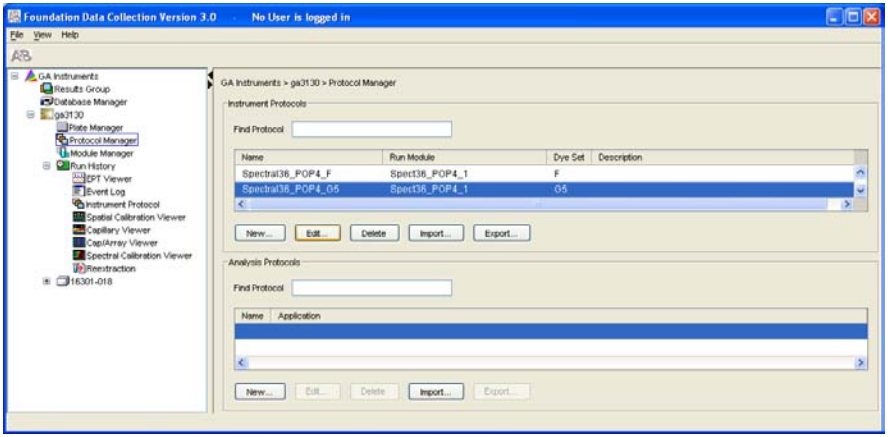
For Dye Set G5

Follow this procedure if you are performing a spectral calibration for kits using a five-dye system, which includes the 6FAM, VIC, NED, PET, and LIZ dyes.

IMPORTANT! For HID applications, the default condition number boundaries for the Dye Set G5 spectral calibration protocol should be edited in Data Collection Software v3.0. Instructions for editing the condition number boundaries are provided below; *you will only need to perform this edit once*.

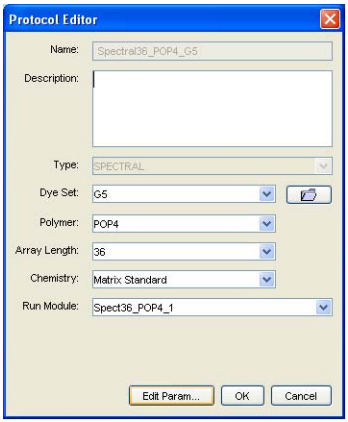
Edit the Dye Set G5 Spectral Calibration Protocol

1. In the tree pane of the Data Collection software, click **GA Instruments** > **ga3130*xl*** or **ga3130** > **Protocol Manager** to open the Protocol Manager window.

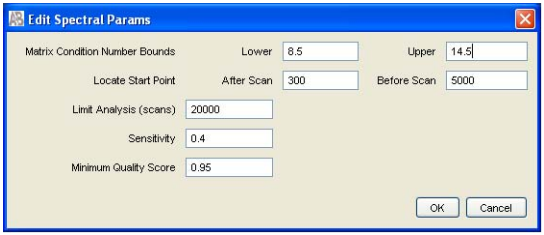


2. In the Instrument Protocols pane, highlight the spectral calibration protocol for Dye Set G5: **Spectral36_POP4_G5**.

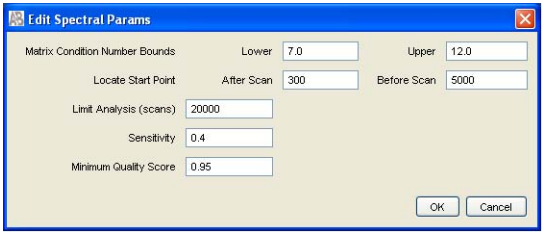
3. Click **Edit** to open the Protocol Editor.



4. Change the condition number boundaries:
   a. Click **Edit Param**. The Edit Spectral Params window opens.



   b. Change the condition number boundary in the Lower field from 8.5 to **7.0**.
   c. Change the condition number boundary in the Upper field from 14.5 to **12.0**.



   d. Click **OK** to return to the Protocol Editor.

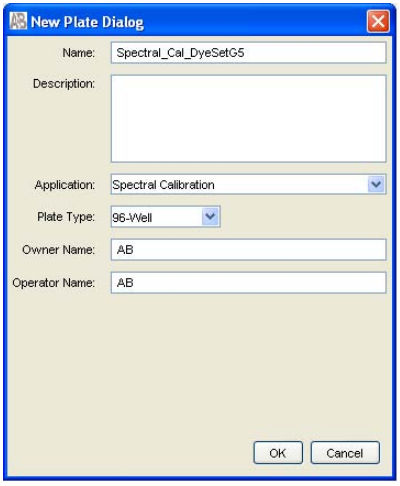
5. Click **OK** to save your changes and exit the Protocol Editor.

   Note: Once you have saved your changes, you will not need to edit the
   Dye Set G5 spectral calibration protocol again. The lower and upper condition number boundaries are now set at 7.0 and 12.0, respectively.

6. Continue with "Select the Dye Set G5 Spectral Calibration Protocol and Create a Plate Record" below.

Select the Dye Set G5 Spectral Calibration Protocol and Create a Plate Record

1. In the tree pane of the Data Collection software, click **GA Instruments** > **ga3130*xl*** or **ga3130** > **Plate Manager** to open the Plate Manager window.
2. Click **New** to open the New Plate dialog box.
3. Complete the New Plate dialog box:



   a. Name: Type a name for the plate.
   b. Description: If desired, type a description for the plate.
   c. Application: Select **Spectral Calibration** from the drop-down list.
   d. Plate Type: Select **96-Well**.

e. Owner Name: Type a name for the owner.

f. Operator Name: Type a name for the operator.

4. Click **OK** to open the Spectral Calibration Plate Editor.

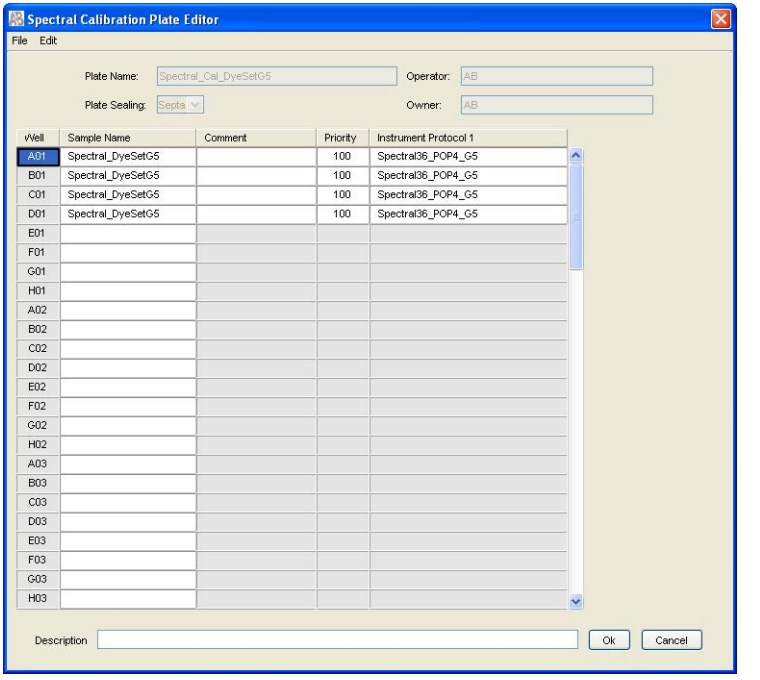
5. Complete the Spectral Calibration Plate Editor.

   a. In the Sample Name column, enter a sample name, then click the next cell. The value 100 automatically displays in the Priority column.

   b. *Optional.* In the Comments column, enter any additional comments or notations for the sample at the corresponding position of the plate.

   c. In the Instrument Protocol 1 column, select **Spectral36_POP4_G5**.

6. Highlight the entire row, then select **Edit > Fill Down Special**. The software automatically fills in the appropriate well numbers for a single run.



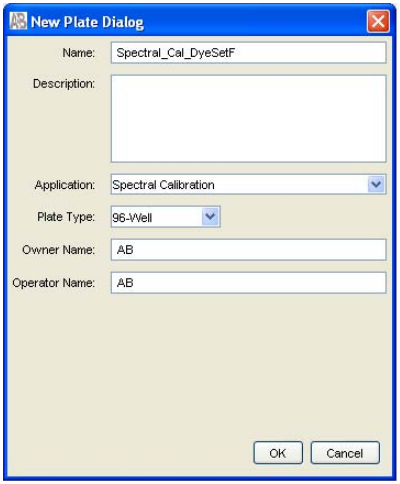
7. Click **OK** to save your changes, then close the plate record.

   IMPORTANT! After clicking **OK** 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.

For Dye Set F

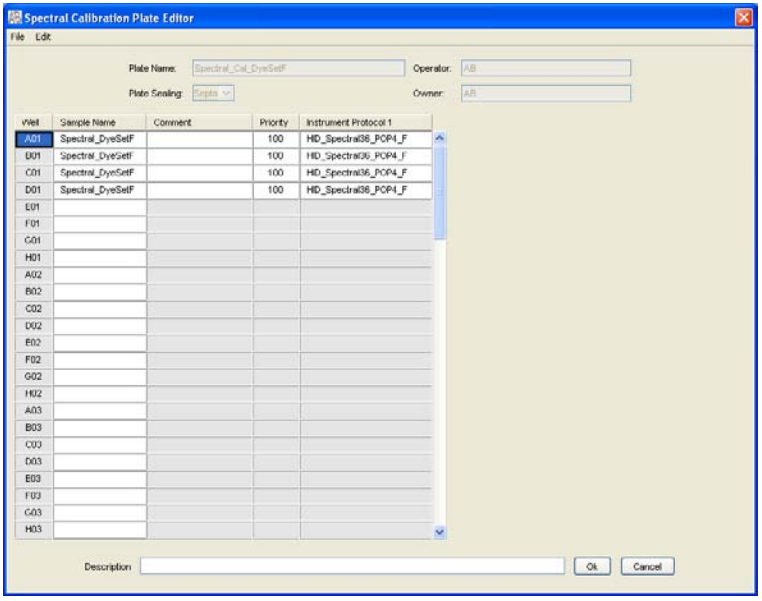
Follow this procedure if you are performing a spectral calibration for kits using a four-dye system, which includes the 5FAM, JOE, NED, and ROX dyes.

1. In the tree pane of the Data Collection software, click **GA Instruments** > **ga3130*xl*** or **ga3130** > **Plate Manager** to open the Plate Manager window.
2. Click **New** to open the New Plate dialog box.
3. Complete the New Plate dialog box.



   a. Name: Type a name for the plate.
   b. Description: If desired, type a description for the plate.
   c. Application: Select **Spectral Calibration** from the drop-down list.
   d. Plate Type: Select **96-Well**.
   e. Owner Name: Type a name for the owner.
   f. Operator Name: Type a name for the operator.

4. Click **OK** to open the Spectral Calibration Plate Editor.
5. Complete the Spectral Calibration Plate Editor.
   a. In the Sample Name column, enter a sample name, then click the next cell. The value 100 automatically displays in the Priority column.
   b. *Optional.* In the Comments column, enter any additional comments or notations for the sample at the corresponding position of the plate.
   c. In the Instrument Protocol 1 column, select **Spectral36_POP4_F**.
6. Highlight the entire row, then select **Edit > Fill Down Special**.



Note: The software automatically fills in the appropriate well numbers for a single run.

7. Click **OK** to save your changes, then close the plate record.

IMPORTANT! After clicking **OK** 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.

Prepare the Spectral Calibration Chemistry

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, which includes the 6-FAM, VIC, NED, PET, and LIZ dyes.

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:

Reagent	Volume (µL) 3130 System	Volume (µL) 3130*xl* System
Matrix Standard Set DS-33	10	10
Hi-Di Formamide	190	190
Final Volume	**200**	**200**

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.

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

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, which includes the 5-FAM, JOE, NED, and ROX dyes.

1. Thoroughly vortex the Matrix Standard Set DS-32 tube for Dye Set F.
2. Spin the tube briefly in a microcentrifuge.
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) 3130 System	Volume (µL) 3130*xl* System
Matrix Standard Set DS-32	10	10
Hi-Di Formamide	190	190
Final Volume	**200**	**200**

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.

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

Troubleshooting: Off-Scale Spectral Calibration Peak Height

New universal matrix standards are available for the multi-capillary electrophoresis instruments (that is, the 3100/3100-*Avant* Genetic Analyzers and the 3130/3130*xl* Genetic Analyzers):

- Matrix Standard Set DS-32 (Dye Set F), PN 4345831
- Matrix Standard Set DS-33 (Dye Set G5), PN 4345833

The new matrix standard sets were designed with improved purity, signal balance, and signal strength. If you obtain off-scale peaks when using the new matrix standards for spectral calibration, follow the procedure below.

Troubleshoot Off-Scale Peak Heights

If the peak height of any one of the matrix standard fragments is saturated (off-scale), re-run the spectral calibration, but use one-half the amount of the matrix standards. The mixture will be twice as dilute as before.

For Dye Set G5

1. Follow the procedure on page 21, "Prepare the Matrix Standards for Dye Set G5."
2. In step 3 on page 21, use the following volumes:

Reagent	Volume (µL) 3130 System	Volume (µL) 3130*xl* System
Matrix Standard Set DS-33	5	5
Hi-Di Formamide	195	195
Final Volume	**200**	**200**

WARNING **CHEMICAL HAZARD. 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: These same instructions are present on the Matrix Standard Set DS-33 product insert.

For Dye Set F

1. Follow the procedure on page 22, "Prepare the Matrix Standards for Dye Set F."
2. In step 3 on page 22, use the following volumes:

Reagent	Volume (µL) 3130 System	Volume (µL) 3130*xl* System
Matrix Standard Set DS-32	5	5
Hi-Di Formamide	195	195
Final Volume	**200**	**200**

CHEMICAL HAZARD. 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: These same instructions are present on the Matrix Standard Set DS-32 product insert.

Performing a Sample Run

In order for data collection and autoanalysis to be successful, each run of samples must have an instrument protocol and results group assigned within a plate record.

Workflow

To perform a run:

Step	Task	See...
1	Set up GeneMapper *ID* Software v3.2 (that is, import panels and bin sets, create analysis methods, etc.). **Note:** This step is only required for autoanalysis	*GeneMapper® ID Software v3.1 and v3.2: Human Identification Analysis Tutorial* (PN 4357520, Rev. A)
2	Create an instrument protocol for HID analysis.	**page 26 of this user bulletin.**
3	Create a results group.	page 115 of the *3130/3130xl Genetic Analyzers GSG.*
4	Create a plate record in GeneMapper *ID* Software v3.2.	**page 28 of this user bulletin.**
5	Prepare your sample chemistry.	**page 32 of this user bulletin.**
6	Load your samples.	**page 33 of this user bulletin.**
7	Place the plate assembly into the instrument.	page 39 of the *3130/3130xl Genetic Analyzers GSG.*
8	Run your sample plate, which includes: • Linking/unlinking a plate • Starting the run • Controlling/monitoring the run • Viewing data	pages 128 to 160 of the *3130/3130xl Genetic Analyzers GSG.*

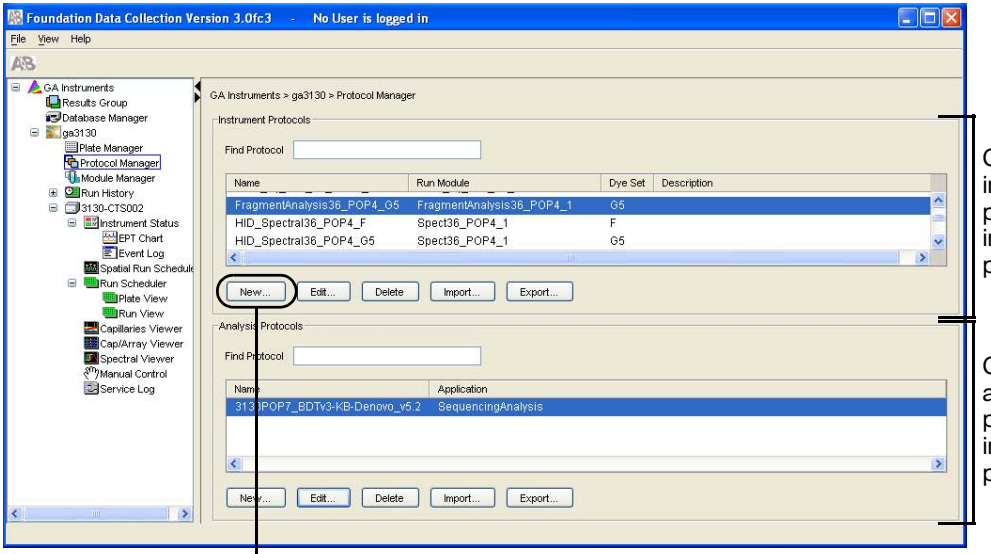
Create an Instrument Protocol

An instrument protocol contains all the settings necessary to run the instrument:

- Protocol name
- Type of run
- Run module
- Dye set

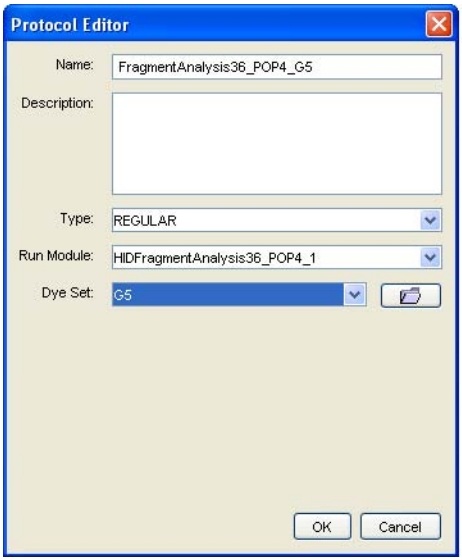
Create an Instrument Protocol

1. In the tree pane of the Data Collection software, click **GA Instruments** > **ga3130*xl*** or **ga3130** > **Protocol Manager** to open the Protocol Manager window.



2. In the Instrument Protocols pane, click **New** to open the Protocol Editor.

3. Complete the Protocol Editor fields:



a. Name: Type a name for the protocol.

b. Description: If desired, type a description for the protocol.

c. Type: Select **REGULAR** from the drop-down list.

d. Run Module: Select **HIDFragmentAnalysis36_POP4_1**.

e. Dye Set: Per the table below, select the correct dye set for your run.

Kit	Dye Set	Matrix Standard Set
• AmpFℓSTR Identifiler PCR Amplification Kit • AmpFℓSTR Yfiler PCR Amplification Kit • AmpFℓSTR SEfiler PCR Amplification Kit	G5	DS-33

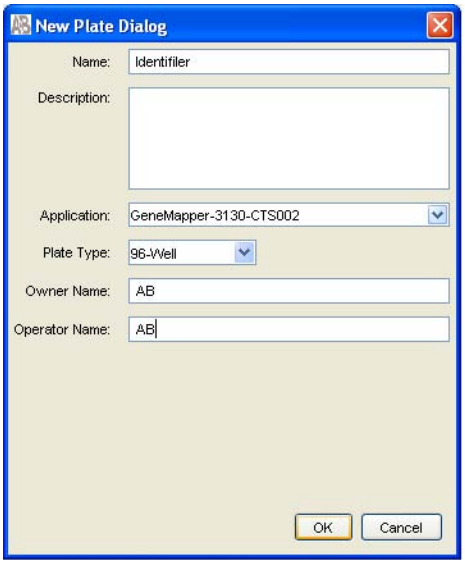
Kit	Dye Set	Matrix Standard Set
• AmpFℓSTR Profiler Plus PCR Amplification Kit • AmpFℓSTR COfiler PCR Amplification Kit • AmpFℓSTR Profiler Plus ID PCR Amplification Kit • AmpFℓSTR SGM Plus PCR Amplification Kit • Other four-dye AmpFℓSTR kits	F	DS-32

4. Click **OK** to save your changes and exit the Protocol Editor.

Create a Plate Record in GeneMapper *ID* Software v3.2

Create a Plate Record

1. In the tree pane of the Data Collection software, click **GA Instruments** > **ga3130*xl*** or **ga3130** > **Plate Manager** to open the Plate Manager window.
2. Click **New** to open the New Plate dialog box.
3. Complete the New Plate dialog box:



a. Name: Type a name for the plate.

b. Description: If desired, type a description for the plate.

c. In the Application drop-down list, select:

- **GeneMapper-Generic** or
- **GeneMapper-<*Computer Name*>**

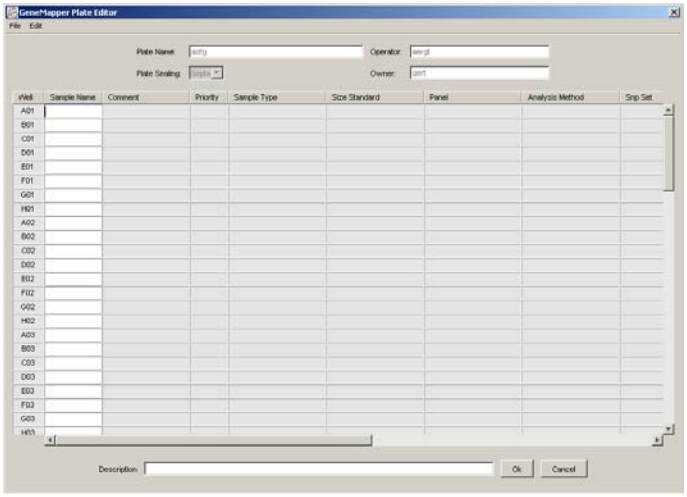
When you are performing autoanalysis, you *must* select the **GeneMapper-<*Computer Name*>** application.

d. Plate Type: Select **96-Well**.

e. Owner Name: Type a name for the owner.

f. Operator Name: Type a name for the operator.

4. Click **OK**. The GeneMapper Plate Editor opens.



5. Continue with one of the following procedures, as appropriate:

- "Complete the Plate Record for Manual Analysis" on page 30
- "Complete the Plate Record for Autoanalysis" on page 30

Complete the Plate Record for Manual Analysis

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. *Optional.* In the Comment column, enter any additional comments or notations for the sample.
3. In the Results Group 1 column, select a group from the drop-down list.
4. In the Instrument Protocol 1 column, select a module from the drop-down list.

   When you use the 3130/3130*xl* Genetic Analyzers with Data Collection Software v3.0 and the AmpFℓSTR kits, Applied Biosystems recommends that you select the **HIDFragmentAnalysis36_POP4_1** module.
5. If you want to perform more than one run:
   a. Select **Edit > Add Sample Run**. Additional Results Group, Instrument Protocol and Analysis Protocol columns are added to the right end of the plate record.
   b. Complete the Results Group and Instrument Protocol columns for the additional runs.
6. Click **OK** to save your changes, then close the plate record.

   IMPORTANT! After clicking **OK** 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.

Complete the Plate Record for Autoanalysis

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:
   - For four-dye AmpFℓSTR kits, select **GS500 ROX**.

- For five-dye AmpFℓSTR kits, select **GS500 LIZ**.

6. In the Panel column, select the appropriate AmpFℓSTR panel from the drop-down list.
7. In the Analysis Method column, select a method from the drop-down list.
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.
11. In the Instrument Protocol 1 column, select a module from the drop-down list.

    When you use the 3130/3130*xl* Genetic Analyzers with Data Collection Software v3.0 and the AmpFℓSTR kits, Applied Biosystems recommends that you select the **HIDFragmentAnalysis36_POP4_1** module.
12. To complete the rest of the plate record based on the samples loaded in your plate, do one of the following:

If the column contains...	Then...
the same sample types and the same protocols	highlight the entire column, then select **Edit > Fill Down Special**. 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.
different sample types and different protocols	complete the plate record manually.

13. If you want to perform more than one run:
    a. Select **Edit > Add Sample Run**. Additional Results Group, Instrument Protocol and Analysis Protocol columns are added to the right end of the plate record.
    b. Complete the columns for the additional runs.

14. Click **OK** to save your changes, then close the plate record.

    IMPORTANT! After clicking **OK** 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.

Prepare Your Sample Chemistry

Perform PCR

To prepare your DNA samples and perform PCR, follow the instructions in the appropriate AmpFℓSTR PCR Amplification Kit user manual.

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.

1. Combine the following in a single microcentrifuge tube:

Preparation Type	Reagent	Volume (µL)	
		Dye Set F	Dye Set G5
Individual sample preparation: For each sample, combine...	GeneScan™-500 ROX™ Size Standard	0.5	—
	GeneScan™-500 LIZ® Size Standard	—	0.3
	Hi-Di™ Formamide	8.5	8.7
Bulk sample preparation: For 16 samples, combine...	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.

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

Load Your Samples

Load the Samples and Allelic Ladder

1. In a 96-well reaction plate:
   - Dispense 9 µL of the formamide:size standard mixture into each well that will contain sample or allelic ladder.
   - Add 10 µL of the formamide to each blank well.

   WARNING **CHEMICAL HAZARD. 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.
2. Load 1 µL of the sample or allelic ladder into the wells.
3. Cover the reaction plate with an appropriate septa. Use:
   - Reservoir septa, or
   - 96-well plate septa if samples for more than one run were prepared
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

1. Assemble the plate assembly:
   a. Place the sample plate into the plate base.
   b. Snap the plate retainer onto the plate and plate base.
2. Verify that the holes of the plate retainer and the septa strip are aligned. If not, re-assemble the plate assembly.

   IMPORTANT! Damage to the array tips will occur if the plate retainer and septa strip holes do not align correctly.

Section 2 Examples of DNA Profiles Generated on the 3130/3130*xl* Genetic Analyzers

This section covers the following topics:

Examples of DNA Profiles Generated on the 3130 Genetic Analyzer

Overview

This section contains examples of data generated on the 3130 Genetic Analyzer during the Verification Study. The experiments conducted on the 3130 Genetic Analyzer are as follows:

- Reproducibility (page 36)
- Mixture Studies (page 38)
- Resolution (page 40)
- Sensitivity (page 41)

All studies were performed using Data Collection Software v3.0 and GeneMapper *ID* Software v3.2. All DNA samples used in the studies were quantitated using the Quantifiler™ Human DNA Quantification Kit.

Reproducibility

Figure 1 shows a comparison of signal intensity across a 3130 instrument capillary array using AmpFℓSTR Control DNA 9948 (1 ng) amplified using the AmpFℓSTR Yfiler PCR Amplification Kit.

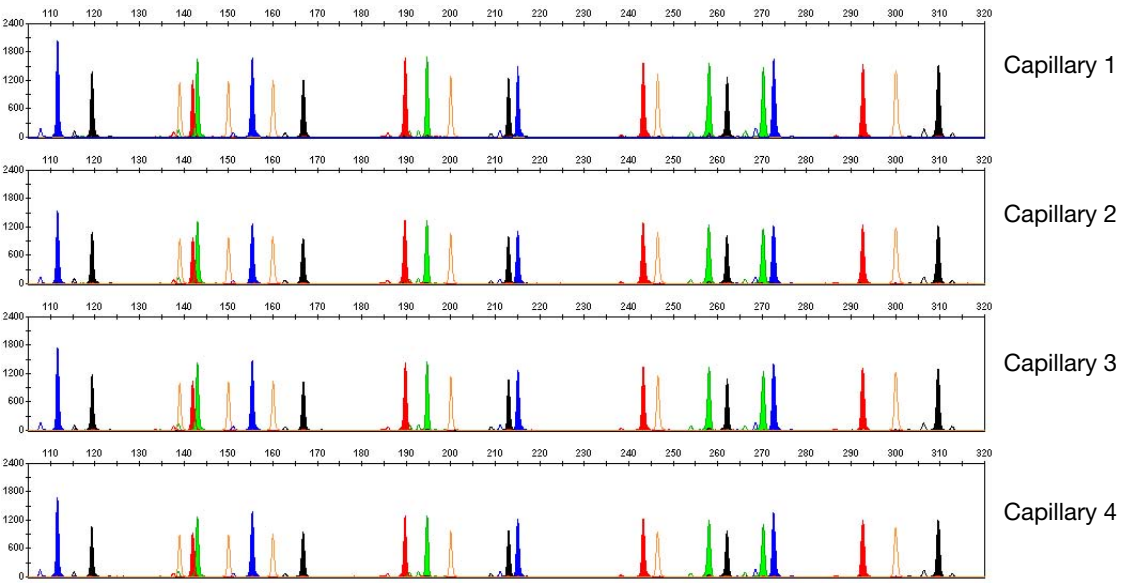


Figure 1 DNA samples amplified using the Yfiler kit

Figure 2 shows a comparison of signal intensity across a 3130 instrument capillary array using AmpFℓSTR Control DNA 9948 (2 ng) amplified using the AmpFℓSTR Profiler Plus PCR Amplification Kit.

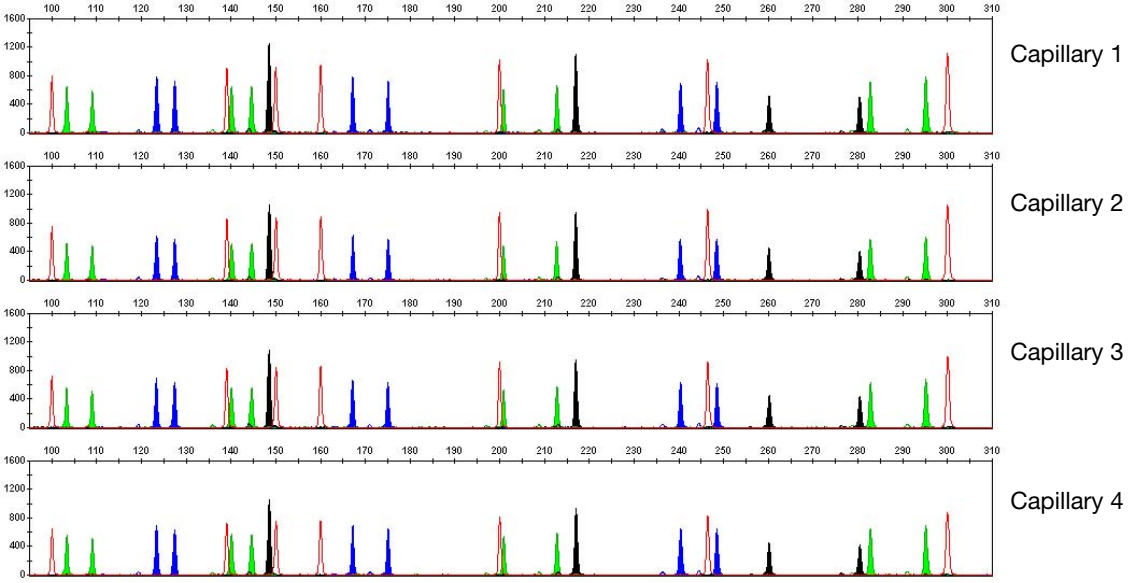


Figure 2 DNA samples amplified using the Profiler Plus kit

Mixture Studies

In Figure 3, the first and last panels display profiles of each DNA sample amplified individually with a total DNA input of 1 ng using the AmpFℓSTR Identifiler PCR Amplification Kit. (The female AmpFℓSTR Control DNA 9947a sample is in the top panel and the male AmpFℓSTR Control DNA 9948 sample is in the bottom panel). The other three panels display the mixtures of DNA at approximate ratios of 1:1, 1:5, and 1:10.

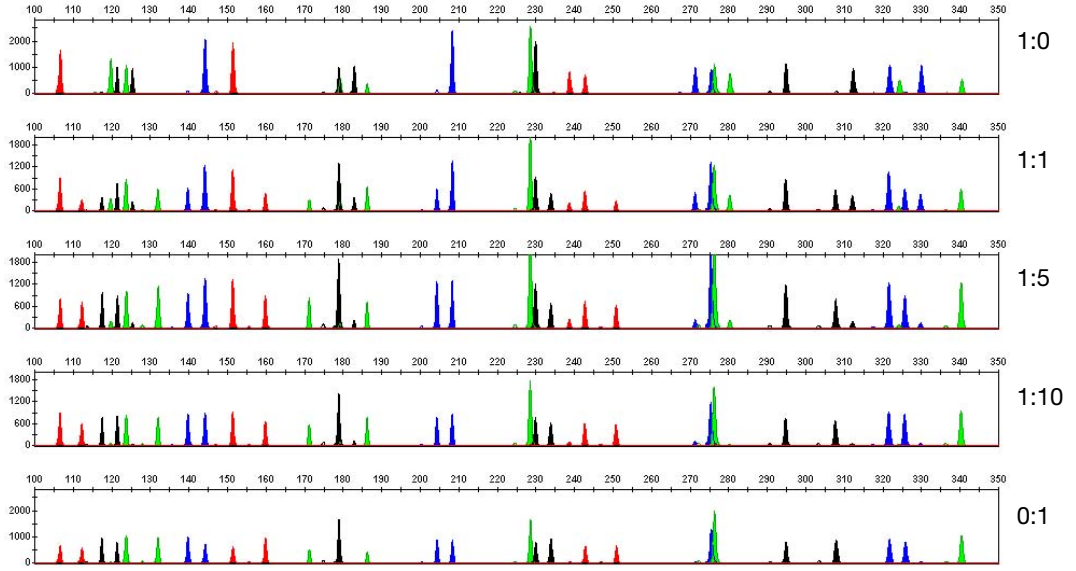


Figure 3 DNA samples amplified using the Identifiler kit

In Figure 4, the first and last panels display profiles of each DNA sample amplified individually with a total DNA input of 2 ng using the AmpFℓSTR Profiler Plus PCR Amplification Kit. (The female AmpFℓSTR Control DNA 9947a sample is in the top panel and the male AmpFℓSTR Control DNA 9948 sample is in the bottom panel). The other three panels display the mixtures of DNA at approximate ratios of 1:1, 1:5, and 1:10.

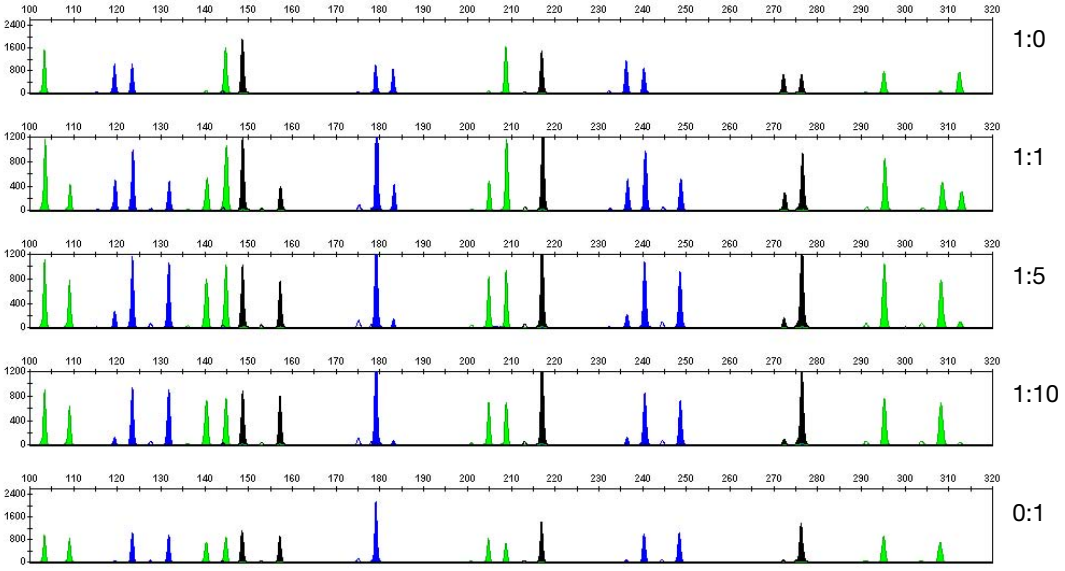


Figure 4 DNA samples amplified using the Profiler Plus kit

Resolution

Figure 5 shows three capillaries of a 3130 instrument 4-capillary array displaying single nucleotide resolution. As shown below, the 9.3 and 10 alleles were resolved and individually detected in all capillaries in which they were run.

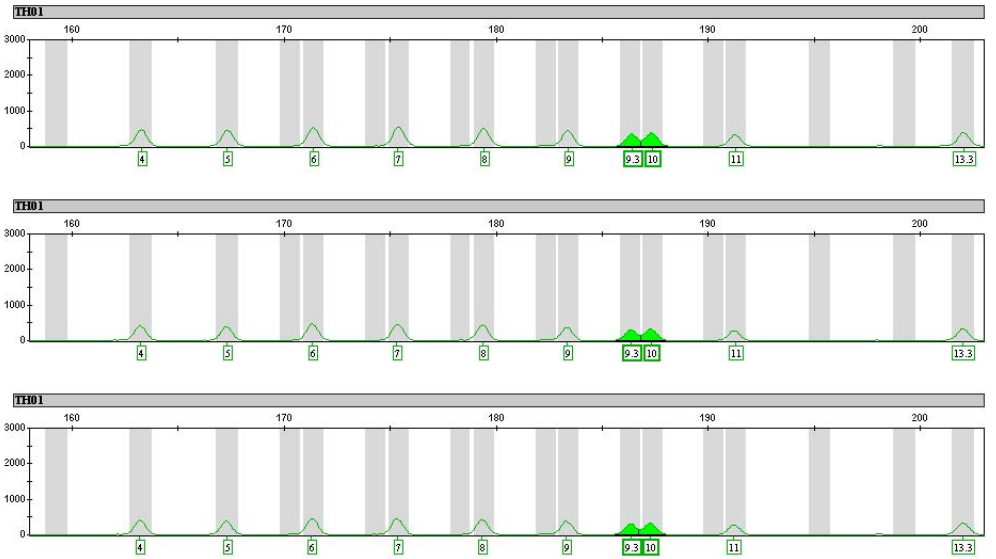


Figure 5 A 4-capillary array displaying single nucleotide resolution

Sensitivity

Figure 6 shows dilutions for the AmpFℓSTR Control DNA 9948 sample quantified using the Quantifiler Human DNA Quantification Kit and amplified using the AmpFℓSTR 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.

With 125 pg of input DNA quantified using the Quantifiler kit, it was observed at the FGA locus that one of the alleles was not detected at a peak amplitude threshold of 50 RFU.

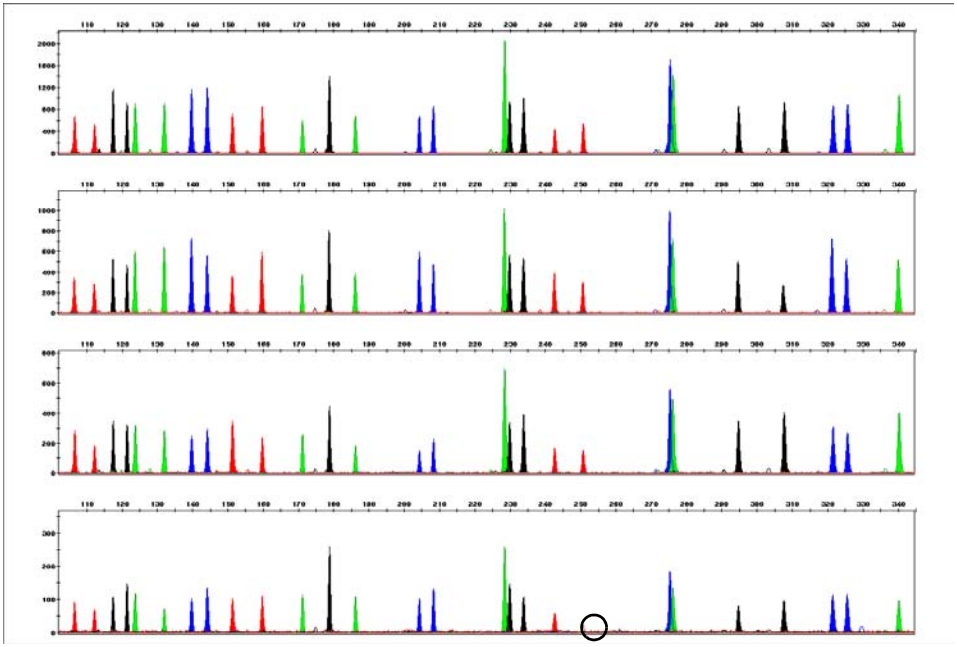


Figure 6 DNA sample amplified using the Identifiler kit

Figure 7 shows dilutions for the AmpFℓSTR Control DNA 9948 sample amplified using the Yfiler 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.

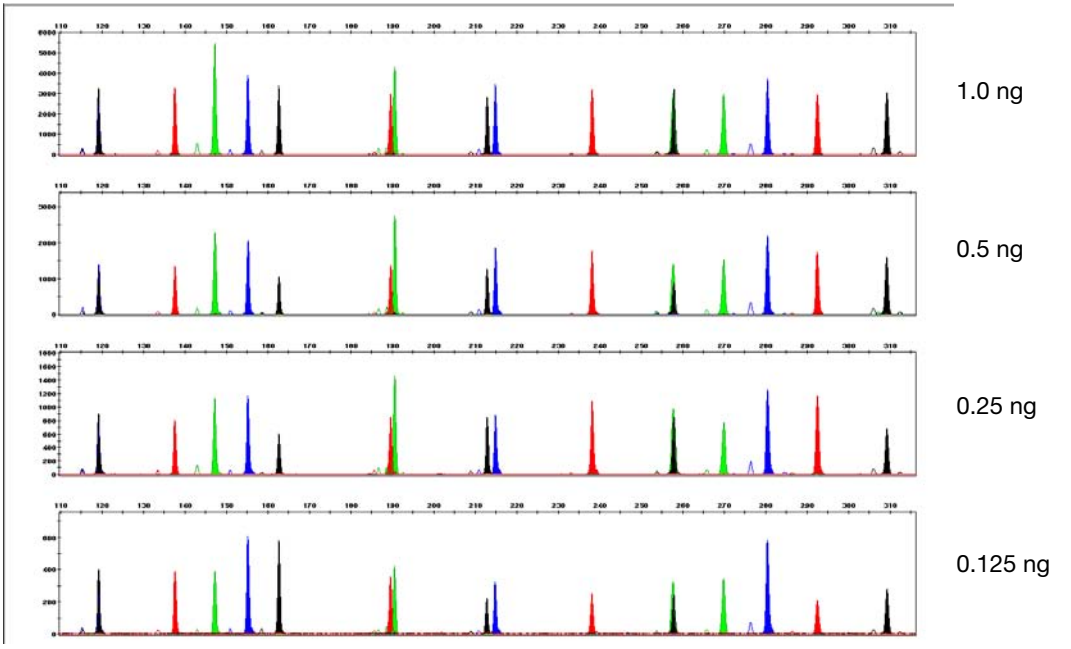


Figure 7 DNA sample amplified using the Yfiler kit

Figure 8 shows dilutions for the AmpFℓSTR Control DNA 9948 sample amplified using the Profiler Plus 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.

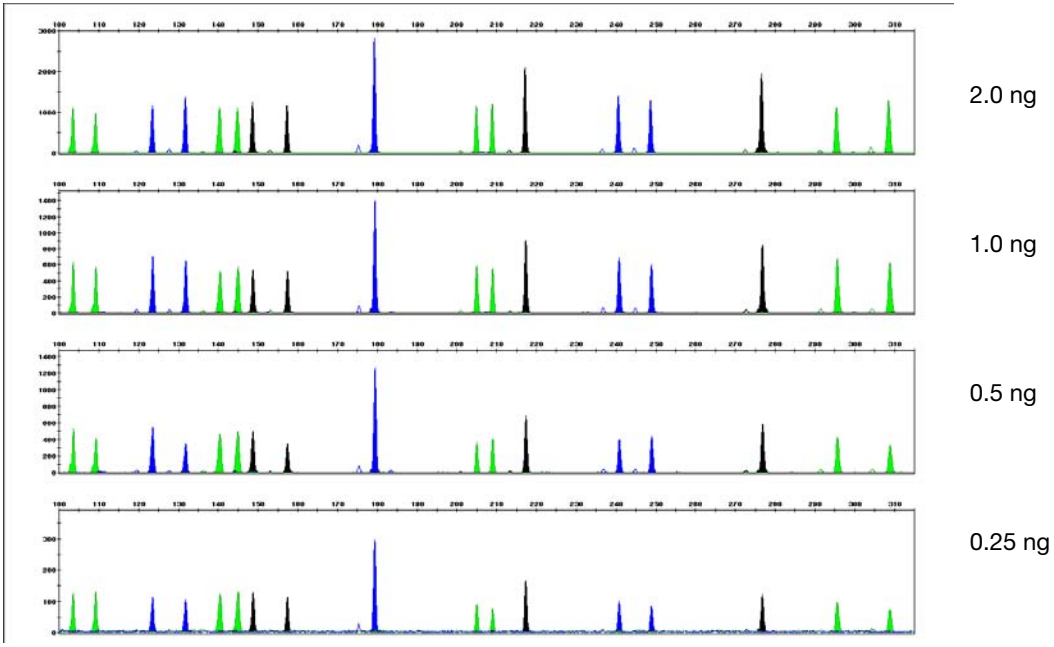


Figure 8 DNA sample amplified using the Profiler Plus kit

Examples of DNA Profiles Generated on the 3130*xl* Genetic Analyzer

Overview

This section contains examples of data generated on the 3130*xl* Genetic Analyzer during the Verification Study. The experiments conducted on the 3130*xl* Genetic Analyzer are as follows:

- Reproducibility (page 44)
- Mixture Studies (page 46)
- Resolution (page 49)
- Sensitivity (page 50)

All studies were performed using Data Collection Software v3.0 and GeneMapper *ID* Software v3.2. All DNA samples used in the studies were quantitated using the Quantifiler Human DNA Quantification Kit.

Reproducibility

Figure 9 shows a comparison of signal intensity across a 3130*xl* instrument capillary array using AmpFℓSTR Control DNA 9948 (1 ng) amplified using the AmpFℓSTR Yfiler PCR Amplification Kit.

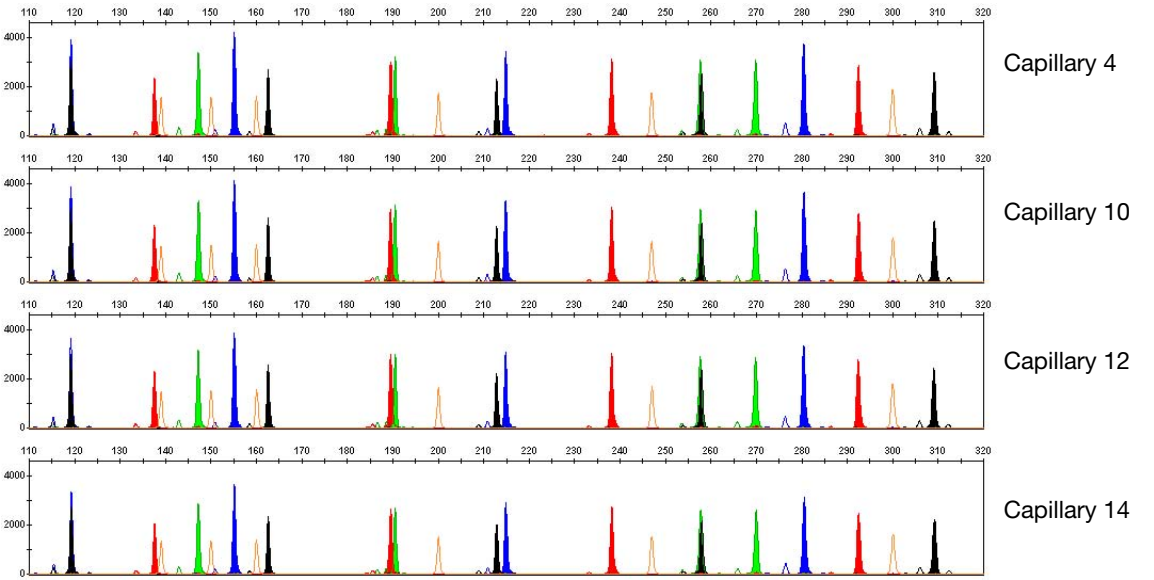


Figure 9 DNA samples amplified using the Yfiler kit

Figure 10 shows a comparison of signal intensity across a 3130*xl* instrument capillary array using AmpFℓSTR Control DNA 9948 (2 ng) amplified using the AmpFℓSTR Profiler Plus PCR Amplification Kit.

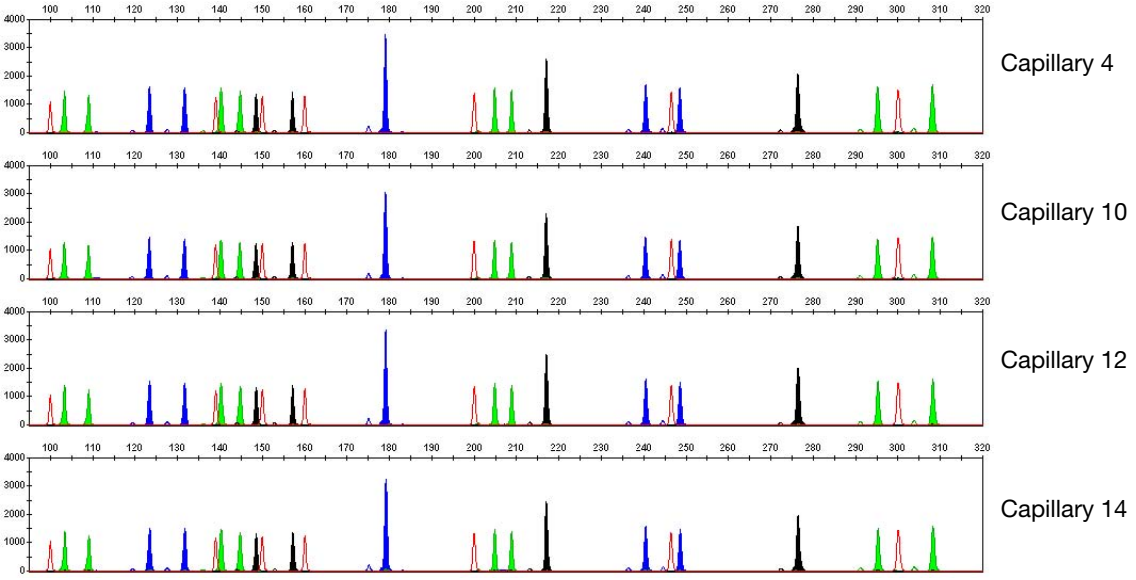


Figure 10 DNA samples amplified using the Profiler Plus kit

Mixture Studies

In Figure 11 on page 47, the first and last panels display profiles of each DNA sample amplified individually with a total DNA input of 1 ng using the AmpFℓSTR Identifiler PCR Amplification Kit. (The female AmpFℓSTR Control DNA 9947a sample is in the top panel and the male AmpFℓSTR Control DNA 9948 sample is in the bottom panel). The other three panels display the mixtures of DNA at approximate ratios of 1:1, 1:5, and 1:10. The panel inset displays the expanded view of the DNA samples mixed at an approximate ratio of 1:10.

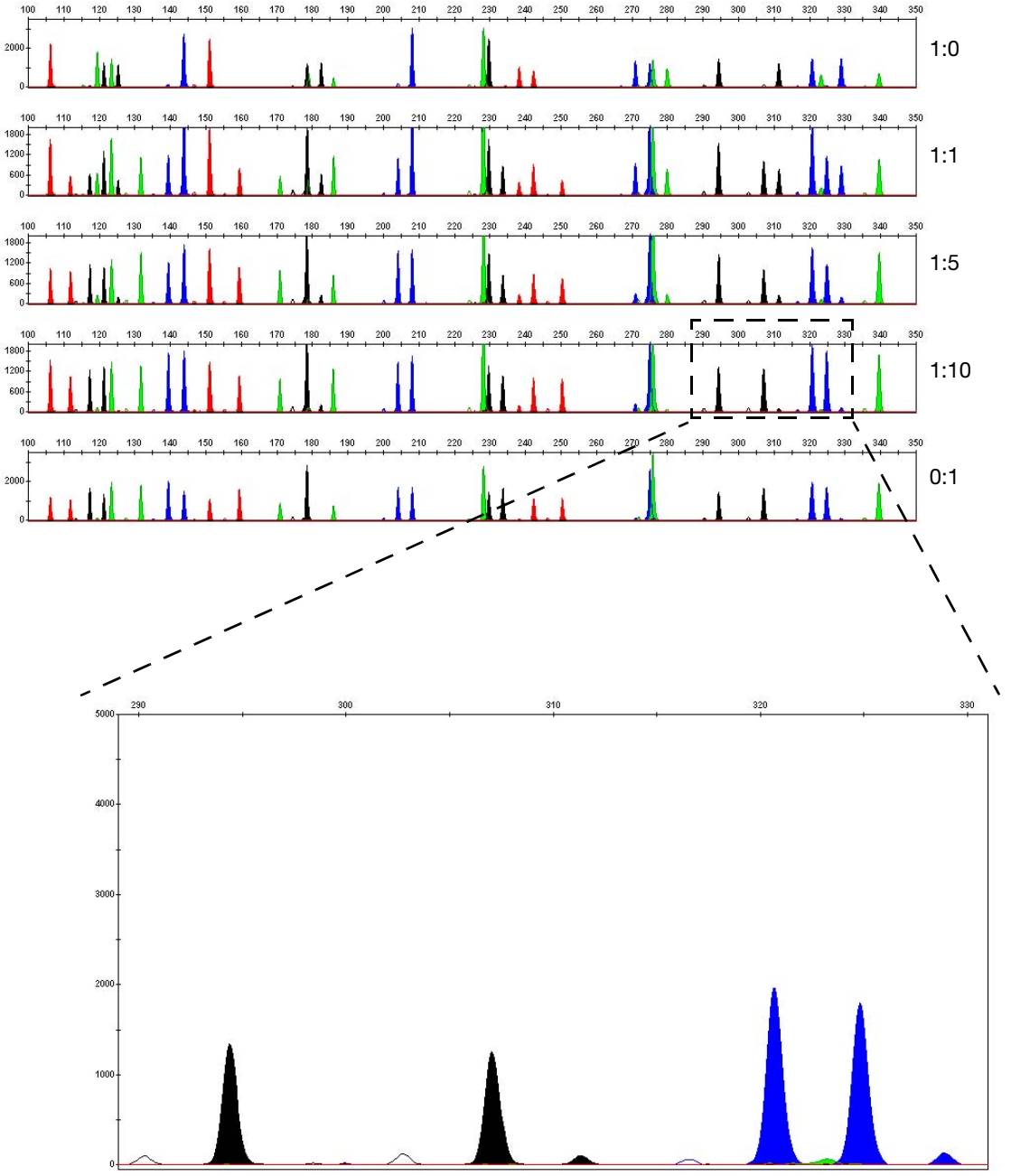


Figure 11 DNA samples amplified using the Identifiler kit

In Figure 12, the first and last panels display profiles of each DNA sample amplified individually with a total DNA input of 2 ng using the AmpFℓSTR Profiler Plus PCR Amplification Kit. (The female AmpFℓSTR Control DNA 9947a sample is in the top panel and the male AmpFℓSTR Control DNA 9948 sample is in the bottom panel). The other three panels display the mixtures of DNA at approximate ratios of 1:1, 1:5, and 1:10.

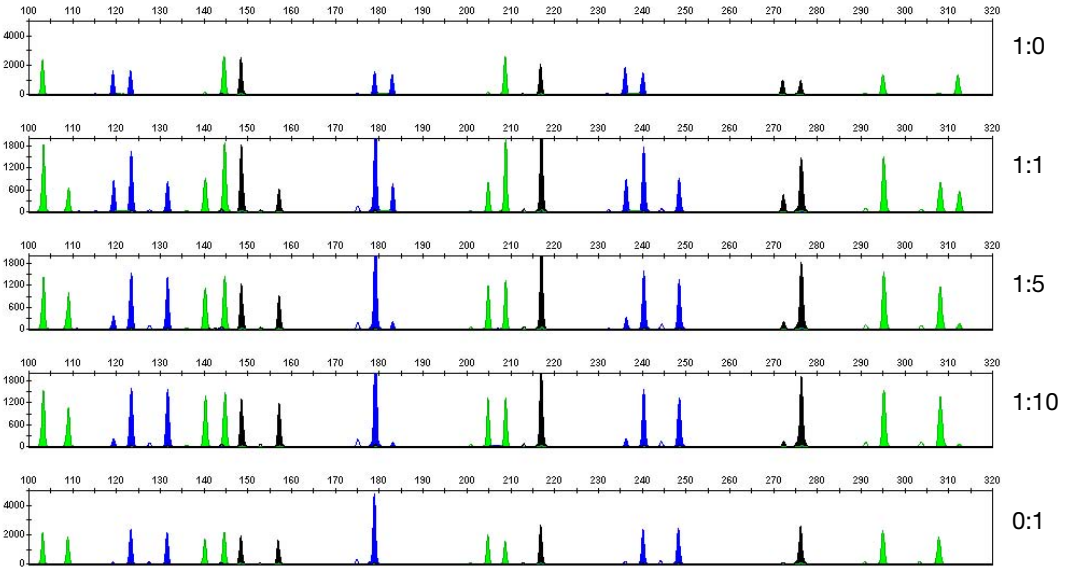


Figure 12 DNA samples amplified using the Profiler Plus kit

Resolution

Figure 13 shows three capillaries of a 3130xl instrument 16-capillary array displaying single nucleotide resolution. As shown below, the 9.3 and 10 alleles were resolved and individually detected in all capillaries in which they were run.

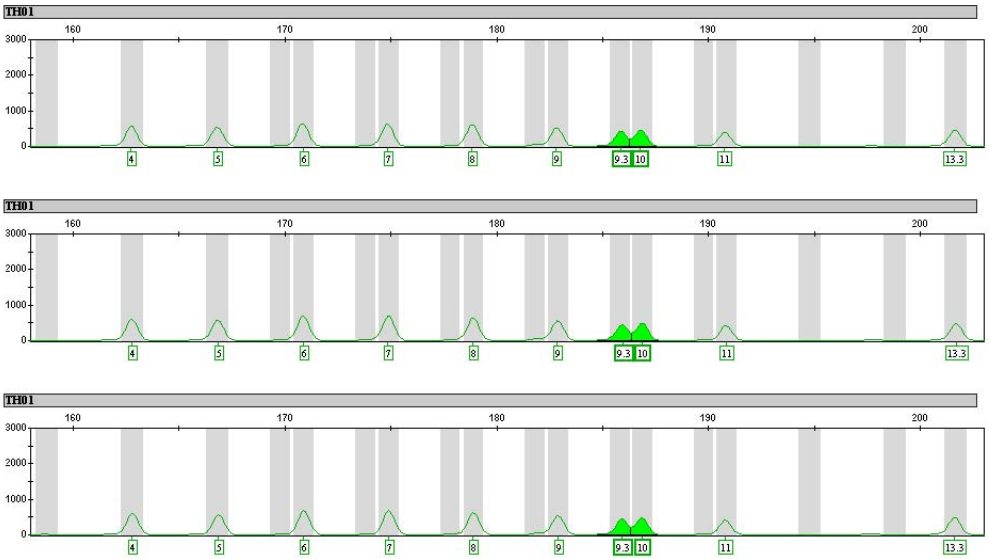


Figure 13 A 16-capillary array displaying single nucleotide resolution

Sensitivity

Figure 14 shows dilutions for the AmpFℓSTR Control DNA 9948 sample amplified using the AmpFℓSTR 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.

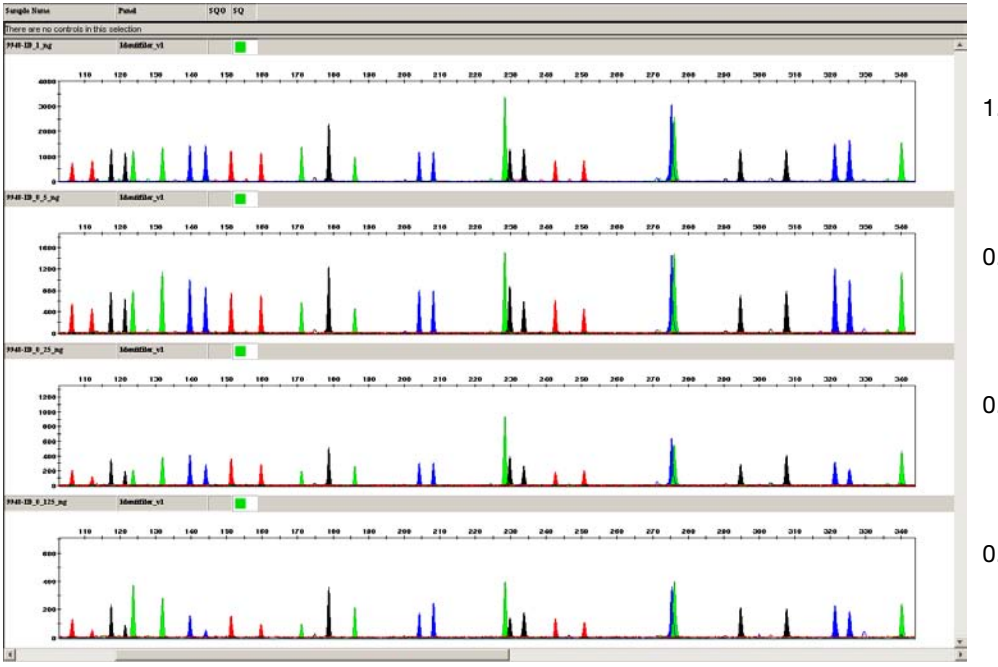


Figure 14 DNA sample amplified using the Identifiler kit

Figure 15 shows dilutions for the AmpFℓSTR Control DNA 9948 sample amplified using the Yfiler 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.

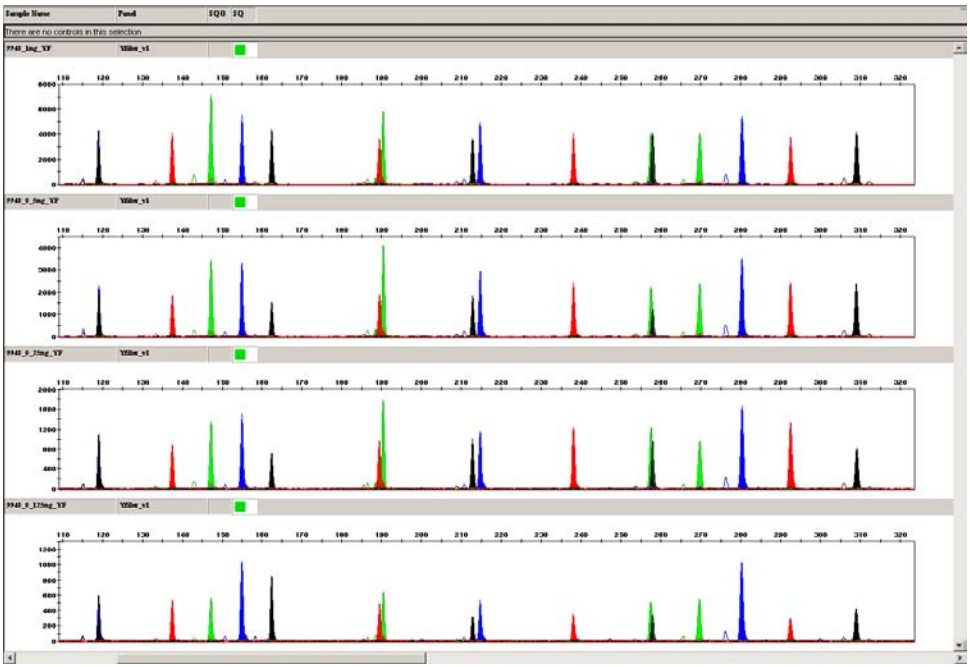


Figure 15 DNA sample amplified using the Yfiler kit

Figure 16 shows dilutions for the AmpFℓSTR Control DNA 9948 sample amplified using the Profiler Plus 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.

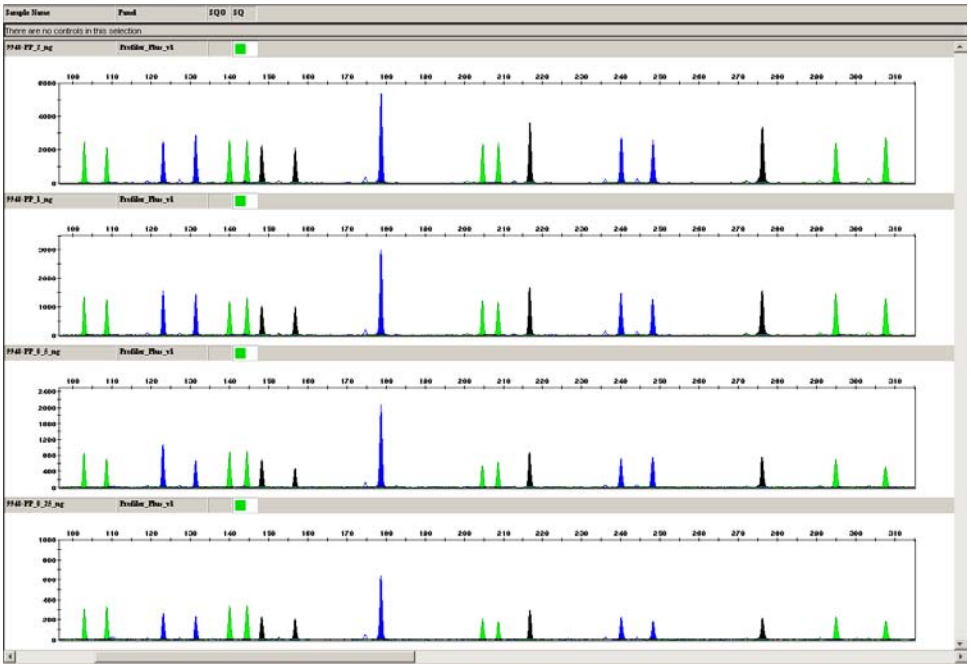


Figure 16 DNA sample amplified using the Profiler Plus kit

Genotype Concordance

The data summarized in Table 1 below were obtained by amplifying the positive Control DNA 007, 9947a, and 9948 samples using the AmpFℓSTR Identifiler and Profiler Plus PCR Amplification Kits. Additionally, Control DNA 007 and 9948 samples were amplified with the AmpFℓSTR Yfiler PCR Amplification Kit.

The samples were prepared for electrophoresis by combining the following:

- For the four-dye kit: 1 µL of PCR product with 8.5 µL of Hi-Di™ formamide and 0.5 µL of GeneScan™-500 ROX™ Size Standard
- For the five-dye kits: 1 µL of PCR product with 8.7 µL of Hi-Di™ formamide and 0.3 µL of GeneScan™-500 LIZ® Size Standard

Each DNA sample was replicated six times on the plate and injected twice on one 3130 Genetic Analyzer and two 3130*xl* Genetic Analyzers, for a total of 36 datapoints per allele. The Data Collection Software v3.0 run module for fragment analysis (HIDFragmentAnalysis36_POP4_1) was used to run the samples. Data were analyzed using GeneMapper *ID* Software v3.2 to evaluate genotype concordance.

Table 1 Genotype concordance

Control DNA Sample	AmpFℓSTR Kit	Total Number of Alleles Evaluated	Concordant Alleles
007	Identifiler kit	1044	100%
007	Yfiler kit	612	100%
007	Profiler Plus kit	648	100%
9947a	Identifiler kit	936	100%
9947a	Profiler Plus kit	540	100%
9948	Identifiler kit	972	100%
9948	Yfiler kit	612	100%
9948	Profiler Plus kit	612	100%

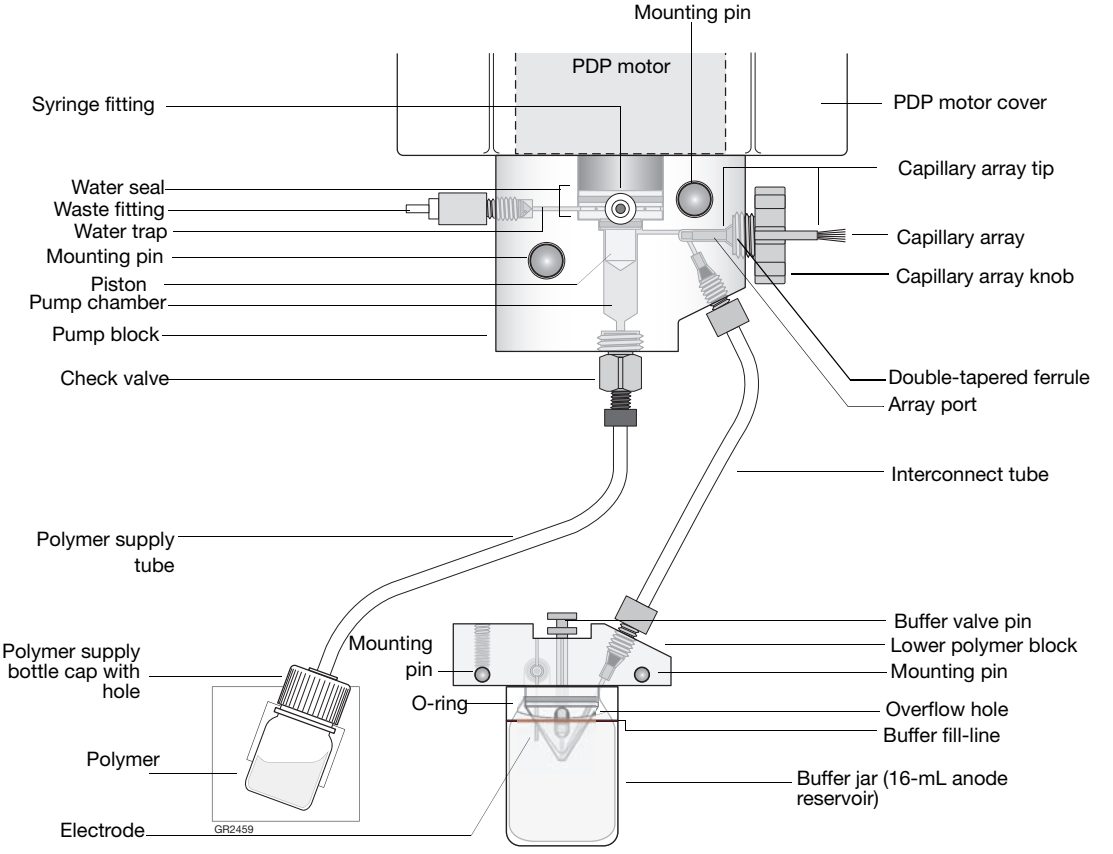
Appendix A Maintenance

Note: For your convenience, this appendix is reproduced from the *Applied Biosystems 3130/3130xl Genetic Analyzers Maintenance, Troubleshooting, and Reference Guide* (PN 4352716, Rev. B).

This appendix covers the following topics:

Polymer Delivery Pump

Components of the polymer delivery pump (PDP) are identified in the drawing below.



Performing Maintenance Tasks

This section lists common tasks required to maintain your Applied Biosystems 3130/3130*xl* Genetic Analyzers in good working condition. The tasks are divided into tables based on how often you should perform each task.

WARNING Wear appropriate protection, including gloves, laboratory goggles, and coat whenever you work with the fluids used on this instrument, or parts that may come into contact with these fluids.

Daily Tasks

Perform these tasks at least once per day.

Maintenance Task	Frequency
Ensure adequate levels of buffer and water in reservoirs.	Before each run
Ensure the plate assemblies are properly assembled. **IMPORTANT!** The holes in the plate retainer must align with the holes in the septa, or the capillary tips will be damaged.	Before each run
Ensure the plate assemblies are positioned on the plate deck properly. Plates should sit snugly on the deck. **IMPORTANT!** Never use warped plates.	Before each run
Check the level of buffer in the buffer jar. Ensure that the overflow hole faces the front of the instrument and is not occluded.	Before each run
Replace the water and 1X running buffer in the reservoirs on the instrument and make sure that the outside of the assemblies are dry.	Every 48 hours
Check for bubbles in the pump block, lower polymer block, interconnect tube, polymer supply tube, and channels. Remove all bubbles with the Bubble Remove wizard.	Daily or before each run
Check the loading-end header to ensure the capillary tips are not crushed or damaged.	Daily or before each run
Check the level of polymer in the bottle to ensure sufficient volume for runs.	Daily or before each run

Maintenance Task	Frequency
Check the pump block and the lower polymer block to ensure they fit securely on the instrument.	Daily
Clean the instrument surfaces.	Daily
Check for leaks around the array knob, interconnecting tube nuts, and check valve.	Daily

Weekly Tasks

Perform these tasks at least once per week.

Maintenance Task	Frequency
Replace the polymer using the Replenish Polymer Wizard.	Weekly or as needed
Check the storage conditions of the used arrays.	Weekly
Restart the computer and instrument.	Weekly

Monthly Tasks

Perform these tasks at least once per month.

Maintenance Task	Frequency
Run the Water Wash Wizard. Flush the array port during this wizard, whether or not bubbles are present in the array port.	Monthly or as needed
Flush the water trap. See “Flushing and Filling the Water Trap” on page 68.	Monthly or as needed
Defragment the hard drive.	Monthly

As-Needed Tasks

Perform these tasks as needed.

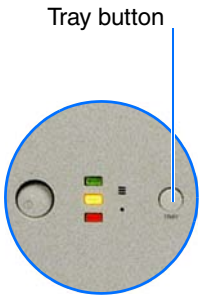
Maintenance Task	Frequency
Clean the drip tray.	As needed
Change the array.	As needed
Remove any dried polymer from the capillary tips. Use a lint-free wipe moistened with deionized water.	As needed

Routine Cleaning

General Cleaning

1. Ensure the oven and instrument doors are closed.
2. Press the Tray button on the front of the instrument to move the autosampler to the forward position.

   IMPORTANT! Never use organic solvents to clean the instrument.



3. Wipe off any liquid on or around the autosampler using a lint-free tissue.
4. Clean off any polymer build-up (crystals) on the instrument including the capillary tips and the stripper plate with deionized water and lint-free tissue.
5. Clean the array port knob, plug, or opening threads of these parts with moistened lab wipes.
6. Clean out the drip trays with deionized water and lint-free tissue.

Resetting the Instrument

Reset the instrument when:

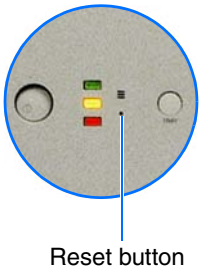
- A fatal error as indicated by the red status light
- The instrument does not respond to the Data Collection software

Two procedures can reset the instrument:

- Press the reset button through the pin hole on the front of the instrument to dump and reload the firmware and to reset the electronics. Try this method first.
- Shut down and restart the computer and the instrument.

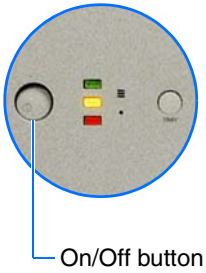
Resetting With the Reset Button

1. Close the instrument doors.
2. Using a long narrow implement, such as a straightened paper clip, press the reset button on the front of the instrument.



Resetting by Powering Down

1. Close the instrument doors.
2. Power off the instrument by pressing the on/off button on the front of the instrument.



3. Restart the computer.
    a. Select **Start** > **Turn off Computer**.

b. In the dialog box, select **Restart**, then click **OK**.

IMPORTANT! Wait until the computer has completely restarted before proceeding.

4. Turn on the instrument, then wait for the solid green light.
5. Launch the Data Collection software (Service Console applications start automatically).

Moving and Leveling the Instrument

CAUTION **PHYSICAL INJURY HAZARD**. Do not attempt to lift the instrument or any other heavy objects unless you have received related training. Incorrect lifting can cause painful and sometimes permanent back injury. Use proper lifting techniques when lifting or moving the instrument. Two or three people are required to lift the instrument, depending upon instrument weight.

6. Remove the following components from the instrument:
   - Any plate assemblies from the autosampler.
   - Water and buffer reservoirs from the autosampler.
   - Capillary array, by selecting Instrument Shutdown Wizard. (See "Performing a Long-Term Shutdown" on page 65.)
   - Anode buffer reservoir.
7. Switch off the breaker on the back of the instrument.
8. Disconnect the power cord and the Ethernet cable.

   IMPORTANT! While moving the instrument, avoid any shock or vibration.
9. Move the instrument.
10. Place the bubble level on the autosampler deck.
11. Turn the instrument legs to level the instrument.

To move the instrument corner ...	Turn the leg ...
up	right (clockwise)
down	left (counterclockwise)

Shutting Down the Instrument

Perform the appropriate shutdown procedure based on the information in the following table:

If the instrument will be unattended for ...	Perform this shutdown procedure ...
no more than 1 week with a full buffer reservoir	Short-term **IMPORTANT!** The key to a successful short-term shutdown is keeping the capillary array in 1× running buffer. This prevents the polymer from drying in the capillaries. **CAUTION** **CHEMICAL HAZARD. 1× 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.
for more than 1 week	Long-term

Performing a Short-Term Shutdown

Fill the Capillary With Fresh Polymer Using Manual Control

1. Ensure the oven and instrument doors are closed.
2. Collect polymer waste**:**
   a. Click **GA Instruments** > **ga3130** or **ga3130*xl***> *instrument name*> **Manual Control**.
   b. In the Send Defined Command drop-down menu, select **Autosampler**.
   c. In the Command Name drop-down menu, select **Move autosampler to site**.
   d. In the Value menu, select **Waste**.
   e. Click **Send Command**. Wait for the autosampler to stop moving and Send Command becomes active, before proceeding.
3. Fill the capillaries:
   a. In the Send Defined Command for drop-down menu, select **Polymer Delivery Pump**.
   b. In the Command Name, select the appropriate Fill <length> cm capillary array length.
   c. Click **Send Command**.The array fill is finished when Send Command becomes active.
   d. Return the buffer reservoir to the capillaries.

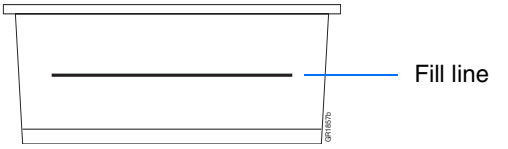
Cleaning the Reservoirs

1. Press the Tray button to move the autosampler forward.
2. Open the doors, then remove the:
   - Plates
   - Cathode buffer reservoir and water reservoirs
3. Dispose of remaining fluids and rinse out the reservoirs with deionized water.

   Note: Follow your company's waste disposal practices for appropriate disposal procedures.

4. Rinse the cathode reservoir with 1× running buffer, and then fill to the line with 1× running buffer (about 16 mL).

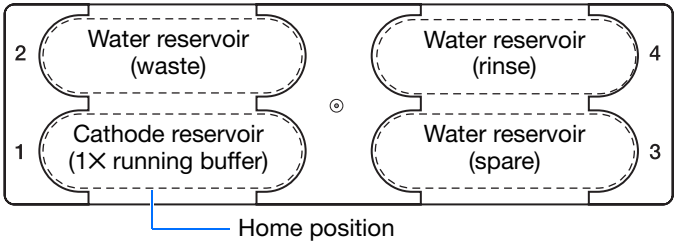
CAUTION **CHEMICAL HAZARD. 1× Genetic Analyzer Buffer with EDTA** may cause eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.



5. Fill the three water reservoirs to the line with quality deionized water (about 16 mL).

CAUTION Be sure that the septa fit snugly and flush on the tops of the reservoirs in order to prevent damaging the capillary tips.

6. Place a clean reservoir septa on each reservoir, and dry the outside of the reservoirs using a lint-free wipe.
7. Place the reservoirs into position on the autosampler as shown below.



8. Close the instrument doors.

   Note: Closing the doors returns the autosampler to the home position, placing the tips of the capillaries in buffer.

9. Shut down the computer and turn off the instrument.

Performing a Long-Term Shutdown

IMPORTANT! Make sure all parts are completely dry before long-term storage.

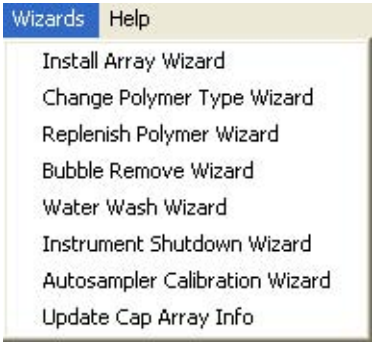
Select **Instrument Shutdown Wizard** and follow the prompts.

Wizards

Accessing Wizards

In the tree pane of the Data Collection software, click **GA Instruments** > **ga3130** or **ga3130*xl*** > *instrument name* or any topic name below instrument name to see Wizards in the menu bar.

The wizards in the Data Collection software guide you through several maintenance procedures.



If plates are linked in the Run Scheduler and you complete a wizard, the plates automatically unlink. You will get a warning dialog box. Click OK, and then relink the plate if applicable.

General Use Guidelines

The following table lists the wizards and when to use them.

Wizard	Use to...
Install Array	• Install a capillary array: – On a new instrument – To reactivate an instrument that has been shut down • Replace an installed capillary array with another capillary array

Wizard	Use to...
Change Polymer Type	• Change to a different polymer type than the one presently being used
Replenish Polymer	• Replenish the polymer supply • Replace the polymer in the PDP with polymer of the same or different lot • Enter polymer information when Data Collection software is installed or upgraded
Bubble Remove	Remove bubbles in the PDP chamber, channels, and tubing
Water Wash	• Wash the PDP chamber, lower polymer block[a], channels, and tubing with water: – As part of a monthly maintenance protocol – To remove any suspected contaminants in the PDP – To remove persistent bubbles (followed by the Bubble Remove Wizard, if needed) – To replace old polymer in the PDP
Instrument Shutdown	Prepare the instrument for a period of disuse of greater than one week
Autosampler Calibration	Calibrate the autosampler positions
Update Cap Array Info	• Update the capillary array information and the serial number • Correct an entry mistake after using a wizard

a. The lower polymer block is cleaned on the instrument using this wizard and should not be removed.

Flushing and Filling the Water Trap

The PDP water trap should be flushed with either distilled or deionized water at least once per month to wash out any diluted polymer and to clear bubbles. Leave the trap filled with either distilled or deionized water.

To flush the water seal trap:

1. Fill the supplied 20 mL, all-plastic Luer lock syringe (PN 4324463) with distilled or deionized water. Expel any bubbles from the syringe.

   IMPORTANT! Do not use a syringe smaller than 20 mL. Doing so may generate excessive pressure within the trap.

2. Attach the syringe to the forward-facing Luer fitting at the top of the pump block. Hold the fitting with one hand while threading the syringe onto the fitting with the other hand.

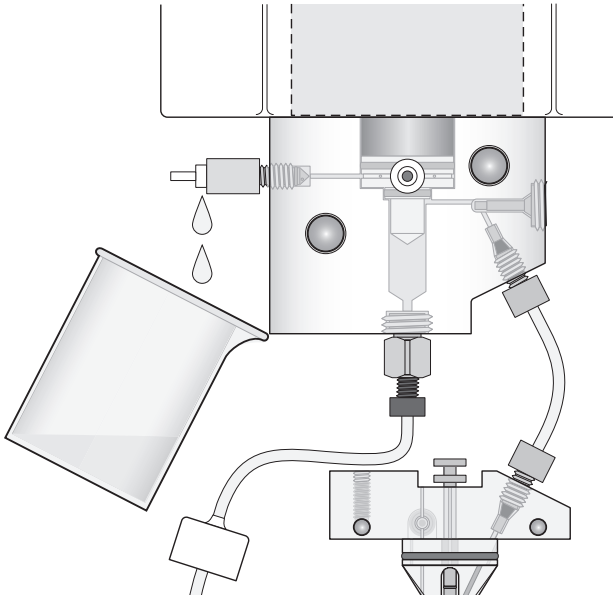
3. Open the Luer fitting by grasping the body of the fitting and turning it and the attached syringe approximately one-half turn counterclockwise.

4. Open the exit fitting at the top left side of the pump block by turning it approximately one-half turn counterclockwise.

5. Hold an empty tube or beaker under the exit fitting to receive approximately 5 mL of waste. Flush the trap by pushing steadily on the syringe plunger.

   IMPORTANT! DO NOT USE EXCESSIVE FORCE when you push the syringe plunger as this may damage the trap seals. Take approximately 30 seconds to flush 5 mL of either distilled or deionized water through the trap.

   Note: Because the water trap volume is approximately 325 µL, a relatively small volume of water is adequate for complete flushing. However, a larger volume only improves flushing as long as force and flow rate are kept within the limits given above.



6. Close the fittings in this order by turning each clockwise until the fittings seal against the block:
   a. Luer fitting
   b. Exit fitting

   IMPORTANT! Do not over-tighten the fittings. Very little pressure develops within the trap during pump operation, so the fittings require only enough tightening to prevent water leaks. Excessive tightening can damage the fittings.

   c. Remove the syringe from the Luer fitting. Hold the fitting with one hand while turning the syringe counterclockwise with the other hand.

Fluids and Waste

Buffer

When to Change the Buffer

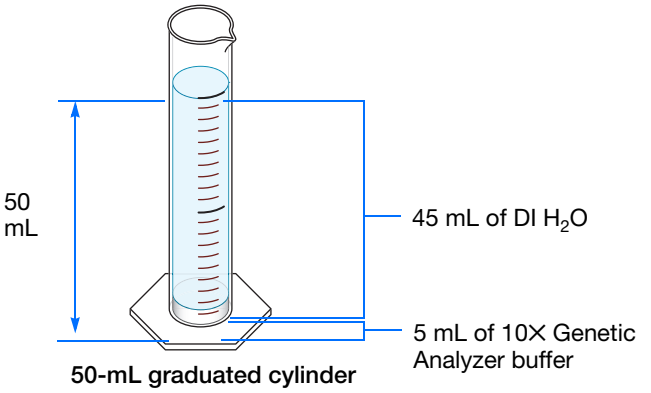
CAUTION CHEMICAL HAZARD. 10× Genetic Analyzer Buffer with EDTA may cause eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Change the buffer before each batch of runs or at least every 24 hours.

Making Buffer for a Single Run

To prepare 50 mL of 1× running buffer:

1. Into a graduated cylinder, add:
   a. 5 mL of 10× Genetic Analyzer buffer
   b. 45 mL of deionized water to bring the total volume up to 50 mL.

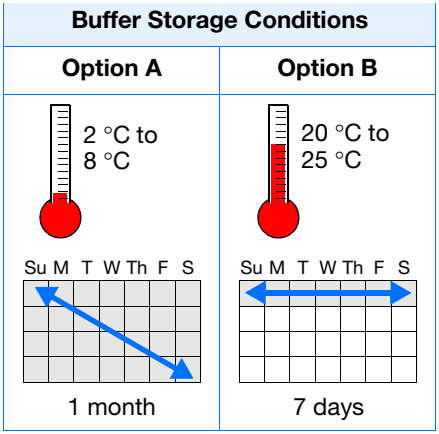


2. Mix well.

Storing the Buffer

The 1× running buffer can be stored at:

- 2 to 8 °C for up to 1 month
- Room temperature for 1 week



Polymer

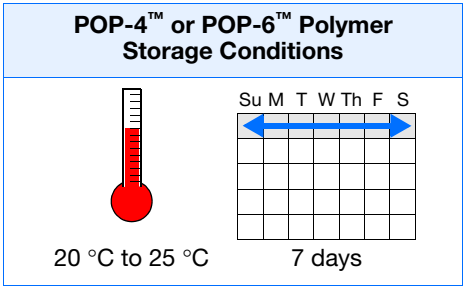
Storing Polymer

Store any remaining POP™ polymer at 2 to 8 °C until the expiration date printed on the jar.

Note: Excessively hot environments may shorten the working life of the polymer.

When to Change the Polymer

Change the polymer weekly. The polymer is good at 25 °C for about 7 days.



Before Using the Polymer

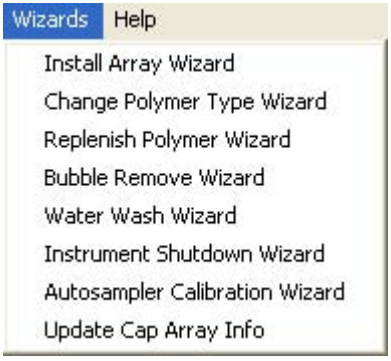
1. Remove the polymer from 4 °C storage.
2. Loosen the cap and bring the polymer to room temperature.
3. To dissolve deposits, tighten the cap and gently swirl the polymer.

Replenishing the Polymer

WARNING **CHEMICAL HAZARD. POP™ polymers** cause eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

IMPORTANT! Wear gloves when you handle the polymer.

1. Select **Wizards** > **Replenish Polymer Wizard**.



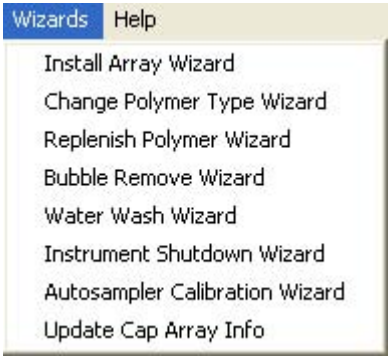
2. Follow the directions given in the wizard to load fresh polymer on the instrument.
3. Relink plate(s), if applicable.

Changing to a Different Polymer Type

WARNING **CHEMICAL HAZARD. POP™ polymers** cause eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

IMPORTANT! Wear gloves when you handle the polymer.

1. Select **Wizards** > **Change Polymer Type Wizard**.



2. Follow the wizard prompts.

Capillary Array

When to Change a Capillary Array

A capillary array should last approximately 100 runs.

The following problems may indicate that a new capillary array is required:

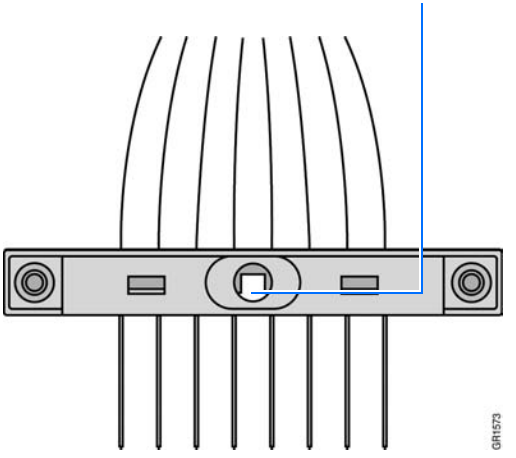
- Poor sizing precision or allele calling
- Poor resolution and/or decreased signal intensity

Checking the Cathode Bar

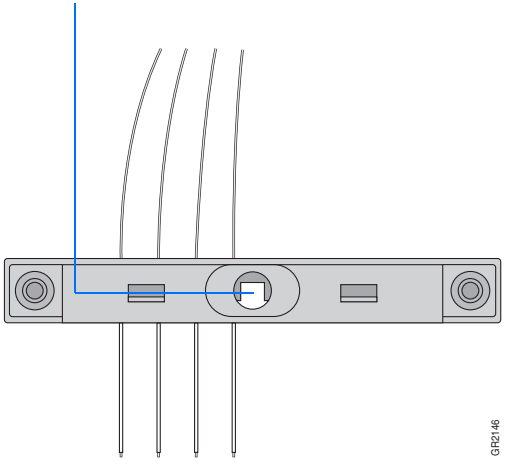
WARNING **ELECTRICAL SHOCK/FIRE HAZARD.** Do not leave liquid on the cathode header. This can lead to electric shock or even fire if not properly maintained.

When placing a used array back on the instrument, be sure that the cathode bar is dry (see figure below). A wet bar could lead to arcing.

Back view: Ensure the cathode header is dry – especially in the center



3130*xl* instrument capillary array



3130 instrument capillary array

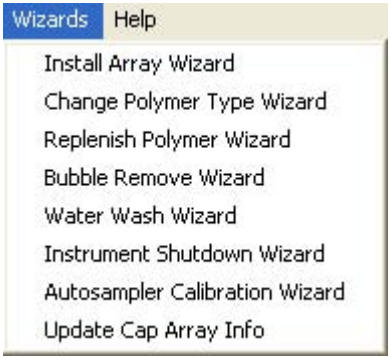
Installing, Removing, or Replacing a Capillary Array

IMPORTANT! Wear gloves when you handle the polymer blocks.

WARNING CHEMICAL HAZARD. POP™ polymers cause eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

1. Close the oven and instrument doors, and then press the Tray button.
2. Select **Wizards** > **Install Array Wizard**.

   IMPORTANT! The capillary array length defined in the wizard must match the array length you are using.



3. Open instrument and oven doors.
4. Follow the directions given in the wizard to install or replace an array.
5. Click Finish when done.
6. Close and lock the oven door, then close the instrument doors.

   IMPORTANT! If you installed or replaced an array that is a different length than the one you were using, you must reset the active spectral calibration or create a new spectral calibration for the dye set and array length combination (see "Activating a Spectral Calibration" in the *Applied Biosystems 3130/3130xl Genetic Analyzers Getting Started Guide*).

7. Relink plate(s), if applicable.

Updating Capillary Array Information

Use the Update Cap Array Info wizard to:

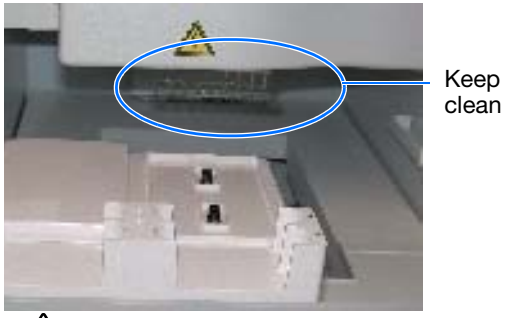
- Update the capillary array length and serial number information into the database
- Correct an entry error after using another wizard

IMPORTANT! The capillary array length defined in the wizard must match the array length you are using.

Caring for the Capillary Array and Work Area

Follow these guidelines to properly care for the capillary array and area:

- Wear gloves and handle the capillary array gently.
- Do not touch the detection cell.
- Keep the ends of the capillary array wet at all times.
- Do not overtighten the capillary array knob.
- Clean off any polymer buildup (crystals) on the instrument, including the capillary electrodes and the stripper plate, with deionized water and lint-free tissue.



WARNING **CHEMICAL HAZARD. POP™ polymers** cause eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Note: When cleaning the capillary electrodes, be careful not to bend them out of position. If the electrodes do get bent, follow the procedure "Storing Capillary Arrays" on page 77.

IMPORTANT! Never use organic solvents to clean the instrument.

Filling the Capillary Array Using Manual Control

See "Fill the Capillary With Fresh Polymer Using Manual Control" on page 63.

Storing Capillary Arrays

Storing a Capillary Array on the Instrument

Store the capillary array on the instrument only when the capillary array will be unused for less than 1 week.

To store the capillary array on the instrument, follow the instructions to perform a short-term shutdown described on page 63.

Storing a Capillary Array off the Instrument

Store the capillary array off of the instrument when the capillary array will be unused for longer than 1 week.

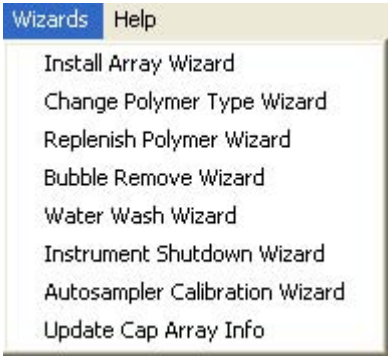
IMPORTANT! Before storing the capillary array for long periods, fill the capillaries with fresh polymer.

IMPORTANT! Wear gloves while performing the following procedure, and any other time you handle the capillary array, septa, or buffer reservoirs.

WARNING **CHEMICAL HAZARD. POP™ polymers** cause eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

CAUTION **CHEMICAL HAZARD. 1× 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.

1. Remove the capillary array from the instrument by selecting **Install Array Wizard**.



2. Select **Store Array** and follow the prompts.
3. Replace the cover over the detection cell.
4. Fill a buffer reservoir with fresh 1× running buffer and cover with a septa strip. Insert the capillary tips into the buffer.
5. Fill the shipping vial with fresh 1× running buffer and insert the detection end of the capillary array.
6. Store the capillary array upright.
7. Check the 1× running buffer level in the reservoir and tube weekly and replenish the buffer as needed.

Removing Bubbles from the Polymer Blocks

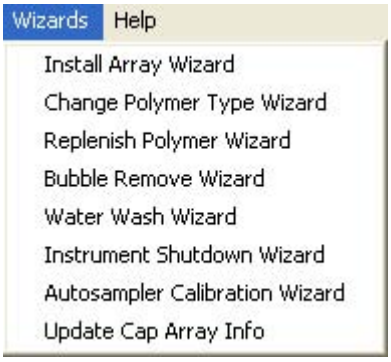
Bubbles may be found in the polymer system, especially after a polymer change or array installation.

Remove the bubbles from all parts of the polymer system including the pump chamber, the pump block channel, polymer supply and interconnect tubing and the lower polymer block channel.

To clear bubbles:

1. Select **Wizards** > **Bubble Remove Wizard** to clear bubbles.

   IMPORTANT! Remove bubbles from the interconnect tubing and the channel of the lower polymer block. These areas are part of the electrophoresis current path. Absence of bubbles in the current path is important for problem-free electrophoresis.



2. Replace the 1× running buffer if excess polymer is expelled into the anode buffer jar during bubble removal.

   CAUTION CHEMICAL HAZARD. 1× 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.

Autosampler Calibration

When to Calibrate the Autosampler

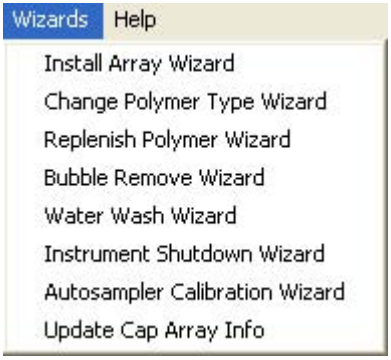
Calibrate the autosampler only as needed.

Symptoms of autosampler alignment problems may include:

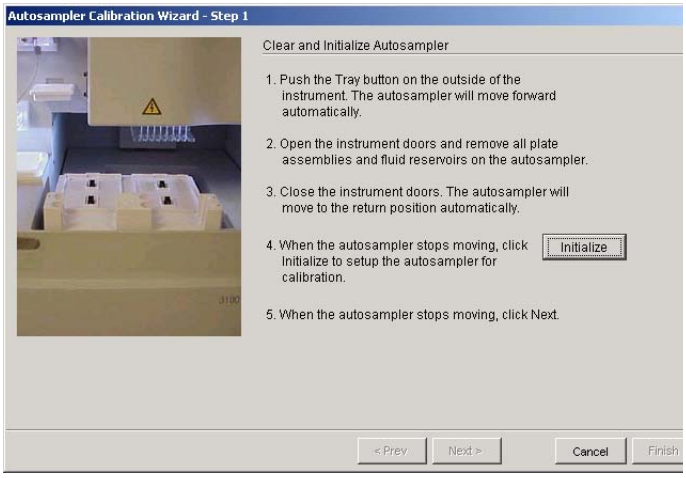
- Poor injection for a small number of capillaries
- Low signal strength
- No evidence of sample

Calibrating the Autosampler

1. Close the oven and instrument doors.
2. Select **Wizards** > **Autosampler Calibration Wizard**.



3. If plates are linked in the Run Scheduler, the plates automatically are unlinked. In the Warning dialog box, click OK.
4. Follow the directions given in the wizard to calibrate the autosampler.



5. Click Finish.
6. Turn the instrument power off for 10 sec, then on.

Manual Control

Note: Manual control is active only if the oven and instrument doors are closed.

Table of Commands

The following table displays the manual control options in the Data Collection software.

Command Function	Command Options	Value
Electrophoresis	Turn On/Off power supply	• On • Off
	Set voltage	A number between 0.0 and 15 kV
	Read voltage	N/A
	Read current	
Laser	Set laser state	• Idle • On • Off
	Set laser power	A number between 0 and 25 mW
	Read laser power setting	N/A
	Read laser power	
	Read laser current	
	Open/Close shutter	• Open • Closed
Oven	Set state	• On • Off
	Set temperature	A number between 18 and 65 °C

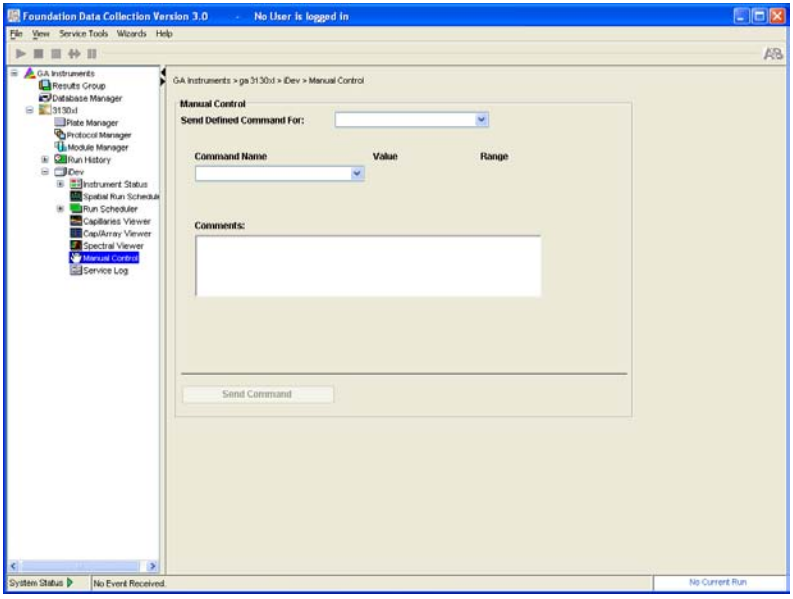
Command Function	Command Options	Value
Autosampler	Initialize autosampler	N/A
	Bring autosampler to forward position	
	Initialize and return to previous position	
	Move autosampler up/down	A number between –500 and 500 steps
	Move autosampler to site	• Buffer (left, front for 1× running buffer), home position • Water1 (left, rear for deionized water) • Water2 (right, front for deionized water) • Waste (right, rear for deionized water)
Polymer Deliver Pump	Initialize polymer delivery pump	N/A
	Home piston	
	Read piston position	
	Move piston down	1 to 38000 counts
	Move piston up	1 to 38000 counts
	Fill capillary array	1 to 38000 counts
Buffer Valve	Close /Open buffer valve	• Close • Open
Detection Cell Heater	Turn On/Off detection cell heater	• On • Off
	Read detection cell heater temperature	N/A

Command Function	Command Options	Value
Oven	Turn On/Off oven	• On • Off
	Set oven temperature	A number between 18 and 70 °C
	Read oven temperature	N/A

Using Manual Control

Note: Manual control functions cannot be used during a run.

1. In the tree pane of the Data Collection software, click **GA Instruments** > **ga3130** or **ga3130*xl***> *instrument name*> **Manual Control**.



2. In the Send Defined Command For drop-down list, select a function.

3. In the Command Name drop-down list, select a command and enter a value, if required.

   Note: The command names are filtered based the function selected in step 2.

4. Click Send ....

Information in this document is subject to change without notice. Applied Biosystems assumes no responsibility for any errors that may appear in this document. This document is believed to be complete and accurate at the time of publication. In no event shall Applied Biosystems be liable for incidental, special, multiple, or consequential damages in connection with or arising from the use of this document.

Applied Biosystems 3130/3130*xl* Genetic Analyzers:

For Research Use Only. Not for use in diagnostic procedures.

NOTICE TO PURCHASER:

This instrument is Authorized for use in DNA sequencing and fragment analysis. This authorization is included in the purchase price of this instrument and corresponds to the up-front fee component of a license under process claims of U.S. patents and under all process claims for DNA sequence and fragment analysis of U.S. patents now or hereafter owned or licensable by Applied Biosystems for which an Authorization is required, and under corresponding process claims in foreign counterparts of the foregoing for which an Authorization is required. The running royalty component of licenses may be purchased from Applied Biosystems or obtained by using Authorized reagents purchased from Authorized suppliers in accordance with the label rights accompanying such reagents. Purchase of this instrument does not itself convey to the purchaser a complete license or right to perform the above processes. This instrument is also licensed under U.S. patents and apparatus and system claims in foreign counterparts thereof. No rights are granted expressly, by implication or by estoppel under composition claims or under other process or system claims owned or licensable by Applied Biosystems. For more information regarding licenses, please contact the Director of Licensing at Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.

NOTICE TO PURCHASER:

The purchase price of the Applied Biosystems 3130/3130*xl* Genetic Analyzers includes a grant of a limited, non-transferable license under U.S. Patent and method claims of its foreign counterparts, to use this particular instrument for electrophoresis methods employing fluorescence as a means of detection. No other licenses or rights are hereby conveyed either expressly, by implication, or estoppel including, but not limited to, any claims to a composition.

The Applied Biosystems 3130/3130*xl* Genetic Analyzers includes patented technology licensed from Hitachi, Ltd. as part of a strategic partnership between Applied Biosystems and Hitachi, Ltd., as well as patented technology of Applied Biosystems.

GeneMapper® *ID* Software:

For Research, Forensic or Paternity Use Only. Not for use in diagnostic procedures.

NOTICE TO PURCHASER: LICENSE DISCLAIMER

Purchase of this software product alone does not imply any license under any process, instrument or other apparatus, system, composition, reagent or kit rights under patent claims owned or otherwise controlled by Applied Biosystems, either expressly or by estoppel.

GeneMapper *ID* Software version 3.2 has undergone a verification process defined by Applied Biosystems. However, human identification laboratories analyzing forensic, paternity, databasing, and single-source samples that choose to use GeneMapper *ID* Software for data analysis should perform their own appropriate validation studies.

TRADEMARKS:

ABI PRISM, AmpF*l*STR, Applied Biosystems, COfiler, GeneMapper, Identifiler, LIZ, PET, Profiler Plus, SGM Plus, and VIC are registered trademarks and AB (Design), Applera, GeneScan, Hi-Di, iScience (Design), JOE, NED, POP, POP4, POP-6, POP-7, Quantifiler, ROX, SEfiler, Yfiler, 5FAM, and 6FAM are trademarks of Applied Biosystems or its subsidiaries in the U.S. and/or certain other countries.

All other trademarks are the sole property of their respective owners.

iScience. To better understand the complex interaction of biological systems, life scientists are developing revolutionary approaches to discovery that unite technology, informatics, and traditional laboratory research**.** In partnership with our customers, Applied Biosystems provides the innovative products, services, and knowledge resources that make this new, **Integrated Science** possible**.**

Worldwide Sales and Support

Applied Biosystems' vast distribution and service network, composed of highly trained support and applications personnel, reaches 150 countries on six continents. For sales office locations and technical support, please call our local office or refer to our Web site at **www.appliedbiosystems.com**.

Applied Biosystems is committed to providing the world's leading technology and information for life scientists.

Headquarters
850 Lincoln Centre Drive
Foster City, CA 94404 USA
Phone: +1 650.638.5800
Toll Free (In North America): +1 800.345.5224
Fax: +1 650.638.5884

Applied Biosystems

Part Number 4363787 Rev. B