User Bulletin

ABI PRISM® 310 Genetic Analyzer

February 7, 2003

SUBJECT: **Protocols for Processing AmpFℓSTR® PCR Amplification Kit Products with the Microsoft® Windows NT® Operating System**

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Before You Begin

Applicable AmpFℓSTR Kits

PCR products generated from any of the AmpFℓSTR kits may be used with the protocols described in this user bulletin.

Software Requirements

The protocols in this user bulletin require the following software and files to be installed on your computer:

- Microsoft Windows NT operating system
- 310 Data Collection Software v3.0 (PN 4326986)
- ABI PRISM® GeneScan® Analysis Software v3.7.1
- ABI PRISM® Genotyper® Software v3.7
- Appropriate run module file
  - GS STR POP4 (1 mL) G5v2 module file or GS STR POP4 (1 mL) G5 module file
  - GS STR POP4 (1 mL) F module file

Note: For more information about the G5v2 module, see the *ABI PRISM® 310 Genetic Analyzer User Bulletin: G5v2 Module For Use with Dye Set 33 (DS-33)* (PN 4339367).

Applied Biosystems

Product Usage

Use the software products as shown in Table 1.

Note: Wherever G5v2 appears in this document, both G5v2 and G5 apply.

Table 1 Product usage

Product	Usage
310 Data Collection Software v3.0	• Running AmpFℓSTR PCR Amplification Kit products • Collecting five-dye and four-dye data
Filter Set G5v2 module files	Running samples, controls, and matrix standards
Matrix Standard Set DS-33 (6-FAM™, VIC®, NED™, PET™, and LIZ® dyes) for the 310/377 systems using the GS STR POP4 (1 mL) G5v2 module	Creating the required matrix file for Dye Set 33 (DS-33)
Filter Set F module files	Running samples, controls, and matrix standards
Matrix Standard Set DS-32 (5-FAM™, JOE™, NED™, and ROX™ dyes) for the 310/377 systems using the GS STR POP4 (1 mL) F module	Creating the required matrix file for Dye Set 32 (DS-32)
ABI PRISM® GeneScan® Analysis Software for the Windows NT operating system, v3.7.1	Detecting peaks and sizing data
ABI PRISM® Genotyper® Software v3.7	Analyzing and genotyping kit data

Filter Set G5v2 Module Files Requirements

The GS STR POP4 (1 mL) G5v2 module file must be used for Dye Set 33 (DS-33) (6-FAM, VIC, NED, PET, and LIZ dyes) on the ABI PRISM 310 Genetic Analyzer.

Note: Wherever Filter Set G5v2 appears in this document, both Filter Set G5v2 and Filter Set G5 apply.

Processing AmpFℓSTR PCR Amplification Kit products that use 6-FAM, VIC, NED, PET, and LIZ dyes requires:

1. Installing the Filter Set G5v2 module file on the instrument computer, if not already present.
2. Creating a matrix file using the Matrix Standard Set DS-33 (6-FAM, VIC, NED, PET, and LIZ dyes) for the 310/377 system, using the GS STR POP4 (1 mL) G5v2 module.
3. Running samples on the 310 instrument using Filter Set G5v2.
4. Analyzing samples with the matrix file created using Filter Set G5v2.

IMPORTANT! Do not apply the G5v2 matrix file to data analyzed using the G5 module. A matrix file produced using the G5v2 module does not use the same virtual filter settings as a matrix file produced using the G5 module. When using the G5v2 module to collect sample data, use a matrix file created using the G5v2 module.

Filter Set F Module Files Requirements

The GS STR POP4 (1 mL) F module file must be used for Dye Set 32 (DS-32) (5-FAM, JOE, NED, and ROX dyes) on the ABI PRISM 310 Genetic Analyzer.

Processing AmpFℓSTR PCR Amplification Kit products that use 5-FAM, JOE, NED, and ROX dyes requires:

1. Creating a matrix file using the Matrix Standard Set DS-32 (5-FAM, JOE, NED, and ROX dyes) for the 310/377 system, using the GS STR POP4 (1 mL) F module.
2. Running samples on the 310 instrument using Filter Set F.
3. Analyzing samples with the matrix file created using Filter Set F.

Dedicated Equipment and Supplies

Equipment

The equipment and supplies necessary or recommended for running AmpFℓSTR kit data on the ABI PRISM 310 Genetic Analyzer are listed in the tables below. Unless otherwise noted, many of the items listed are available from major laboratory suppliers (MLS).

Note: Amplified DNA, equipment, and supplies used to handle amplified DNA should not be taken out of the amplified DNA work area. Samples that have not yet been amplified should never come into contact with these supplies and equipment.

Table 2 Equipment

Equipment	Source
Instruments	
ABI PRISM 310 Genetic Analyzer	See your Applied Biosystems sales representative
GeneAmp® PCR System 9700	
ABI PRISM 310 Genetic Analyzer Accessories	
1.0 mL Glass Syringe	Applied Biosystems (PN 4304471)
310 Capillaries, 47 cm × 50 µm i.d. (internally uncoated)	Applied Biosystems (PN 402839)
Genetic Analyzer Buffer Vials, 4.0 mL	Applied Biosystems (PN 401955)
Genetic Analyzer Sample Tubes (0.5 mL)	Applied Biosystems (PN 401957)
Genetic Analyzer Septa for 0.5-mL Sample Tubes	Applied Biosystems (PN 401956)
Miscellaneous Equipment	
Benchkote absorbent protector sheets	MLS
Freezer, –15 to –25 °C, non-frost-free	MLS
Glassware	MLS

Table 2 Equipment *(continued)*

Equipment	Source
Gloves, disposable, powder-free	MLS
Ice bucket	MLS
Lab coat	MLS
Lint-free tissues	MLS
Microcentrifuge tubes, 1.5-mL	MLS
Microtube racks	MLS
Nalgene filter apparatus, 150–mL, 0.2-µm CN filter	MLS
Permanent ink pen	MLS
Pipet bulb	MLS
Pipet tips, sterile, disposable hydrophobic filter-plugged	MLS
Pipets, serological	MLS
Pipettors, adjustable, 1–10 µL, 2–20 µL, 20–200 µL, and 200–1000 µL	MLS
Refrigerator	MLS
Repeat pipettor and Combitips that dispense 25–125 µL (optional)	MLS
Syringe, 35-cc (optional)	MLS
Tape	MLS
Tube, 50 mL Falcon	MLS
Tube decapper, autoclavable	MLS

Reagents

Table 3 Reagents

Reagent	Source
ABI PRISM 310 10X Genetic Analyzer Buffer with EDTA	Applied Biosystems (PN 402824)
AmpFℓSTR Blue™ PCR Amplification Kit	Applied Biosystems (PN 402800)
AmpFℓSTR® COfiler® PCR Amplification Kit	Applied Biosystems (PN 4305246)
AmpFℓSTR Green™ I PCR Amplification Kit	Applied Biosystems (PN 4307133)
AmpFℓSTR® Identifiler® PCR Amplification Kit	Applied Biosystems (PN 4322288)
AmpFℓSTR® Profiler Plus® and AmpFℓSTR® COfiler® PCR Amplification Kits	Applied Biosystems (PN 4305979)
AmpFℓSTR® Profiler Plus® *ID* PCR Amplification Kit	Applied Biosystems (PN 4330284)
AmpFℓSTR® Profiler Plus® PCR Amplification Kit	Applied Biosystems (PN 4303326)
AmpFℓSTR® SEfiler™ PCR Amplification Kit	Applied Biosystems (PN 4335129)
AmpFℓSTR® SGM Plus® PCR Amplification Kit	Applied Biosystems (PN 4307133)
GeneScan™-500 LIZ® Size Standard	Applied Biosystems (PN 4322682)
GeneScan™-500 ROX™ Size Standard	Applied Biosystems (PN 401734)
Hi-Di™ Formamide	Applied Biosystems (PN 4311320)
Matrix Standard Set DS-32 [5-FAM, JOE, NED, ROX dyes] for use with the 310/377 system	Applied Biosystems (PN 4312131)
Matrix Standard Set DS-33 [6-FAM, VIC, NED, PET, LIZ dyes] for use with the 310/377 system	Applied Biosystems (PN 4318159)

Table 3 Reagents *(continued)*

Reagent	Source
POP-4™ Performance Optimized Polymer	Applied Biosystems (PN 402838)
Deionized water, PCR grade	MLS

Documents

Table 4 Applied Biosystems documents

Document	Part Number
ABI PRISM® 310 Genetic Analyzer User Bulletin: G5v2 Module for Use with Dye Set 33 (DS-33)	4339367
ABI PRISM® 310 Genetic Analyzer User Guide	4317588
ABI PRISM® GeneScan® Analysis Software for the Windows NT® Operating System User Bulletin: Overview of the Analysis Parameters and Size Caller	4335617
ABI PRISM® GeneScan® Analysis Software Version 3.7 User's Manual	4308923
ABI PRISM® Genotyper® 3.7 NT Software User's Manual	4309947
AmpFℓSTR Blue™ PCR Amplification Kit User's Manual	402827
AmpFℓSTR Green™ I PCR Amplification Kit User's Manual	402944
AmpFℓSTR® COfiler® PCR Amplification Kit User's Manual	4305469
AmpFℓSTR® Identifiler® PCR Amplification Kit User's Manual	4323291
AmpFℓSTR® Profiler Plus® ID PCR Amplification Kit User Bulletin	4330429
AmpFℓSTR® Profiler Plus® PCR Amplification Kit User's Manual	4303501

Table 4 Applied Biosystems documents *(continued)*

Document	Part Number
AmpFℓSTR® Profiler® PCR Amplification Kit User's Manual	402945
AmpFℓSTR® SEfiler™ PCR Amplification Kit User's Manual	4337410
AmpFℓSTR® SGM Plus® PCR Amplification Kit User's Manual	4309589

Safety

Safety Alert Words

Four safety alert words appear in Applied Biosystems user documentation. Each word implies a particular level of observation or action, as described below:

IMPORTANT! Indicates information that is necessary for proper instrument operation, accurate chemistry kit use, or safe use of a chemical.

CAUTION Indicates a potentially hazardous situation that, if not avoided, can result in minor or moderate injury. It can also alert against unsafe practices, damage to an instrument, or loss of data.

WARNING Indicates a potentially hazardous situation that, if not avoided, can result in serious injury or death.

DANGER Indicates an imminently hazardous situation that, if not avoided, will result in serious injury or death. This signal word is to be limited to the most extreme situations.

Chemical Hazard Warning

WARNING **CHEMICAL HAZARD**. Some of the chemicals used with Applied Biosystems instruments and protocols are potentially hazardous and can cause injury, illness, or death.

Chemical Safety Guidelines

To minimize the hazards of chemicals:

- Read and understand the MSDSs provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. See “About MSDSs.”
- Minimize contact with chemicals. When handling chemicals, wear appropriate personal protective equipment such as safety glasses, gloves, and protective clothing. For additional safety guidelines, consult the MSDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, a fume hood). For additional safety guidelines, consult the MSDS.

- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the cleanup procedures recommended in the MSDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

About MSDSs

Chemical manufacturers supply current Material Safety Data Sheets (MSDSs) with shipments of hazardous chemicals to *new* customers. They also provide MSDSs with the first shipment of a hazardous chemical to a customer after an MSDS has been updated. MSDSs provide the safety information you need to store, handle, transport, and dispose of the chemicals safely.

Each time you receive a new MSDS packaged with a hazardous chemical, be sure to replace the appropriate MSDS in your files.

Obtaining MSDSs

You can obtain from Applied Biosystems the MSDS for any chemical supplied by Applied Biosystems. This service is free and available 24 hours a day.

To obtain MSDSs:

1. Go to **https://docs.appliedbiosystems.com/msdssearch.html**
2. In the Search field, type in the chemical name, part number, or other information that appears in the MSDS of interest. Select the language of your choice, then click **Search**.
3. Find the document of interest, right-click the document title, then select any of the following:
   - **Open** – To view the document
   - **Print Target** – To print the document
   - **Save Target As** – To download a PDF version of the document to a destination that you choose
4. To have a copy of a document sent by fax or e-mail, select **Fax** or **Email** to the left of the document title in the Search Results page, then click **RETRIEVE DOCUMENTS** at the end of the document list.
5. After you enter the required information, click **View/Deliver Selected Documents Now**.

Chemical Waste Hazard Warning

WARNING **CHEMICAL WASTE HAZARD.** Some wastes produced by the operation of the instrument or system are potentially hazardous and can cause injury, illness, or death.

Chemical Waste Guidelines

To minimize the hazards of chemical waste:

- Read and understand the MSDSs for the chemicals in a waste container before you store, handle, or dispose of chemical waste.
- Provide primary and secondary waste containers
- A primary waste container holds the immediate waste. A secondary container contains spills or leaks that may occur from the primary container. Both must be compatible with the waste material and meet national, state/provincial, and local requirements for container storage.
- Minimize contact with and inhalation of chemical waste. When handling chemicals, wear appropriate personal protective equipment such as safety glasses, gloves, and protective clothing.
- Handle chemical wastes in a fume hood.
- After you empty a chemical waste container, seal it with the cap provided.
- Dispose of the contents of a waste container in accordance with good laboratory practices and local, state/provincial, and/or national environmental and health regulations.

Site Preparation and Safety Guide

A site preparation and safety guide is a separate document sent to all customers who have purchased an Applied Biosystems instrument. Refer to the guide written for your instrument for information on site preparation, instrument safety, chemical safety, and waste profiles.

Waste profiles help you plan for the handling and disposal of waste generated by operation of the instrument. Read the waste profiles and all applicable MSDSs for your instrument before handling or disposing of chemical waste.

Waste Disposal

If potentially hazardous waste is generated when you operate the instrument:

- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure the health and safety of all personnel in your laboratory.
- Ensure that the instrument waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.

Note: Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological Hazard Safety

BIOHAZARD. Biological samples such as tissues, body fluids, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective eyewear, clothing, and gloves. Read and follow the guidelines in these publications:

- U.S. Department of Health and Human Services guidelines published in *Biosafety in Microbiological and Biomedical Laboratories* (stock no. 017-040-00547-4, http://bmbl.od.nih.gov)
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR §1910.1030, http://www.access.gpo.gov/nara/cfr/waisidx_01/29cfr1910a_01.html)

Additional information about biohazard guidelines is available at: **http://www.cdc.gov**

Matrix File Creation

Purpose

The GeneScan Analysis software requires the matrix file to address spectral overlap of the dyes when performing multicomponent analysis of samples with multiple markers and colors in one injection.

When to Prepare Matrix Files

Prepare a new matrix file:

- For each instrument
- For a particular set of run conditions
- At least one time each month to use with AmpFℓSTR products
- After changing the lot of polymer, capillaries, and/or buffer
- After service of the optics or other associated hardware

Procedural Overview

Matrix file creation involves:

1. Choosing the dye set, run module, and matrix standard
2. Preparing the matrix standards
3. Performing a matrix file run
4. Creating the matrix file
5. Verifying the matrix file

Choosing the Dye Set, Run Module, and Matrix Standard

Choose the appropriate dye set, run module, and matrix standard for the AmpFℓSTR kit you are using as shown in Table 5.

Table 5 Dye sets, run modules, and matrix standards for AmpFℓSTR kits

Kit	Dye Set	Run Module	Matrix Standard
• AmpFℓSTR® Identifiler® PCR Amplification Kit • AmpFℓSTR® SEfiler™ PCR Amplification Kit	Dye Set 33: • 6-FAM™ dye • VIC® dye • NED™ dye • PET™ dye • LIZ® dye	Filter Set G5v2	Matrix Standard Set DS-33 (PN 4318159)
• AmpFℓSTR® COfiler® PCR Amplification Kit • AmpFℓSTR® Profiler® PCR Amplification Kit • AmpFℓSTR® Profiler Plus® PCR Amplification Kit • AmpFℓSTR® Profiler Plus® *ID* PCR Amplification Kit • AmpFℓSTR® SGM Plus® PCR Amplification Kit • AmpFℓSTR Blue™ PCR Amplification Kit • AmpFℓSTR Green™ I PCR Amplification Kit	Dye Set 32: • 5-FAM™ dye • JOE™ dye • NED™ dye • ROX™ dye	Filter Set F	Matrix Standard Set DS-32 (PN 4312131)

Preparing the Matrix Standards

To prepare the matrix standards:

1.	Vortex the matrix standard set tubes thoroughly and then centrifuge them briefly. • For Dye Set 33: Use the Matrix Standard Set DS-33 • For Dye Set 32: Use the Matrix Standard Set DS-32
2.	Prepare one tube for each matrix standard sample by combining in each tube: • Matrix standard: 1 µL • Hi-Di™ Formamide: 24 µL **WARNING** **CHEMICAL HAZARD. Formamide** is harmful if absorbed through the skin and may cause irritation to the eyes, skin, and respiratory tract. It may cause damage to the central nervous system and the male and female reproductive systems, and is a possible birth defect hazard. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eye wear, clothing, and gloves. **IMPORTANT!** Do not include the size standard in the preparation of the matrix standards.
3.	Heat the tubes at 95 °C for 3 minutes to denature the DNA.
4.	Immediately place the tube on ice for 3 minutes.
5.	Place tubes in the sample tray. **IMPORTANT!** Make sure that you do not carry over any water on the outside of the tubes. Water on the autosampler tray may promote arcing.

Performing a Matrix File Run

After you create a satisfactory matrix file, you can apply this matrix file to subsequent runs. It is not necessary to run matrix standard samples for each new capillary.

To perform a matrix file run:

1.	Start the 310 Data Collection Software v3.0.
2.	Create a sample sheet for the matrix standard samples: a. Select **File** > **New** and click the appropriate GeneScan sample sheet icon. b. Enter the sample names/numbers for each row in the **Sample Name** column to identify which sample is in which tube of the sample tray. c. Close and save the sample sheet. **Note:** For more information, refer to the *ABI PRISM® 310 Genetic Analyzer User Guide* (PN 4317588).
3.	Create an injection list for each matrix standard sample: a. Select **File** > **New** and click the GeneScan Injection List icon. b. In the Injection List, select the appropriate sample sheet from the Sample Sheet pop-up menu. c. For every injection, select: • **Module** > **GS STR POP4 (1 mL) G5v2** for Dye Set 33 • **Module** > **GS STR POP4 (1 mL) F** for Dye Set 32 d. In the Matrix File column, select **None**.
4.	Click **Run**.

Creating the Matrix File

When the injections are completed, create the matrix using the GeneScan Analysis Software.

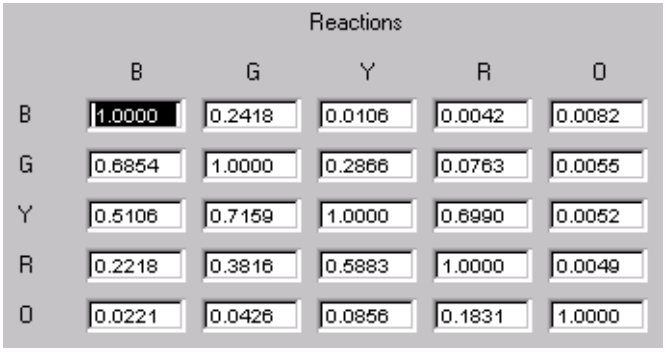
To create the matrix file:

1.	Start the GeneScan Analysis Software v3.7.1.
2.	Select **File** > **New**.
3.	Click the **Matrix** icon and select from the number of dyes pop-up window: • **Five dyes** for Dye Set 33 • **Four dyes** for Dye Set 32
4.	In the window that opens: a. Indicate the sample files that correspond to each matrix standard dye color. b. Select starting scan numbers for each sample to exclude the primer peak. c. Select the number of points so that the matrix standard peaks are contained in the scanned region (approximately 2500 scan data points). Avoid spikes or artifacts, if possible, when selecting the range. **Note:** Review data of each matrix standard. Reinject if necessary.
5.	Click **OK** to create the matrix and open the matrix file table.
6.	Save the matrix file in the Matrix folder: `D:\AppliedBio\Shared\Analysis\SizeCaller\Matrix`

Verifying the Matrix File

To verify the matrix file:

1. Select **File** > **Open** > **Matrix** and verify that the values on the diagonal from top left to bottom right are 1.000.



Reactions

	B	G	Y	R	O
B	1.0000	0.2418	0.0106	0.0042	0.0082
G	0.6854	1.0000	0.2866	0.0763	0.0055
Y	0.5106	0.7159	1.0000	0.6990	0.0052
R	0.2218	0.3816	0.5883	1.0000	0.0049
O	0.0221	0.0426	0.0856	0.1831	1.0000

Note: The values obtained are unique for each instrument, for each virtual filter set, and for each specific set of run conditions.

2. Apply the new matrix file to the Matrix Standard Sample Files:
   a. In the Analysis Control window, highlight the **Sample File** column by clicking in the **Sample File** title row.
   b. Select **Sample** > **Install New Matrix**.
   c. Select the new matrix file located in the Matrix folder:

      `D:\AppliedBio\Shared\Analysis\SizeCaller\Matrix`

   d. Click **Open**.

3. Analyze all of the matrix standard samples.
   a. Select **Settings** > **Analysis Parameters**, and verify that the settings are correct.
   b. In the Analysis Control window, select:
      - For Dye Set 33: All five colors (B, G, Y, R, and O)
      - For Dye Set 32: Four colors (B, G, Y, and R)
   c. Click **Analyze**.

To verify the matrix file: *(continued)*

4.	In the Results Control window, examine the results for all colors for each of the matrix standard samples. • In a five-dye system, the 6-FAM matrix standard results should have peaks for blue. Evaluate the baseline. A pattern of pronounced peaks or dips in any of the other four colors indicates that the color separation may not be optimal. • In a four-dye system, the 5-FAM matrix standard results should have peaks for blue. Evaluate the baseline. A pattern of pronounced peaks or dips in any of the other three colors indicates that the color separation may not be optimal.

Instrument Setup

Procedural Overview

Instrument setup involves:

1. Setting the data collection software preferences
2. Setting the run temperature
3. Creating a sample sheet and injection list

Setting the Data Collection Software Preferences

To set data collection software preferences:

1.	Start the 310 Data Collection Software v3.0.
2.	Select **Window** > **Preferences**.
3.	**Note:** This is an optional step. Set the standard color. a. Select the **GeneScan Sample Sheet Defaults** tab. b. Set the Size Standard Dye Color-5 Dye to **O** (Orange). c. Set the Size Standard Dye Color-4 Dye to **R** (Red). 

To set data collection software preferences: *(continued)*

4.	Select the **GeneScan Injection List Defaults** tab and make the following selections: a. Select the appropriate module. • 5 Dye Module: **GS STR POP4 (1 mL) G5v2** • 4 Dye Module: **GS STR POP4 (1 mL) F** b. Select an appropriate matrix file. c. Select the Autoanalyze with file. • If you plan to autoanalyze, select **AnalyzeGSSample.bat**. • If you do not plan to autoanalyze your data, select **<none>**. **Note:** When you create a new sample sheet, a portion of the form is automatically completed for you. You can modify the automatic defaults in the Preferences file.
5.	Click **OK** to save your changes.

Setting the Run Temperature

Setting the run temperature prior to starting a run is optional; however, this step saves time. This heating step occurs automatically at the beginning of the run modules.

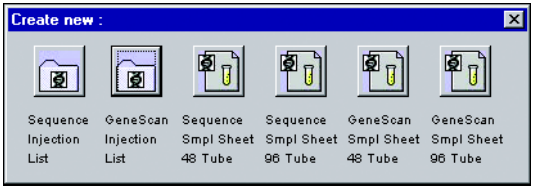
To set the run temperature:

1.	Close the instrument doors.
2.	Return to the 310 Data Collection Software v3.0.
3.	Set the temperature: a. Select **Window** > **Manual Control**. b. Select **Temperature Set** from the pop-up menu. c. Set the temperature to 60 °C. d. Click **Execute**. **Note:** It takes up to 30 min for the instrument to reach the 60 °C run temperature. You can prepare samples while the instrument is heating.

Creating a Sample Sheet and Injection List

The sample sheet can be prepared at any time before the preparation of samples and saved in the Sample Sheet folder.

To create a sample sheet and injection list:

1.	Using 310 Data Collection Software v3.0, select **File** > **New** and click the appropriate GeneScan Sample Sheet icon. 
2.	Make a selection from the drop-down menu: • For five-dye samples: **5-dyes** • For four-dye samples: **4-dyes**
3.	Enter sample names and numbers for each injection in the Sample Name column. This column indicates which sample is in which tube of the sample tray.

To create a sample sheet and injection list: *(continued)*

4. For each sample, enter the sample description for each row in the Sample Info column.
   - For five-dye samples: Blue, Green, Yellow, and Red
   - For four-dye samples: Blue, Green, and Yellow

   Note: Sample descriptions are necessary for the AmpFℓSTR® template file to build tables containing the genotypes for each sample.

   Note: If you are using Profiler Plus and COfiler PCR Amplification Kits together with Genotyper Software v3.7 and the combined COPP (COfiler and Profiler Plus) template files, enter the information as follows:

AmpFℓSTR Kit Used	Sample Info[a]	Comments
Profiler Plus PCR Amplification Kit	Unique sample description	Profiler Plus
COfiler PCR Amplification Kit		COfiler

[a]For each sample, enter the same Sample Info for both kits used.

To create a sample sheet and injection list: *(continued)*

5. For each AmpFℓSTR Allelic Ladder injection, type **ladder** in the Sample Info column.
   - For five-dye samples: Blue, Green, Yellow, and Red
   - For four-dye samples: Blue, Green, and Yellow

   Note: The Genotyper software requires the word "ladder."

   Note: If you are using Profiler Plus and COfiler PCR Amplification Kits together with Genotyper Software v3.7 and the combined COPP (COfiler and Profiler Plus) template files, enter the information as follows:

AmpFℓSTR Allelic Ladder Used	Sample Info	Comments[a]
Profiler Plus	ladder	PPL
COfiler		COL

   [a]Entries are case sensitive.

6. Make sure that the diamond symbol in the Std column indicates the appropriate standard:
   - For five-dye samples: Orange
   - For four-dye samples: Red

7. Select **File** > **New** and click the GeneScan Injection List icon.

8. Select the appropriate sample sheet from the **Sample Sheet** pop-up menu (at the top left of the Injection List window).

To create a sample sheet and injection list: *(continued)*

9.	Select the module file for every injection: a. Click the arrow in the Module column for the first sample/injection to view the pop-up menu and select: • For Dye Set 33: **GS STR POP4 (1 mL) G5v2** module file • For Dye Set 32: **GS STR POP4 (1 mL) F** module file b. Select the entire Module column by clicking the **Module** column heading, then select **Edit** > **Fill Down**. **Note:** You do not need to perform this step if the preferences were set as described in "Setting the Data Collection Software Preferences" on page 20.
10.	Select matrix files for the injections: a. From the Matrix file pop-up menu, select the appropriate matrix file for each injection. b. Click the arrow in the Matrix column for the first sample/injection to view the pop-up menu and select the appropriate matrix file. Select the entire Matrix column by clicking the Matrix column heading, then select **Edit > Fill Down**. **IMPORTANT!** The matrix file must be one that was made using the appropriate matrix standards and filter set module. To autoanalyze, place a copy of the matrix file in the Matrix folder: `D:\AppliedBio\Shared\Analysis\SizeCaller\Matrix` **Note:** You do not need to perform this step if the preferences were set as described in "Setting the Data Collection Software Preferences" on page 20.

Electrophoresis

Procedural Overview

Electrophoresis involves:

1. Preparing samples and AmpFℓSTR Allelic Ladder
2. Loading and running samples

Preparing Samples and AmpFℓSTR Allelic Ladder

To prepare the samples:

1.	For the master mix, combine the necessary amount of Hi-Di Formamide and size standard in a single microcentrifuge tube. **IMPORTANT!** Be sure to include at least one injection of AmpFℓSTR Allelic Ladder per run in the calculations. • For five-dye samples: – (Number of samples + 2) × 24.5 µL Hi-Di Formamide – (Number of samples + 2) × 0.5 µL GeneScan-500 LIZ Size Standard • For four-dye samples: – (Number of samples + 2) × 24 µL Hi-Di Formamide – (Number of samples + 2) × 1 µL GeneScan-500 ROX Size Standard **Note:** If you are using a multi-channel pipettor or processing many samples, you may need to prepare additional master mix. **WARNING** **CHEMICAL HAZARD. Formamide** is harmful if absorbed through the skin and may cause irritation to the eyes, skin, and respiratory tract. It may cause damage to the central nervous system and the male and female reproductive systems, and is a possible birth defect hazard. Read the MSDS, and follow the handling instructions. Wear appropriate protective eye wear, clothing, and gloves.
2.	Vortex the tube to mix and then centrifuge it briefly.

To prepare the samples: *(continued)*

3.	Aliquot the Hi-Di Formamide/size standard mixture: a. Label 0.2-mL or 0.5-mL Genetic Analyzer sample tubes appropriately. b. Aliquot 25 µL of the Hi-Di Formamide/size standard mixture into the labeled tubes. **Note:** To pipet the Hi-Di Formamide/size standard mixture, Applied Biosystems recommends using a repeating pipettor.
4.	Add 1.5 µL of PCR product or AmpFℓSTR Allelic Ladder per tube and mix by pipetting up and down.
5.	Seal each tube with a septum.
6.	Vortex the sample tray and then centrifuge the tray briefly. **Note:** Make sure that there are no bubbles.
7.	Denature each sample for 3 minutes at 95 °C.
8.	Chill tubes for at least 3 minutes on ice. **Note:** Be careful not to carry over any water on the outside of the tubes. Water on the autosampler tray may promote arcing.

Loading and Running Samples

To load and run samples:

1.	Open the instrument doors and press the **Tray** button to present the autosampler.
2.	Place a 48-well or 96-well sample tray on the autosampler. **Note:** For a 48-well autosampler tray, place tube #1 into sample tray position A1, tube #2 into sample tray position A3, and so on. **Note:** For a 96-well autosampler tray, place tube #1 into sample tray position A1, tube #2 into sample tray position A2, and so on.

To load and run samples: *(continued)*

3.	Press the **Tray** button on the instrument to retract the autosampler.
4.	Close the instrument doors.
5.	Click **Run**. **Note:** If you have not preheated the heat plate, the module has an initial step in which the plate is heated to 60 °C before running the first sample. This step takes up to 30 min. After the plate reaches 60 °C, the run will begin.

Data Analysis

Procedural Overview

Data analysis involves:

1. Setting analysis preferences
2. Analyzing data with GeneScan software
3. Displaying results
4. Analyzing data with Genotyper software

Setting Analysis Preferences

For more information about the analysis parameters, refer to the *ABI PRISM® GeneScan® Analysis Software Version 3.7 User's Manual* (PN 4308923) and the *ABI PRISM® GeneScan Analysis Software for the Windows NT® Operating System User Bulletin: Overview of the Analysis Parameters and Size Caller* (PN 4335617).

Analyzing Data with GeneScan Software

To analyze data with GeneScan software:

1.	Start the GeneScan Analysis Software v3.7.1.
2.	Ctrl-click the arrow in the Size Standard column for a sample file to view the pop-up menu and select **New**. **Note:** Do **not** assign a size for the 250-bp peak for data generated on the 310 instrument. Skip a row or assign a size of zero. This peak can be used as an indicator of precision within a run.

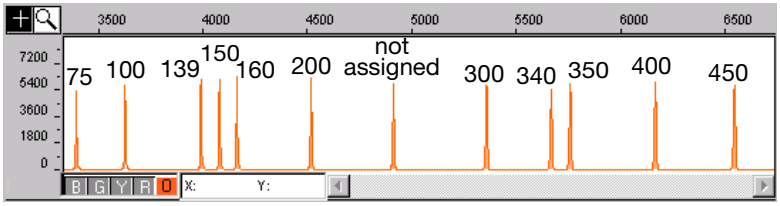


Figure 1 GeneScan-500 LIZ Size Standard peaks

Note: In Figure 1, the 450-bp peak is assigned when using the GeneScan-500 LIZ Size Standard. When using the GeneScan-500 ROX Size Standard (figure not shown), do not assign the 450-bp peak.

To analyze data with GeneScan software: *(continued)*

3.	Save the size standard in the SizeStandards folder: `D:\AppliedBio\Shared\Analysis\SizeCaller\SizeStandards`
4.	To apply the size standard to all injections, select the appropriate standard in the Size Standard column header (above sample 1) in the Analysis Control window.
5.	Analyze sample files: a. Highlight the appropriate columns: • For Dye Set 33: Blue, green, yellow, red, and orange • For Dye Set 32: Blue, green, yellow, and red b. Confirm that a diamond symbol appears in all appropriate boxes where a size standard is included with the sample files. • For five-dye samples: Confirm that the orange box is the standard. • For four-dye samples: Confirm that the red box is the standard. **Note:** If the diamond symbol does not appear in the appropriate boxes, Ctrl-click to place a diamond in the box. c. Click **Analyze**.

To analyze data with GeneScan software: *(continued)*

6. After the analysis is complete, confirm that the sizes for the size standard peaks are correctly assigned:

   a. Select **Window > Results Control** and examine the appropriate size standard peaks in overlapping groups of 16 samples (Quick Tile Off).
      - For five-dye samples: Examine the orange GeneScan-500 LIZ Size Standard peaks.
      - For four-dye samples: Examine the red GeneScan-500 ROX Size Standard peaks.

      Note: Be sure to select **View > Align By Size**.

   b. While the samples are tiled, check the 250-bp peaks (sized as approximately 246 bp) in the enlarged view window. Remember that this peak was not defined in the size standard. The tiled 250-bp peaks should size consistently and should all overlap. In a typical run, the 250-bp peaks all fall within a size window of approximately 1 bp.

      Note: Laboratory temperature variations can cause size variations >1 bp. If the temperature of the laboratory varies, try injecting the AmpFℓSTR Allelic Ladder approximately every 10 injections, or 5 hours.

   c. Scroll through the tables to verify correct size standard peak assignments.

   d. Check the size standard peaks in the remaining samples, taking note of which samples (if any) have incorrect peak assignments.

Displaying Results

For more information about displaying the results, refer to the *ABI PRISM® GeneScan® Analysis Software Version 3.7 User's Manual* (PN 4308923).

Analyzing Data with Genotyper Software

For more information about analyzing data with Genotyper software, refer to the *ABI PRISM® Genotyper® 3.7 NT Software User's Manual* (PN 4309947).

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