User Bulletin

GeneMapper® Software Version 4.0 and Version 3.7

September 2005

SUBJECT: **Installing the GeneMapper® Software and Creating a Matrix on the ABI PRISM® 310 Genetic Analyzer**

In This User Bulletin

This user bulletin covers the following topics:

Overview

This user bulletin supplements the *GeneMapper® Software v4.0 Installation and Administration Guide*. It contains information that is specific to installing the GeneMapper Software on an ABI PRISM® 310 Genetic Analyzer.

When installing the GeneMapper Software v4.0 or v3.7 on a 310 instrument, be aware of the following:

- **Stand-alone configuration** – The GeneMapper Software v4.0 and v3.7 only supports the *Stand-Alone* configuration on a 310 instrument (see "GeneMapper Software Configuration" on page 2).
- **Autoanalysis** – The GeneMapper Software v4.0 and v3.7 *does not* automatically analyze 310 instrument sample files (see "GeneMapper Software Configuration" on page 2).
- **Co-installation** – The GeneMapper Software v4.0 or v3.7 can be co-installed on the same computer as the 310 Data Collection Software v3.0 or v3.1 as long as you use the computer provided by Applied Biosystems after December 2003.
- **Matrix file** – After installing the GeneMapper Software v4.0 or v3.7, you must create a matrix file using the GeneMapper Software, then export it for use in the 310 Data Collection Software (see "Creating and Using a Matrix File with the GeneMapper® Software" on page 5).

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Installing the GeneMapper® Software v4.0 or v3.7 on the ABI PRISM® 310 Genetic Analyzer

GeneMapper Software Configuration

To install the GeneMapper Software on the 310 instrument, you must perform a *full installation* and select the **Stand-Alone** configuration.

Note: The 310 Data Collection Software v3.1 or v3.0 and the GeneMapper Software v4.0 or v3.7 can be co-installed on the same computer, as long as you use the computer provided by Applied Biosystems after December 2003.

For installation instructions:

- **GeneMapper Software v4.0** – Refer to the *GeneMapper® Software v4.0 Installation and Administration Guide*, PN 4363080
- **GeneMapper Software v3.7** – Refer to the *GeneMapper® Software v3.7 Installation Guide*, PN 4359289

IMPORTANT! The GeneMapper Software v4.0 and v3.7 *does not* perform automatic analysis (autoanalysis) of 310 instrument sample files. You must import 310 instrument sample files into the GeneMapper Software manually.

Minimum Computer Configurations

Table 1 shows the minimum computer configurations required for installing and operating the GeneMapper Software v4.0 and v3.7.

Note: The *GeneMapper® Software v4.0 Installation and Administration Guide* states that two 120-GB hard drive are "a mandatory requirement when the Data Collection Software and the GeneMapper Software are co-installed on the same workstation." This requirement does not apply to the 310 instrument computer, because the 310 Instrument Data Collection Software does not require this hard drive configuration. Installing the GeneMapper Software on the same computer with the 310 Instrument Data Collection Software requires that you use the computer provided by Applied Biosystems after December 2003.

Note: Minimum requirements are the lowest specifications that will permit the installation of the GeneMapper Software. They may not provide optimal performance. Applied Biosystems does not support the GeneMapper Software installed in the minimum installable environment.

Table 1 GeneMapper® Software computer requirements

Component	GeneMapper Software Version 4.0 Minimum Configuration	GeneMapper Software Version 3.7 Minimum Configuration
Computer **Note:** The full installation of the GeneMapper Software requires a hard drive with at least 7 GB of available free space.	• Intel Pentium® processor, 733 MHz • 512 MB of RAM • 20/48× IDE CD-ROM • 10/100 NIC with RWU (internal)	• Intel Pentium® III processor, 733 MHz • 512 MB of RAM • 20/48× IDE CD-ROM • 10/100 NIC with RWU (internal)
Monitor	• 800 × 600 pixel resolution • 17-inch color monitor	• 800 × 600 pixel resolution • 17-inch color monitor
Operating System	Microsoft® Windows® 2000 Professional Operating System, Service Pack 4‡	Microsoft® Windows® 2000 Professional Operating System, Service Pack 3‡

‡ The GeneMapper Software can be installed and operated on a computer running the Microsoft® Windows® XP operating system, Service Pack 1 or later.

Software and Sample File Compatibility

Table 2 shows the version(s) of the Data Collection Software, Microsoft® Windows® operating system, and sample data files supported by the GeneMapper Software for the 310 instrument.

Table 2 ABI PRISM® 310 Genetic Analyzer software and sample file compatibility

Data Collection Software	Operating System and Service Pack	Sample Data Compatibility
Data Collection v3.0	Windows NT‡ 4.0, SP 3, 4, 5, and 6a	Sample Files
Data Collection v3.0	Windows 2000, SP 3 and 4	• Sample Files • Co-installation
Data Collection v3.1	Windows XP, SP 1 or later	

‡ The GeneMapper Software v4.0 and v3.7 *cannot* be installed on a computer running the Microsoft® Windows NT® operating system.

Compatible Data Collection Software

This column displays the version(s) of the Data Collection Software supported by the GeneMapper Software for the 310 instrument.

Compatible Operating System Column

This column displays the version(s) of Windows operating system, and any associated service pack(s) supported by the Data Collection Software for the 310 instrument.

Sample Data Compatibility Column

This column indicates the methods by which the GeneMapper Software can process sample data produced by the associated combination of 310 instrument, Data Collection Software, and Windows operating system.

- **Sample Files** – The software can import sample files created by the specified combination of the Data Collection Software and Windows operating system.
- **Co-installation** – The software can be installed on the same computer that contains the specified combination of the Data Collection Software and Windows operating system.

Creating and Using a Matrix File with the GeneMapper® Software

Overview

When you install a 310 instrument, verify that the instrument operates in accordance to Applied Biosystems installation specifications.

Matrix Standards

Before creating a matrix file using the GeneMapper Software, perform a run with an appropriate matrix standard (see Table 3).

Note: Refer to the product inserts and the *ABI PRISM® 310 Genetic Analyzer User Guide* for more information about how to perform runs using the matrix standards.

Table 3 Matrix standards for the ABI PRISM® 310 Genetic Analyzer

Matrix standard set	Dye set components	Part Number
DS-02	dR110, dR6G, dTAMRA™, dROX™, LIZ®	4323050
DS-20	5-FAM™, JOE™, TAMRA™, ROX™	401114
DS-30	6-FAM™, TET™, HEX™, TAMRA™, ROX™	401546
	NED™ Matrix Standard	402996
DS-31	6-FAM™, TET™, HEX™, TAMRA™, ROX™	401546
	NED™ Matrix Standard	402996
	VIC® Matrix Standard	4313939
DS-32	5-FAM™, JOE™, NED™, ROX™	4312131
DS-33	6-FAM™, VIC®, NED™, PET®, LIZ®	4318159
DS-33	GeneScan Installation Standard	4330397
DS-34	6-FAM™, TET™, HEX™, TAMRA™	401546

Procedures for Creating a Matrix File

Creating a matrix file in GeneMapper requires that you:

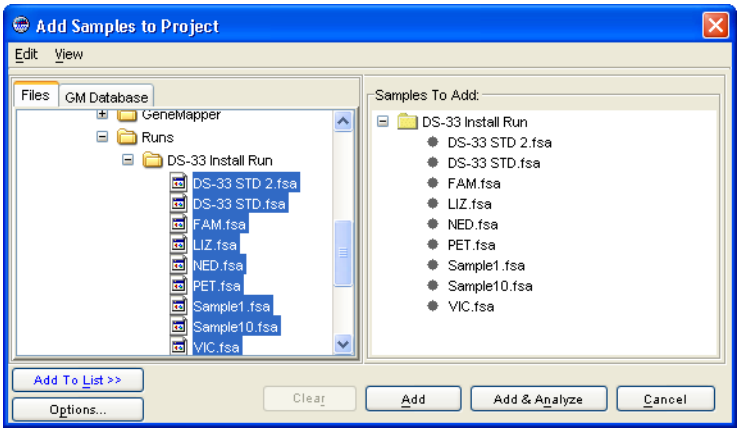
Procedure	See page
1. Perform a run using an appropriate matrix standard (see Table 3 on page 5). Refer to the product inserts and the *ABI PRISM® 310 Genetic Analyzer User Guide* for more information about how to perform the run. After performing the run, create a matrix file using the GeneMapper Software as described in the following procedures.	—
2. Create a GeneMapper Software project that contains the matrix sample files.	7
3. View the raw data of each matrix sample and select an appropriate start point.	8
4. Create the matrix file using the GeneMapper Software.	10
5. Export the matrix file for use in the Data Collection Software.	13
6. Analyze samples.	14

About the Examples in the Following Procedures

The following procedures illustrate creating a matrix file using sample files generated using the DS-33 Matrix Standards as an example. Depending on the various applications you use and other combinations of fluorescent dyes or pH buffer conditions, you may need to create different matrix files. See Table 3 on page 5 for a list of matrix standards.

Creating a GeneMapper Project

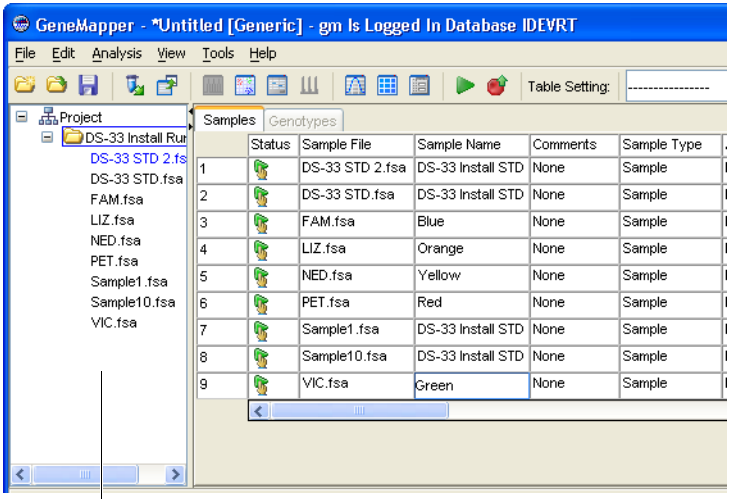
1. Start the GeneMapper Software either by double-clicking the desktop icon or selecting **Start ▸ All Programs ▸ Applied Biosystems ▸ GeneMapper ▸ GeneMapper v*X.X***.
2. In the GeneMapper window, import Samples by selecting **File ▸ Add Samples to Project**.
3. Navigate to the disk/directory containing the files from the DS-33 Install Run, then select the matrix sample files you wish to use.



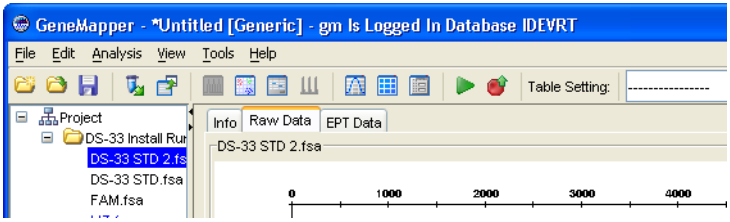
4. Click **Add to List**. A folder containing the files you selected appears in the Samples To Add pane on the right side of the window.
5. Click **Add** to import the files into a new project.

Viewing the Raw Matrix Data

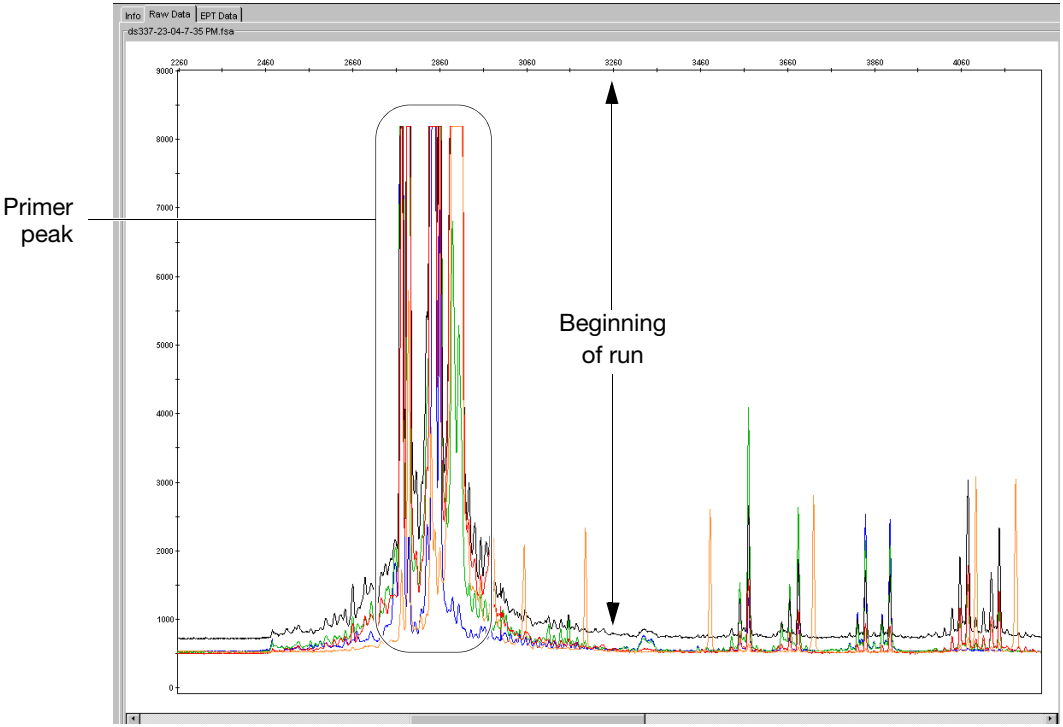
1. In the Navigation Pane, expand the folder containing the sample files.
2. Select a matrix sample file in the Navigation Pane.



3. Select the **Raw Data** tab.



4. In the Raw Data tab, move the cursor well away from the primer peak to a region at the beginning of the run, in a flat part of the baseline. Record the data point values for both the start and stop points in the flat part of the baseline of the data point range. You will enter the start point and the difference of the stop point minus the start point when generating the new matrix.



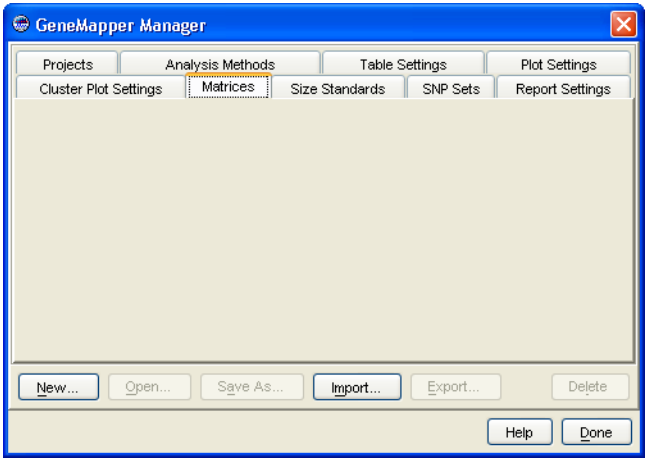
Note: When choosing the start point, do not include primer peaks in the data point range.

Note: To create a good matrix, you need at least five fragments in each color.

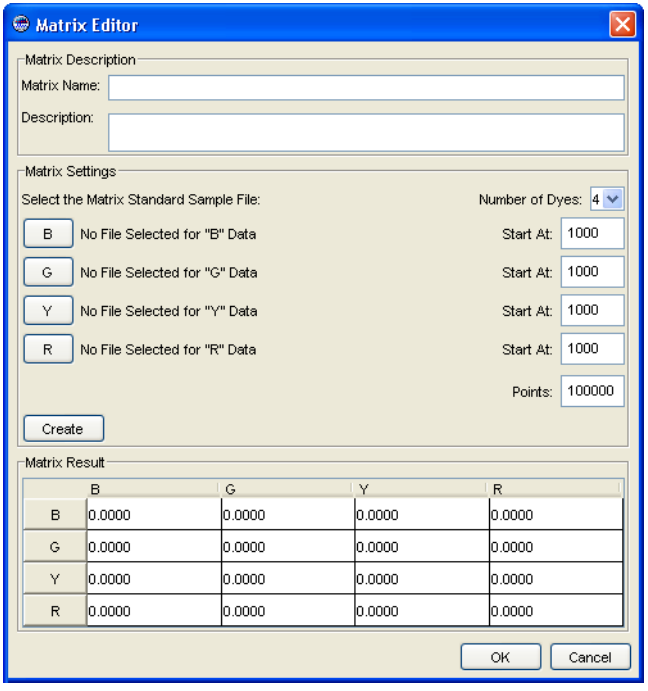
5. Repeat steps 2 to 4 for each matrix sample file. Examine the installation standard at this time and select an appropriate analysis start point.
6. Return to the Samples view by selecting **View ▸ Samples**.

Creating a Matrix Using the GeneMapper Software

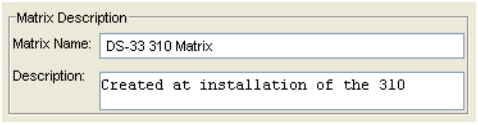
1. Select **Tools ▸ GeneMapper Manager**.
2. Select the **Matrices** tab to access the Matrices page.



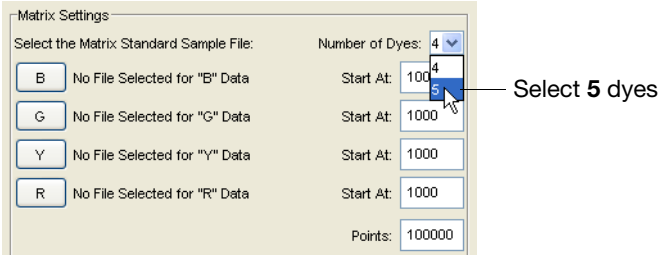
3. Click **New** on the Matrices tab to open the Matrix Editor dialog box.



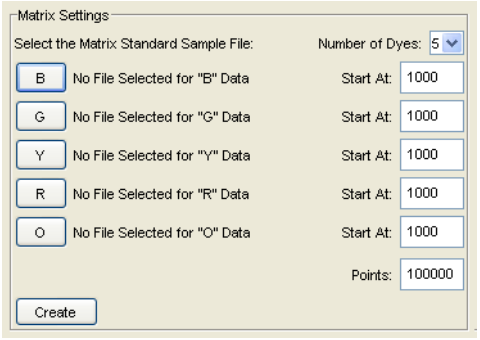
4. Fill out the Matrix Name and Description in the Matrix Description area of the Matrix Editor, as shown below:



5. In the Matrix Settings area of the Matrix Editor:
   a. Select **5** as the number of dyes in the Number of Dyes drop-down list.



   b. Select the sample files for the matrix as follows:



      - Click **B** (for the blue dye), and in the Open dialog box that appears, locate and select the sample file representing the blue dye (FAM dye).
      - Select files for the G (VIC dye), Y (NED dye), R (PET dye), and O (LIZ dye) dyes.

   c. For each dye, enter the start point that you determined in step 4 on page 8 into the Start At field.

d. Enter the total number of data points to include when calculating the matrix in the Points field. In most cases, leave the default value, unless you need to exclude a portion of your data because of artifacts or bleed-through.

Note: To calculate the total number of data points, subtract the start point from the stop point that you recorded on page 8 as follows:

stop point – start point = total number of points

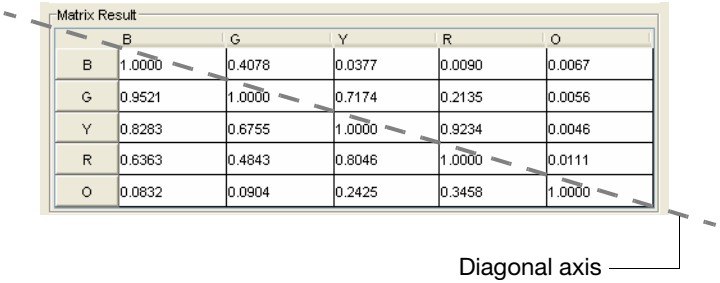
Note: You must have at least five peaks to make a matrix.

e. Click **Create** to generate a new matrix.

6. Review the matrix values in the Matrix Result area at the bottom of the Matrix Editor window to assess values in the new matrix file.

A successfully created matrix displays the value "1.0000" on the diagonal axis and generally decreasing numbers as you move away from the diagonal axis, as shown below. If your matrix values do not conform to this pattern, contact Technical Support.

Note: This pattern does not apply to Filter Set C.



	B	G	Y	R	O
B	1.0000	0.4078	0.0377	0.0090	0.0067
G	0.9521	1.0000	0.7174	0.2135	0.0056
Y	0.8283	0.6755	1.0000	0.9234	0.0046
R	0.6363	0.4843	0.8046	1.0000	0.0111
O	0.0832	0.0904	0.2425	0.3458	1.0000

7. Click **OK** to save and close the Matrix Editor and return to the GeneMapper Manager, then click **Done** to close the GeneMapper Manager and return to the GeneMapper window.

8. Select the matrix sample files in the Samples tab, then select **Edit ▸ Delete from Project**.

Exporting the Matrix File

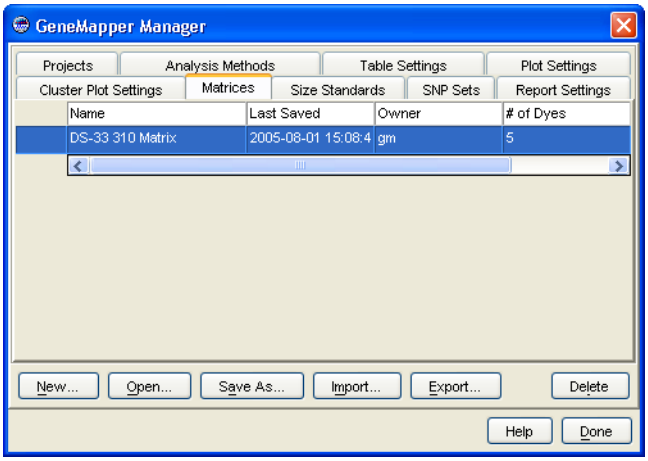
You must export the matrix file manually for it to appear in the matrix field of the sample sheet in the Data Collection Software.

Note: For information about setting up sample sheets, refer to the *ABI PRISM® 310 Genetic Analyzer User Guide*.

To export the matrix file:

IMPORTANT! Be sure to close the 310 Data Collection Software before performing the following procedures.

1. Select **Tools ▸ GeneMapper Manager**.
2. Select the **Matrices** tab to access the Matrices page.



3. Select **DS-33 310 Matrix**, then click **Export**.
4. In the Exporting Matrix window, navigate to:

   D:\AppliedBio\Shared\Analysis\Sizecaller\Matrix
5. Click **Save**.
6. Click **Done** to close the GeneMapper Manager window.

Analyzing Samples

Before performing an analysis using the GeneMapper Software, you must select DS-33 310 Matrix as the analysis method for all the samples in the project.

1. In the GeneMapper Software, open the GeneMapper project that contains the samples you want to analyze.
2. In the Samples tab of the GeneMapper window, select the first sample in the Matrix column.
3. In the drop down list, select **DS-33 310 Matrix**.
4. Highlight the Matrix column, then use the **Edit ▸ Fill Down** feature to assign the DS-33 310 Matrix to all the samples in the project.

The matrix is now applied to your samples. This matrix will be available in the drop-down list when you add samples in the future, Refer to the appropriate GeneMapper Software Getting Started Guide to continue your analysis.

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