



# **Quick Reference Guide**

# In This Guide

This quick reference guide describes example analysis workflows for the GeneMapper<sup>®</sup> Software Version 4.1. It also summarizes the version(s) of the Data Collection Software, Microsoft<sup>®</sup> Windows<sup>®</sup> operating system, and sample data files that are supported by the GeneMapper Software for specified instruments.

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# **For More Information**

For more information about the workflows described in this guide, refer to the:

- GeneMapper<sup>®</sup> Software Version 4.1 Getting Started Guides Shipped with the software, as well as available on the GeneMapper<sup>®</sup> Software Version 4.1 Documentation CD as portable document format (PDF) files.
- GeneMapper<sup>®</sup> Software Version 4.1 Online Help Accessible from the GeneMapper Software by doing one of the following:
  - Clicking 🕜 in the toolbar
  - Selecting Help 
     Contents and Index
  - Pressing F1

# **Basic Sizing/Peak Detection Analysis Workflow**

### Overview

Use the method described in the following workflow to perform a basic sizing and peak detection analysis using the GeneMapper Software.

**Note:** This workflow does not include instructions for performing allele or genotype calling.

#### Set Up the Analysis

- 1. Create a new project.
- 2. Add samples to the project.
- 3. Specify the analysis parameters in the Samples tab.
- 4. (Optional) Specify the table settings.

#### Analyze and Examine the Data

- 1. Analyze the samples in the project.
- 2. Review the size quality and size-calling data.
- 3. Examine the data.

#### Print or Export the Results

If necessary:

- •Print or export views, plots, and tables for use in reports or presentations.
- •Export views, plots, and tables for further analysis by third-party software.

#### Setting Up the Analysis

- **1.** Create a new project:
  - a. In the GeneMapper window toolbar, click 📴.
  - b. Select the appropriate project type.
  - c. Click OK.
- 2. Click 🗓 to add samples to the project.
- **3.** In the Samples tab, specify the analysis parameters for the samples in the project:

**Note:** You can use the fill-down function to apply the parameters to all the samples at one time.

a. In the Analysis Method column, select or create an analysis method with the following settings:

| Tab              | Parameter                      | Setting   |
|------------------|--------------------------------|---|
| Allele           | Bin Set                        | Select None.  |
| Peak<br>Detector | Peak<br>Detection<br>Algorithm | Select the appropriate<br>algorithm ( <b>Basic</b> ,<br><b>Advanced</b> , or <b>Classic</b> ) and<br>the appropriate peak sizing<br>method (as applicable). |
|                  |                                | IMPORTANT! If you use<br>the Classic peak detection<br>algorithm, you must select<br>a size standard created in<br>Classic mode.                            |

- b. In the Panel column, select None.
- c. In the Size Standard column, select the appropriate size standard.
- **4.** (Optional) In the toolbar, select or create table settings for the analysis.

# Analyzing and Examining the Data

- **1.** Click **b** to analyze the samples in the project.
- 2. Review the size quality and size-calling data.
- 3. Examine the data.

**Note:** In the plot windows, the sizing table shows information about the detected peaks.

# **Printing or Exporting the Results**

If necessary, print or export the results.

To print the results:

- 1. In the GeneMapper window, select the desired tab.
- 2. Select File ▶ Print.

- 1. Select the desired table setting.
- 2. Select File > Export Table.

# **AFLP®** Analysis Workflow

## Overview

Use the method described in the following workflow to study band patterns from multiple amplified fragment length polymorphisms (AFLP<sup>®</sup>) simultaneously.

#### Set Up the AFLP<sup>®</sup> Analysis

- 1. Create a new AFLP project.
- 2. Add samples to the project.
- 3. Specify the analysis parameters in the Samples tab.
- 4. (Optional) Specify the table settings.

#### Analyze and Examine the Data

- 1. Analyze the project.
- 2. Review the SQ PQV in the Samples tab.
- 3. Review the PQV in the Genotypes tab.
- 4. Open the Samples Plot and examine the data.
- 5. (Optional) Export the generated panel.

#### Print or Export the Results

If necessary:

- •Print or export views, plots, and tables for use in reports or presentations.
- •Export views, plots, and tables for further analysis by third-party software.

# Automated Generation of AFLP® Panels

The GeneMapper Software can generate panel data algorithmically from AFLP sample files added to a project. The generated panel, which consists of the bin definitions for the peaks of all samples in the project, can be exported for use in other projects after data analysis.

**IMPORTANT!** The Auto Bin function cannot generate bins from AFLP data. To generate panels from AFLP samples, use the automated panel function of the AFLP analysis method described in this workflow.

# Setting Up the AFLP® Analysis

- Click is to create a new project and select AFLP for the project type.
- 2. Click 🗓 to add samples to the project.
- **3.** In the Samples tab, specify the analysis parameters for the samples in the project:

**Note:** You can use the fill-down function to apply the parameters to all the samples at one time.

a. In the Analysis Method column, select or create an AFLP analysis method with the following settings:

**IMPORTANT!** All samples must use the same analysis method.

| Tab   | Parameter  | Setting   |
|---|--|---|
| Allele  | Bin Set  | Select <b>None</b> to generate the panel<br>automatically, or select an<br>appropriate bin set if you are using<br>an existing panel.                       |
|   | Analyze<br>Dyes  | Select the dyes that you want to analyze.   |
|   | Analysis<br>Range  | Use the default settings or enter a desired range (bps).  |
|   | Panel  | Select Generate panel using<br>samples to have the software<br>generate the panel automatically. <sup>‡</sup>   |
|   | Allele<br>Calling  | Select an appropriate naming<br>method (Name alleles using bin<br>names or Name alleles using<br>labels).   |
| Peak<br>Detector  | Peak<br>Detection<br>Algorithm   | Use the default settings, or select<br>the appropriate algorithm (Basic,<br>Advanced, or Classic) and an<br>appropriate peak-sizing method.                 |
|   |  | <b>IMPORTANT!</b> If you use the<br>Classic peak detection algorithm,<br>you must select a size standard<br>definition that was created in<br>Classic mode. |
| PeakUse the default settings or enter valueQualityMax Peak Width and Pull-Up Ratio. |  | ault settings or enter values for the<br>Vidth and Pull-Up Ratio.   |
| Quality<br>Flags  | Use the default settings or enter values for the Quality Flag Settings and PQV Thresholds. |   |

‡ When configured to generate a panel automatically, the software creates bins from the collective peaks present in all samples of the project, not for each sample individually. **b.** In the Panel column, select **None** (the analysis method is configured to generate the panel automatically).

**IMPORTANT!** The analysis method and panel that you select must use the same bin set.

- c. In the Size Standard column, select the appropriate size standard.
- 4. (Optional) In the toolbar, select or create table settings and plot settings for the analysis.

**IMPORTANT!** If you create a table setting, select the following options in the Genotype tab of the Table Settings Editor:

- Limit the number of displayed alleles shown to the number of bins.
- Show only binned alleles in their respective bin positions.

# Analyzing and Examining the Results

- **1.** Click **b** to analyze the project.
- 2. In the Samples tab, review the Size Quality (SQ) Process Quality Value (PQV).

If necessary, fix failed size standards in the Size Match Editor, then reanalyze the samples that failed sizing. Samples that display (low quality) fail sizing and are not genotyped. Samples that display (check) are genotyped but may have lower Genotype Quality (GQ) values.

- **3.** In the Genotypes tab, review the PQV (BD, OS, and SPU).
- 4. Open the Samples Plot and examine the data:
  - a. (Optional) Overlay and verify the concordance of the size standards data.
  - **b.** Overlay the sample data and review the electropherograms for polymorphisms.
  - c. If necessary, edit the genotype calls and generated bins.
- 5. (Optional) If the software generated a panel for the project:
  - a. In the GeneMapper window, select
     File > Export Project Panel to export the panel from the project.
  - b. In the Panel Manager, select File ►
     Import Panels to import the panel for use in later projects.

# Printing and Exporting the Results

If necessary, print or export the results.

To print the results:

- 1. In the GeneMapper window, select the desired tab.
- 2. Select File > Print.
- To export the results:
- 1. Select the desired table setting.
- 2. Select File > Export Table.

# LOH Microsatellite Analysis Workflow

## Overview

Use the method described in the following workflow to perform microsatellite-based relative peak height comparisons, such as loss of heterozygosity (LOH) or replication error (RER).

#### Set Up the LOH Microsatellite Analysis

- 1. Create a kit and a panel for the project.
- 2. Create a new microsatellite project.
- 3. Add samples to the project.
- 4. Specify the analysis parameters in the Samples tab.
- 5. Perform an initial analysis.
- 6. Create a new bin set.
- 7. Generate bins.

#### Analyze and Examine the Data

- 1. Edit the analysis method to specify a bin set.
- 2. Specify the analysis parameters in the Samples tab.
- 3. (Optional) Specify the table settings.
- 4. Analyze the samples in the project.
- 5. Examine the data.

#### Sort Data and Evaluate Loss of Heterozygosity

- 1. Sort the data in the Genotypes tab by sample file and marker.
- 2. Generate a report.

#### Print or Export the Results

If necessary:

- •Print or export the desired views, plots, and tables for use in reports or presentations.
- •Export desired views, plots, and tables for further analysis by third-party software.

#### Sample-Naming Conventions

When you create sample sheets for LOH samples, Applied Biosystems recommends that you use a consistent sample-naming convention that distinguishes the controls from the test samples for each individual in your data set. Such a naming convention enables the GeneMapper Software to sort the analyzed data to take advantage of report settings that evaluate for LOH.

# Setting Up the LOH Microsatellite Analysis

- 1. Create a kit and a panel for the project:
  - a. Click 🔠 to open the Panel Manager.
  - b. Create or select a kit.
  - c. Create or import panels with marker information.
  - d. Close the Panel Manager.
- Click i to create a new project and select Microsatellite for the project type.
- 3. Click 🗓 to add samples to the project.
- 4. In the Samples tab, specify the analysis parameters for the samples in the project:

**Note:** You can use the fill-down function to apply the parameters to all the samples at one time.

a. In the Analysis Method column, select or create a microsatellite analysis method with the following settings:

| Tab              | Parameter                           | Setting   |  |
|------------------|-------------------------------------|---|--|
| Allele           | Bin Set                             | Select None.  |  |
| Peak<br>Detector | ctor Peak<br>Detection<br>Algorithm | Select the appropriate<br>algorithm ( <b>Basic</b> ,<br><b>Advanced</b> , or <b>Classic</b> ) and<br>the appropriate peak sizing<br>method (as applicable). |  |
|                  |                                     | IMPORTANT! If you use<br>the Classic peak detection<br>algorithm, you must select<br>a size standard created in<br>Classic mode.                            |  |

- b. In the Panel column, select the panel created in step 1.
- c. In the Size Standard column, select the appropriate size standard.

- Click b to perform the initial analysis on the sample files in the project so they will be available as reference data.
- 6. Create a bin set to associate with the panel:
  - **a.** Click **III** to open the Panel Manager.
  - **b.** In the Navigation Pane, select the panel created in step 1 on page 6.
  - c. Select Bins > New Bin Set to create a bin set.
  - d. Select Bins > Add Reference Data to add the analyzed reference data to the panel.

**Note:** You can add reference samples collectively in a folder or individually using the Ctrl-click function.

- 7. Generate bins for the panel and bin set:
  - a. In the Navigation Pane, select the panel created in step 1 on page 6.
  - b. In the Bin Set drop-down list, select the bin set created in step 6, above.
  - c. Select Bins ▶ Auto Bin to generate the bin set automatically.
  - d. Select the markers, then review the bins. Edit if necessary.
  - e. (Optional) For faster loading of the panel in the future, remove the reference samples from the panel.
  - f. Close the Panel Manager.

## Analyzing and Examining the Data

- 1. Edit the analysis method to use the bin set created in step 6 of "Setting Up the LOH Microsatellite Analysis," above:
  - a. Click 🔚 to open the GeneMapper Manager.
  - b. Select the Analysis Method tab.
  - c. Select the analysis method created in step 4a on page 6, then click Open.
  - d. In the Allele tab, select the bin set created in step 6, above.
  - e. Click OK, then click Done.
- 2. In the Samples tab, specify the analysis parameters for the samples in the project created in step 2 of "Setting Up the LOH Microsatellite Analysis" on page 6:
  - a. In the Sample Type column, select the appropriate sample type (Sample, Positive Control, or Negative Control).

 b. Leave the settings for the Analysis Method, Panel, and Size Standard columns the same as those selected in step 4 of "Setting Up the LOH Microsatellite Analysis" on page 6.

**IMPORTANT!** The selected analysis method and panel must use the same bin set.

- **3.** (Optional) In the toolbar, select or create table settings for the analysis.
- 4. Click be to analyze the samples in the project.
- 5. Examine the data:
  - a. In the Samples tab, examine the Size Quality (SQ) scores and the size standards.
  - b. In the Genotypes tab, review the Process Quality Value (PQV) columns (BD, BIN, CC, LPH, OBA, OS, PHR, SHP, SP, SPA, SPU, and XTLK).
  - c. Display the samples and genotypes plots.

# Sorting Data and Evaluating LOH

1. In the Genotypes tab, sort the data by sample file and marker.

**Note:** Depending on your sample-naming convention, control (healthy) and test (tumor) samples from the same individual may be listed consecutively, or all control samples may be listed first, followed by all test samples.

- 2. Generate a report:
  - a. Create a report setting that is appropriate for the order in which your data is sorted (this is determined by your sample-naming convention) or use the "LOH Default" report setting.
  - b. Select the samples to include in the report.
  - c. Select Analysis ► Report Manager, then select the appropriate report setting.

# **Printing or Exporting the Results**

If necessary, print or export the results.

To print the results:

- 1. In the GeneMapper window, select the desired tab.
- 2. Select File ▶ Print.
- To export the results:
- 1. Select the desired table setting.
- 2. Select File > Export Table.

# **Microsatellite Analysis Workflow**

#### Overview

Use the method described in the following workflow to analyze polymorphic DNA loci that contain a repeated nucleotide sequence (usually 2 to 7 base pair repeats).

#### Set Up the Microsatellite Analysis

- 1. Create a kit and a panel for the project.
- 2. Create a new microsatellite project.
- 3. Add samples to the project.
- 4. Specify the analysis parameters in the Samples tab.
- 5. Perform an initial analysis.
- 6. Create a new bin set.
- 7. Generate bins.

#### Analyze and Examine the Data

- 1. Edit the analysis method to specify a bin set.
- 2. Specify the analysis parameters in the Samples tab.
- 3. (Optional) Specify the table settings.
- 4. Analyze the samples in the project.
- 5. Examine the data.

#### Print or Export the Results

If necessary:

- •Print or export the desired views, plots, and tables for use in reports or presentations.
- •Export desired views, plots, and tables for further analysis by third-party software.

### Setting Up the Microsatellite Analysis

- 1. Create a kit and a panel for the project:
  - a. Click 🔠 to open the Panel Manager.
  - b. Create or select a kit.
  - c. Create or import panels with marker information.
  - d. Close the Panel Manager.
- Click i to create a new project and select Microsatellite for the project type.
- **3.** Click **5** to add samples to the project.
- 4. In the Samples tab, specify the analysis parameters for the samples in the project:

**Note:** You can use the fill-down function to apply the parameters to all the samples at one time.

a. In the Analysis Method column, select or create a microsatellite analysis method with the following properties:

| Tab              | Parameter                      | Setting   |
|------------------|--------------------------------|---|
| Allele           | Bin Set                        | Select None.  |
| Peak<br>Detector | Peak<br>Detection<br>Algorithm | Select the appropriate<br>algorithm ( <b>Basic</b> ,<br><b>Advanced</b> , or <b>Classic</b> ) and<br>the appropriate peak sizing<br>method (as applicable). |
|                  |                                | <b>IMPORTANT!</b> If you use<br>the Classic peak detection<br>algorithm, you must select<br>a size standard created in<br>Classic mode.                     |

- b. In the Panel column, select the panel created in step 1.
- c. In the Size Standard column, select the appropriate size standard.
- Click to perform the initial analysis on the sample files in the project so they will be available as reference data.
- 6. Create a bin set to associate with the panel:
  - a. Click 🔠 to open the Panel Manager.
  - b. In the Navigation Pane, select the panel created in step 1.
  - c. Select Bins ▶ New Bin Set to create a bin set.

d. Select Bins ► Add Reference Data to add the analyzed reference data to the panel.

**Note:** You can add reference samples collectively in a folder or individually using the Ctrl-click function.

- 7. Generate bins for the panel and bin set:
  - a. In the Navigation Pane, select the panel created in step 1 on page 8.
  - **b.** In the Bin Set drop-down list, select the bin set created in step 6 on page 8.
  - c. Select Bins ▶ Auto Bin to generate the bin set automatically.
  - d. Select the markers and review the bins. Edit if necessary.
  - e. (Optional) For faster loading of the panel in the future, remove the reference samples from the panel.
  - f. Close the Panel Manager.

# Analyzing and Examining the Data

- 1. Edit the analysis method to use the bin set created in step 6 of "Setting Up the Microsatellite Analysis" on page 8:
  - a. Click 📰 to open the GeneMapper Manager.
  - b. Select the Analysis Method tab.
  - c. Select the analysis method created in step 4 on page 8, then click Open.
  - d. In the Allele tab, select the bin set created in step 6 on page 8.
  - e. Click OK, then click Done.
- 2. In the Samples tab, specify the analysis parameters for the samples in the project created in step 2 of "Setting Up the Microsatellite Analysis" on page 8:

**Note:** You can use the fill-down function to apply the parameters to all the samples at one time.

a. In the Sample Type column, select the appropriate sample type (Sample, Positive Control, or Negative Control).

**b.** Leave the settings for the Analysis Method, Panel, and Size Standard columns the same as those selected in step 4 on page 8.

**IMPORTANT!** The selected analysis method and panel must use the same bin set.

- **3.** (Optional) In the toolbar, select or create table settings for the analysis.
- 4. Click be to analyze the samples in the project.
- 5. Examine the data:
  - a. In the Samples tab, examine the Size Quality (SQ) scores and the size standards.
  - b. In the Genotypes tab, review the Process Quality Value (PQV) columns (BD, BIN, CC, LPH, OBA, OS, PHR, SHP, SP, SPA, SPU, and XTLK).
  - c. Display the samples and genotypes plots.

# **Printing or Exporting the Results**

If necessary, print or export the results.

#### To print the results:

- 1. In the GeneMapper window, select the desired tab.
- 2. Select File ▶ Print.

- 1. Select the desired table setting.
- 2. Select File > Export Table.

# SNaPshot<sup>®</sup> Kit Analysis Workflow

#### Overview

Use the method described in the following workflow to analyze multiplexed, single-base-extension SNP sample data. The workflow for the analysis varies, depending on the chemistry used to prepare the samples.

#### Create Panels and Bin Sets Using Reference Data

- 1. Create a new SNaPshot kit project.
- 2. Add reference samples to the project.
- 3. Specify the analysis parameters in the Samples tab.
- 4. Perform a sizing-only analysis on the project.
- 5. Examine the SQ scores and size standards in the Samples tab.
- 6. Create the kit, panel, and bin sets for the project.

#### Create Panels and Bin Sets Using Primer Focus<sup>®</sup> KIt Data

- 1. Create a new SNaPshot kit project.
- 2. Add Primer Focus kit samples to the project.
- 3. Specify the analysis parameters in the Samples tab.
- 4. Perform a sizing-only analysis on the project.
- 5. Create the kit, panel, and bin sets for the project.

#### Set Up the SNaPshot<sup>®</sup> Kit Analysis

- 1. Create a new SNaPshot kit project.
- 2. Add samples to the project.
- 3. Specify the analysis parameters in the Samples tab.
- 4. (Optional) Specify the table settings.

#### Analyze and Examine the Data

- 1. Analyze the samples in the project.
- 2. Examine the data.

#### Print or Export the Results

If necessary:

- •Print or export the desired views, plots, and tables for use in reports or presentations.
- •Export desired views, plots, and tables for further analysis by third-party software.

# Creating Panels and Bin Sets Using Reference Data

- 1. Click 🚰 to create a new project and select SNaPshot<sup>®</sup> for the project type.
- Click 1/2 to add reference sample files to the project.
- **3.** In the Samples tab, specify the analysis parameters for the project:
  - a. In the Analysis Method column, select or create a SNaPshot kit analysis method with the following settings:

| Tab              | Parameter                      | Setting   |
|------------------|--------------------------------|---|
| Allele           | Bin Set                        | Select None.  |
| Peak<br>Detector | Peak<br>Detection<br>Algorithm | Select the appropriate<br>algorithm ( <b>Basic</b> ,<br><b>Advanced</b> , or <b>Classic</b> ) and<br>the appropriate peak sizing<br>method (as applicable). |
|                  |                                | <b>IMPORTANT!</b> If you use<br>the Classic peak detection<br>algorithm, you must select<br>a size standard created in<br>Classic mode.                     |

- b. In the Panel column, select None.
- c. In the Size Standard column, select the appropriate size standard.
- Click b to perform a sizing-only analysis on the project.

**Note:** The GeneMapper Software only sizes samples during the analysis. It does not make allele or genotype calls.

- 5. In the Samples tab, examine the size quality (SQ) scores and the size standards.
- 6. Create the kit, panel, and bin sets for the project:
  - a. Click 🔠 to open the Panel Manager.
  - **b.** Click 📑 to create a new kit.
  - c. For kit type, select SNP.
  - d. Click if to create a panel for the SNP kit.
  - e. Select Bins > New Bin Set to create a bin set for the SNP kit.
  - f. Select Bins > Add Reference Data to add the analyzed reference data to the SNP kit.

- g. Select Bins > Panel Reference Data to add the analyzed reference data to the SNP panel.
- h. Using the reference sample files, manually create and adjust SNP markers and associated bins.
- i. Create SNP markers as needed.
- j. Using the imported data as a reference, use the tools of the Plot tab to adjust the SNP marker and associated bins.
- 7. Set up and perform the analysis as described in "Setting Up the SNaPshot<sup>®</sup> Kit Analysis" on page 12.

# Creating Panels and Bin Sets Using Primer Focus<sup>®</sup> Kit Data

**IMPORTANT!** The Auto Panel function can be used to generate panels for samples prepared using Primer Focus Kit chemistry only.

- Click G to create a new project and select SNaPshot<sup>®</sup> for the project type.
- Click J to add Primer Focus kit sample files to the project.
- **3.** In the Samples tab, specify the analysis parameters for the project:
  - a. In the Sample Type column, select Primer Focus.
  - b. In the Analysis Method column, select (or create) a SNaPshot kit analysis method with the following settings:

| Tab              | Parameter                      | Setting  |
|------------------|--------------------------------|--|
| Allele           | Bin Set                        | Select None.   |
| Peak<br>Detector | Peak<br>Detection<br>Algorithm | Select the appropriate<br>algorithm (Basic,<br>Advanced, or Classic) and<br>the appropriate peak sizing<br>method (as applicable). |
|                  |                                | IMPORTANT! If you use<br>the Classic peak detection<br>algorithm, you must select<br>a size standard created in<br>Classic mode.   |

- c. In the Panel column, select None.
- d. In the Size Standard column, select the appropriate size standard.

 Click to perform a sizing-only analysis on the project.

**Note:** The GeneMapper Software only sizes samples during the analysis. It does not make allele or genotype calls.

- 5. Create the kit, panel, and bin sets for the project:
  - a. Click 🔠 to open the Panel Manager.
  - **b.** Click 📑 to create a new kit.
  - c. For kit type, select SNP.
  - d. Select Bins ► New Bin Set to create a bin set for the SNP kit.
  - e. Select Bins Add Reference Data to add the Primer Focus kit data to the SNP kit.
  - f. Select Bins ► Auto Panel to create the panel, markers, and bin set automatically.
  - g. Review the panels and bins created with the Auto Panel function.
- Set up and perform the analysis as described in "Setting Up the SNaPshot<sup>®</sup> Kit Analysis," below.

# Setting Up the SNaPshot<sup>®</sup> Kit Analysis

- 1. Click 🚰 to create a new project and select SNaPshot<sup>®</sup> for the project type.
- 2. Click 🗓 to add sample files to the project.
- 3. In the Samples tab, specify the analysis parameters for the project:

**Note:** You can use the fill down function to apply the parameters to all the samples at one time.

a. In the Sample Type column, select the appropriate sample type (Sample, Positive Control, or Negative Control).

 b. In the Analysis Method column, select or create a SNaPshot kit analysis method with the following settings:

| Tab              | Parameter                      | Setting   |
|------------------|--------------------------------|---|
| Allele           | Bin Set                        | Select the bin set created<br>in either of the previous<br>procedures.  |
| Peak<br>Detector | Peak<br>Detection<br>Algorithm | Select the appropriate<br>algorithm ( <b>Basic</b> ,<br><b>Advanced</b> , or <b>Classic</b> ) and<br>the appropriate peak sizing<br>method (as applicable). |
|                  |                                | <b>IMPORTANT!</b> If you use<br>the Classic peak detection<br>algorithm, you must select<br>a size standard created in<br>Classic mode.                     |

c. In the Panel column, select the panel created in either of the previous procedures.

**IMPORTANT!** The selected analysis method and panel must use the same bin set.

d. In the Size Standard column, select the appropriate size standard.

**Note:** If you imported analysis parameters from the sample sheet, verify that the settings are correct.

4. (Optional) In the toolbar, select or create table settings for the analysis.

# Analyzing and Examining the Results

- 1. Click be to analyze the samples in the project.
- 2. Examine the data:
  - a. In the Samples tab, examine the Size Quality (SQ) score and the size standards.
  - b. In the Genotypes tab, review the Process Quality Value (PQV) columns (AN, BD, CC, DP, LPH, NB, OS, PHR, and SPU).
  - c. Display and examine the samples and genotypes plots.

# Printing or Exporting the Results

If necessary, print or export the results.

To print the results:

- **1.** In the GeneMapper window, select the desired tab.
- 2. Select File > Print.

- 1. Select the desired table setting.
- 2. Select File > Export Table.

# **SNPlex<sup>™</sup> System Analysis Workflow**

#### Overview

Use the method described in the following workflow to analyze multiplexed, oligonucleotide ligation/PCR assay (OLA/PCR) SNPs prepared using the Applied Biosystems SNPlex<sup>™</sup> System chemistries.

#### Set Up the SNPlex<sup>™</sup> System for Analysis

If necessary:

- 1. Import the panels and bins for the SNPlex System.
- 2. Import the AIF.

#### Create and Analyze a Project

- 1. Create a new SNPlex<sup>™</sup> System project.
- 2. Add sample files to the project.
- 3. Specify the analysis parameters in the Samples tab.
- 4. Confirm or edit the settings for sample type, panel, size standard, and SNP set.
- 5. Analyze the project.
- 6. Examine the data.

#### Create and Analyze a Study

- 1. Create a new study.
- 2. Add projects to the study.
- Review the IQC data.
- 4. Review the SQC data.
- 5. Review the SNP data as needed.

#### **Export and Print the Results**

If necessary:

- •Print or export the desired views, plots, and tables for use in reports or presentations.
- •Export desired views, plots, and tables for further analysis by third-party software.

# Setting Up the SNPlex<sup>™</sup> System for Analysis

- 1. If you have not already done so, import the panels and bins for the SNPlex System:
  - a. Click 🔠 to open the Panel Manager.
  - b. In the Navigation Pane, select Panel Manager, then import the SNPlex\_48plex\_3730\_Panels.txt or the SNPlex\_48plex\_3130\_Panels.txt file.
  - c. In the Navigation Pane, select the SNPlex\_48\_plex folder, then import the SNPlex\_48plex\_3730\_Bins.txt or the SNPlex\_48plex\_3130\_Bins.txt file.
  - d. Close the Panel Manager.
- If you have not already done so, use the GeneMapper Manager to import the Assay Information File (AIF) found on the CD that was shipped with your SNPlex System chemistry kit.

# **Creating and Analyzing a Project**

For each run (electrokinetic injection) performed by the compatible Applied Biosystems electrophoresis instrument:

- Click C to create a new project and select SNPlex<sup>™</sup> for the project type.
- Click J to add the samples of a single run folder to the project.
- **3.** In the Samples tab, specify the analysis parameters and table settings for the project:

**Note:** You can use the fill-down function to apply the parameters to all the samples at one time.

- a. In the Sample Type column, select the appropriate sample type.
- b. In the Analysis Method column, select the appropriate SNPlex System analysis method (SNPlex\_Model\_3730, SNPlex\_Rules\_3730, or SNPlex\_Rules\_3130).
- c. In the Panel column, select the SNPlex System panel.
- d. In the Size Standard column, select the SNPlex\_48\_plex\_v1 size standard definition.
- e. Confirm the sample type (Sample, Positive Control, Allelic Ladder, or Negative Control)
- f. In the SNP Set column, select the SNPlex System SNP set.

- 4. Click **b** to analyze the project.
- 5. Examine the data:
  - a. Examine the size standards.
  - **b.** Display and examine the cluster plots (using the cluster plot settings).
  - c. (Optional) Display and examine the samples and genotypes plots.

### Creating and Analyzing a Study

After you create a sufficient number of projects:

- 1. Select Tools > Study Manager to open the Study Manager.
- 2. Click 🚰 to create a new study.
- **3.** Click 🗓 to add projects to the study.
- 4. Review the Initial QC (IQC) data:
  - a. Review the Sizing IQC data and resolve any sizing-related issues.
  - **b.** Review the Allelic Ladder IQC data and resolve any binning-related issues.
  - c. Review the Positive Hybridization Control (PHC) and Assay IQC signal data (normalized and otherwise) to identify and diagnose any process-related problems.
  - d. If necessary, override the IQC status of suspect runs.
- 5. Review the Secondary QC (SQC) data.
  - a. Review the SQC metrics applicable to your analysis and review the test results of runs exhibiting potential problems.
  - b. If necessary, override the SQC status of suspect runs.
- 6. Review the genotype (SNP) data as needed.

## **Exporting and Printing the Results**

If necessary, print or export the results.

#### To print the results:

- 1. In the GeneMapper window, select the desired tab.
- 2. Select File ▶ Print.

- 1. Select the desired table setting.
- 2. Select File > Export Table.

# Instrument, Software, and Data Compatibility

| Genetic Analysis<br>Instrument |  | Compatible Data Collection Software<br>and Operating System  | Sample Data<br>Compatibility  |  |
|--------------------------------|--|--|---|--|
|                                | Applied Biosystems<br>3500/3500xL<br>Genetic Analyzer            | <ul> <li>Data Collection v1.0</li> <li>Windows Vista<sup>®</sup> Business edition, 32-bit, SP1</li> </ul>  | <ul><li>Sample files</li><li>Co-installation</li><li>Automation</li></ul> |  |
| - 0                            |  | <ul> <li>Data Collection v1.0</li> <li>Windows<sup>®</sup> 2000, SP 2</li> </ul>   |   |  |
|                                | Applied Biosystems   | <ul> <li>Data Collection v2.0</li> <li>Windows<sup>®</sup> 2000, SP 3 and 4</li> </ul>   | Sample files  |  |
|                                | 3730/3730x/<br>DNA Analyzer                                      | <ul> <li>Data Collection v3.0</li> <li>Windows<sup>®</sup> XP Professional, SP 1 and 2</li> </ul>  |   |  |
|                                |  | <ul> <li>Data Collection v3.1</li> <li>Windows<sup>®</sup> XP Professional, SP 2 and 3</li> <li>Windows Vista<sup>®</sup> Business edition, 32-bit, SP1</li> </ul> | <ul><li>Sample files</li><li>Co-installation</li><li>Automation</li></ul> |  |
|                                | Applied Biosystems   | <ul> <li>Data Collection v3.0</li> <li>Windows<sup>®</sup> XP Professional, SP 1 and 2</li> </ul>  | Sample files  |  |
|                                | 3130/3130 <i>xl</i><br>Genetic Analyzer                          | <ul> <li>Data Collection v3.1</li> <li>Windows<sup>®</sup> XP Professional, SP 2 and 3</li> <li>Windows Vista<sup>®</sup> Business edition, 32-bit, SP1</li> </ul> | <ul><li>Sample files</li><li>Co-installation</li><li>Automation</li></ul> |  |
|                                | ABI PRISM <sup>®</sup> 3100                                      | <ul> <li>Data Collection v1.0/1.0.1/1.1</li> <li>Windows<sup>®</sup> NT, SP 5</li> </ul>   |   |  |
|                                | Genetic Analyzer   | <ul> <li>Data Collection v2.0</li> <li>Windows<sup>®</sup> 2000, SP 3 and 4</li> </ul>   | • Comple files  |  |
|                                | ABI PRISM <sup>®</sup><br>3100- <i>Avant</i><br>Genetic Analyzer | <ul> <li>Data Collection v1.0</li> <li>Windows<sup>®</sup> NT, SP 5</li> </ul>   |   |  |
|                                |  | <ul> <li>Data Collection v2.0</li> <li>Windows<sup>®</sup> 2000, SP 3 and 4</li> </ul>   |   |  |
|                                | ABI Prism <sup>®</sup> 310                                       | <ul> <li>Data Collection v1.0/1.0.1/1.1/3.0</li> <li>Windows<sup>®</sup> NT 4.0, SP 3, 4, 5, and 6a</li> </ul>   | Sampla filos  |  |
| -                              |  | <ul> <li>Data Collection v3.0</li> <li>Windows<sup>®</sup> 2000, SP 3 and 4</li> </ul>   |   |  |
|                                | Genetic Analyzer   | <ul> <li>Data Collection v3.1</li> <li>Windows<sup>®</sup> XP Professional, SP 2 and 3</li> </ul>  | Sample files  |  |
|                                |  | <ul> <li>Data Collection v3.1 Vista Patch</li> <li>Windows Vista<sup>®</sup> Business edition, 32-bit, SP1</li> </ul>  | Co-installation   |  |

## **Compatible Data Collection Software and Operating System Column**

This column displays the version(s) of the Data Collection Software, Windows operating system, and any associated service pack(s) supported by the GeneMapper Software Version 4.1 for the specified instrument.

## Sample Data Compatibility Column

This column indicates the methods by which the GeneMapper Software Version 4.1 can process sample data produced by the associated combination of instrument, Data Collection Software, and Windows operating system.

- **Sample Files** The software can import sample files created by the indicated combination of the Data Collection Software and Windows operating system.
- **Co-installation** The software can be installed on the same computer that contains the indicated combination of the Data Collection Software and Windows operating system.
- Automation The software can automatically retrieve and analyze data generated by the indicated combination of the Data Collection Software and Windows operating system.

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