User Bulletin

ABI PRISM® 3100 Genetic Analyzer

November 12, 2001

SUBJECT: ABI PRISM 3100 22-cm Capillary Array for High Throughput Microsatellite and SNP Genotyping

About This Bulletin

High throughput Microsatellite and SNP Genotyping is now possible on the 3100 Genetic Analyzer by using the 22-cm ABI PRISM® 3100 Capillary Array and ABI PRISM® 3100 POP-4™ polymer.

IMPORTANT The 3100 22-cm capillary array is not recommended or supported for HID/forensics applications.

In This Bulletin

This user bulletin contains the following topics:

Topic	See Page
Safety	2
Microsatellites	4
ABI PRISM SNaPshot Multiplex System	5
Chemistry Information	6
Software Information	7
Preparing the Instrument	8
Calibrating the Instrument	9
Preparing For a Run	11
Starting the Run	14
Analyzing the Data	15
Troubleshooting	17
Ordering Information	18

Applied Biosystems

Safety

Documentation User Attention Words

Five user attention words appear in the text of all Applied Biosystems user documentation. Each word implies a particular level of observation or action as described below.

Note Calls attention to useful information.

IMPORTANT Indicates information that is necessary for proper instrument operation.

⚠ CAUTION Indicates a potentially hazardous situation which, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.

⚠ WARNING Indicates a potentially hazardous situation which, if not avoided, could result in death or serious injury.

⚠ DANGER Indicates an imminently hazardous situation which, if not avoided, will result in death or serious injury. 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.

- Read and understand the material safety data sheets (MSDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (*e.g.,* safety glasses, gloves, or 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 (*e.g.*, fume hood). For additional safety guidelines, consult the MSDS.
- Do not leave chemical containers open. Use only with adequate ventilation.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended on the MSDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

Chemical Waste Hazard Warning

⚠ WARNING CHEMICAL WASTE HAZARD. Wastes produced by Applied Biosystems instruments are potentially hazardous and can cause injury, illness, or death.

- Read and understand the material safety data sheets (MSDSs) provided by the manufacturers of the chemicals in the waste container before you store, handle, or dispose of chemical waste.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (*e.g.,* safety glasses, gloves, or 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 (*e.g.*, fume hood). For additional safety guidelines, consult the MSDS.
- Handle chemical wastes in a fume hood.

- After emptying the waste container, seal it with the cap provided.
- Dispose of the contents of the waste tray and waste bottle in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.

Ordering MSDSs

You can order free additional copies of MSDSs for chemicals manufactured or distributed by Applied Biosystems using the contact information below.

<table>
<tr><th>To order MSDSs...</th><th>Then...</th></tr>
<tr><td>Over the Internet</td><td>a. Go to our Web site at www.appliedbiosystems.com/techsupp
b. Click MSDSs

<table><tr><th>If you have...</th><th>Then...</th></tr><tr><td>The MSDS document number or the Document on Demand index number</td><td>Enter one of these numbers in the appropriate field on this page.</td></tr><tr><td>The product part number</td><td rowspan="2">Select Click Here, then enter the part number or keyword(s) in the field on this page.</td></tr><tr><td>Keyword(s)</td></tr></table>
c. You can open and download a PDF (using Adobe® Acrobat® Reader™) of the document by selecting it, or you can choose to have the document sent to you by fax or email.</td></tr>
<tr><td>By automated telephone service</td><td>Use “To Obtain Documents on Demand” under “Technical Support.”</td></tr>
<tr><td>By telephone in the United States</td><td>Dial 1-800-327-3002, then press 1.</td></tr>
<tr><td>By telephone from Canada</td><td><table><tr><th>To order in...</th><th>Dial 1-800-668-6913 and...</th></tr><tr><td>English</td><td>Press 1, then 2, then 1 again</td></tr><tr><td>French</td><td>Press 2, then 2, then 1</td></tr></table></td></tr>
</table>

For chemicals not manufactured or distributed by Applied Biosystems, call the chemical manufacturer.

Microsatellites

Overview of Microsatellites

Microsatellites can be used for a variety of applications such as human disease research, mouse mapping, population genetics, etc.

The ABI PRISM® Linkage Mapping Set is an example of microsatellite analysis.The compilation of 811 highly informative dinucleotide markers can be used for linkage disequilibrium, association studies, and population genetics. Between 15 to 20 loci can be multiplexed post PCR amplification and co-electrophoresed in one capillary. For optimal resolution, as in the case of fine mapping, Applied Biosystems recommends using the 36-cm capillary array. However, the 22-cm capillary array can be used to rapidly scan the genome when using markers less than 360 bp.

Throughput

There is a greater than two-fold increase in throughput using the 22-cm capillary array.

Array Length	Number of Dyes	Run Time (Min)	Runs/ 24 Hours	Number of Capillaries	Number of Loci	Genotypes/ 24 Hours
22-cm	4	21	72	16	15[a]	17,280
	5	21	72	16	20[b]	23,040
36-cm	4	45	32	16	15	7,680
	5	45	32	16	20	10,240

a. five loci/color x three colors

b. five loci/color x four colors

Limitations

- Peak Height

  Due to the shorter separation, the 22-cm capillary array shows a 50–60% increase in peak height. It is recommended that users re-optimize dilution and pooling ratios to avoid generating offscale data (Y-axis value greater than 8192 RFUs).

- Precision

  The sizing precision is similar to the 36-cm capillary array; yielding ±0.15 bp standard deviation for up to 350 bp.

- Sizing

  When compared to the 36-cm capillary array, all fragments sized on a 22-cm capillary array demonstrated an increased interpolated size between 0.4–0.6 bp. This is due to the mobility shifts.

- Size Range

  For dinucleotide repeats, Applied Biosystems recommends restricting marker size range between 75–350 bp. Tri- and tetranucleotide repeats can contain alleles between 75–400 bp.

- Multiplexing

  Between 15–20 loci can be multiplexed depending on the dye set utilized (four versus five dyes). Applied Biosystems recommends a minimum of 10 bp spacing between loci in the same color.

- Resolution

  The resolution is 1 bp up to 250 bp, and 2 bp up to 360 bp.

ABI PRISM SNaPshot Multiplex System

Overview of SNaPshot Multiplex Kit

The ABI PRISM® SNaPshot™ Multiplex Kit is a tool to rapidly validate suspected single nucleotides polymorphisms (SNPs). It is designed to interrogate up to ten SNPs in one capillary thereby increasing throughput.

The chemistry is based on dideoxy single-base extension of an unlabeled olignucleotide primer (or primers).

- Each SNP primer binds to a complementary template in the presence of fluorescently labeled ddNTPs and AmpliTaq® DNA Polymerase, FS.
- The polymerase extends the primer by one nucleotide, adding a single ddNTP to its 3´ end.

Dye Assignments

The fluorescent dyes are assigned to the individual ddNTPs as follows:

ddNTP	Dye Label	Color of Analyzed Data
A	dR6G	Green
C	dTAMRA™	Yellow (Black)
G	dR110	Blue
T (U)	dROX™	Red
–	LIZ™	Orange

Throughput

The 22-cm capillary array allows 2X increase in throughput without compromising precision, resolution or multiplexing capability.

Array Length	Run Time (Min)	Runs/ 24 Hours	Number of Capillaries	Number of Loci	Genotypes/ 24 Hours
22-cm	15	96	16	10	15,360
36-cm	30	48	16	10	7,680

Limitations

- Peak Height

  Due to the shorter separation, the 22-cm capillary array shows a 40–50% increase in peak height. It is recommended that users load 0.5 μL SNaPshot Multiplex product per capillary to avoid generating offscale data (Y-axis value greater than 8192 RFUs).

- Precision

  Using the SNaPshot Multiplex protocol, Applied Biosystems supports ±0.5 bp standard deviation.

- Size Range and Multiplex Scheme

  For SNaPshot Multiplex, the rules for the 36-cm capillary array apply to the 22-cm capillary array. This includes restricting SNaPshot Multiplex product between 20–105 bp. Additionally, loci less than 36 bp should be spaced a minimum of 6 bp apart, while loci greater than 36 bp should be spaced 4 bp apart.

Chemistry Information

Required Reagents

The procedures require the following:

Description	Part Number
10X Genetic Analyzer Buffer with EDTA	402824
22-cm Capillary Array	4319898
Hi-Di™ Formamide	4311320
3100 POP-4 Polymer	4316355

For kit and reagent part numbers, and information on custom oligos, refer to “Ordering Information” on page 18.

Chemical Hazards

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

⚠ WARNING **CHEMICAL HAZARD**. **Formamide** causes eye, skin, and respiratory tract irritation. It is a possible reproductive and birth defect hazard. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

⚠ CAUTION **CHEMICAL HAZARD**. **POP-4 polymer** may cause eye, skin, and respiratory tract irritation. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Use for research and development purposes only.

Supported Applications

Dye Set	Kit	Application
DS-02	SNaPshot™ Multiplex Kit	High throughput SNP scoring
DS-30	Custom oligos	Microsatellites only
DS-31	Linkage Mapping Set, mouse and custom oligos	
DS-33	5-Dye Linkage Mapping Set and custom oligos	

Software Information

Software Prerequisites

The three software requirements for using the 22-cm capillary array are:

- ABI PRISM® 3100 Data Collection Software, version 1.0.1
- ABI PRISM® DNA GeneScan™ Analysis Software, version 3.7
- ABI PRISM 3100 22-cm Array Software Support Files CD-ROM (P/N 4331860)

Contents of the CD

To use the 22-cm capillary array on the 3100 instrument, new modules are required. These files are located on the CD-ROM.

The CD contains:

- ABI PRISM® 3100 22-cm Array ReadMe file
- 22-cm method files:
  - Spect22_POP4.mtd
  - SNP22_POP4.mtd
  - GeneScan22_POP4.mtd

Installing the New Files

To install the new files:

Step	Action
1	Start or restart the computer. Make sure the OrbixWeb Daemon is running.
2	Insert the ABI PRISM 3100 Genetic Analyzer Software Support Files CD-ROM. The installer starts automatically.
3	In the 22-cm Array Support Files Installer window, click **Next**.
4	In the next window, open the OrbixWeb Daemon if you have not done so in step 1. Click **Next**. The methods are automatically stored on the D drive and are then imported into the database.
5	Click **Finish** to complete the installation.
6	Remove the CD-ROM.
7	Restart the computer.

When the installation is complete, the method files are placed in the storage location listed below. Confirm proper installation by navigating to the following directory:

File	Storage Location
Spect22_POP4.mtd[a]	D:\appliedbio\Support Files\Data Collection Support Files\Method Files
SNP22_POP4.mtd	
GeneScan22_POP4.mtd	

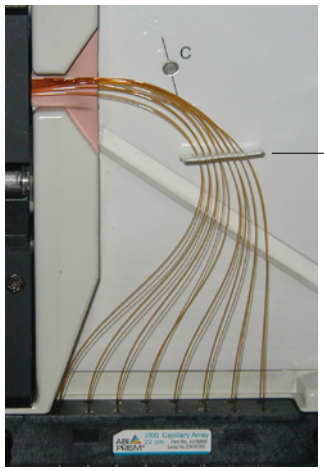
a. A script contained on the CD automatically converts the method files into module files, and imports the module files into the database.

Preparing the Instrument

Using the Install Array Wizard

The current Install Array wizard does not include the 22-cm capillary array length option button. Use the 36-cm capillary array length option button instead.

To install the capillary array:

Step	Action
1	Make sure your polymer blocks, tubing, and syringes are clean and dry. **Note** A Polymer Block Cleaning Kit (P/N 432291) is available to more easily clean the upper and lower polymer blocks.
2	Place the clean upper and lower polymer block on the 3100 instrument, and connect the blocks with the polymer tubing.
3	With the instrument doors closed, press the Tray button.
4	From the **Tools** menu, select **Install Capillary Array Wizard**.
5	Follow the directions in the wizard to: a. Input the capillary's length and serial number. **Note** Use the **36-cm** option button and type in the serial number of your 22-cm array. b. Install the capillary array. **Note** There is no capillary array holder for the 22-cm array, therefore position the comb as shown below. Do not remove the comb as it spaces the capillaries apart and allows for minimal overlap between adjacent capillaries.  Position the comb as shown here c. Fill the reservoirs and anode reservoir. d. Fill the syringes with 3100 POP-4 polymer and install the syringes onto the 3100 instrument. e. Prime the polymer blocks with polymer and remove all bubbles. f. Fill the capillary array with polymer. g. Replace the buffer in the anode reservoir.

Calibrating the Instrument

Performing a Spatial Calibration

Perform a spatial calibration as usual. Refer to Chapter 4 in the *ABI PRISM 3100 Genetic Analyzer User Guide* (P/N 4315834) for details.

Performing a Spectral Calibration Using Matrix Standards

Determining Which Matrix Standard and Dye Sets to Use

Use the table below to determine which matrix standards to use and what dye set to select.

Application	Dye Set	Matrix Standard Kit	Dyes
SNaPshot Multiplex	E5	DS-02	dR110, dR6G, dTAMRA™, dROX™, LIZ™
Custom oligos	D	DS-30	6-FAM, HEX, NED™, ROX
♦ LMS v2.5 ♦ Mouse ♦ Custom oligos	D	DS-31	6-FAM, VIC™, NED, ROX
♦ 5-Dye LMS v2.5 ♦ Custom Oligos	G5	DS-33	6-FAM, VIC, NED, PET™, LIZ

Performing the Spectral Calibration

To perform a spectral calibration using matrix standards:

Step	Action
1	Thoroughly mix the matrix standards.
2	Prepare the matrix standards according to the instructions in the product insert for the specific dye set you are using. **⚠ WARNING CHEMICAL HAZARD. Formamide** causes eye, skin, and respiratory tract irritation. It is a possible reproductive and birth defect hazard. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
3	Dispense 10 µL of the standard and formamide mixture into a 96-well plate, using wells A1 through H2.
4	Assemble the plate and place the plate assembly onto the autosampler.
5	Within the **Plate View** page of the 3100 Data Collection software, click **New**. a. In the **Plate Name** text box, type a name for the plate. **IMPORTANT** Use letters and numbers and the following punctuation only: -_()#.+. Do not use spaces. b. For the **Application**, select **Spectral Calibration**. c. For the **Plate Type**, select **96-Well**. d. Click **Finish**. This opens the Plate Editor spreadsheet.

To perform a spectral calibration using matrix standards: *(continued)*

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>6</td><td>To complete the Plate Editor spreadsheet:
a. In the A1 cell, type a name for the samples.
IMPORTANT Use letters and numbers and the following punctuation only: -_()#.+. Do not use spaces.
b. Select the rest of the options using the table below.
<table>
<tr><th>Column Heading</th><th>Application</th><th>Select…</th></tr>
<tr><td rowspan="3">Dye Set</td><td>SNaPshot Multiplex</td><td>E5</td></tr>
<tr><td>♦ LMS v2.5
♦ Mouse
♦ Custom oligos</td><td>D</td></tr>
<tr><td>♦ 5-Dye LMS v2.5
♦ Custom Oligos</td><td>G5</td></tr>
<tr><td>Run Module</td><td>All</td><td>Spect22_POP4DefaultModule</td></tr>
<tr><td rowspan="3">Spectral Parameter</td><td>SNaPshot Multiplex</td><td>MtxStd{GeneScan-SetE5}.par</td></tr>
<tr><td>♦ LMS v2.5
♦ Mouse
♦ Custom oligos</td><td>MtxStd{GeneScan-SetD}.par</td></tr>
<tr><td>♦ 5-Dye LMS v2.5
♦ Custom Oligos</td><td>MtxStd{GeneScan-SetG5}.par</td></tr>
</table>
c. Fill down each column to H2.
d. Click OK.</td></tr>
<tr><td>7</td><td>In the Pending Plate Records table, select the plate record that you just created.</td></tr>
<tr><td>8</td><td>Click the plate graphic that corresponds to the plate you are linking.
This links the plate record to the plate position. The plate position indicator changes from yellow to green and the entry for the plate record moves to the Linked Plate Records table.</td></tr>
<tr><td>9</td><td>Click the Run button.</td></tr>
<tr><td>10</td><td>Review the quality of your spectral calibration.</td></tr>
<tr><td>11</td><td>Proceed with “Preparing For a Run” on page 11.</td></tr>
</table>

Preparing For a Run

Preparing and Loading Samples

To prepare and load samples:

Step	Action
1	Pool the PCR/SNP products according to the kit protocol.
2	Combine the following: **Application** / **Product** / **Size Standard** / **Hi-Di Formamide** PCR / 1 µL / 0.5 µL / 10 µL SNP / 0.5 µL / 0.25 µL / 9.25 µL **⚠ WARNING** **CHEMICAL HAZARD**. **Formamide** causes eye, skin, and respiratory tract irritation. It is a possible reproductive and birth defect hazard. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
3	Heat denature the samples at 95 °C for 5 min.
4	Immediately place the samples on ice.
5	Load the samples in the plate and assemble the plate.
6	Place the plate assembly onto the autosampler.

Creating a Plate Record and Linking a Plate

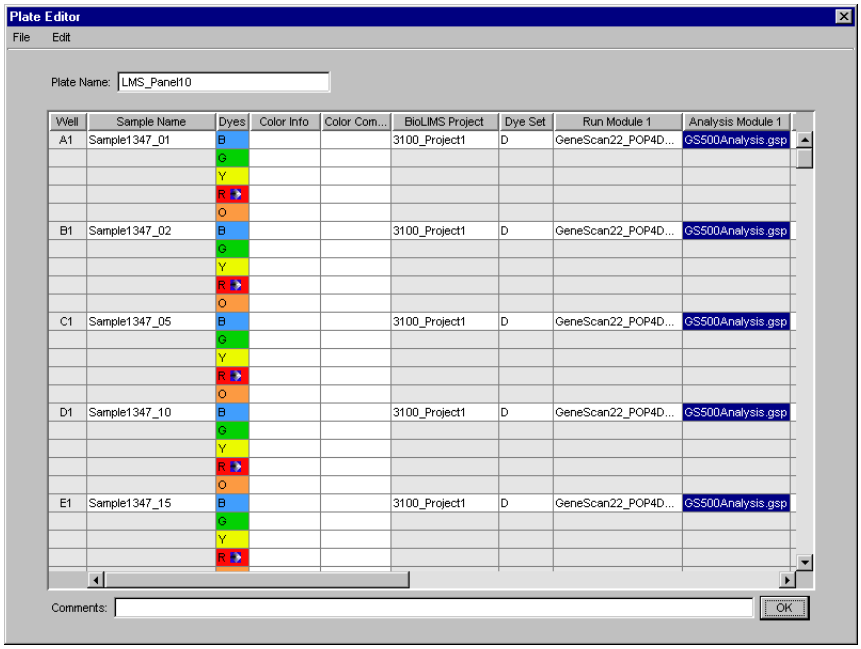
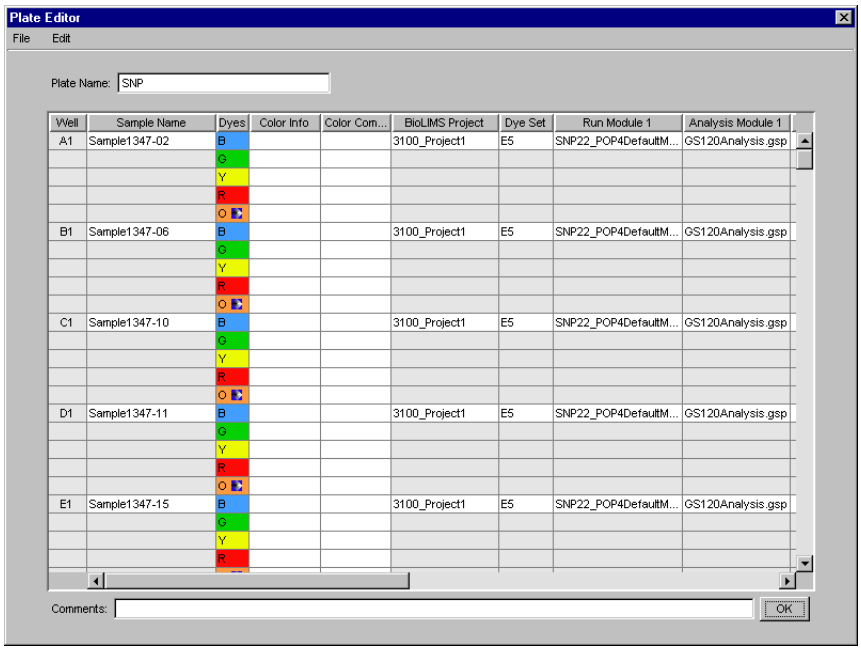
To create a plate record and to link the plate:

Step	Action
1	From the **Plate View** page of the 3100 Data Collection software, click **New**. This opens the **Plate Editor** dialog box.
2	In the **Plate Editor** dialog box, enter the plate name, the application, and plate type. a. In the **Plate Name** text box, type a name for the plate. **IMPORTANT** Use letters and numbers and the following punctuation only: -_()#.+. Do not use spaces. b. Use the default selection, **GeneScan**, for the **Application**. c. In the **Plate Type** drop-down list, select the appropriate plate size. d. Click **Finish**. This opens the Plate Editor spreadsheet.

To create a plate record and to link the plate: *(continued)*

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>3</td><td>Use the table below to complete the columns of the plate editor spreadsheet.
<table>
<tr><th>For…</th><th>Column Heading</th><th>Select…</th></tr>
<tr><td rowspan="8">Dye Set E5</td><td>Sample Name</td><td>Type in the names of all samples</td></tr>
<tr><td>Dye</td><td>O (orange)</td></tr>
<tr><td>Color Info</td><td>User defined</td></tr>
<tr><td>Color Comment</td><td>User defined</td></tr>
<tr><td>BioLIMS Project</td><td>3100_Project1</td></tr>
<tr><td>Dye Set</td><td>E5</td></tr>
<tr><td>Run Module</td><td>SNP22_POP4DefaultModule</td></tr>
<tr><td>Analysis Module</td><td>GS120Analysis.gsp</td></tr>
<tr><td rowspan="8">Dye Set D</td><td>Sample Name</td><td>Type in the names of all samples</td></tr>
<tr><td>Dye</td><td>R (red)</td></tr>
<tr><td>Color Info</td><td>User defined</td></tr>
<tr><td>Color Comment</td><td>User defined</td></tr>
<tr><td>BioLIMS Project</td><td>3100_Project1</td></tr>
<tr><td>Dye Set</td><td>D</td></tr>
<tr><td>Run Module</td><td>GeneScan22_POP4DefaultModule</td></tr>
<tr><td>Analysis Module</td><td>GS400HDAnalysis.gsp or
GS500Analysis.gsp</td></tr>
<tr><td rowspan="8">Dye Set G5</td><td>Sample Name</td><td>Type in the names of all samples</td></tr>
<tr><td>Dye</td><td>O (orange)</td></tr>
<tr><td>Color Info</td><td>User defined</td></tr>
<tr><td>Color Comment</td><td>User defined</td></tr>
<tr><td>BioLIMS Project</td><td>3100_Project1</td></tr>
<tr><td>Dye Set</td><td>G5</td></tr>
<tr><td>Run Module</td><td>GeneScan22_POP4DefaultModule</td></tr>
<tr><td>Analysis Module</td><td>GS500Analysis.gsp</td></tr>
</table>
</td></tr>
</table>

To create a plate record and to link the plate: *(continued)*

Step	Action
4	♦ An example of a completed linkage mapping plate record is shown below.  ♦ An example of a completed SNP plate record is shown below.  Click **OK** when you are done.

To create a plate record and to link the plate: *(continued)*

Step	Action
5	In the **Pending Plate Records** table, select the plate record that you just created.
6	Click the plate graphic that corresponds to the plate you are linking. This links the plate record to the plate position. The plate position indicator changes from yellow to green and the entry for the plate record moves to the **Linked Plate Records** table.

Starting the Run

Starting the Run

To start the run:

Step	Action
1	Click the **Run** button.
2	Click the **Status View** tab and monitor the status of the instrument. The run time for: ♦ Microsatellites is 21 min ♦ SNP is 15 min **IMPORTANT** To prevent screen refresh problems, do not leave the **Array View** or **Capillary View** pages open for extended periods during a run.

Analyzing the Data

Software Required

Conduct data analysis using the following:

- ABI PRISM® GeneScan Analysis Software version 3.7
- Analysis parameters and size standards

Application	Size Standard	Analysis Modules
SNaPshot Multiplex	GS 120.szs	GS120Analysis.gsp
Custom oligos	GS 400HD.szs	GS400HDAnalysis.gsp
♦ LMS v2.5 ♦ Mouse ♦ Custom oligos	GS 400HD.szs or GS500-250.szs	GS400HDAnalysis.gsp or GS500-250Analysis.gsp or custom
♦ 5-Dye LMS v2.5 ♦ Custom Oligos	GS500.szs or GS500-250.szs	GS500Analysis.gsp or custom

- ABI PRISM® GenoTyper™ Software version 3.7 or
- ABI PRISM® GeneMapper™ Software version 2.0

Conducting Data Analysis

Refer to the *ABI PRISM DNA GeneScan Analysis Software version 3.7 Users Guide* (P/N 4308923) for instructions on how to analyze data from a GeneScan run.

SNaPshot data can be analyzed using ABI PRISM GenoTyper version 3.7. Refer to the *GenoTyper for SNaPshot User Bulletin* for details.

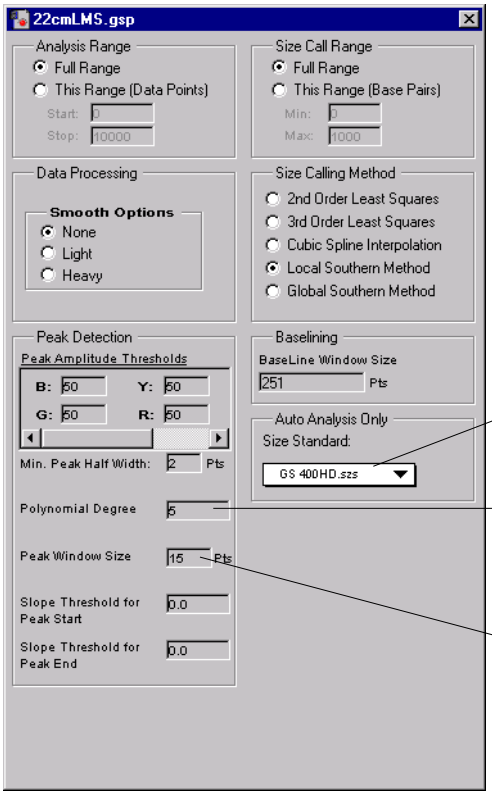
Editing the Analysis Parameters

Some applications involving di-, tri- and tetranucleotide repeats may require the Analysis Parameters in the analysis module to be modified for proper analysis.

To edit the analysis parameters:

Step	Action
1	Open the ABI PRISM® GeneScan™ Analysis software.
2	From the **File** menu, select **Open**.
3	Select the **Analysis Parameters** icon.
4	a. Select the analysis module you want to edit. The analysis modules are stored in the following directory: D:\appliedbio\Shared\Analysis\Sizecaller\Params b. Click **Open**.

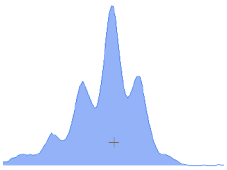
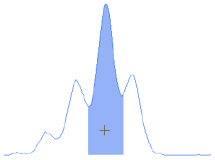
To edit the analysis parameters: *(continued)*

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>5</td><td>The analysis module opens. Select the appropriate settings:
<table>
<tr><th>For…</th><th>Value for Polynomial Degree</th><th>Size Standard</th><th>Peak Window Size</th></tr>
<tr><td>Dinucleotides repeats</td><td>5</td><td rowspan="2">Select the appropriate size standard: GS400HD.szs, GS500.szs or GS500-250.szs</td><td>15</td></tr>
<tr><td>Tri- and tetranucleotide repeats</td><td>3</td><td>19</td></tr>
</table>

</td></tr>
<tr><td>6</td><td>Save the changes as a new analysis module.
a. From the File menu, select Save As.
b. Assign a unique name and click OK.

IMPORTANT Store the new analysis modules in the following directory:
D:\appliedbio\Shared\Analysis\Sizecaller\Params</td></tr>
<tr><td>7</td><td>Reanalyze your data with the new analysis parameter file.</td></tr>
</table>

Troubleshooting

Troubleshooting Table

Topic	Symptom	Solution
Resolution	Single base pair resolution does not exceed 260 bp. Is the array bad?	Short array lengths lead to less resolution, therefore the array is only supported for 2 applications.
		The 22-cm array is guaranteed for 100 runs.
Peak Detection	Adjacent peaks are clearly separated peaks; however the software calls the entire cluster as on peak.  One peak for the cluster, polynomial degree = 3	Create a new analysis parameter, refer to “Editing the Analysis Parameters” on page 15. For dinucleotide repeats increase the polynomial degree to 5 and change peak window size to 15.  One peak, polynomial degree = 5
Comb Placement	The comb does not fit into the comb holder for the 36-cm array.	There is no designated holder for the 22-cm array. Do not remove the comb. Refer to “Using the Install Array Wizard” on page 8.
Signal	Peak heights are significantly greater than the 36-cm array sometimes yielding offscale data.	Review the raw data for fragments greater than 8000 RFUs. These products should be diluted to prevent offscale data. If the product is from a: ♦ Microsatellite application, then adjust the pooling ratios. ♦ SNaPshot multiplex, then reduce the amount of PCR product or SNP primer in the SNP reaction.

Ordering Information

SNaPshot Multiplex Kit and Reagents

Description	Part Number
SNaPshot Multiplex Kit, 100 reactions	4323151
SNaPshot Multiplex Kit, 1000 reactions	4323154
SNaPshot Multiplex Kit, 5000 reactions	4323155
GeneScan™-120 LIZ™ Size Standard	4324287
Matrix Standard Set DS-02	4323014

Linkage Mapping Set v2.5-MD10 and Mouse Reagents

Description	Part Number
Linkage Mapping Set, 50 reactions	4329186
Linkage Mapping Set, 300 reactions	4329185
Linkage Mapping Set, 1200 reactions	4329184
GeneScan™-500-LIZ™ Size Standard	402985
Matrix Standard Set DS-33	4323016
GeneScan®-400HD ROX™ Size Standard	402985
Matrix Standard Set DS-30	4316100
VIC™ Matrix Standard	4323022

Custom Oligos

Applied Biosystems, the leading supplier of instruments, reagents, and software systems for life science research, also provides custom nucleic acid synthesis services. This includes: ABI PRISM® primers, Custom ABI PRISM® Sequencing Kits, and ABI PRISM® Linkage Mapping Sets.These products are available via Applied Biosystems Web site, e-mail, or facsimile, depending on the country.

P/N 4331535, Rev. A, Stock No. 106UB25-01