User Bulletin

ABI PRISM® 310 Genetic Analyzer

Issue Date – 2/06/03

SUBJECT: G5v2 Module for Use with Dye Set 33 (DS-33)

In This User Bulletin

This user bulletin describes how to install the G5v2 module for the ABI PRISM® 310 Genetic Analyzer and compares data analyzed using the G5 and G5v2 modules.

This user bulletin includes the following topics:

Overview

The ABI PRISM 310 Genetic Analyzer collects fluorescence from five non-overlapping regions, referred to as virtual filters, on the CCD camera. Each virtual filter corresponds to a wavelength range that contains, or is close to, the emission maximum of an ABI PRISM dye.

The G5v2 module for the ABI PRISM 310 Genetic Analyzer provides modified virtual filters that minimize the disparity in the collected fluorescence from each of the fluorophores in Dye Set 33 (DS-33), which include 6-FAM™, VIC®, NED™, PET™, and LIZ® dyes. The adjustment of the relative signals of the collected fluorescence improves intercolor balance in DS-33 applications such as:

- AmpFℓSTR® Identifiler® PCR Amplification Kit
- AmpFℓSTR® SEfiler™ PCR Amplification Kit
- ABI PRISM® Linkage Mapping Set version 2.5

The degree of intercolor balance varies from instrument to instrument and is dependent upon the intercolor balance of sample amplification.

Applied Biosystems

Kits Used with the G5v2 Module

The G5v2 module can be used with kits that use 5-dye technology, such as:

Kit	Dyes	Matrix Standard Set
AmpFℓSTR Identifiler PCR Amplification Kit AmpFℓSTR SEfiler PCR Amplification Kit	• 6-FAM • VIC • NED • PET • LIZ	DS-33 for the 310/377 systems (P/N 4318159)
ABI PRISM Linkage Mapping Set version 2.5	• 6-FAM • VIC • NED • PET* • LIZ *Optionally available for custom-primer labeling	DS-33 for the 310/377 systems (P/N 4318159)

Installing the G5v2 Module

The G5v2 module software is available on CD or can be downloaded from the Applied Biosystems Internet web site.

Downloading From the Internet

1. Navigate to the Applied Biosystems Services and Support website at http://www.appliedbiosystems.com/support/software/.
2. From the Select Product Software pull-down menu, select **ABI PRISM® 310 Genetic Analyzer**.
3. On the Software Download page, select **Module Files** from the Select Software Type pull-down menu.
4. On the ABI PRISM® 310 Genetic Analyzer Modules page, select the **G5v2 Module**, then follow the instructions for download.
5. Install the module as described below.

Installing on the Microsoft Windows NT® Operating System

Note: To run the 5-dye chemistry on a Microsoft Windows NT system, the instrument must be operating with the ABI PRISM 310 Genetic Analyzer Data Collection v 3.0 software. Contact your local sales representative if you do not have this version of data collection software.

1. Close all windows and applications. If you downloaded the G5v2 module from the Applied Biosystems internet web site, skip to step 4.
2. Insert the G5v2 Module Software CD into the computer CD-ROM drive.
3. Double-click **My Computer**, then double-click the CD-ROM drive to open the CD.
4. Double-click **My Computer**, then access the D:\ drive (the default location for the data collection software).
5. On the D:\ drive, navigate to the 310 Data Collection Modules folder (default path D:\AppliedBio\310\Modules), then double-click the folder.
6. Drag the **GS STR POP4 (1mL) G5v2.md5** file from the CD or from its download location to the Modules folder in the 310 Data Collection Modules folder on the hard drive.
7. Close all files, then eject the G5v2 Module CD.

Installing on the Macintosh® Operating System

Note: To run the 5-dye chemistry on a Macintosh system, the instrument must be operating with the ABI PRISM 310 Genetic Analyzer Data Collection v 2.1 software. Contact your local sales representative if you do not have this version of data collection software.

1. Close all windows and applications. If you downloaded the G5v2 module from the Applied Biosystems internet web site, skip to step 4.
2. Insert the G5v2 Module Software CD into the computer CD-ROM drive.
3. On the desktop, double-click the CD icon to open the G5v2 Module CD.
4. On the desktop, double-click the hard drive icon.
5. On the hard drive, navigate to the ABI PRISM 310 folder, then double-click the folder.

6. Drag the **GS STR POP4 (1mL) G5v2** module file from the CD or from its download location to the Modules folder in the ABI PRISM 310 folder on the hard drive.
7. Close all windows, then drag the CD icon to the Trash to eject the G5v2 Module CD.

New Matrix File Required

After you install the new G5v2 module, you must create a new matrix file using the new module.

For information on creating a new matrix file, refer to the procedure in the documentation appropriate for your system.

If your system runs on a Windows NT Operating System, you need these documents:

- *ABI PRISM 310 Genetic Analyzer User's Manual*, P/N 4317588
- *DS-33 Matrix Standard Product Insert,* P/N 4318173
- *AmpFℓSTR Identifiler PCR Amplification Kit User's Manual*, P/N 4323291 or *AmpFℓSTR SEfiler PCR Amplification Kit User's Manual*, P/N 4335145

If your system runs on a Macintosh Operating System, you need these documents:

- *ABI PRISM 310 Genetic Analyzer User's Manual*, P/N 903565
- *User Bulletin: ABI PRISM 310 Data Collection Software, v2.0 and v2.1:Installation and Setup*, P/N 4323951
- *DS-33 Matrix Standard Product Insert,* P/N 4318173
- *AmpFℓSTR Identifiler PCR Amplification Kit User's Manual*, P/N 4323291 or *AmpFℓSTR SEfiler PCR Amplification Kit User's Manual*, P/N 4335145

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.

Examples of Data Analyzed on the ABI PRISM 310 Genetic Analyzer Using the G5 and G5v2 Modules

The figures in this section are examples of data analyzed using the AmpFℓSTR Identifiler PCR Amplification Kit and the ABI PRISM Linkage Mapping Set version 2.5. These figures compare data analyzed using the G5 and G5v2 modules.

The data support that the use of the G5v2 module significantly improves color balance ($p<0.05$) compared to the G5 module, with no change to pull-up ($p>0.05$).

If you are using the AmpFℓSTR Identifiler PCR Amplification Kit or the AmpFℓSTR SEfiler PCR Amplification Kit, perform appropriate validation studies.

Data Analyzed Using the AmpFℓSTR Identifiler PCR Amplification Kit

Data shown in this section were analyzed on the ABI PRISM 310 Genetic Analyzer using the AmpFℓSTR Identifiler PCR Amplification Kit and DS-33.

Note: The G5v2 module does not alter the emission spectra in the LIZ dye region.

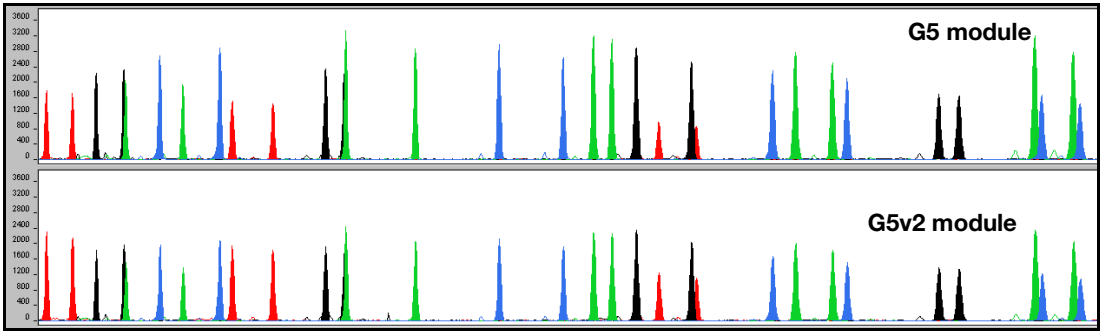

Figure 1 Comparison of emission intensity of a representative profile of a DNA sample amplified with the AmpFℓSTR Identifiler PCR Amplification Kit. Data were analyzed using the G5 and G5v2 modules on a 310 system with Microsoft Windows NT Operating System.

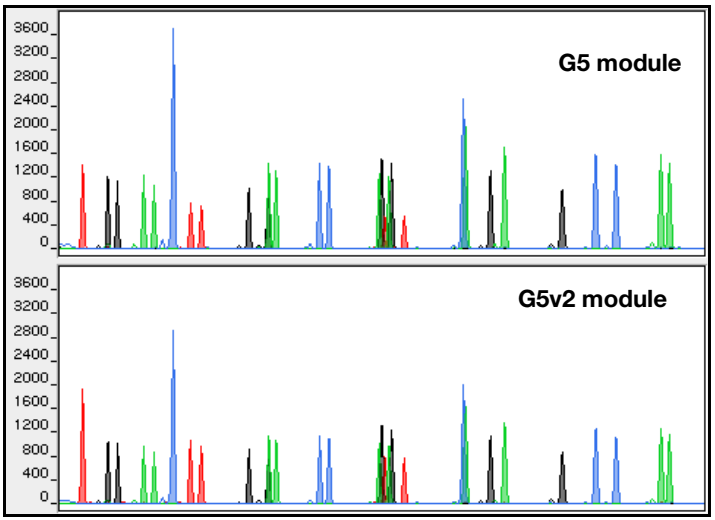

Figure 2 Comparison of emission intensity of a representative profile of a DNA sample amplified with the AmpFℓSTR Identifiler PCR Amplification Kit. Data were analyzed using the G5 and G5v2 modules on a 310 system with Macintosh Operating System.

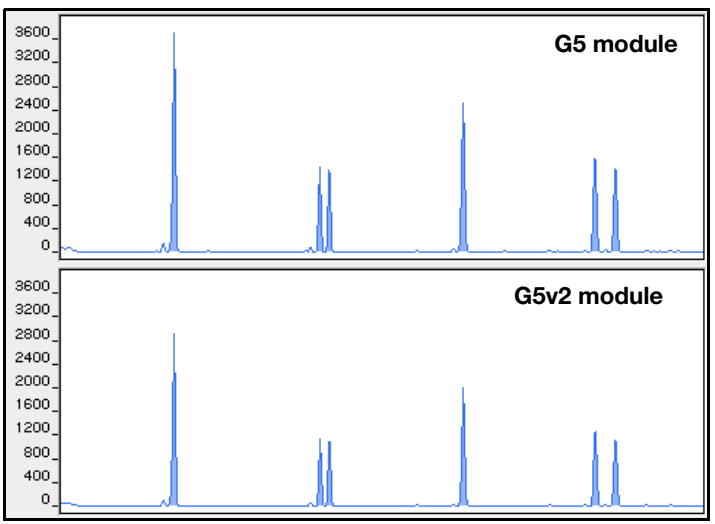

Figure 3 Comparison of the emission intensity of 6-FAM dye data analyzed using G5 and G5v2 modules on a 310 system with Macintosh Operating System. The average signal of the 6-FAM dye is decreased using the G5v2 module relative to the average signal using the G5 module.

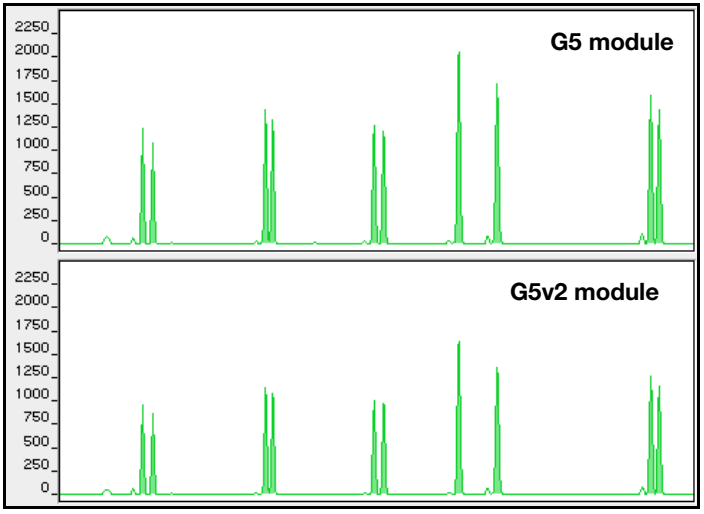

Figure 4 Comparison of the emission intensity of VIC dye data analyzed using the G5 and G5v2 modules on a 310 system with Macintosh Operating System. The average signal of the VIC dye is decreased using the G5v2 module relative to the average signal using the G5 module.

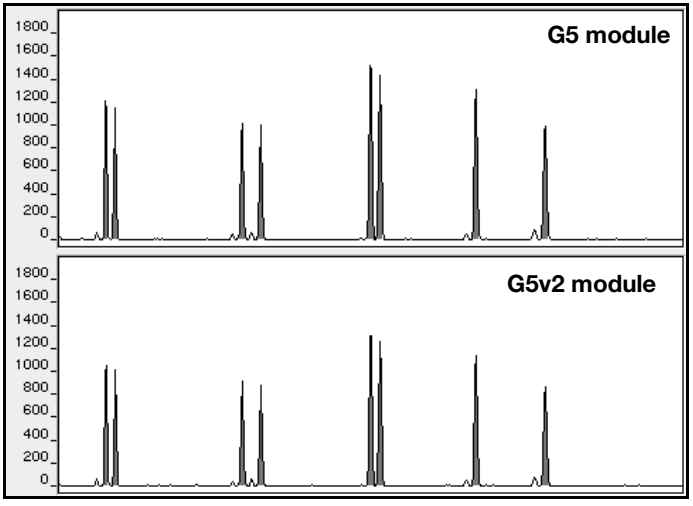

Figure 5 Comparison of the emission intensity of NED dye data analyzed using the G5 and G5v2 modules on a 310 system with Macintosh Operating System. The average signal of the NED dye is slightly decreased using the G5v2 module using the G5v2 module relative to the average signal using the G5 module.

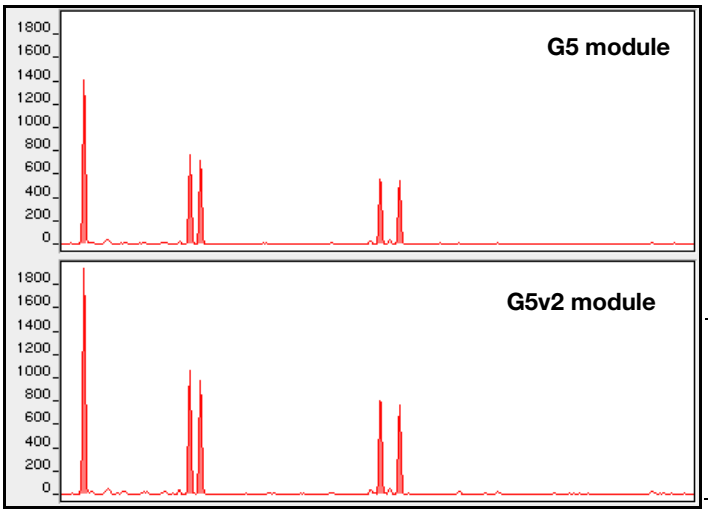

Figure 6 Comparison of the emission intensity of PET dye data analyzed using the G5 and G5v2 modules on a 310 system with Macintosh Operating System. The average signal of the PET dye is increased using the G5v2 module using the G5v2 module relative to the average signal using the G5 module.

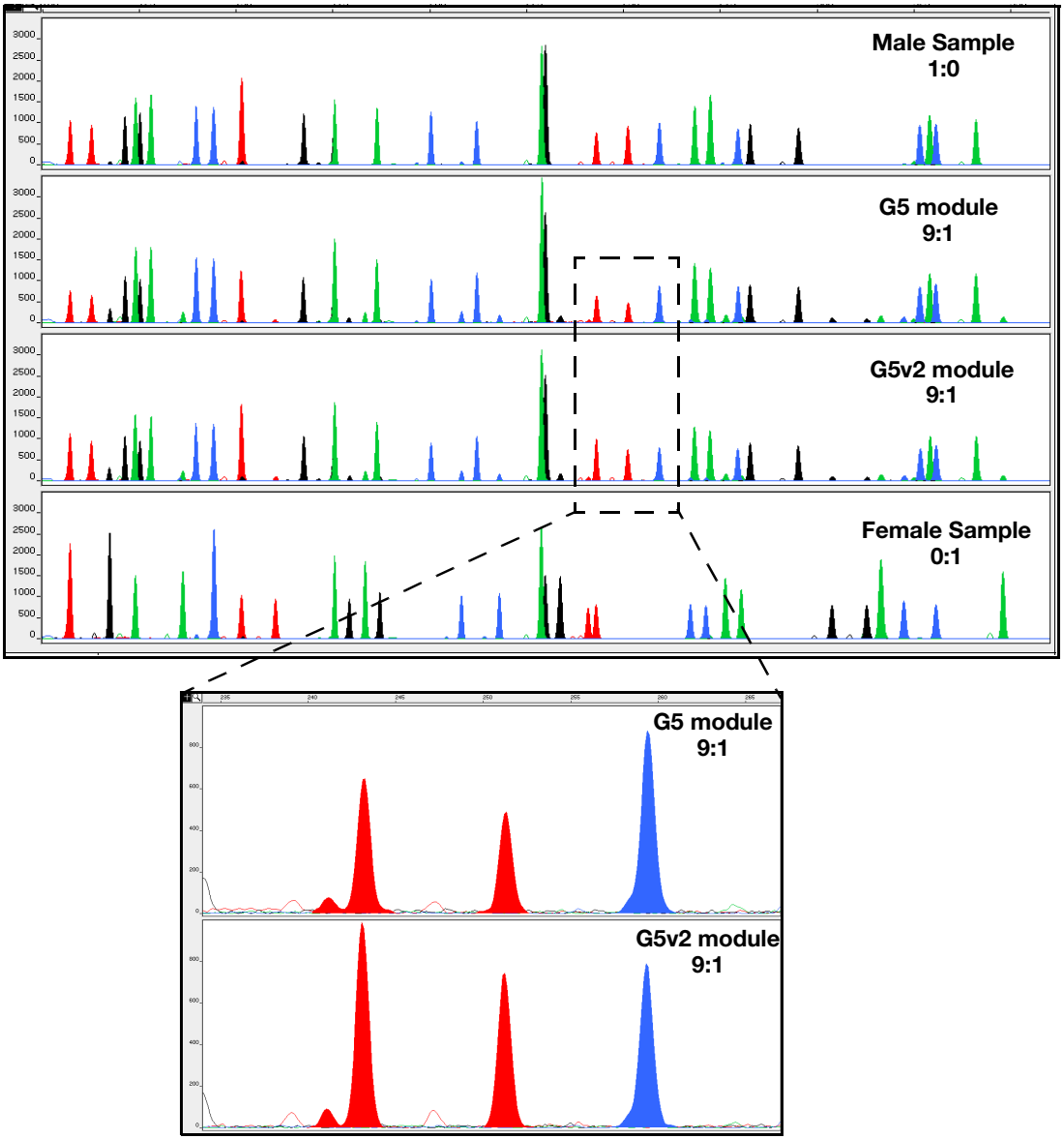

Figure 7 Comparison of mixture study of male and female sample data amplified with the AmpFℓSTR Identifiler PCR Amplification Kit and analyzed using G5 and G5v2 modules on a 310 system with Macintosh Operating System. In the top figure, the first and fourth panels display the profiles of male and female DNA samples amplified individually with 1ng DNA using the AmpFℓSTR Identifiler PCR Amplification Kit. The second and third panels display the data for a mixture of these DNA samples (approximate ratios of 9:1) analyzed using the G5 and G5v2 modules. The bottom figure (panel inset) displays the expanded view of the DNA sample data at FGA (red) and D7S820 (blue) at an approximate ratio of 9:1 with the corresponding module.

Data Analyzed Using the ABI PRISM Linkage Mapping Set Version 2.5

Historically, the recommended pooling ratios for linkage mapping applications using DS-33 on the ABI PRISM 310 Genetic Analyzer have been 1:1:3:4.

Using the G5v2 module, the modified intercolor balance allows you to decrease the amount of sample added when optimizing pooling ratios. The figure below shows examples of possible pooling ratios that can be used with the new G5v2 module. Adjust the pooling ratios as needed for your application.

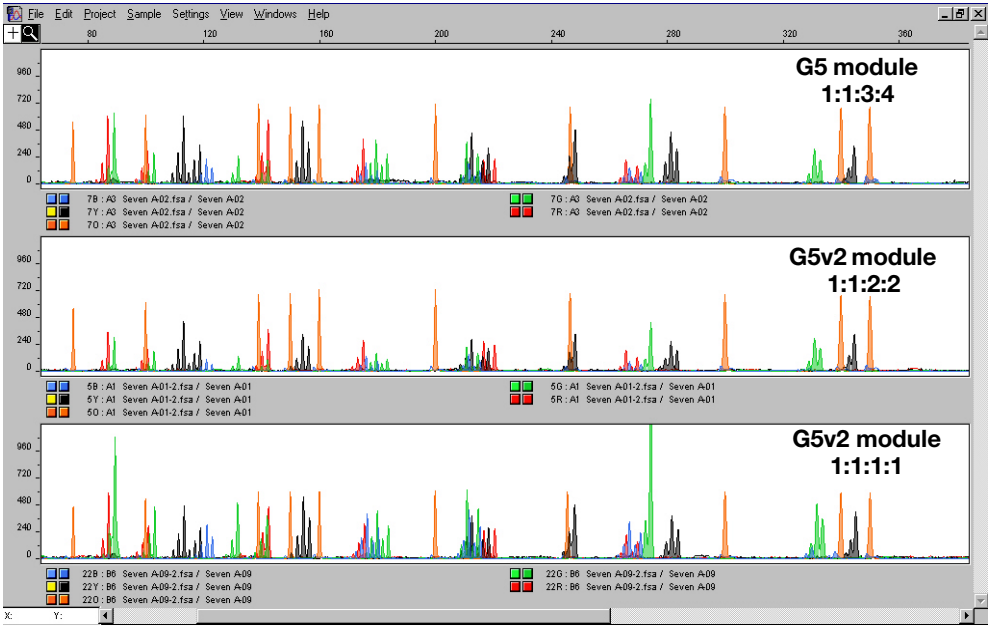

Figure 8 22 Microsatellite Markers. These markers are from the ABI PRISM Linkage Mapping Set v2.5, labeled in 6-FAM, VIC, NED and PET dyes, run on the ABI PRISM 310 Genetic Analyzer with POP-4™ Polymer and the GeneScan™-500 LIZ Size Standard.

Obtaining Technical Support

Applied Biosystems Web Site

A services and support page is available on the Applied Biosystems Web site. To access this, go to:

http://www.appliedbiosystems.com

and click the link for services and support.

At the services and support page, you can:

- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support
- Order Applied Biosystems user documents, MSDSs, certificates of analysis, and other related documents
- Download PDF documents
- Obtain information about customer training
- Download software updates and patches

In addition, the services and support page provides worldwide telephone and fax numbers to contact Applied Biosystems Technical Support and Sales facilities.

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