



SOFTGENETICS®
Software PowerTools for Genetic Analysis

GeneMarker®HID Software for Spectrum CE Systems User Manual

Instructions for Use with Products CE3001, CE3010 and CE3011

Sold and supported by Promega Corporation.
For Technical Services support, e-mail: genetic@promega.com
or visit www.promega.com

Table of Contents

1	Description.....	5
	1.A. Product Listing	5
2	Installing GeneMarker®HID Software for Spectrum CE Systems	6
	2.A. Computer System Requirements	6
	2.B. Installation and Licensing Options	6
	2.C. Managing Program Template Files	30
	2.D. Uninstalling the Software	31
3	Preparing for STR Analysis.....	32
	3.A. Getting Started: Overview	32
	3.B. Importing Data Files	33
	3.C. Raw Data Analysis	36
4	Processing Data	47
	4.A. Template Selection	47
	4.B. Data Process – HID Analysis	55
	4.C. Additional Settings - HID Analysis	61
	4.D. Process Data	64

All technical literature is available at: www.promega.com/protocols/

Visit the web site to verify that you are using the most current version of this Technical Manual.

E-mail Promega Technical Services if you have questions on use of this system: genetic@promega.com

5	Reviewing Processed Data.....	65
5.A.	Navigating the Sample File Tree	66
5.B.	Navigating the Electropherograms with Peak Table	67
5.C.	Navigating the 'All Color Browser' Window	75
5.D.	Navigating the Profile Comparison View	76
5.E.	Navigating the Report Table	79
5.F.	Re-Analyzing Processed Data	92
6	Main Menu Options.....	94
6.A.	'File' Menu	94
6.B.	'View' Menu	95
6.C.	'Project' Menu	107
6.D.	'Applications' Menu	108
6.E.	'Tools' Menu	111
6.F.	'Help' Menu	118
7	Fragment Sizing Standards.....	122
7.A.	Navigating the Size Template Editor	122
7.B.	Navigating the Calibration Charts	134
8	Advanced Use of the Panel Editor.....	140
8.A.	Navigating the Panel Editor	142
8.B.	Editing Existing Panels	150
8.C.	Adjusting Panels to Data—Manual and Automated Panel Calibration	154
8.D.	Custom Panel Creation	155

9	Printing	160
	9.A. Printing from the 'Main Analysis' Window	160
	9.B. Printing from the All Color Browser	170
	9.C. Printing from the 'Calibration Charts' Window	172
	9.D. Printing from the 'Show Edit History' Window	174
	9.E. Printing from the 'User Manager' Window	175
10	Quality Assessment	176
	10.A. Profile Quality	176
	10.B. Contamination Check	187
11	Database Management.....	192
	11.A. Submitting Genotypes to a Database	192
	11.B. Database Editors	195
12	File Name Grouping and Profile Comparisons.....	197
	12.A. File Name Group Editor	197
	12.B. Project Comparison Tool	202
	12.C. Replicate Comparison Tool	206
13	Validation Assistance Tools	213
	13.A. Performing an LOD Calculation	213
	13.B. Performing Stutter Ratio Calculations	216
	13.C. Performing Height Ratio Calculations	218
	13.D. Performing Migration Precision Calculations	219
14	Summary of Changes.....	221

1 Description

GeneMarker®HID Software for Spectrum CE Systems (GMHID-Spectrum) is designed for analysis of short tandem repeat (STR) data generated by capillary electrophoresis (CE) systems, including the Spectrum CE Systems*. GMHID-Spectrum allows genotyping using an allelic ladder and is optimized for human identification applications including forensic casework analysis, databasing, missing persons and relationship testing. GMHID-Spectrum provides a suite of integrated post-genotyping applications, including:

- expert system review
- database creation and profile searching
- contamination check
- kinship analysis
- population statistics calculation

GMHID-Spectrum is compatible with commercial STR chemistries, including PowerPlex® and *GenePrint*® Systems from Promega and custom STR chemistries. The software supports CE instrument data files from numerous platforms, including Promega (.promega, .fsa) and Applied Biosystems (.fsa, .hid). GMHID-Spectrum is developed by SoftGenetics, LLC, and is sold and supported by Promega Corporation. Please review the end user license agreement (EULA) during installation or view it at: www.softgenetics.com/gmhid-spectrumeula

*GMHID-Spectrum also supports analysis of STR data from Applied Biosystems 3130, 3130xl, 3500 and 3500xL Genetic Analyzers.

1.A. Product Listing

PRODUCT	CAT.#
GeneMarker®HID Software for Spectrum CE Systems, Local	CE3001
GeneMarker®HID Software for Spectrum CE Systems, Network	CE3010
GeneMarker®HID Software for Spectrum CE Systems, Client	CE3011

2 Installing GeneMarker®HID Software for Spectrum CE Systems

2.A. Computer System Requirements

Promega recommends the following computer configuration for installation and use of GMHID-Spectrum:

- Windows® 10 Operating System
- Dual-core processor
- ≥4GB RAM and 256GB of available disk storage

Computer systems with lower specifications can operate GMHID-Spectrum but have not been tested at Promega.

2.B. Installation and Licensing Options

GMHID-Spectrum is available for download at: www.promega.com/gmhid/

A Software Registration Document will be provided following the purchase of GMHID-Spectrum. The document will provide instructions to download the software along with license password(s) to register the software following installation.

There are two licensing options available for GMHID-Spectrum: local and network (with client installation(s)). With a local license, each license is installed on and registered to a specific computer. With a network license, GMHID-Spectrum client installations share their licensing information with a central License Server Manager over a network. Further information and installation instructions for each setup are described below.

GMHID-Spectrum can be installed as a 35-day trial version without purchasing a license.

2.B.1 Installing and Activating GMHID-Spectrum in 35-Day Trial Version

Installing GMHID-Spectrum

1. The software will download as a compressed folder. Extract the contents of the folder to the desired location, then open the folder.
2. Double-click the **GeneMarker HID [VX.x.x] Spectrum Installer executable file (.exe)**.
3. The setup wizard will launch, displaying the 'License Agreement' window (Figure 1).

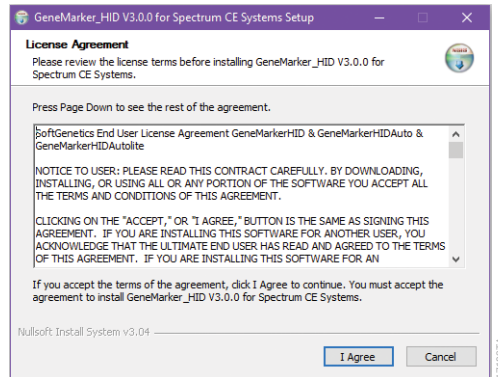


Figure 1. 'License Agreement' window.

4. Read the SoftGenetics End User License Agreement (EULA) and select the **I Agree** button. The EULA can also be reviewed at: www.softgenetics.com/gmhid-spectrumeula
5. Select **Main** in the 'Select components to install' field of the 'Choose Components' window (Figure 2), then select **Next**.

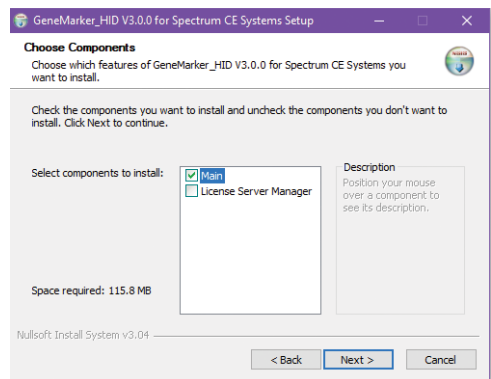


Figure 2. Selecting Main in the 'Choose Components' window.

6. Select the Destination Folder for the software in the 'Choose Install Location' window (Figure 3), then select **Next**.

Note: The default location is **C:\Program Files (x86)\SoftGenetics\GeneMarker_HID\ [VX.x.x]**. To choose a different location, select the **Browse...** button and navigate to the desired location.

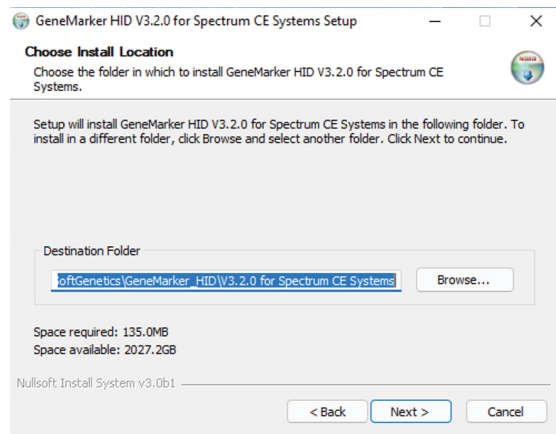


Figure 3. 'Choose Install Location' window.

7. Choose the Start Menu Folder for the software's shortcuts in the 'Choose Start Menu Folder' window (Figure 4), then select **Install**.

Note: If you do not want software shortcuts, select the "Do not create shortcuts" check box.

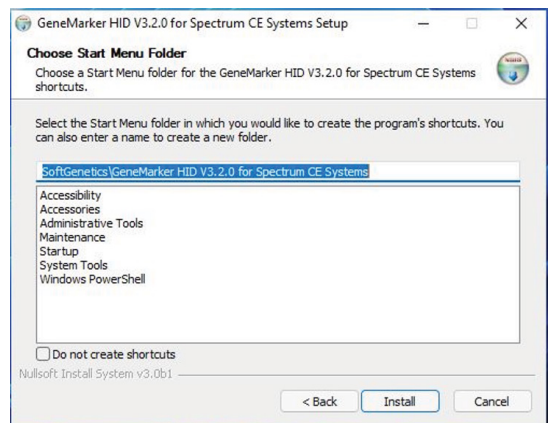


Figure 4. 'Choose Start Menu Folder' window.

8. Select **Close** in the 'Installation Complete' window (Figure 5).

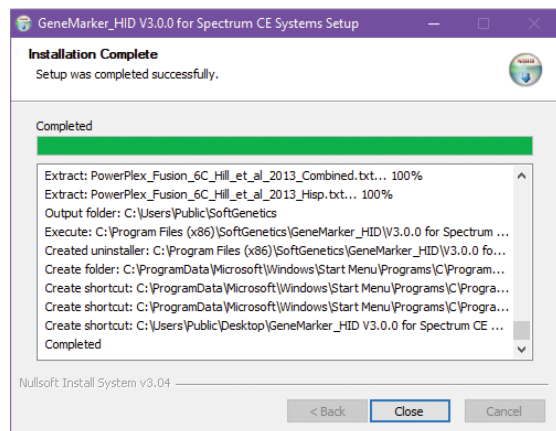


Figure 5. 'Installation Complete' window.

Activating a 35-Day Trial Period

1. Double-click the **GeneMarkerHID** desktop icon or navigate to the destination folder location selected during installation and double-click **GeneMarker_HID.exe** to launch the software.
2. Select **Run Validation** in the 'Configure' window (Figure 6).

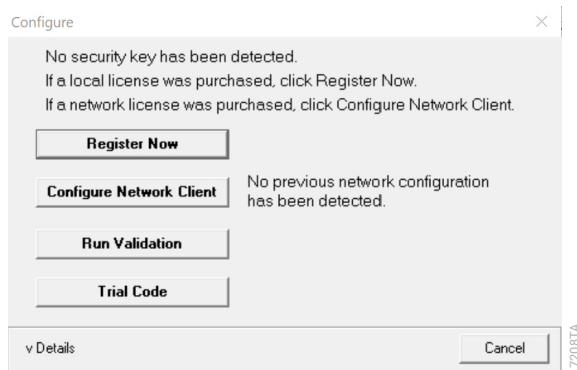


Figure 6. 'Configure' window.

A 'Trial License' window indicating the number of trial days remaining, is displayed each time the software is launched during the trial period (Figure 7). At the end of the trial period, the 'Trial License' window will indicate the trial period is over and the software will not open without registration of a local or network license. To purchase a GMHID-Spectrum license to continue use after the trial period, please contact your Promega representative or e-mail:

genetic@promega.com

Once you receive your license password, follow the registration instructions for your purchased license setup (see Section 2.B.2 for a local license or Section 2.B.3 for a network license).

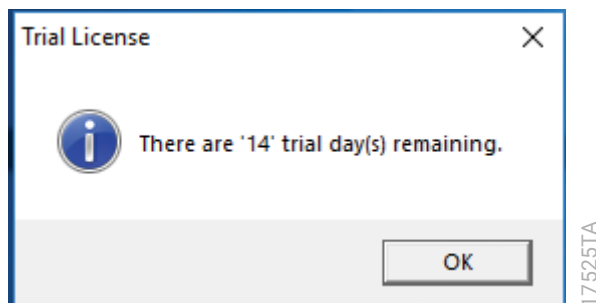


Figure 7. 'Trial License' window.

2.B.2 Installing and Registering GMHID-Spectrum with a Local License

Installing a Local License

1. The software will download as a compressed folder. Extract the contents of the folder to the desired location, then open the folder.

2. Double-click **GeneMarker_HID [VX.x.x] for Spectrum CE Systems_setup executable file (.exe)**.
3. The setup wizard will launch, displaying the 'License Agreement' window (Figure 8).

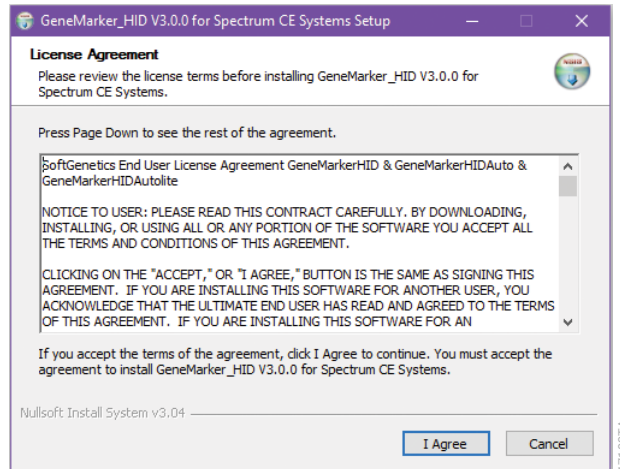


Figure 8. 'License Agreement' window.

4. Read the SoftGenetics End User License Agreement (EULA) and select the **I Agree** button. The EULA can also be reviewed at: www.softgenetics.com/gmhid-spectrumeula
5. Select **Main** in the 'Select components to install' field of the 'Choose Components' window (Figure 9), then select **Next**.

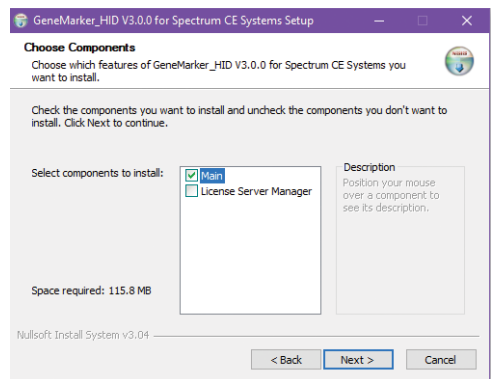


Figure 9. Selecting Main in the 'Choose Components' window.

6. Select the Destination Folder for the software in the 'Choose Install Location' window (Figure 10), then select **Next**.

Note: The default location is **C:\Program Files (x86)\SoftGenetics\GeneMarker_HID\ [VX.x.x]**. To select a different location, select the **Browse...** button and navigate to the desired location.

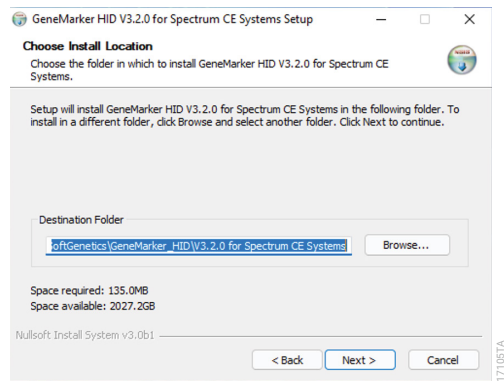


Figure 10. 'Choose Install Location' window.

7. Choose the Start Menu Folder for the software's shortcuts in the 'Choose Start Menu Folder' window (Figure 11), then select **Install**.

Note: If you do not want software shortcuts, select the "Do not create shortcuts" check box.

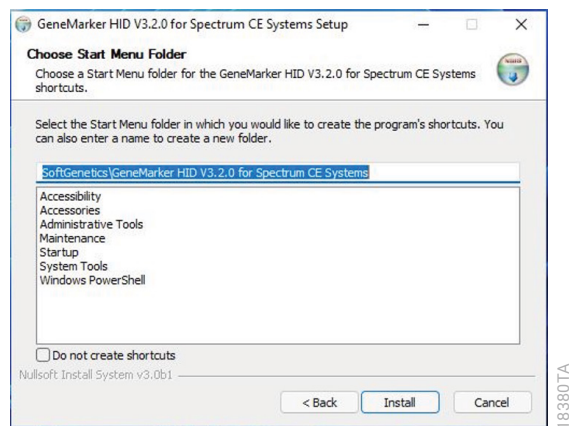


Figure 11. 'Choose Start Menu Folder' window.

8. Select **Close** in the 'Installation Complete' window (Figure 12).

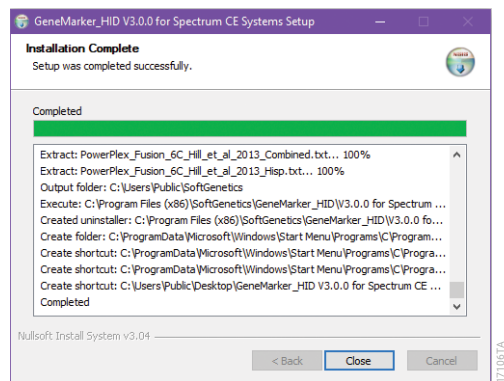


Figure 12. 'Installation Complete' window.

Registering a Local License

1. Double-click the **GeneMarkerHID** desktop icon or navigate to the destination folder location selected during installation and double-click **GeneMarker_HID.exe** to launch the software.
2. Select **Register Now** in the 'Configure' window (Figure 13).

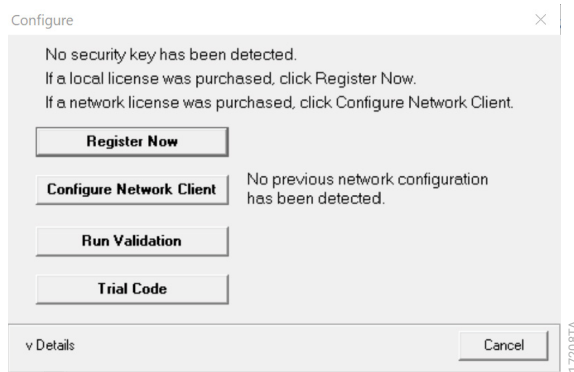


Figure 13. 'Configure' window.

3. Select **Register Local Text-based Key** in the 'Choose Registration Method' window (Figure 14). This opens the 'GeneMarker HID Registration' window (Figure 15). The Request Code is automatically generated by the software.

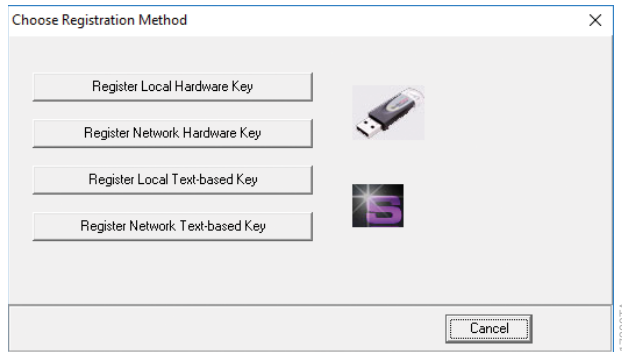


Figure 14. 'Choose Registration Method' window.

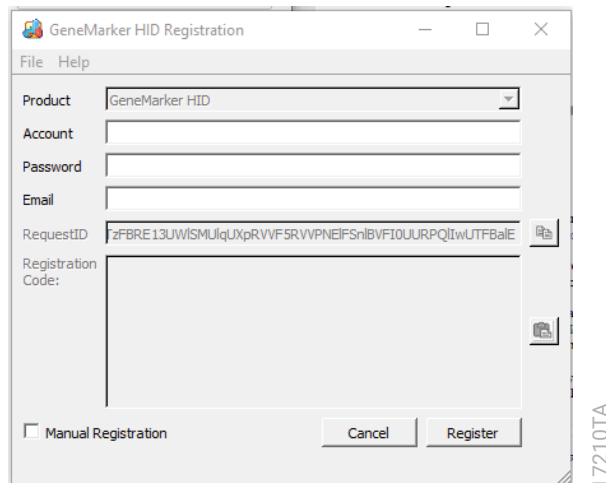


Figure 15. 'GeneMarkerHID Registration' window.

4. Locate the password in your Software Registration Document.

Online Registration

1. If you installed GMHID-Spectrum on a computer with an internet connection, enter your Account (GMHIDSPECTRUM), password and e-mail address, then select **Register**.
2. A confirmation e-mail will be sent once registration is complete.
3. A 'Registration Success' window appears upon successful completion of registration. Select **OK** to close the window.

Notes:

- a. Some characters can commonly be misread. If you get an error trying to register, check for possible confusion between number "1" and lowercase letter "l" or number "0" and uppercase letter "O".
- b. For internet connections that use a proxy server, proxy information can be entered to aid connection for Online Registration.

1. Select **Proxy Settings** from the File menu of the 'GeneMarker HID Registration' window (Figure 16) to open the 'Proxy Settings' window (Figure 17).

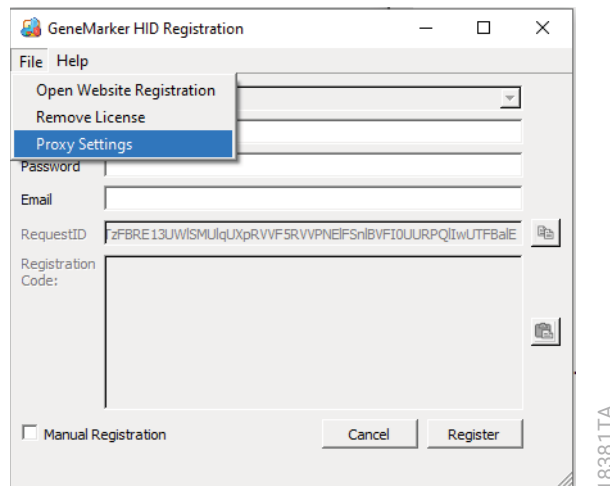


Figure 16. Accessing the 'Proxy Settings' window from the 'GeneMarker HID Registration' window.

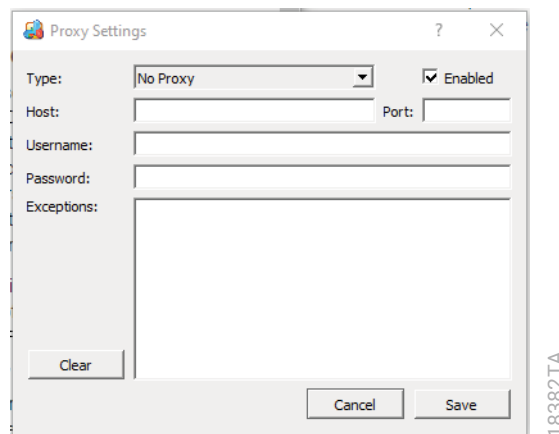


Figure 17. 'Proxy Settings' window.

2. Select the “Enabled” check box to activate proxy connection for registration.
3. Select the proxy type from the drop-down list. Selecting “Default Proxy” will use the proxy settings for the Windows® connection and does not require the other settings in this window to be completed.
4. Enter the host, port, username, password and any needed exceptions for the proxy connection.
5. Select **Save** to apply the proxy settings and return to the ‘GeneMarker HID Registration’ window.

Manual Registration

If the Online Registration process failed, select the “Manual Registration” check box in the ‘GeneMarker HID Registration’ window (Figure 18).

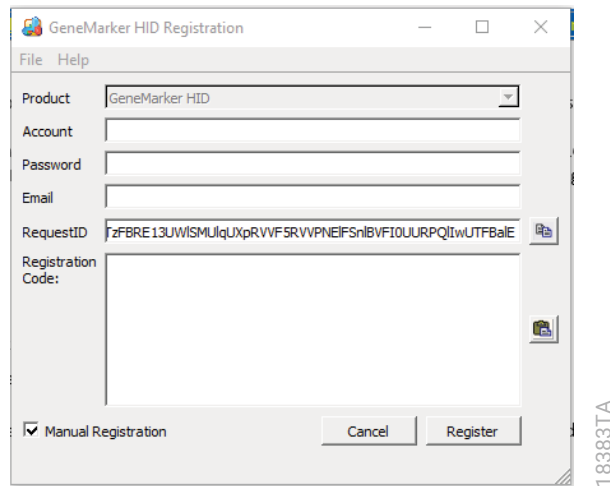


Figure 18. Selecting ‘Manual Registration’ from the ‘GeneMarker HID Registration’ window.

1. If the computer does not have an internet connection, select the **Copy** button to the right of the RequestID field. Then, from a computer with internet connection, navigate to the web site: https://softgenetics.com/releasereg/ng_register.jsp to access the Website Registration form (Figure 19).

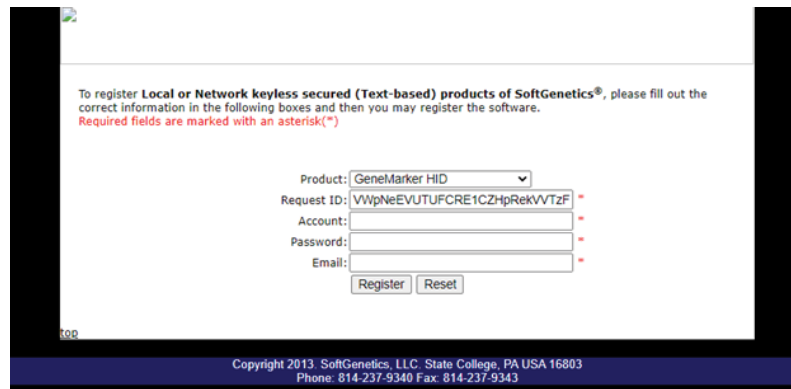


Figure 19. The Website Registration form.

Alternatively, if the computer does have an internet connection, select **Open Website Registration** from the File menu of the 'GeneMarker HID Registration' window (Figure 20) to open the Website Registration form in an internet browser (Figure 19).

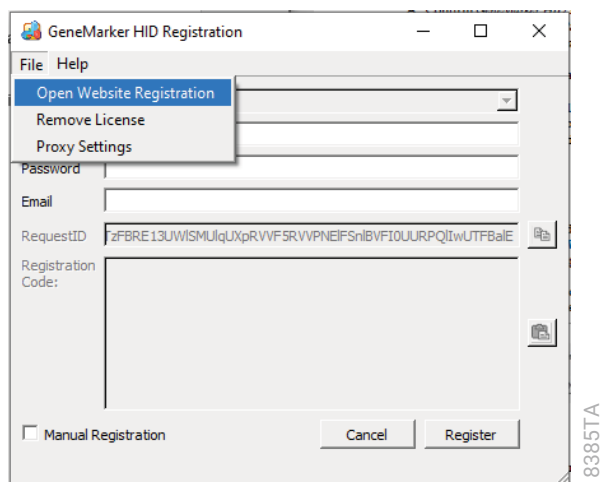


Figure 20. Accessing Website Registration from the 'GeneMarker HID Registration' window.

2. Select **GeneMarker HID** from the Product drop-down field. Paste the Request ID into the form, type your Account (GMHIDSPECTRUM), password and e-mail address, then select **Register**.

Note: The Product name and Request ID are automatically populated when the Open Website Registration command is used.

3. A Registration Code will appear on the web page and a confirmation e-mail will be sent.
4. Copy the Registration Code from the web page.
5. Select the **Paste** button to the right of the Registration Code field of the 'GeneMarker HID Registration' window.

Note: When completing Manual Registration, the Account, Password and Email fields of the 'GeneMarker HID Registration' window do not need to be completed.

6. A 'Registration Success' window appears when registration is successfully completed. Select **OK** to close the window.

If registration via the Website Registration form fails, a Registration Code for Manual Registration can be obtained by contacting Soft Genetics' Technical Support.

1. Select the **Copy** button to the right of the RequestID field from the 'GeneMarker HID Registration' window (Figure 18). Paste the ID and type your Account (GMHIDSPECTRUM) and password into the body of an e-mail and send it to: **tech_support@softgenetics.com**
2. A Registration Code will be sent to you, via e-mail, within one business day.
Note: The Registration Code is unique to this registration event and must be used within 4 days.
3. Copy the Registration Code from the e-mail.
4. Select the **Paste** button to the right of the Registration Code field of the 'GeneMarker HID Registration' window.
Note: When completing Manual Registration, the Account, Password and Email fields of the 'GeneMarker HID Registration' window do not need to be completed.
5. A 'Registration Success' window appears when registration is successfully completed. Select **OK** to close the window.

2.B.3 Installing and Registering GMHID-Spectrum for Network and Client Use

With a network/client license, GMHID-Spectrum installations share their licensing information over a local network. GMHID-Spectrum can be installed on multiple computers, but the maximum number of simultaneous users equals the number of purchased client licenses. The computer where the License Server Manager (LSM) Program is installed is considered the Server computer. Computers on the network other than the Server are called Client computers. GMHID-Spectrum must first be installed and registered on the Server computer before it can be installed on Client computers.

Note: The LSM controls which computers can access GMHID-Spectrum. If the computer running the LSM is turned off, no computer will be able to access GMHID-Spectrum, as the LSM will be nonfunctional. For this reason, we recommend that the LSM be installed on a computer that is always on, or always on during working hours.

Installing the LSM will require you to restart the system to complete installation. Save all work and close all applications before installing the LSM. The LSM uses text-based registration—no hardware is required. Both software components are installed from the same executable file (.exe).

2.B.3.A Installing and Registering a Network License

Installing a Network License

1. The software will download as a compressed folder. Extract the contents of the folder to the desired location, then double-click the folder.
2. Double-click **GeneMarker_HID [VX.x.x] for Spectrum CE Systems_setup executable file (.exe)**.
3. The setup wizard will launch, displaying the 'License Agreement' window (Figure 15).

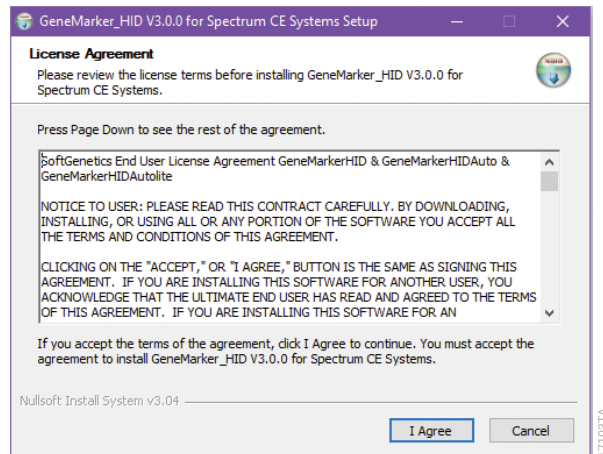


Figure 21. 'License Agreement' window.

4. Read the SoftGenetics End User License Agreement (EULA) and select **I Agree** (Figure 15). The EULA can also be reviewed at: www.softgenetics.com/gmhid-spectrumeula
5. Select **License Server Manager** in the 'Select components to install' field of the 'Choose Components' window (Figure 16), then select **Next**.

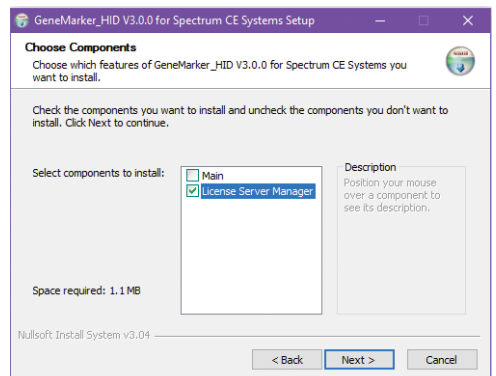


Figure 22. 'Choose Components' window.

6. The setup wizard will first install GMHID-Spectrum. Choose the Destination Folder for the software in the 'Choose Install Location' window (Figure 17), then select **Next**.

Note: The default location is **C:\Program Files (x86)\SoftGenetics\GeneMarker_HID\ [VX.x.x]**. To choose a different location, select the **Browse...** button and navigate to the desired location.

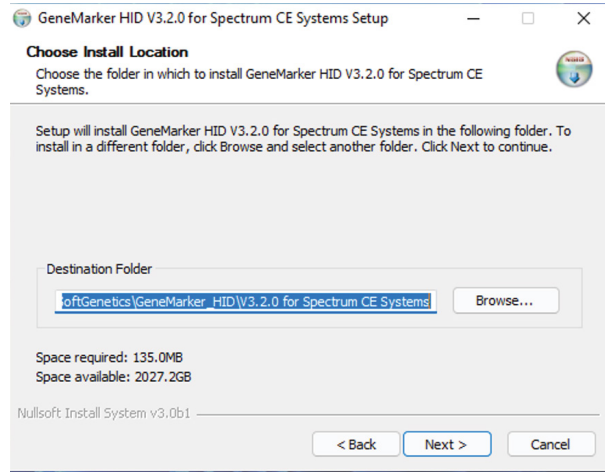


Figure 23. 'Choose Install Location' window.

7. Choose the Start Menu Folder for the software shortcuts in the 'Choose Start Menu Folder' window (Figure 24), then select Install.

Note: If you do not want software shortcuts, select the "Do not create shortcuts" check box.

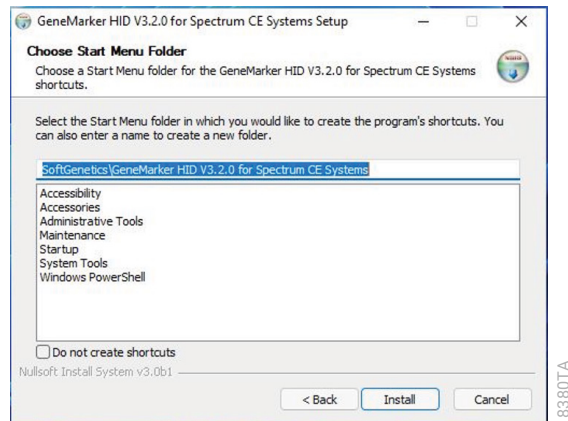


Figure 24. 'Choose Start Menu Folder' window.

8. Select **Close** in the 'Installation Complete' window (Figure 25).

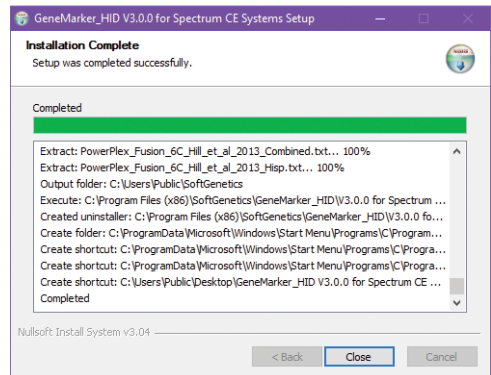


Figure 25. 'Installation Complete' window.

9. The SoftGenetics License Server Setup wizard will launch after GMHID-Spectrum installation is complete (Figure 26). Select **Next**.

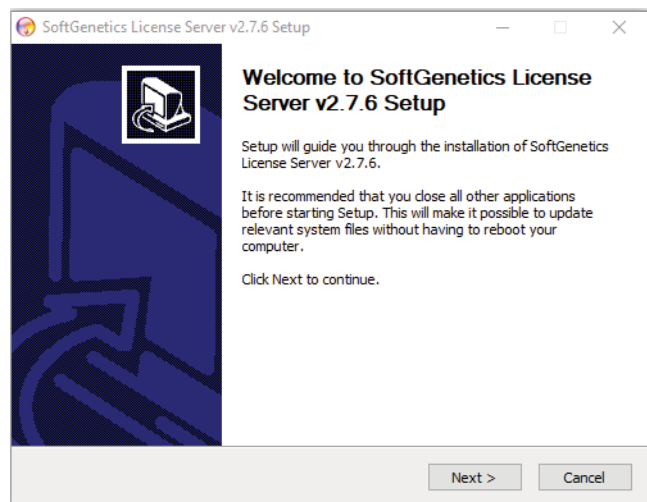
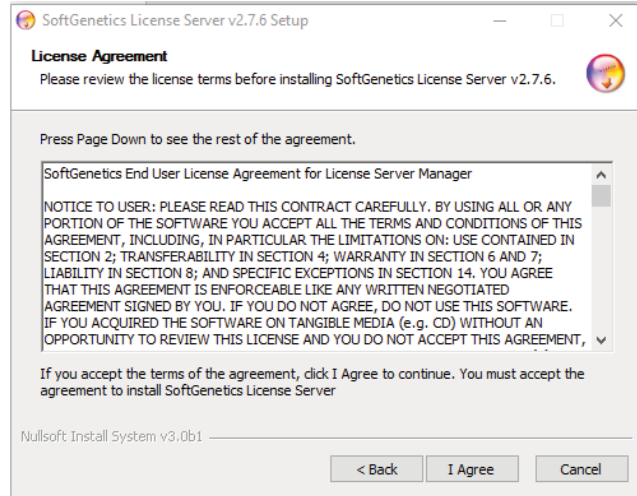


Figure 26. 'Welcome to the SoftGenetics License Server [VX.x.x] Setup' window.

10. Read the SoftGenetics End User License Agreement (EULA) and select the **I Agree** button in the 'License Agreement' window (Figure 27). The EULA can also be reviewed at:
www.softgenetics.com/gmhid-spectrumeula

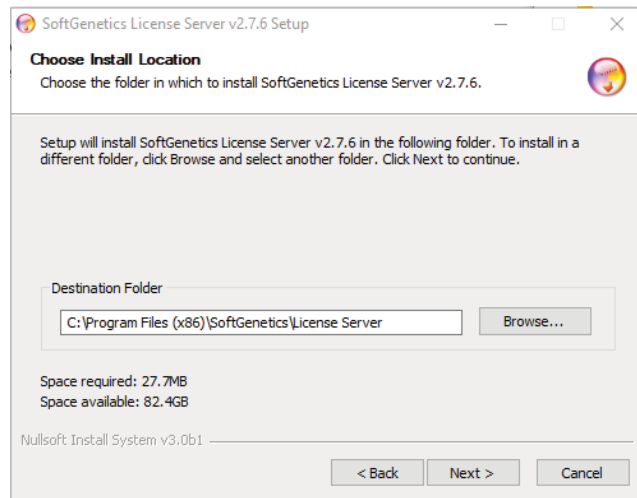


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Figure 27. 'License Agreement' window.

11. Choose the Destination Folder for the software in the 'Choose Install Location' window (Figure 28), then select **Next**.

Note: The default location is C:\Program Files (x86)\SoftGenetics\License Server. To choose a different location, select the **Browse...** button and navigate to the desired location.



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Figure 28. 'Choose Install Location' window.

12. Choose the Start Menu Folder for the software's shortcuts in the 'Choose Start Menu Folder' window (Figure 29), then select **Install**.

Note: If you do not want software shortcuts, select the "Do not create shortcuts" check box.

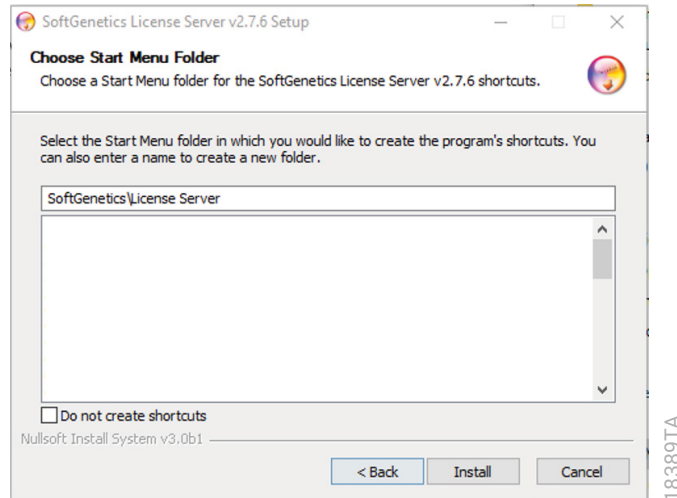


Figure 29. 'Choose Start Menu Folder' window.

13. Select **Next** in the 'Installation Complete' window (Figure 30).

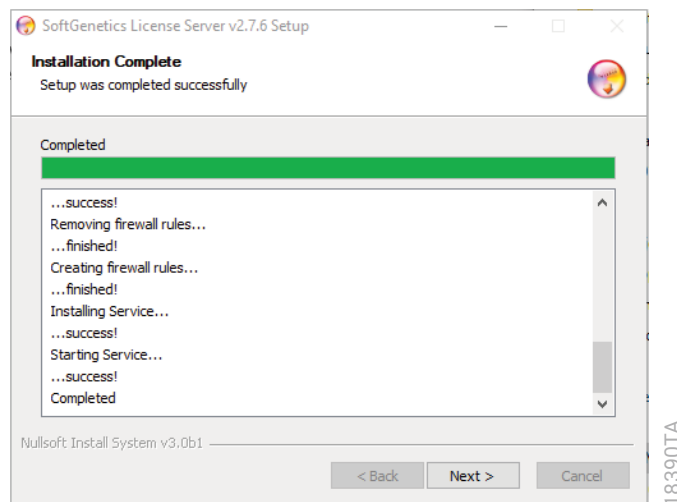


Figure 30. 'Installation Complete' window.

14. Select **Finish** in the 'Completing SoftGenetics License Server [vX.x.x] Setup' window (Figure 31).

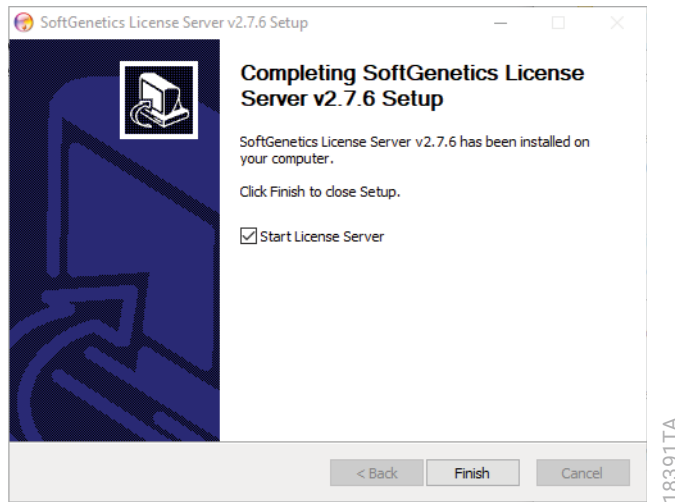


Figure 31. 'Completing SoftGenetics License Server [vX.x.x] Setup' window.

Registering a Network License

1. The LSM should launch after installation. If the LSM did not launch, double-click the **LSM** icon in your system icons tray (Figure 18) or navigate to the destination folder location selected during installation and double-click the **LicenseServer.exe**.



Figure 32. 'LSM' icon.

2. Select the **Register** menu in the 'SoftGenetics License Server Manager' window (Figure 19).

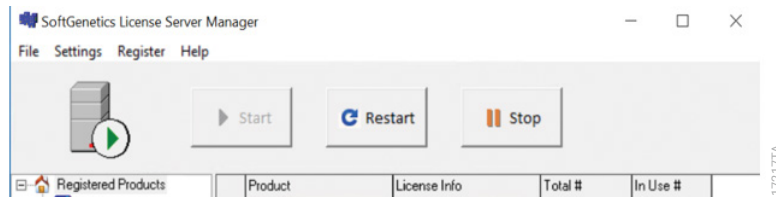


Figure 33. 'SoftGenetics License Server Manager' window.

3. Select **GeneMarker HID** from the 'Register Product Name' drop-down list of the 'SoftGenetics Product Registration' window (Figure 34). The Request Code is automatically generated by the software.
4. Locate the password in your Software Registration Document.

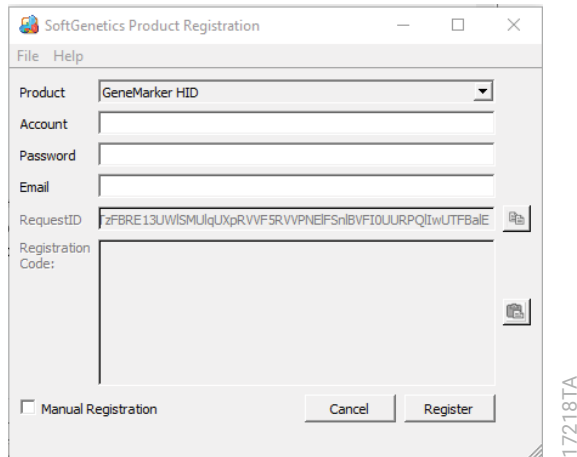


Figure 34. 'SoftGenetics Product Registration' window.

Online Registration

1. If you installed the LSM on a computer with an internet connection, enter your Account (GMHIDSPECTRUM), password and e-mail address under the 'Register Online' tab, then select **Register**.
2. An 'Info' window will appear indicating the registration succeeded. Select **OK**. An 'Action Info' window will appear indicating the LSM will reload the license data. Select **OK**.
3. GeneMarkerHID is now in the 'Registered Products' list of the LSM (Figure 21). The server name or IP address of the computer where the LSM is installed is displayed in the lower left corner of the LSM in the format 'servername or IPaddress:portnumber'. Record the server name or IP address for use in configuring client computers (Section 2.B.3.B).
Note: Some characters can commonly be misread. If you get an error when trying to register, check for possible confusion between the number "1" and lowercase letter "L" or number "0" and uppercase letter "O".

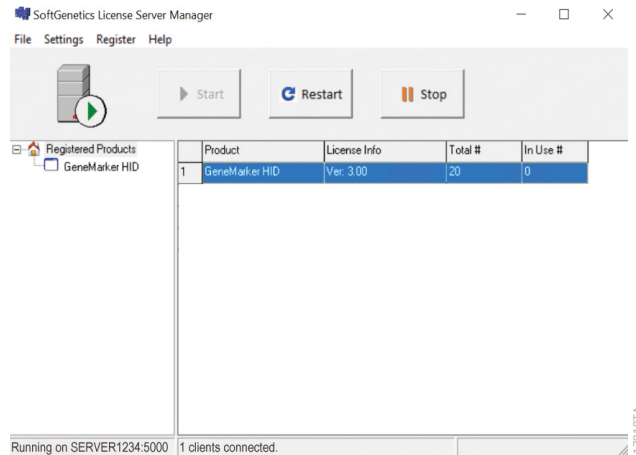


Figure 35. Registered Products and server name or IP address in the 'SoftGenetics License Server Manager' window.

Note: For internet connections that utilize a proxy server, proxy information can be entered to aid connection for Online Registration.

1. Select **Proxy Settings** from the 'File' menu of the 'SoftGenetics Product Registration' window (Figure 36) to open the 'Proxy Settings' window (Figure 37).

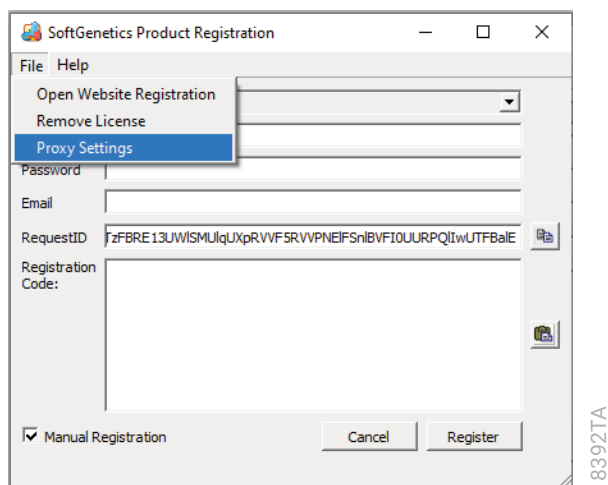


Figure 36. Accessing the 'Proxy Settings' window from the 'SoftGenetics Product Registration' window.

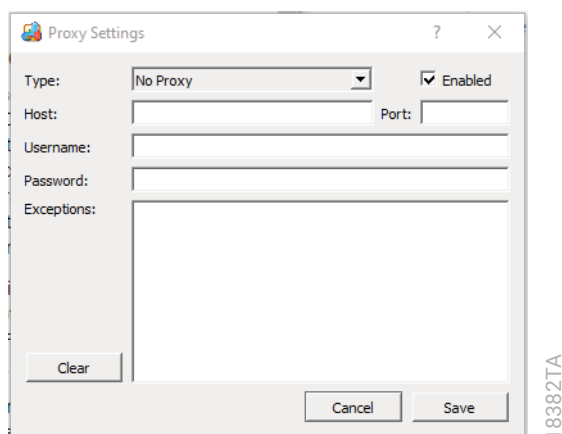


Figure 37. 'Proxy Settings' window.

2. Select the "Enabled" check box to activate proxy connection for registration.
3. Select the proxy type from the drop-down list. Selecting Default Proxy will use the proxy settings for the Windows® connection and does not require the other settings in this window to be completed.
4. Enter the host, port, username, password and any needed exceptions for the proxy connection.
5. Select **Save** to apply the proxy settings and return to the 'SoftGenetics Product Registration' window.

Manual Registration

If the Online Registration process failed, select the “Manual Registration” check box in the ‘SoftGenetics Product Registration’ window (Figure 38).

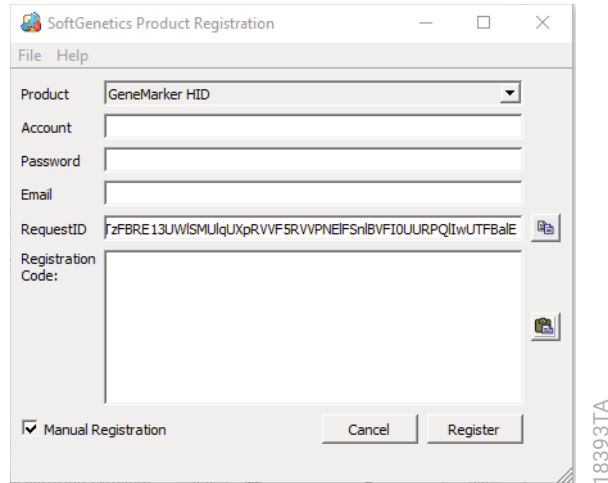


Figure 38. Selecting ‘Manual Registration’ from the ‘SoftGenetics Product Registration’ window.

1. If the computer does not have an internet connection, select the Copy button to the right of the ‘RequestID’ field. Then, from a computer with internet connection, navigate to the website: https://softgenetics.com/releasereg/ng_register.jsp to access the Website Registration form (Figure 39).

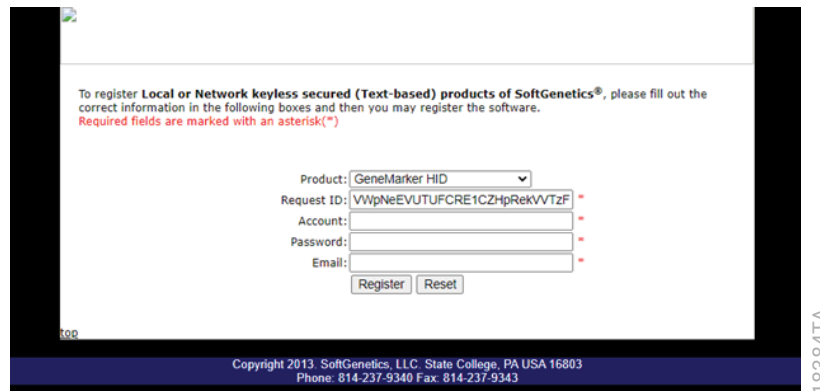


Figure 39. The Website Registration form.

Alternatively, if the computer does have an internet connection, select **Open Website Registration** from the File menu of the 'SoftGenetics Product Registration' window (Figure 40) to open the Website Registration form in an internet browser (Figure 39).

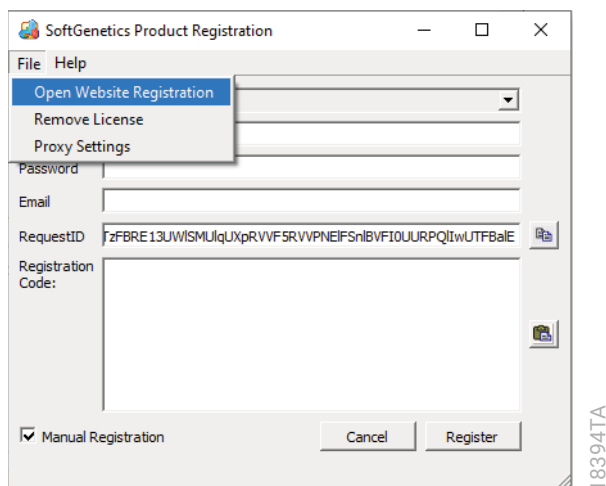


Figure 40. Accessing Website Registration from the 'SoftGenetics Product Registration' window.

2. Select 'GeneMarker HID' from the Product drop-down field. Paste the Request ID into the form, type your Account (GMHIDSPECTRUM), password and e-mail address, then select Register.

Note: The Product name and Request ID are automatically populated when the Open Website Registration command is used.

3. A Registration Code will appear on the webpage and a confirmation e-mail will be sent.
4. Copy the Registration Code from the webpage.
5. Select the **Paste** button to the right of the 'Registration Code' field of the 'SoftGenetics Product Registration' window.

Note: When completing Manual Registration, the Account, Password and Email fields of the 'SoftGenetics Product Registration' window do not need to be completed.

6. A 'Registration Success' window appears upon successful completion of registration. Select **OK** to close the window.

If registration via the Website Registration form fails, a Registration Code for Manual Registration can be obtained by contacting SoftGenetics' Technical Support.

1. Select the **Copy** button to the right of the 'RequestID' field from the 'SoftGenetics Product Registration' window (Figure 38). Paste the ID and type your Account (GMHIDSPECTRUM) and password into the body of an e-mail and send it to: **tech_support@softgenetics.com**
2. A Registration Code will be sent to you, via e-mail, within one business day.

Note: The Registration Code is unique to this registration event and must be used within 4 days from generation.
3. Copy the Registration Code from the e-mail.

4. Select the **Paste** button to the right of the 'Registration Code' field of the 'SoftGenetics Product Registration' window.

Note: When completing Manual Registration, the Account, Password and Email fields of the 'SoftGenetics Product Registration' window do not need to be completed.

5. A 'Registration Success' window appears upon successful completion of registration. Select **OK** to close the window.

2.B.3.B Installing and Configuring on a Client Computer

After an LSM has been installed and registered on a Server computer, GMHID-Spectrum must be installed on client computers, and then configured to the LSM. When configured as a Client, GMHID-Spectrum can be installed on as many computers as desired.

1. Install GMHID-Spectrum on the Client computer as described in Section 2.B.1.
2. Double-click the **GeneMarkerHID** desktop icon or navigate to the destination folder location selected during installation and double-click **GeneMarker_HID.exe** to launch the software.
3. Select **Configure Network Client** in the 'Configure' window (Figure 41).

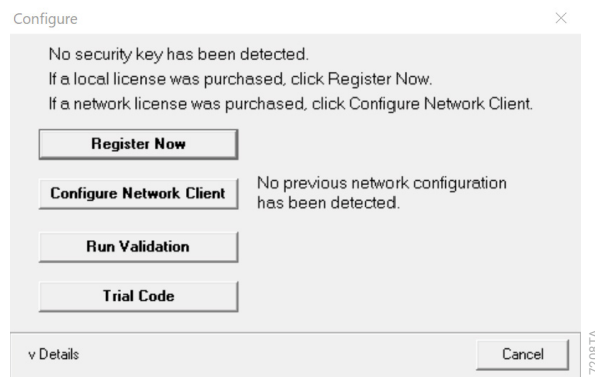


Figure 41. 'Configure' window.

4. Select **Configure Connection to License Server Manager** in the 'Choose Network Configuration' window (Figure 42).

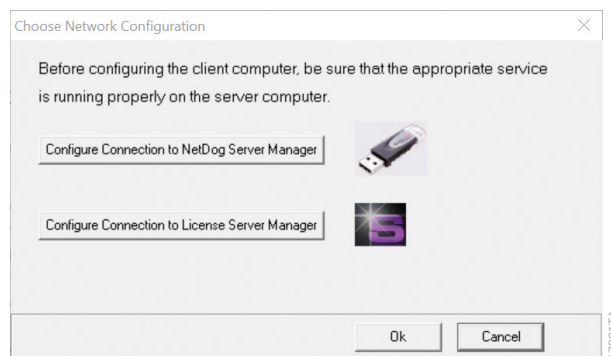


Figure 42. 'Choose Network Configuration' window.

5. Enter the Server Name or Server IP Address of the computer where the LSM is installed (Figure 43).

Note: This information is located in the lower left corner of the 'SoftGenetics License Server Manager' window (Figure 35).

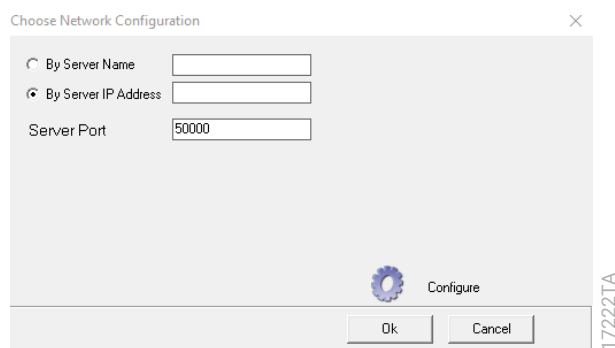


Figure 43. Entering server information in the 'Choose Network Configuration' window.

6. Select the 'Configure' button. An 'Info' window will appear indicating the registration succeeded. Select **OK**. A confirmation window appears if the configuration was successful. Select **OK**.

2.B.4 Updating Software Version

When new versions of GMHID-Spectrum are made available, you can update licenses and install the latest version.

Updating the Software Version of a Local License Installation

To update the version of a local license installation, follow the instructions outlined in "Installing and Registering GMHID-Spectrum with a Local License" using the updated version setup file (Section 2.B.1).

When using a local license, each version of GMHID-Spectrum will be installed in its own version-specific directory. Thus, installing a new version of the program will not overwrite any previous installations. Custom panels, size standards and other files can then be transferred from the old version to the new version, if desired.

Updating the Software Version for a Network/Client License Installation

When using a network license and client installations, the LSM software installation must first be updated before each client computer is updated.

To update the LSM, open the 'License Server Manager' application and follow 'Registering a Network License' (Section 2.B.3.A). After the LSM is updated, update each Client computer by following the instructions outlined in 'Installing and Configuring a Client License' (Section 2.B.3.B). If the network configuration for the LSM has not changed, the client installations of the software should activate without configuring the IP address of the LSM.

User Management of Client Installations

While the number of client installations for a Network License is unlimited, the number of concurrent active users is defined by the number of purchased “seats”. To ensure continued access to the available client seats, we recommend enabling the ‘Overtime Protection’ setting in the User Manager (Section 6.F), which automatically logs out an inactive user after a specified period of time.

2.C. Managing Program Template Files

GMHID-Spectrum uses configuration settings files (.ini) for a series of program templates. These program template files can be shared across software installations for consistency. The software reads and writes to the program template files from their default file location (**C:\Users\Public\SoftGenetics**; see Table 1).

Table 1. Program Template File Locations.

Program Template	Configuration Settings (.ini) file
CODIS CMF (Section 6.D)	codis.ini
Positive Controls (Section 6.E)	PositiveControls.ini
Run Wizard (Section 4.A)	RunWizardTemplates.ini
Sample and Allele Comments (Sections 3.C.1 and 5.B.3)	CommentsTemplates.ini
Preferences (Section 6.B)	Preferences.ini
Print (Section 9)	PrintTemplates.ini

The location of the CODIS CMF, positive controls, Run Wizard, sample and allele comments and print program templates are managed from the ‘Program Data Folders’ section of the ‘Others’ tab of the ‘Preferences’ window (Section 6.B). Copies of the factory-default settings of these five program template files are stored in the **C:\Program Files (x86)\SoftGenetics\GeneMarker_HID\VX.x.x** folder. To restore the factory-default settings of these program template files, copy the appropriate files from the x86 folder and paste them into the ‘Program Data Folders’ location.

Notes:

- The sample and allele comments and print program template files are written to the default file directory the first time a comment and print template are saved in the program.
- You may need to delete the GeneMarker_HID_Spectrum.ini file stored in the **C:\Users\[user name]\AppData\Roaming\SoftGenetics\GeneMarker_HID** folder to reset the Preferences template.

2.D. Uninstalling the Software

To uninstall GMHID-Spectrum navigate to

C:\Program Files (x86)\SoftGenetics\GeneMarker_HID\VX.x.x for Spectrum CE Systems
and run the GeneMarker_HID_Uninstall.exe application. Program template files saved in the
C:\Program Files (x86)\SoftGenetics\GeneMarker_HID\VX.x.x for Spectrum CE Systems
location will be deleted during the uninstall process. Program template files saved outside of
this location will be retained and must be deleted by the user if no longer needed.

3 Preparing for STR Analysis

3.A. Getting Started: Overview

After installing GMHID-Spectrum, a shortcut icon will be displayed on the desktop.

Double-click the icon to open the software. Alternatively, navigate to the 'Start' menu (of the Windows® 10 operating system) and select **GeneMarker®HID Software for Spectrum CE Systems** from the list of programs.

Upon starting GMHID-Spectrum, the launch screen will appear (Figure 44). This screen has a Menu Bar, a Toolbar Ribbon, a File Navigator and a Splash Screen. If the Magic Wizard is enabled as the Run Method for Start up (Section 6.B), the 'Start Your Project' window (Section 3.B) will also be displayed.



Figure 44. GMHID-Spectrum launch screen.

The Menu Bar provides access to the six menu options ('File', 'View', 'Project', 'Applications', 'Tools' and 'Help') that remain static throughout the program and are described in following sections. The Toolbar Ribbon provides shortcut icons to several menu actions. The toolbar icons displayed in the ribbon vary, depending on the state of analysis. Before STR data files are imported, six shortcuts are available for use in the Toolbar Ribbon: Open Data, Show/Hide Navigator, Show/Hide Report, Preferences, Event Log and Magic Wizard. The File Navigator displays an untitled folder when the program is in this state.

3.B. Importing Data Files

To begin fragment analysis, you must upload raw data files to the program. Table 2 shows the list of file types supported by GMHID-Spectrum.

Note: GMHID-Spectrum analyzes one STR chemistry per project. STR data from different chemistries (e.g., PowerPlex® Fusion 6C and PowerPlex® Y23 Systems) must be imported separately into their own projects.

Table 2. File Types Supported by GMHID-Spectrum.

Instrument Provider	File Format
Promega	.fsa, .promega
Applied Biosystems	.fsa, .hid

Data files are imported into the software using the 'Open Data' command. There are three places to access the 'Open Data' command: The 'Start your project' window of the Magic Wizard, the 'Open Data' icon in the Toolbar Ribbon and the 'Open Data' option under the 'File' menu (Figure 45).



Figure 45. 'Open Data' options.

If the Magic Wizard (Section 6.E) is enabled as the Run Method for Start up (Section 6.B), the 'Start your project' window will be displayed when the software is launched. To import data files, select **Open Data** from the 'Start your project' window. Alternatively, **Open Data** can be selected from the toolbar or the 'File' menu. This will launch the 'Open Data Files' window (Figure 46). The 'Open Data Files' window provides several features that streamline the file importing process and customize how the data is processed upon import.

There are four buttons along the right side to define which raw data files will be imported: 'Add', 'Remove', 'Remove All' and 'Add Folder...' (Figure 28). The 'Dyes...' button at the bottom allows you to define dye color names.

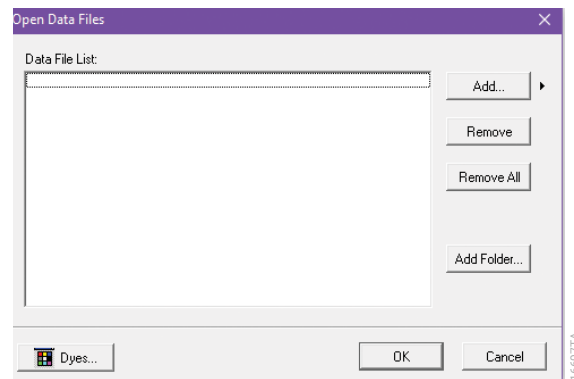


Figure 46. 'Open Data Files' window.

Dyes: Opens the 'Chemistry Dye Set' window (Figure 29), which allows you to define the names for the dye channels in a given kit. The dye names as defined in this window will be displayed in the 'Main Analysis' screen and in any report where the dye display is selected in the print report settings (Section 9, Printing). Chemistry dye sets are organized by the number of dye colors in a kit: 4 Colors, 5 Colors, 6 Colors or 8 Colors. Select the appropriate tab at the top of the window to view or edit chemistry dye sets. The default setting for each tab is 'Universal'. Under this setting the dye names are listed as dye colors. Alternatively, dye names can be defined for specific manufacturers (e.g., AB and Promega) using the drop-down menu under the 'Default' heading (Figure 47).

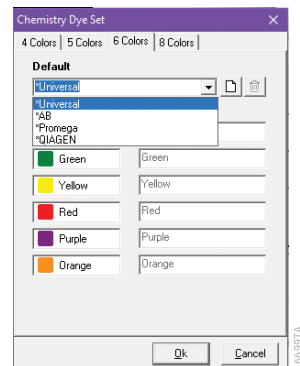


Figure 47. 'Chemistry Dye Set' window showing dye names listed as dye colors.

Alternatively, a new chemistry dye set can be created by selecting the **Create New** icon to the right of the drop-down menu. This opens a 'User Chemistry' window. Enter the chemistry name in the provided field and select **OK**. The new dye set will now appear in the drop-down menu. Enter the appropriate dye names in the 'Name' fields. Edits to the dye names are automatically saved. To delete a user-defined chemistry dye set, select the **chemistry dye set** from the drop-down menu and click the **Delete** button to the right of the drop-down menu.

Note: Only user-defined chemistry dye sets can be edited or deleted. Pre-installed chemistry dye sets, which are marked with an asterisk, cannot be edited or deleted.

Add Folder: Opens a browser window that allows you to navigate to a folder from which raw data will be selected for import. The default folder location is set in Preferences (Section 6.B).

Add: Opens the 'Open' window that allows you to designate a folder from which raw data will be selected for import when the 'Add' button is selected. Select all files with **CTRL + A**, use **CTRL** to select individual files or use **Shift** to select groups of files. Select **Open** to close the browser and add the selected files to the 'Data File List' field in the 'Open Data Files' window. This process can be repeated to import files from different folders to the same project. The default folder location is set in Preferences (Section 6.B). This folder location can also be modified using the 'Add Folder' button.

Note: The four most recently accessed directories can be opened via the arrow next to the 'Add' button.

Remove: Removes selected files from the Data File List so that they are not imported.

Remove All: Removes all files from the Data File List.

Alternatively, you can copy files from a folder outside of GMHID-Spectrum and paste via **Control + V** or right-clicking and selecting **Paste From Clipboard**. You can also remove samples from the Data File List via this right-click menu.

When the desired files are listed in the 'Data File List' field, select **OK** to import the raw data files into the software. A 'Loading Files...' processing window will appear as the files are uploaded. Files that are successfully loaded will be marked **Completed** (Figure 48). Files that are corrupt or cannot be loaded will be marked **Failed**. The 'Loading Files...' window will automatically close once all files are processed.

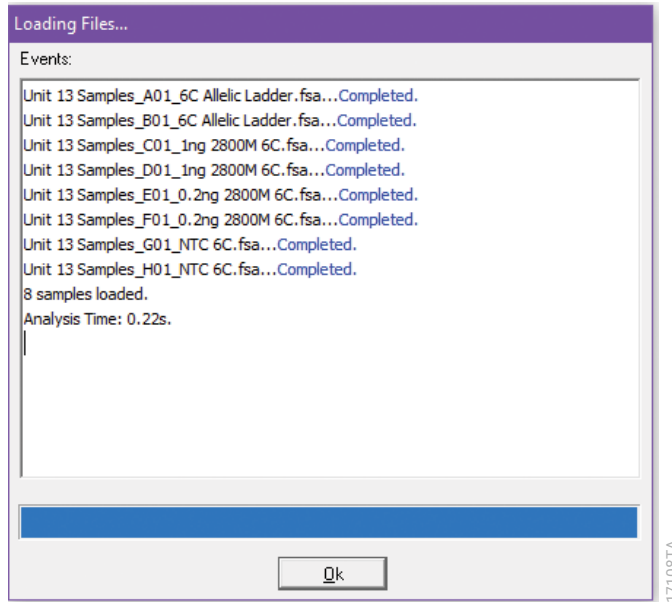


Figure 48. Completed status of 'Loading Files...' window.

3.C. Raw Data Analysis

Once the raw data files are uploaded, the 'Main Analysis' window appears showing raw data (Figure 49). The File Navigator now contains a Sample File Tree listing the imported raw data files. These raw data files remain accessible via the 'Raw Data' folder in the File Navigator throughout the analysis process.

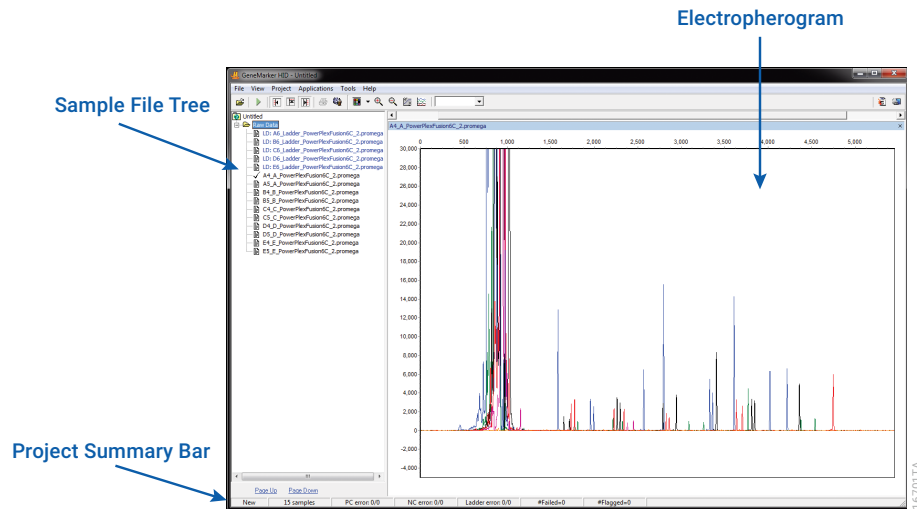









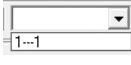




Figure 49. 'Main Analysis' window showing raw data.

The raw data electropherogram charts or electropherograms of selected samples are displayed in the center of the screen. If no samples are selected this area will be blank. A Project Summary Bar that summarizes project information by state, number of samples, and quality status, is now available at the bottom of the screen. Quality metrics are not applied until the data files are analyzed (Section 10). Additional shortcuts are now available in the toolbar ribbon to assist with raw data analysis.

Table 3. Toolbar Ribbon Icons of the 'Raw Data Main Analysis' window.

	Open Data: Opens the 'Open Data Files' window to begin analysis.
	Run Project: Opens the Run Wizard to process the data.
	Show/Hide Toggles: Displays or hides the File Navigator, Gel Image and Report Table, respectively.
	Preferences: Opens the 'Preferences' window of the View menu (Section 6.B).
	Show Dye: Select specific dye channels, show all dye channels or hide all dye channels by selecting the down arrow. To cycle through individual dye channels, click on the icon.
	Zoom In: Zooms in on the center point of the electropherogram.
	Zoom Out: Zooms out from the center point of the electropherogram.
	Set Axis: Provides options to set x and y axes of the electropherogram. The default setting automatically sets the y axis according to the maximum peak intensity (Auto Fit Y) and the x axis according to the analysis range (Auto Fit X). Set specific ranges for the x and y axes by selecting Fixed X and Fixed Y (Figure 38, Section 3.C.2).
	Browse by All Colors: Opens the All Color Browser, which displays sample electropherograms with dye colors separated by individual dye channels (Section 5.C).
	Open Charts Group: Allows the user to toggle between sets of open electropherograms displayed in the 'Main Analysis' window. This option is most useful when the number of samples in the dataset exceeds the computer's ability to display all at once. This typically occurs when several plates of data are uploaded to the project.
	Event Log: Opens the 'Events' window, which displays the status of the last event performed for each sample while loading files and data processing.
	Magic Wizard: Activates the 'Start your project', 'Run' or 'Report' windows, depending on the current stage of data processing (Section 6.E).

3.C.1 Navigating the Sample File Tree

The 'Raw Data' folder in the File Navigator section contains a 'Sample File Tree' list of all imported sample files. By default, the first sample in the tree is selected and its raw data plot is displayed in the electropherogram window. To select or deselect individual samples for review, double-click the samples in the Sample File Tree.

Right-clicking the sample file name provides additional viewing and analysis options (Figure 50).

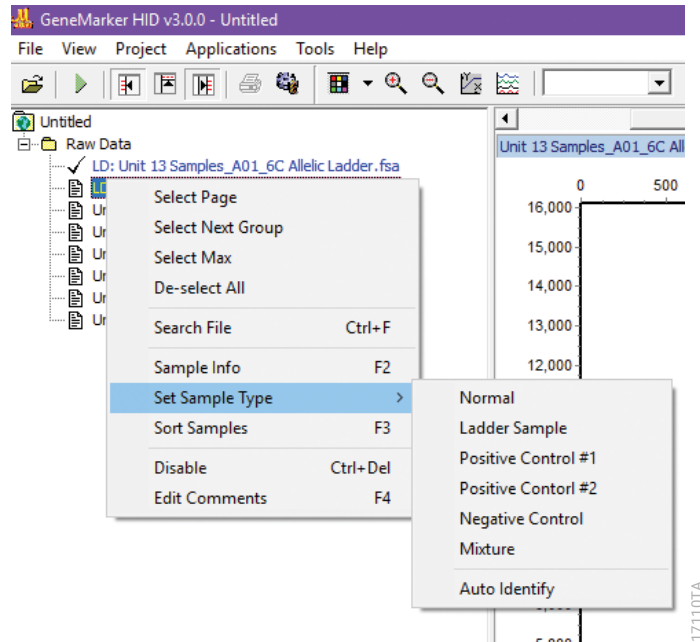


Figure 50. Navigating the Sample File Tree.

Select Page: Opens electropherograms for the number of samples specified in the 'Max # in Page' field in the 'Display' tab of the 'Preferences' window (Section 6.B). Use **Page Up/Page Down** at the bottom of the File Navigator for shortcuts to this process.

Select Next Group: Opens the next group of electropherograms. The number of electropherograms opened as a group is based on the current number of selected samples, regardless of the method of selection (Select Page, Select Max, Sample Grouping (Section 12) or double-clicking individual samples).

Select Max: Opens electropherograms for the number of samples specified in the 'Max # of Open Charts' field in the 'Display' tab of the 'Preferences' window (Section 6.B). If **Max # of Open Charts** is not selected in the 'Preferences' window, all electropherograms in the project will open when **Select Max** is chosen.

De-select All: De-selects all selected samples in the 'Sample File Tree' list and closes the electropherograms.

Search File: Opens the 'File Search' window (Figure 51), which allows you to begin entering a file name to search for the sample. Enter the text to be searched in the 'Match String' field. Check the box next to **Case Sensitive** if a distinction between uppercase and lowercase letters should be applied to the search criteria. Select **Search** to execute the search. Matching files will be displayed in the 'File Name' list. Left-click and use the **Control** or **Shift** key to select samples from the list, then select **Open Selected**. The electropherograms of the selected samples will be displayed.

Use Shortcut = Control + F to launch the 'File Search' window.

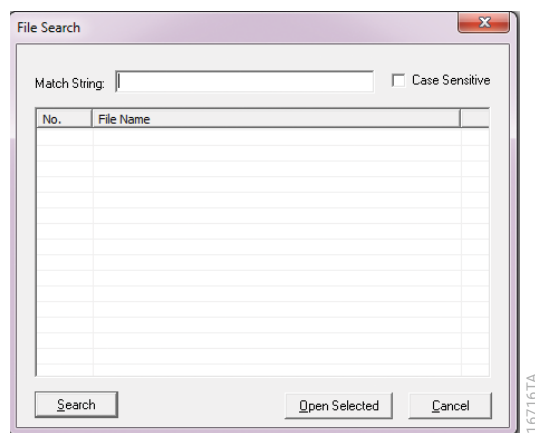


Figure 51. 'File Search' window.

Set Sample Type: Allows you to assign a sample type to the selected sample. Hovering over this option provides a pop-out list of the available sample types (Normal, Ladder Sample, Positive Control #1, Positive Control #2, Negative Control, Mixture). Selecting a sample type will designate the selected sample as that type. You can select a ladder from the dataset from which all samples will be assigned alleles. You can also allow the software to Auto Identify sample types based on specified sample file name identifiers. To use this function, you must complete the 'Sample Type Identifier in Filename' fields in the 'Forensic' tab of the 'Preferences' window (Section 6.B). Alternatively, .promega file identifiers designated in the Spectrum Control Software during sample setup will be retained in GMHID-Spectrum. Ladder and control sample types should be set before data is processed.

Sort Samples: Opens the 'Sort Sample Options' window (Figure 52). First, Second and Third Order sorting options can be selected from the drop-down menu for each field. The options include: Sample Type, File Name, Lane Number, Well ID and Size Score. The check boxes below the 'Order' fields provide additional sorting options:

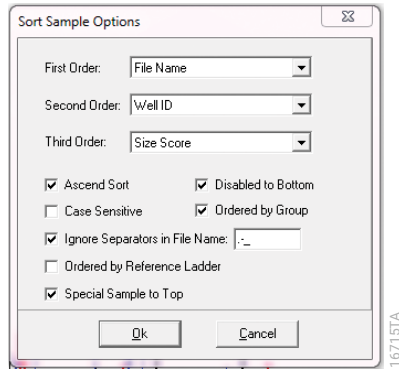


Figure 52. 'Sort Sample Options' window.

Ascend Sort: Sorts samples by ascending order.

Disabled to Bottom: Moves samples marked as Disabled (see below) to the bottom of the list.

Case Sensitive: Makes a distinction between the use of uppercase and lowercase letters when the sort criteria are applied

Ordered by Group: Sorts samples by group when Sample Grouping is applied (Section 12).

Ignore Separators in File Name: Ignores separators entered into the adjacent field when sorting rules are applied.

Ordered by Reference Ladder: Sorts samples by their respective reference ladder (if multiple ladders are included and used for bin offsetting).

Special Sample to Top: Moves samples designated as Ladder, Positive Control or Negative Control to the top of the Sample File Tree.

Note: If this option is selected and these sample designations are made after sorting was applied, sorting will need to be re-applied to bring these samples to the top of the list.

Use Shortcut = F3 to launch the 'Sort Sample Options' window.

Disable (or Enable): Allows you to disable or enable a sample from analysis. When a sample is disabled, the 'Reason Sample is Disabled' window opens (Figure 53), allowing you to enter information in the 'Comments' field. Recent comments will be stored and can be subsequently selected from the 'Reason Sample is Disabled' comments list for future samples. Stored comments can be managed and shared across software installations via the 'Sample Comments' program template file (Section 2.C). A comment is not required to disable a sample. Selecting **OK** marks the sample with a red strike-through. A disabled sample cannot be selected for display in the electropherogram of the 'Main Analysis' window and will not appear in the Report Table if **Show Disabled Samples in Report** in the 'Report' tab of the 'Preferences' window is deselected (Section 6.B).

A disabled sample can be enabled by right-clicking the sample name and selecting **Enable**.

Use Shortcut = Control + Delete to launch the 'Reason Sample is Disabled' window (Figure 53).

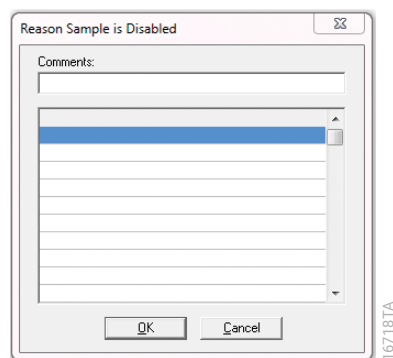


Figure 53. 'Reason Sample is Disabled' window.

Edit Comments: Opens the 'Edit Sample Comments' window, which allows you to enter information in the 'Comments' field. Recent comments will be stored and can be subsequently selected for future samples. Stored comments can be managed and shared across software installations via the Sample Comments program template file (Section 2.C). Comments assigned to a sample are viewable in the 'Sample Comments' column of the Peak Table (Section 5.B.2) of the analyzed data and printable via the 'Print Report' window (Section 9.A) and the 'All Color Browser Setting' window (Section 9.C).

Use Shortcut = F4 to launch the 'Edit Sample Comments' window.

Sample Info: Opens the 'Sample Information' window for the selected sample (Figure 54). There are three tabs in the 'Sample Information' window: 'Sample Info', 'Consumable Report' and 'Log Messages'. The 'Consumable Report' and 'Log Messages' tabs are only populated when data files are in the .promega format from the Spectrum CE System.

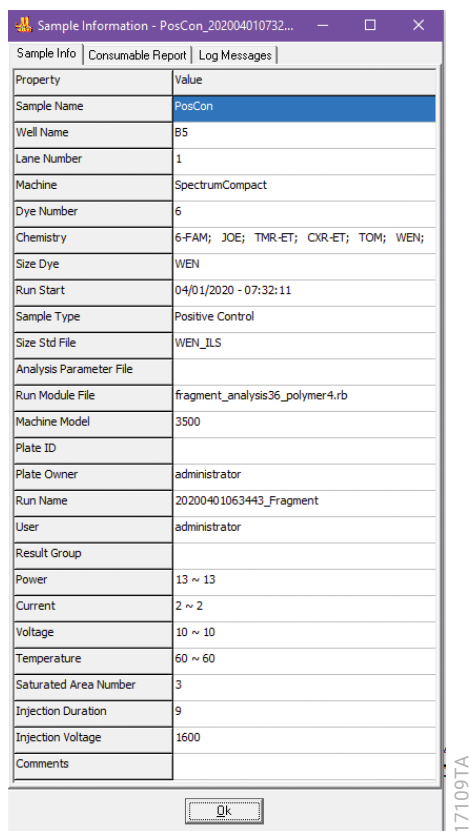


Figure 54. Three tabs in the 'Sample Information' window.

Sample Info: Displays a list of properties collected from the instrument data collection software, including: Sample Name, Well Name, Lane Number (capillary number), Machine (instrument name) and run information. The list of properties varies depending on the file type.

Consumable Report: Displays the list of consumables and their respective RFID information recorded by the instrument at the beginning of a run.

Log Messages: Displays the list of instrument events log messages recorded during the run.

Use Shortcut = F2 to launch the 'Sample Information' window.

3.C.2 Navigating the Electropherogram Charts

Raw data electropherograms for selected samples will be displayed in the electropherogram chart area of the main window. If multiple samples are selected, individual electropherograms can be closed using the X in the upper right corner. The electropherograms display fluorescent signal intensities for the selected dye color(s). The signal intensities, recorded in Relative Fluorescent Units (RFU), are plotted along a camera frame scale for the raw data displayed in the 'Main Analysis' window (Figure 55). The largest sized fragments are on the far right of the plot. The dye channels displayed in the plot can be chosen by using the 'Show Dye' icon in the toolbar.

Select the arrow next to the 'Show Dye' icon and select the dye channels from the drop-down list to display in the electropherogram. Select **Show All** or **Hide All** to view or hide all dye channels, respectively. Alternatively, select the **Show Dye** icon to cycle through dye channels in the order in which they appear in the drop-down list.



Figure 55. Raw data in the 'Main Analysis' window.

To move along the x axis of the selected electropherogram(s), use the top slider bar (below the toolbar) to scroll the image in either direction, or hold down the right mouse button within the electropherogram and drag the plot right or left.

By default, the y-axis scale is set automatically according to the maximum peak intensity of the data within a given view. To adjust the x and/or y axis to a fixed setting, use the 'Fixed X' and 'Fixed Y' functions by selecting the **Set Axis** icon in the toolbar. This will display the 'Set Axis' drop-down menu (Figure 56).

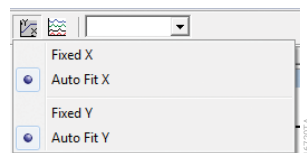


Figure 56. 'Set Axis' menu.

The x- and y-axis position of the mouse pointer in the electropherogram is displayed in the upper right corner of the electropherogram.

Zooming in or out of the electropherogram can be accomplished via two methods. To zoom in to a specific area within the electropherogram, hold down the left mouse button and drag a box from upper left to lower right around the area. To zoom back out, hold down the left mouse button and drag a box in the opposite direction from lower right to upper left. To zoom in to or out from the midpoint of the plot, select the appropriate zoom icon in the toolbar (Table 3).

3.C.3 Navigating the Gel Image

In addition to the electropherogram display, a synthetic gel image may be viewed for all samples in a dataset (Figure 57). To view the image, select the **Show/Hide Gel Image** icon from the toolbar ribbon (Table 3) or select **Show Gel Image** from the 'View' menu.

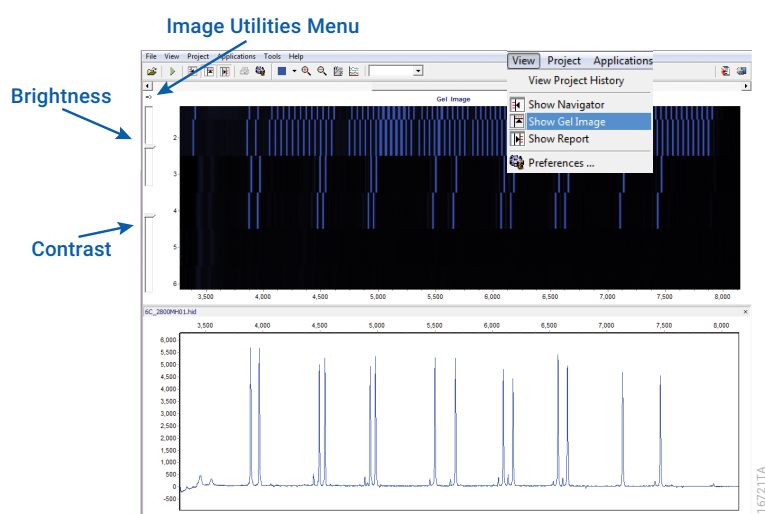


Figure 57. 'Gel Image' window.

The gel image displays the unprocessed data in a traditional gel format with larger fragments located on the right. Each sample within the dataset has its own lane within the gel image. Samples are ordered vertically down the gel based on the order listed in the Sample File Tree. To identify which sample is in a specific lane within the gel image, hover over the lane and a tool tip will display the corresponding file name. Any modifications to the view (e.g., zooming in or out or changing the displayed dye channels) will automatically apply to both the gel image and the electropherogram.

Options to copy, save or view the gel image in a separate window are available in the 'Image Utilities' menu. To access this menu, select the **Image Utilities** icon in the upper left corner of the gel image. A pop-out menu will appear with the following options:

Copy to Clipboard: Copies the gel image to the Windows® clipboard to paste into other applications such as Microsoft® PowerPoint.

Save Image: Allows the user to save the gel image as a .png, .jpeg, .bmp or .pdf image file.


Show In Window: Displays the gel image in its own window within the software.

The brightness and contrast of the bands within the gel image can be adjusted using the slide bars on the left side of the gel image (Figure 39). The upper slide bar adjusts the brightness of the gel image, with the top of the slide being the brightest. The lower slide bar adjusts the contrast of the gel image, with the top of the slide being the highest contrast.

The gel image can be further adjusted by navigating to the 'Gel Image' section of the 'Display' tab of the 'Preferences' window.

Select **Gray for Single Dye** to change the single dye gel image to black and white when only a single dye color is selected (when multiple dye colors are selected the fragments will appear in their respective colors). Select the **Background in White** option to reverse the black and white exposure for single-dye color gel images.

3.C.4 Navigating the 'All Color Browser' Window

In addition to the electropherogram within the main window, which provides an overlay view of the plots when multiple colors are selected, the 'All Color Browser' window provides a view of the same data with separate charts for each selected color (i.e., dye channel charts). To access the 'All Color Browser' window, select the **Browse by All Color** icon  in the toolbar ribbon.

The 'All Color Browser' window contains its own toolbar ribbon that provides options to change the display (Table 4). The displayed dye channels can be individually selected or deselected using the 'Show Dye' icon in the toolbar. Zooming in and out of the data occurs in the same manner as in the 'Main Analysis' window. Right-clicking in the electropherogram provides a shortcut for switching between Fixed Y and Auto Fit Y.






When the Mouse Cross Lines are shown (Table 4), grid lines for the x and y axes will be shown as the cursor moves over the plot. For raw data, the numbers displayed correspond to the frame (x axis) and peak intensity (y axis). All imported samples are displayed in this window whether or not they are selected in the Sample File Tree in the 'Main Analysis' window. To display only those samples that are selected in the 'Main Analysis' window, check the box in the toolbar next to "Show Selected Samples".

You can select specific samples using the drop-down list in the toolbar. Alternatively, the scrollbar on the right can be used to scroll through samples. When the 'Max Chart Numbers' field (Table 4) is set to a value less than the total number of dyes in a kit (e.g., four charts for a six-dye kit), a second, inner scrollbar is made available. This inner scrollbar allows the user to scroll within a sample, while the outer scrollbar maintains the function of scrolling through samples.


To close out of the 'All Color Browser' window and return to the 'Main Analysis' window, select the **[X]** in the upper right-hand corner.

Note: The software will not close if the 'All Color Browser' window is open and minimized.

Table 4. Toolbar Ribbon of the 'All Color Browser' Window.

	<p>Show Dye: Allows you to select the displayed dye channels.</p>
	<p>Zoom In: Zooms in on the center point of the electropherogram</p>
	<p>Zoom Out: Zooms out from the center point of the electropherogram</p>
	<p>Set Axis: Provides options for setting the x and y axes of the electropherogram charts. The default setting automatically sets the y axis according to the maximum peak intensity of the samples (Auto Fit Y) and the x axis analysis range (Auto Fit X). You can also set specific ranges for the x and y axes by selecting Fixed X and Fixed Y.</p>
	<p>Show/Hide Mouse Cross Lines: When selected, displays x- and y-axis grid lines at the tip of the cursor along with the cursor position on both the x and y axes.</p>
<p>Max Chart Numbers: <input type="text" value="6"/></p>	<p>Max Chart Numbers: Provides a choice of how many dye channels to display in the window.</p>
<p><input type="checkbox"/> Show Selected Samples</p>	<p>Show Selected Samples: When checked, displays only the sample(s) selected in the Sample File Tree of the 'Main Analysis' window in the All Color Browser.</p>
<p>Amp 1_A1_1_Run197_Fusion6C. Ladder 1_B1_2_Run197_Fusion6 Ladder 2_D1_4_Run197_Fusion6 Amp 1_A1_1_Run197_Fusion6C. Amp 2_C1_3_Run197_Fusion6C. Amp 3_E1_5_Run197_Fusion6C. Amp 4_G1_7_Run197_Fusion6C.</p>	<p>Sample Selection Drop-down List: Provides a list of available samples to view in the All Color Browser.</p>

4 Processing Data

After the raw data files are uploaded to GMHID-Spectrum, you can conduct a brief review of the raw data as described in Section 3 or proceed directly to data processing. The processing step includes applying a sizing standard and analytical parameters, as well as comparing sample data to a known allelic panel. GMHID-Spectrum combines all of these steps into one tool called the Run Wizard. To access the Run Wizard, select the **Run Project** icon  in the main toolbar ribbon. Alternatively, select **Run** from the 'Project' menu or select the **Magic Wizard** icon (Section 6.E) from the toolbar ribbon.

Note: Ensure all ladder and control samples are appropriately designated before proceeding with data processing.

4.A. Template Selection

The first screen of the Run Wizard is the 'Template Selection' window (Figure 58) where an analysis run template can be selected or created. These templates store all of the parameters set in the Run Wizard that are needed to process raw data files. GMHID-Spectrum contains preloaded templates for many Promega STR kits. Run Wizard templates are saved in the Run Wizard program template file (Section 2.C). Templates can be deleted by selecting a template from the list and selecting **Delete** at the bottom of the window. Templates saved outside of the Program Data Folder location in the 'Others' tab of the 'Preferences' window (Section 6.B.6), including pre-installed templates, can be restored following the instructions in Section 2.C. To exit the Run Wizard at any time without applying analysis settings, select **Cancel** in the lower right corner of the window.

The 'Template Selection' window allows editing of the Template Name as well as selection of the Analysis Panel, Size Standard Template and Size Standard Color. Preloaded and saved templates include this information.

Before analyzing STR data, verify that the correct panel and size standard templates exist for your specific STR kit(s). These files contain information needed to perform data analysis such as: marker size range and dye channel, stutter percentages, peak amplitude thresholds and sizes of size standard fragments.

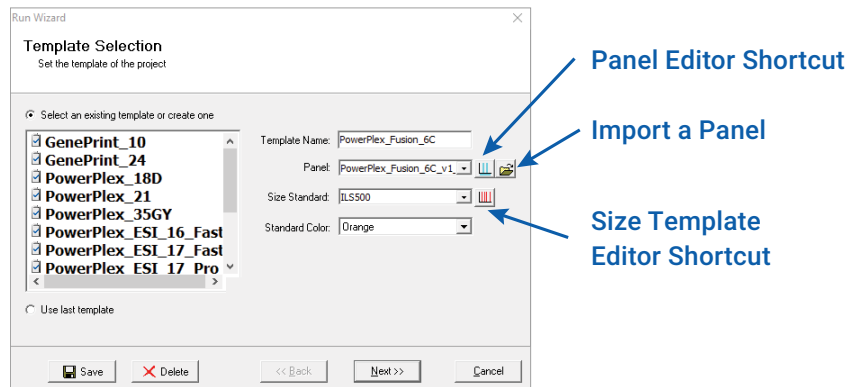


Figure 58. 'Template Selection' window of the Run Wizard.

4.A.1 Panels

To assign an allele designation to a peak, the software uses a panel file, which defines the dye channel and expected size range for each marker in an STR kit. Within each marker, the panel defines the expected bin size range for each allele within that marker. Panels are also where peak amplitude thresholds and stutter filters are defined. Preloaded and saved panel files are known as panel templates.

The Panel Editor is where all panel templates are managed. GMHID-Spectrum comes preloaded with panel templates for many Promega STR kits. To view or edit an existing panel from the Run Wizard, select an analysis panel using the drop-down list in the panel field, then select the **Panel Editor** icon to the right of the panel selection drop-down list (Figure 58). This will open the Panel Editor with the selected 'Project Panel' highlighted.

Note: Do not edit the Project Panel. Edits should be made to the Panel Templates.

Outside of the Run Wizard, the Panel Editor can also be accessed from the 'Tools' menu (Section 8).

If you are using a Promega STR kit and you do not see a preloaded panel, contact Promega: **genetic@promega.com**. If a panel for a kit from another vendor is needed, contact SoftGenetics: **tech_support@softgenetics.com**

To import a panel from the 'Template Selection' window of the Run Wizard, use the 'Import A Panel' tool next to the 'Panel' field (Figure 58). This tool can also be accessed from the 'File' menu of the Panel Editor (Section 8.D).

The following section describes selecting, viewing and editing panels for sample analysis. A more detailed description of the Panel Editor, including creating custom panels, is provided in Section 8.D.

Viewing and Editing Panel Templates for Analysis

The Panel Editor has a Menu Bar, Toolbar Ribbon, Panel File Navigator, Chart Overlay, Sample File Tree and Panel Table (Figure 59).

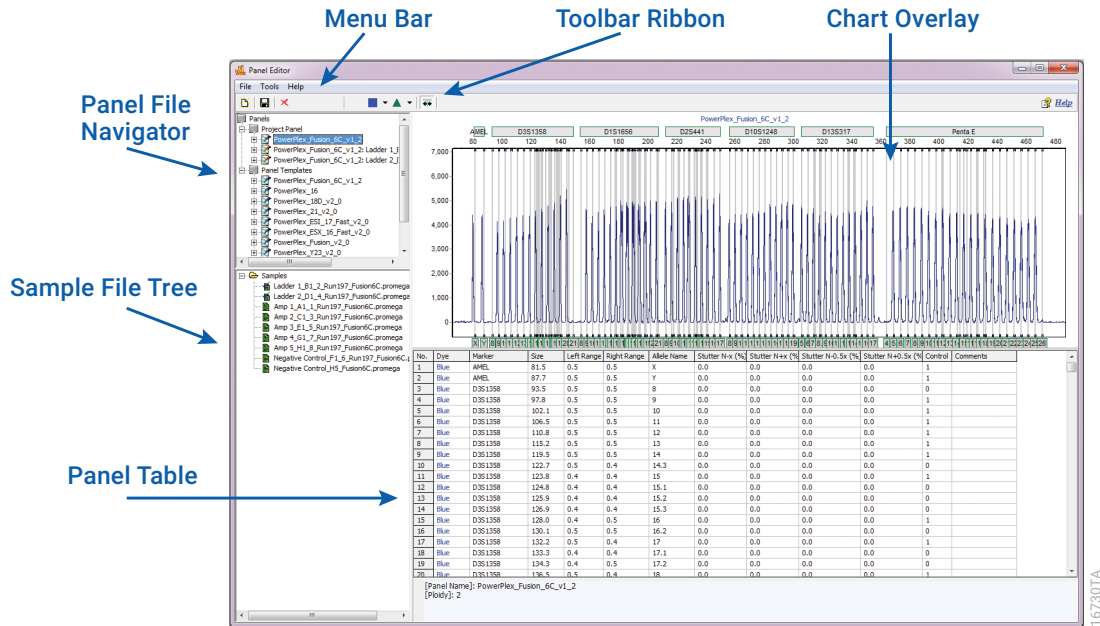


Figure 59. 'Panel Editor' window.

The 'Panel File Navigator' window includes a list of Project Panels and Panel Templates. The Project Panels include the panel assigned for the currently open project. Editing a panel listed in the 'Project Panel' tree will only apply changes to the open project. To make changes that will apply to all future uses of the selected panel, edit the corresponding panel in the 'Panel Templates' list.

Analysis parameters of a panel are set at the marker level. To view or edit these parameters for a selected panel, double-click the **Marker Bar** above the electropherogram in the Chart Overlay section or right-click the **Marker Bar** and select **Edit Marker** (Figure 60).

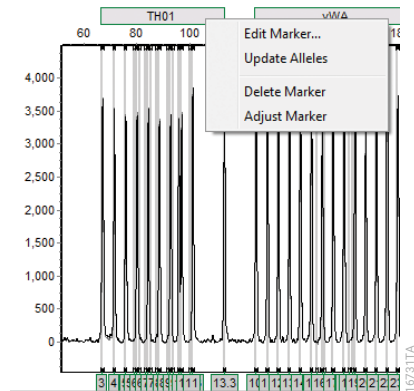


Figure 60. Accessing 'Edit Marker' from the Chart Overlay.

Alternatively, the 'Edit Marker' window can be accessed from the Panel File Navigator. To do this, expand the desired panel in the Panel File Navigator by selecting the **+** button next to the panel name. This will reveal the list of all markers in the kit. Select a marker name, then right-click and select **Edit** (Figure 61) to open the 'Edit Marker' window (Figure 62).

The 'Edit Marker' window contains information about the selected marker (Name, Nucleotide Repeats and Boundary) as well as analysis parameters that will be applied to peaks that fall into the selected marker size range (Figure 62).

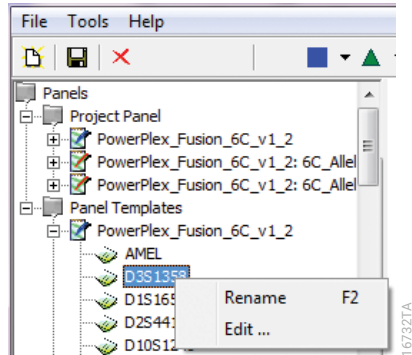


Figure 61. Accessing the 'Edit Marker' window from the Panel File Navigator.

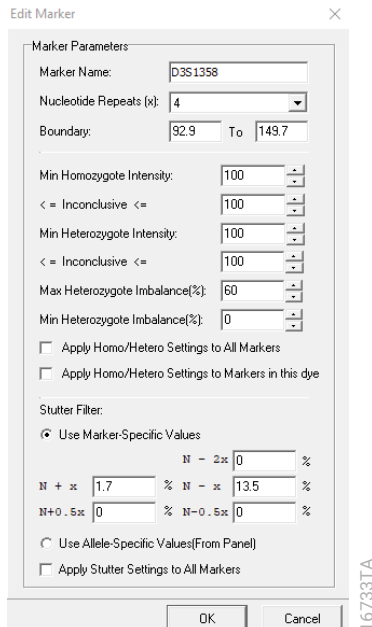


Figure 62. 'Edit Marker' window.

Marker Name: Displays how the marker will be labeled in the panel.

Nucleotide Repeats: Displays the length of the repeat motif for the marker.

Boundary: Displays the left and right boundaries (in base pairs) of the marker.

The Marker Name, Nucleotide Repeat and Boundary fields are defined when a panel is created and are generally not edited before applying a panel to a dataset.

Peak amplitude thresholds are set separately for homozygous and heterozygous events (Figure 62). The minimum intensity of both can be set to the same value.

Min Homozygote Intensity: Sets the minimum RFU value at which the software will call a peak if it is the only peak in a marker. The number of peaks in a marker is the number of peaks above the Min Heterozygote Intensity threshold.

Min Heterozygote Intensity: Sets the minimum RFU value at which the software will call peaks if there is more than one peak in a marker. The number of peaks in a marker is the number of peaks above this minimum intensity threshold.

Inconclusive Range (Stochastic Range) Flagging: Like the minimum intensity thresholds, this value is set separately for homozygous and heterozygous events, and the inconclusive value of both can be the same. For both homozygous and heterozygous conditions, peaks above the minimum intensity threshold but below the inconclusive range, will be flagged with the quality reason 'Inconclusive Heterozygous' (IHE) or 'Inconclusive Homozygous' (IHO) (Section 10). If you do not wish to use this quality flag, set the inconclusive values to match the minimum intensity thresholds for the homozygous and heterozygous events/conditions.

Note: If the minimum homozygote intensity and minimum heterozygote intensity thresholds are different from each other, a single peak above the 'Min Heterozygote Intensity' but below 'Min Homozygote Intensity' will be called and labeled with a yellow "Check" quality flag, with the quality reason of "Low Intensity" (LO) (Section 10) if a second peak is detected above the 'N-x Stutter Filter' value. The second peak will not be called, however, because the peak is below the 'Min Heterozygote Intensity' threshold. In the example shown in Figure 63, the 16 allele is above the 'Min Heterozygote Intensity' threshold of 200RFU but lower than the 'Min Homozygous Intensity' threshold. This allele is marked with a yellow "Check" quality flag with the quality reason of 'Low Intensity' (LO). The other peaks in the marker are not labeled as they fall below the 'Min Heterozygote Intensity' threshold.

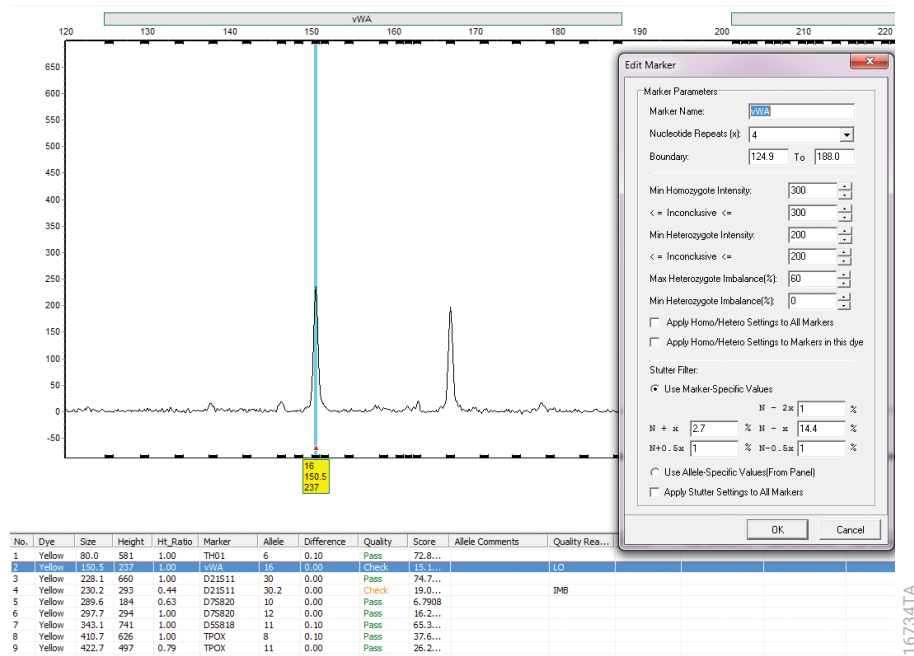


Figure 63. Example of 'Min Heterozygote Intensity' exception.

Max Heterozygote Imbalance Flagging: Flags peaks within a locus that do not meet the laboratory's minimum peak height ratio for a heterozygous locus. All peaks in a locus are compared to the peak height of the highest peak found in the locus and a ratio is calculated. Peaks that fall below the maximum imbalance setting are marked with a yellow 'Check Quality' flag with a quality reason of 'Imbalance' (IMB) (Section 10). If you do not wish to use this quality flag, set both the maximum and minimum heterozygote imbalance settings to zero.

Note: The value set for the Max Heterozygote Imbalance Flagging must be greater than that of the Min Heterozygote Imbalance Filter.

Min Heterozygote Imbalance Filter: Applies a minimum heterozygote imbalance filter to a locus. All peaks in a locus are compared to the height of the highest peak found in the locus and a ratio is calculated. Peaks that fall below the minimum heterozygote imbalance filter are not called. This filter acts as a global filter at the marker level, such that no peaks with ratios below the calculated ratio will be called as alleles.

Note: All minimum intensity, inconclusive and imbalance settings for one marker can be applied to all markers within a dye channel or all markers within a kit by using the respective check boxes below the imbalance settings (Figure 62).

Stutter Filter: Forward and reverse stutter peaks commonly produced in PCR amplification can be filtered. Stutter filters can be defined for the following positions: $N - 2x$, $N + x$, $N - x$, $N + 0.5x$ and $N - 0.5x$, where N is the primary peak and x is the number of nucleotide repeats for the selected marker. Peaks in these positions are compared to the peak height of the peak at the N position and a ratio is calculated. Peaks that fall below the stutter filter for that position will not be called. If stutter filters are not desired, set the values for all positions to 0.

Note: Partial stutter filters ($N + 0.5x$ and $N - 0.5x$) do not apply to trinucleotide and penta-nucleotide repeat markers.

There are two options for setting stutter filters: marker-specific and allele-specific.

To use marker-specific stutter filters, select **Use Marker-Specific Values** and enter the appropriate stutter filters for the listed positions of the 'Edit Marker' window (Figure 62).

Note: Stutter filters for one marker can be applied to all markers in a kit by checking the box 'Apply Stutter Settings to All Markers'.

To use allele-specific values, select the radio button next to **Use Allele-Specific Values (From Panel)** in the 'Edit Marker' window, then enter the appropriate stutter filters in the Panel Table of the 'Panel Editor' window (Figure 64).

Note: Allele-specific stutter is additive. If a peak is in a shared stutter position between two other peaks, the stutter peak must exceed the total stutter filter for the flanking alleles before it is called. Peaks that are within the inconclusive range or are themselves filtered out as stutter will not be used to influence stutter determination of other peaks.

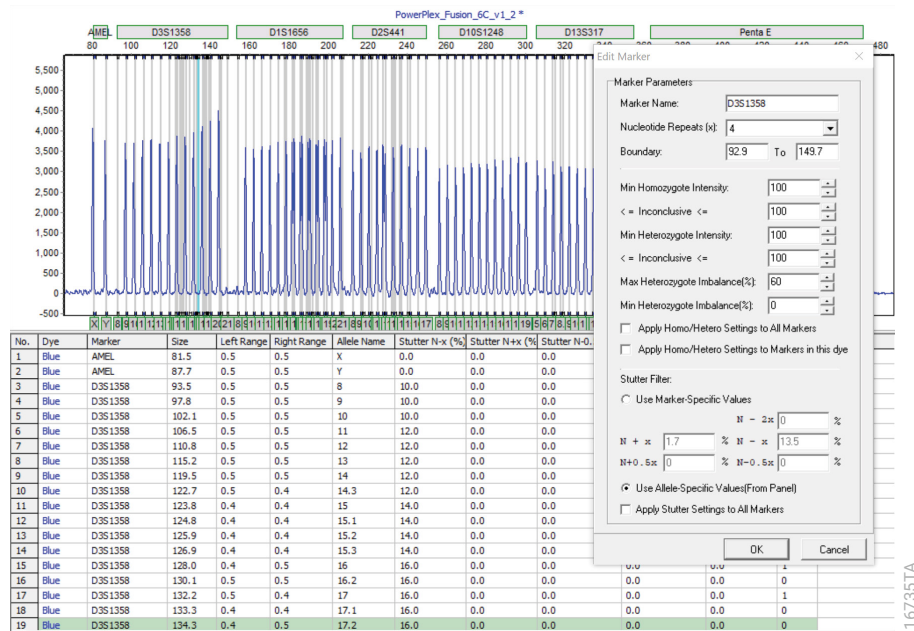


Figure 64. Allele-specific stutter values in the 'Panel Editor' window.

When the marker parameters are set to the desired values, select **OK** to close the 'Edit Marker' window and return to the Panel Editor. When the parameters for all markers in a panel are set to the desired values, the user may **Save Changes** to the selected panel or **Save As New Panel** via the 'File' menu. To return to the Run Wizard or main screen, close the Panel Editor.

For additional Panel Editor functions, refer to Section 8.

4.A.2 Size Standards

A size standard template is needed for each internal size standard that will be used for data analysis. Each size standard template defines the fragment sizes for a specific internal size standard.

GMHID-Spectrum comes preloaded with size standard templates for Promega internal size standards. All size standard templates are managed from the 'Size Template Editor' window (Figure 66). The 'Size Template Editor' window can be accessed through the Run Wizard or from the 'Main Analysis' window menu bar under 'Tools'. To view or edit an existing size standard template from the Run Wizard, select it using the drop-down list in the 'Size Standard' field, then select the **Size Template Editor** icon to the right of the 'Size Standard' field (Figure 65). This will open the 'Size Template Editor' window with the selected size standard highlighted and displayed (Figure 66).



Figure 65. Size Template Editor accessed from the Run Wizard.

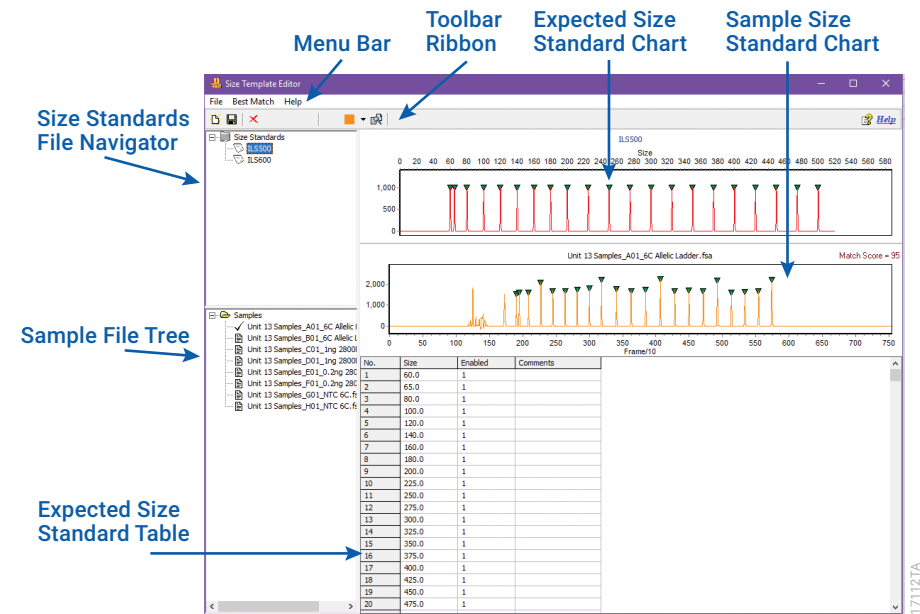


Figure 66. 'Size Template Editor' window.

If the size standard you are using is not present, a new size standard template can be created (Section 7) or imported. If you are using a Promega STR kit and you do not see a preloaded size standard template, contact Promega: genetic@promega.com If a size standard template for a kit from another vendor is needed, contact SoftGenetics: tech_support@softgenetics.com To import a size standard, use the 'Import Size Standard' option from the 'File' menu (Section 7.A.5) in the 'Size Template Editor' window.

Viewing Size Templates for Analysis

The 'Size Template Editor' window has a Menu Bar, Toolbar Ribbon, Size Standards File Navigator, Expected Size Standard Chart, Sample Size Standard Chart, Sample File Tree and Expected Size Standard Table (Figure 66).

The Size Standards File Navigator includes a list of size standard templates. For more information on managing size standard templates, including creating new templates, editing existing templates, and deleting templates, see Section 7.

The dye channel that contains the size standard to be used for analysis is set in the 'Template Selection' window of the Run Wizard (Figure 58). When the appropriate size standard template is verified, close the 'Size Template Editor' window to return to the Run Wizard.

4.B. Data Process – HID Analysis

After a 'Run Template' is selected, select **Next** on the 'Template Selection' window (Section 4.A, Figure 58) to proceed to the 'Data Process - HID Analysis' window (Figure 67). The 'Data Process – HID Analysis' window includes the 'Raw Data Analysis', 'Size Call' and 'Allele Call' parameters that can be reviewed or edited. You can also return to the 'Template Selection' window by selecting **Back** at the bottom of the window.

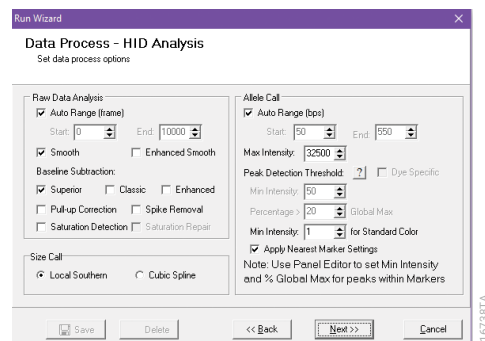


Figure 67. 'Data Process - HID Analysis' window of the Run Wizard.

4.B.1 Raw Data Analysis

Raw Data Analysis Field: Allows you to define the camera frame range used to analyze the raw data, the degree of smoothing applied to the baseline and the amount of baseline subtraction and correction.

Auto Range (frame): When selected, the software automatically determines the range of camera frames that include data and uses that range to analyze the raw data. If 'Auto Range' is not selected, you can manually enter the start and end frame numbers of the dataset for analysis. Frame numbers are displayed in the raw data electropherograms.

Note: If the Auto Range function fails due to high saturation of the primer peak, deselect **Auto Range** and manually input the required data range to exclude that area of saturation.

Smoothing

There are two options for peak smoothing, 'Smooth' and 'Enhanced Smooth'. The result of both options is reduced sharpness of peaks, thus smoothing the baseline by eliminating low-level noise peaks.

Smooth: Applies a standard amount of smoothing, resulting in a smoother baseline with minimal loss of resolution. This is the setting used for typical data analysis.

Enhanced Smooth: Applies an increased amount of smoothing, resulting in an even smoother baseline but also an increased loss of resolution. This setting is typically only used in research applications and is used for select samples to compensate for excess noise. This setting is not recommended for forensic casework samples.

Note: Peaks with low peak heights may be lost when this setting is applied since the broadening of peaks caused by smoothing can lead to loss of resolution.

Baseline Subtraction

There are three options for baseline subtraction (Figure 68 and Figure 69). The settings differ by the camera frames used in the subtraction calculation. If none of these options are selected, no baseline subtraction is applied to the raw data, resulting in higher peak heights in the processed data along with higher baseline noise.

Superior: Sets the baseline to 20% of the lowest value detected in 100 camera frame sections to perform baseline subtraction. This setting is used for typical data analysis and is the recommended baseline subtraction setting.

Classic: Sets the baseline to 20% of the lowest value detected in 500–600 camera frame sections to perform baseline subtraction. This setting removes the baseline completely so that the y axis will be raised above the noise level.

Enhanced: Uses the second derivative of the absolute value for every 30 data points in 300 camera frame sections to perform baseline subtraction. This setting is used only in cases where the data have excessive baseline noise in one or more of the dye channels or the primer peaks slope into the region of lower-molecular-weight markers, interfering with baseline subtraction.

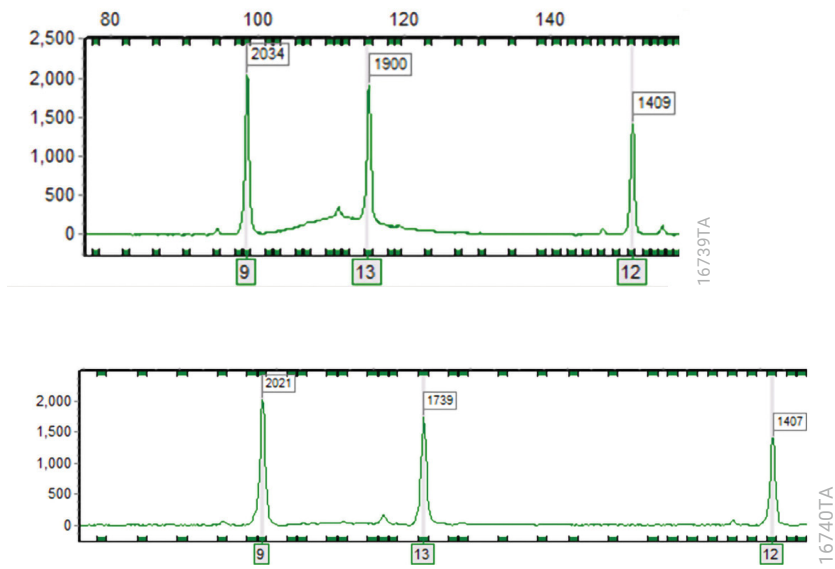


Figure 68. Comparison of classic baseline subtraction (top panel) and superior baseline subtraction (bottom panel) for a sample with raised baseline.

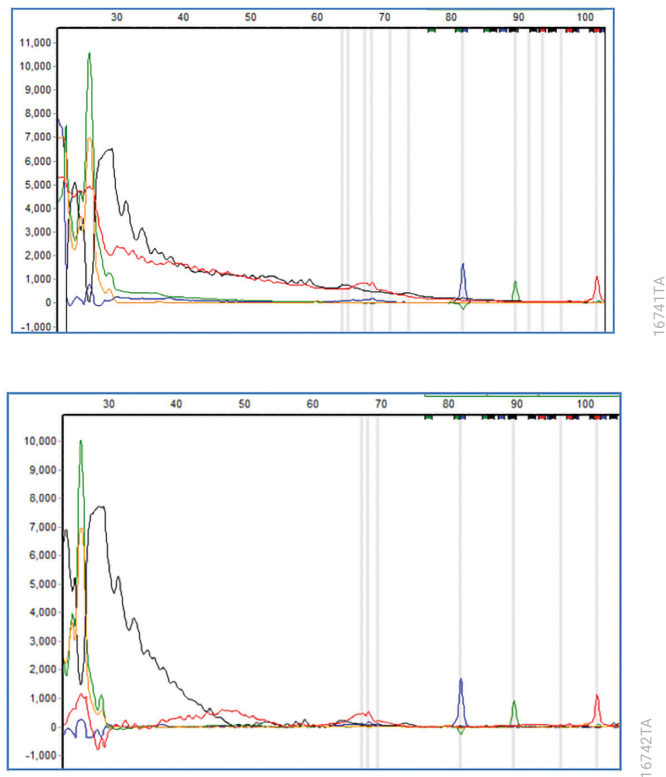


Figure 69. Comparison of classic baseline subtraction (top panel) and superior baseline subtraction (bottom panel) for a sample with sloping primer peaks.

Corrections

There are four settings for detection and correction of common artifacts: Pull-Up Correction, Spike Removal, Saturation Detection and Saturation Repair. The Pull-Up Correction, Spike Removal and Saturation Repair settings are **not** recommended for forensic casework samples.

Pull-Up Correction: Removes peaks caused by bleedthrough (pull-up) of signal from one dye channel into another. Only pull-up peaks with peak heights up to 20% of the main peak are removed.

Spike Removal: Removes peaks from voltage spikes caused by micro-air bubbles or debris in the laser path. Spikes are typically less than a base pair wide.

Saturation Detection: Applies an algorithm to the data to identify areas of potential signal saturation beyond what is detected by the data collection software. To flag saturation detected by the collection software, select **Show Saturation Alert Line** from the 'Chart settings' section of the 'Display' tab in the 'Preferences' window (Section 6.B).

Saturation Repair: Analyzes saturated data points and creates a synthetic estimate of the peak shape based on the peak height and shape of pull-up peaks under the saturated peak. The results will be less accurate than that for nonsaturated peaks for DNA quantification, such as when evaluating mixtures by peak ratios. Saturation Repair is only active if **Saturation Detection** is selected.

In the example shown in Figure 70, the top panel shows saturation detected, but not repaired. The saturated peak in the blue dye channel is split at the top and a pull-up peak is present in the green dye channel. The bottom panel shows the same data where saturation is both detected and repaired. The pull-up peak in the green dye channel has been removed and its peak height added to saturated peak in the blue dye channel. The top of the peak in the blue dye channel is no longer split.

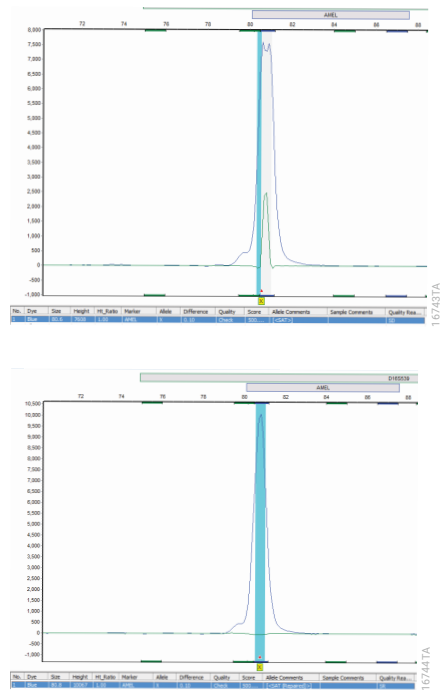


Figure 70. Saturation detection and repair. The **top panel** shows a peak where saturation is detected. The **bottom panel** shows the same peak detected but repaired.

4.B.2 Size Call

GMHID-Spectrum offers two sizing methods:

Local Southern: Commonly used sizing algorithm for STR analysis. This method overlays a plot of size versus time and a plot of size versus 1/time graph to determine sizing linearity. The size of the two peaks on either side of an unknown peak are used to calculate the size of the unknown peak. For unknown peaks that do not have two size standard peaks on either side, a modified Local Southern method is applied. (Southern, E.M. (1979) Measurement of DNA Length by Gel Electrophoresis. *Anal. Biochem.* **100**, 319–23.)

Cubic Spline: An alternative method, similar to the Local Southern method; however, the sizing curve is fit to all known points. This method uses a cubic equation to connect known points on the size versus time graph. (Akerlof, C. *et al.* (1994) An example of a cubic equation: ax^3+bx^2+cx+d . *Astrophys. J.* **436** 787–94.)

4.B.3 Allele Call

The 'Allele Call' field allows you to set the allele-calling range as well as the detection thresholds and filters for peaks outside the panel ranges.

Auto Range (bps): When selected, the software automatically locates the data range in base pairs used to identify peaks. If **Auto Range** is not selected, you can manually enter the start and end range of the dataset for peak calling. Peaks outside this set range will not be called.

Max Intensity: Peaks above this intensity (in RFU) will have the quality designation of 'Pass', but the quality reason of 'High Intensity' (HI; Section 10).

Peak Detection Threshold

Note: The 'Peak Detection Threshold' parameters are only applied to peaks outside of the panel marker ranges. To adjust settings for peaks within panel marker ranges, see Section 4.A.1.

Min Intensity: Minimum peak height at which the software will call a peak that migrates outside of a panel marker range.

Percentage > Global Max: All peaks that migrate outside of a panel marker range will be compared to the peak height of the 5th highest peak in the corresponding dye channel. Peaks that fall below the percentage global max setting will not be called.

Min Intensity for Standard Color: Minimum peak height at which the software will call a peak in the size standard dye channel.

Apply Nearest Marker Settings: The software applies the allele-calling threshold settings of the nearest marker (defined in the panel selected in the 'Template Selection' window) to peaks that migrate outside of panel marker ranges.

Dye Specific: The user defines the peak detection threshold by individual dye channel. When selected, a table with fields for each dye channel is displayed in the 'Allele Call' section of the Run Wizard (Figure 71). The 'Min RFU' column allows the Min Intensity (see above) to be applied by dye channel. The 'Min RFU%' column allows the Percentage Global Max (see above) to be applied by dye channel.

Note: 'Min Intensity', 'Percentage > Global Max' and 'Dye Specific' options are only available if **Apply Nearest Marker Settings** is not selected.

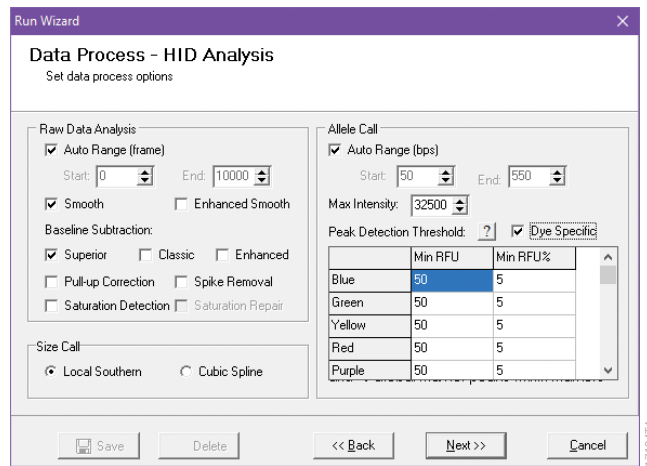


Figure 71. Setting dye-specific peak detection threshold in the Run Wizard.

4.C. Additional Settings - HID Analysis

Select **Next** on the 'Data Process - HID Analysis' window to proceed to the 'Additional Settings - HID Analysis' window (Figure 72).

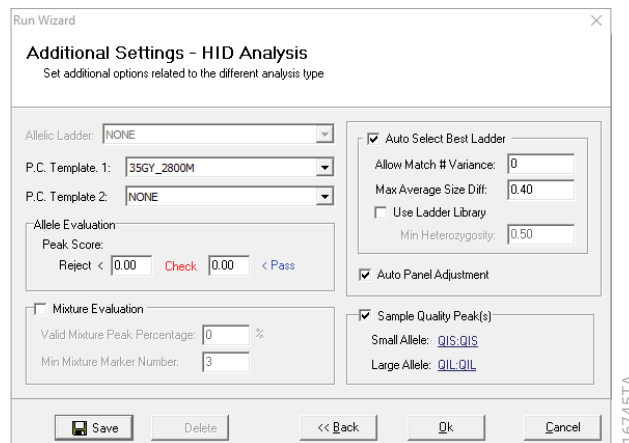


Figure 72. 'Additional Settings - HID Analysis' window of the Run Wizard.

4.C.1 Allelic Ladder

One allelic ladder from the dataset can be selected from the 'Allelic Ladder' drop-down list for use in assigning alleles to all samples in the project. Alternatively, GMHID-Spectrum can automatically select the best ladder for each sample in the dataset by checking the box next to **Auto Select Best Ladder**. Allelic Ladder files must be designated as ladders for this function to work (Section 3.C.1).

Allow Match # Variance: Sets the number of unmatched bins allowed between a sample and ladder. The recommended setting is zero. Increasing this number decreases the matching stringency applied when passing ladders are compared to each sample.

Max Average Size Diff: Sets the allowable average size difference between a peak in a sample and the center of the corresponding bin. The recommended setting is 0.40. Decreasing this number increases the matching stringency between a sample and a ladder.

Use Ladder Library: When this box is checked, the 'Min Heterozygosity' field is available. The 'Min Heterozygosity' field defines the minimum number of detected peaks per marker for a sample to be matched to a ladder by the auto select process. This setting may be needed if a ladder library, a set of ladders not run concurrently with the imported sample set, will be used to auto-select the best ladder. The default setting is 0.5, which requires a sample to have a minimum of one peak per marker to be automatically matched to a ladder.

4.C.2 Auto Panel Adjustment

Choosing this feature allows the software to automatically align the markers and bins of the chosen panel with the peak positions of the ladder fragments within a dataset (within a 5 base pair shift). This will allow for migration differences due to electrophoresis conditions (e.g., room temperature, polymer age).

4.C.3 Positive Control Template

Positive control files must be designated as controls for this function to work (Section 3.C.1). Select the corresponding template from the 'P.C. Template 1' drop-down list (Figure 72). If a second positive control template is needed, select the appropriate template from the 'P.C. Template 2' drop-down list. A positive control template lists the expected alleles of the positive control samples for profile accuracy. GMHID-Spectrum is preloaded with the positive control profiles for many Promega STR kits. If the control DNA or chemistry is not present in the template list, you can create a new positive control template using the Positive Control Template Editor (Section 6.E).

4.C.4 Allele Evaluation

The 'Allele Evaluation' field allows the user to define a confidence level of an allele call using the peak score. Peak score is an algorithm that takes into account signal-to-noise ratio and peak morphology (Section 5.B.2). You should evaluate data to determine the appropriate values for rejecting and passing peak scores. Samples that fall between these values will be marked with a yellow "Check" quality flag with a quality reason of 'Low Score' (LS). Rejected peaks within bin ranges will be marked with a red "Undetermined" quality flag and a quality reason of 'Low Score' (LS). Rejected peaks outside of bin ranges will not be labeled. Peaks with a passing peak score will not be marked (Section 10).

In the example in Figure 73, the 'Reject <' peak score was set to 1.00 and the '< Pass' peak score was set to 3.00. As a result, the peaks with a peak score below 1.00 have allele labels displayed in red and are marked "Undetermined" in the 'Quality' column. Peaks with scores between 1.00 and 3.00 have allele labels displayed in yellow and are marked "Check" in the 'Quality' column. In both instances, the quality reason is 'LS'. The allele call for the peak with a score above 3.00 is displayed in gray and marked "Pass" in the 'Quality' column.

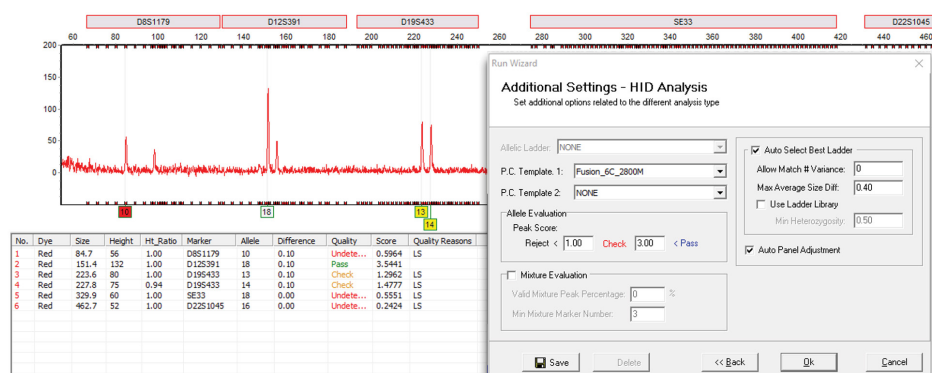


Figure 73. Allele Evaluation in the 'Additional Settings - HID Analysis' window.

4.C.5 Mixture Evaluation

You can define criteria to identify the presence of potential mixture samples. To activate and edit the two Mixture Evaluation criteria fields, check the **Mixture Evaluation** box.

Valid Mixture Peak Percentage: Applies a filter to samples with the mixture sample type designation. All peaks in a locus will be compared to the peak height of the second highest peak found in the locus and a ratio will be calculated. Peaks which fall below the valid mixture peak percentage will not be called. This filter acts as a global filter at the marker level, meaning that all peaks, including stutter peaks, that do not reach this percentage will not be called. This overwrites the 'Min Heterozygote Imbalance' set in the applied panel (Section 4.A.1).

Min Mixture Marker Number: Defines the minimum number of markers that meet the mixture criteria before the whole profile is flagged as a mixture. These criteria are determined by the Min and Max Heterozygote Imbalance and ploidy settings in the selected panel. Profiles that meet or exceed the minimum number of markers identified as possible mixtures will be flagged as a mixture sample in the Sample File Tree (MX:[filename]).

4.C.6 Sample Quality Peak(s)

The 'Sample Quality Peak(s)' field allows you to define quality indicator peaks for STR kits that contain up to two synthetic DNA templates to aide in sample quality assessment. To activate and select quality indicator peaks, check the "Sample Quality Peak(s)" box.

Small Allele: Left-click on the field and select the locus and allele for the small allele quality indicator from the pop-out list. If the STR kit does not contain a small allele quality indicator, leave the field blank.

Large Allele: Left-click on the field and select the locus and allele for the large allele quality indicator from the pop-out list. If the STR kit does not contain a large allele quality indicator, leave the field blank.

The threshold for quality indicator flagging is defined in the 'Preferences' window (Section 6.B).

Note: The 'Sample Quality Peak(s)' option is available in GMHID-Spectrum software version 3.2.0 or later.

4.D. Process Data

When each of the three screens of the Run Wizard have been reviewed and the desired settings applied, select the **Save** button in the bottom left to save any changes to the selected analysis template (selected on the 'Template Selection' window of the Run Wizard). To apply the Run Wizard settings to the imported data, select **OK**.

A 'Data Processing Event Log' window will appear and record the analysis events as the software applies the analysis settings to the imported data. Samples that are successfully processed are labeled "Completed" in blue. Samples that fail the process are labeled "Failed" in red. Further review of all samples is done in the 'Main Analysis' window. The total number of samples processed and the analysis time are recorded at the end of the Event Log (Figure 74). When analysis is complete, select **OK** to proceed to the 'Main Analysis' window.

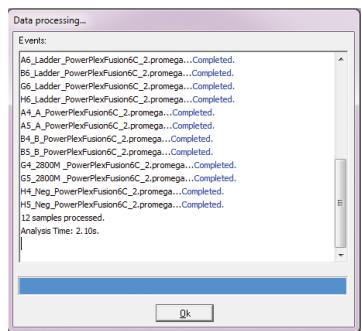


Figure 74. 'Data Processing Event Log' window.

5 Reviewing Processed Data

Once the raw data files are processed through the Run Wizard, the 'Main Analysis' window displays processed data (Figure 75). The Sample File Tree displays a listing of the processed data files in the 'Allele Call' folder.

The **Electropherogram Chart** (also Electropherogram) displays the processed data of selected samples. If no samples are selected this area will be blank.

The **Project Summary Bar** at the bottom of the screen is updated with a summary of the quality metrics applied to the data.

A **Report Table** is now available on the right side of the screen. This displays the allele table for the project samples.

Additional shortcuts are now available in the toolbar ribbon to assist with processed data analysis (Table 5).

Many of the features and functions of the 'Main Analysis' window for raw data (Section 3.C) are available in the 'Main Analysis' window for processed data. Features only available in the 'Main Analysis' window for processed data are described below.

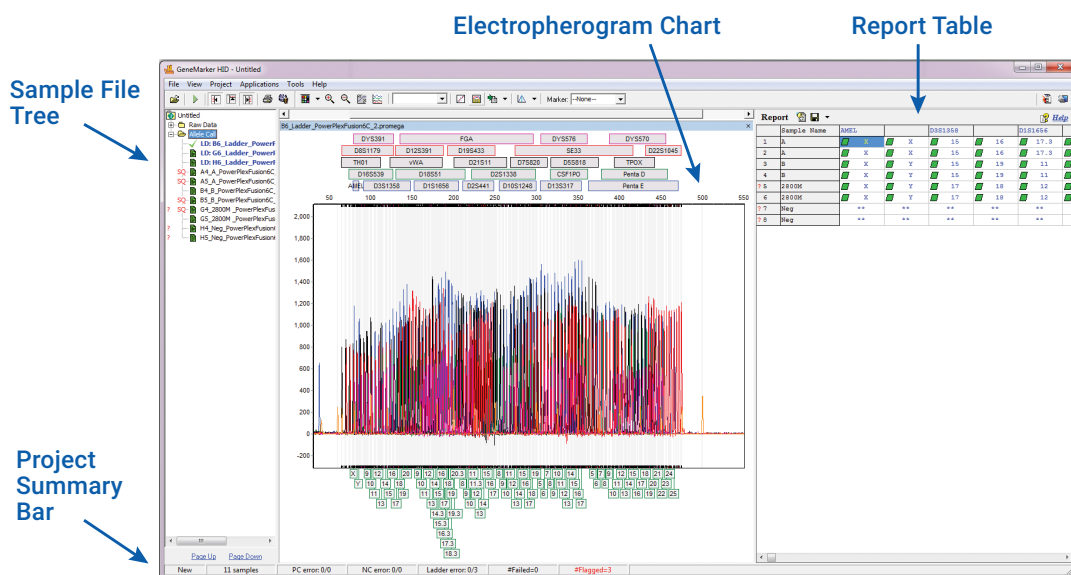





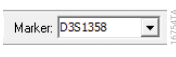


Figure 75. 'Main Analysis' window with processed data.

Table 5. Additional 'Main Analysis' Toolbar Icons for Processed Data.





	Print Report: Opens the 'Print Report' window, which allows a choice of print display options (Section 9).
	Size Calibration: Opens the 'Calibration Charts' window, which allows review and editing of size standard calibrations for the dataset (Section 7.B).
	Show Chart/Table: Toggles the display window to show only the Peak Table, the Peak Table and Electropherogram or just the Electropherogram.
	Save Peak Table: Exports the Peak Table as an Excel (.xlsx), tab-delimited text (.txt) or CSV (.csv) file. The arrow allows selection or deselection of two table options. 'Filter Delete Peaks' removes deleted peaks from the saved table. 'Exclude Report Header' removes the report header from the saved table.
	Call Allele: Opens the 'Recall Allele' window, which allows slight modifications to the samples without having to activate the Run Wizard again. The drop-down menu allows you to define how the re-analysis will be applied: by sample(s), by marker or by dyes (Section 5.F).
	Marker Drop-Down Menu: Displays the selected marker. This menu is available after samples have been compared to a panel.

5.A. Navigating the Sample File Tree

After raw data files are processed, the Sample File Tree will contain a second folder, 'Allele Call', in addition to the 'Raw Data' folder (Section 3.C.1). The 'Allele Call' folder contains a list of all project samples in their processed form. By default, the first sample in the tree is selected and its processed data electropherogram is displayed in the electropherogram window. The same viewing and analysis options available in the 'Raw Data' view are available in the 'Processed Data' view.

Samples listed in the 'Allele Call' folder are assessed for quality measures applied during data processing and flagged if necessary. These flags (Table 6) provide a quick assessment of profile quality.

Table 6. Quality Measure Flags in the Sample File Tree.

	Size Match Score: Provides an assessment of the size standard compared to the applied size standard template (Section 10.A.1).
	Quality Indicator (Q): Indicates the Quality Intensity Ratio is below the minimum value set in the 'Preferences' window (Section 6.B). This flag is only applied when the "Sample Quality Peak(s)" box is selected in the 'Additional Settings - HID Analysis' window of the Run Wizard (Section 4.C.6).
	Analysis Quality: Indicates one or more analysis parameters were not met for that sample. Details of the parameter(s) not met, including quality reasons, are listed in the allele label and the Peak Table (Section 10). This indicator also marks samples with null marker(s) and samples with amelogenin and Y-STR marker conflict(s). The amelogenin and Y-STR marker conflict assessment is only applied when set in the 'Preferences' window (Section 6.B).
	Size Quality (SQ): Indicates a sample with one or more low-quality peaks in the size standard (e.g., assessing broad peaks) or samples in which at least one expected size fragment is not called. This flag is only applied when set in the 'Preferences' window (Section 6.B). The 'Size Quality' flag is described in detail in Section 10.A.2.

5.B. Navigating the Electropherograms with Peak Table

Electropherograms for selected samples will be displayed in the electropherogram chart area of the 'Main Analysis' window. The sample file name will be displayed at the top of each electropherogram. The allelic ladder used to determine allele calls for the selected sample will be displayed in parentheses after the sample name. A Peak Table (Section 5.B.2) can be added to the electropherogram plot view of the 'Main Analysis' window. This table contains information for all called peaks displayed in the selected dye channel(s) of the electropherogram.

5.B.1 Electropherogram Features

The electropherograms display fluorescent signal intensities as a single line plot for the selected dye color(s). The signal intensities, recorded in RFU, are plotted along a base pair scale. The 'Show Dye' icon in the toolbar can be used to view different dye channels as explained in Section 3.C.2.

The x axis of the electropherogram is set to reflect the range defined in the 'Data Process' window of the Run Wizard (Section 4.B). By default, the y axis is set to auto scale according to the maximum peak intensity of the displayed data (Figure 76). The 'Fixed X/Y' and 'Auto Fit X/Y' functions described in Section 3.C.2 are available in the 'Main Analysis' window for processed data. The Auto Fit Y By Allele feature automatically adjusts the y axis to reflect the highest intensity allele regardless of the height of peaks outside of the marker ranges (i.e., the primer peaks).



Figure 76. 'Set Axis' menu.

Just as with the raw data, you can zoom in or out of the electropherogram using the mouse or the **Zoom In** and **Zoom Out** icons in the toolbar.

The marker, bin and allele settings of the selected analysis panel are applied to the processed data and labeled in the electropherogram (Figure 75). The marker ranges associated with the applied analysis panel are displayed in gray bars above the region for each marker. Bin ranges appear as dye-colored brackets at the top and bottom of the electropherogram area. Allele labels appear below the electropherogram and correspond to the center of each called peak, which is also marked by a light gray vertical line in the electropherogram. The color of the allele label reflects the Analysis Quality assessment of the peak as Pass (no color), Check (yellow) and Undetermined (red). Samples with a Q and/or SQ flag will have a Q and/or SQ label in the upper left corner of the electropherogram. Hovering the mouse pointer over the Q or SQ label will display the quality reason for the flag. The Q flag is only applied when the "Sample Quality Peak(s)" box is selected in the 'Additional Settings - HID Analysis' window of the Run Wizard (Section 4.C). The SQ flag is only applied when set in the 'Preferences' window (Section 6.B). For more details on Quality Assessment, see Section 10.

If a panel was not applied during data analysis, then marker and bin regions are not labeled and alleles will only be labeled with the peak size (in base pairs).

Electropherograms can be reviewed by marker. Scroll through individual markers by selecting a marker from the 'Marker' drop-down list in the main toolbar. To view subsequent markers, use the **Up** or **Down** arrow keys on the keyboard. Use either the mouse or **Zoom Out** icon to return to full range view.

5.B.2 Features of the Peak Table

The Peak Table can be added to the chart area of the 'Main Analysis' window to provide detailed information on all peaks displayed in the selected dye color(s) of the electropherogram. To show the Peak Table, select the **Show Chart/Table** icon in the toolbar (Table 5). Use this icon to scroll through views showing both the electropherogram and Peak Table, just the Peak Table or just the electropherogram. Each peak in a dye color is listed in the Peak Table by size from smallest to largest and counted by number, which is displayed in the No. column of the table (Figure 77). The No. column is fixed and always displayed. There are 21 other columns of information available for display in the Peak Table. Each column is described below. To display certain columns, right-click in the Peak Table, select **Show Columns** and then select the desired columns from the list. Alternatively, expanding the headers next to hidden columns will display the hidden columns.



Figure 77. 'Peak Table' menu options.

File Name: Displays the full file name of the data file.

Sample Name: Displays the sample name recorded in the data file.

Dye: Indicates the dye name of the peak as defined in the 'Chemistry Dye Set' window (Figure 47).

Size: Indicates the size of the peak in base pairs (x axis).

Height: Indicates the peak height in RFUs (y axis).

Height Ratio: Displays the value obtained when the peak height is divided by the height of the highest peak in the dye color (when a panel is not applied) or marker (when a panel is applied).

FWHM (Full Width Half Max): Displays the width of a peak (in base pairs) at half the maximum height of the peak.

Area: Displays the area under the curve of the peak. The area calculation begins and ends along the x axis as indicated by the values in the 'Start' and 'End' columns of the Peak Table, respectively.

Area Ratio: Displays the value obtained when the peak area is divided by the area of the highest peak in the dye color (when a panel is not applied) or marker (when a panel is applied).

Score: Displays the peak quality score calculated based on signal-to-noise ratio and peak shape or morphology. The score value is based on an exponential curve. A score of 500 represents a perfect score. Lower scores indicate poorer quality peaks. You should evaluate the quality of peaks with these lower scores (typically less than 1.0000) to determine if a peak score filter should be applied (Section 4.C.4).

Start: Indicates the beginning of the area calculation for the peak.

End: Indicates the end of the area calculation for the peak.

Allele Comments: Displays user comments applied at the allele level in the electropherogram or Peak Table (Section 5.B.3).

Sample Comments: Displays user comments applied at the sample level in the Sample File Tree (Section 3.C.1).

Comment: Displays comments recorded for the sample in the instrument collection software.

The following columns are available only when a panel is applied to data:

Marker: Indicates the marker (locus) in which the peak is located.

Allele: Indicates the bin in which the peak is located.

Difference: Indicates the absolute value in base pairs of the distance between the peak center and bin center.

Quality: Displays a "Pass", "Check" or "Undetermined" quality designation for each peak based on the peak score filter (if applied) in the Run Wizard (Section 4.C.4), the Analysis Quality parameters (Section 10) and/or software editing of the original raw data, such as correction of saturated peaks (SAT Repaired; Section 4.B.1).

Quality Reasons: Displays the two or three letter code quality reason (Section 10) for peaks that are assigned a "Check" or "Undetermined" quality designation.

Note: Peaks that exceed the maximum peak intensity threshold set in the 'Data Process - HID Analysis' window of the Run Wizard (Section 4.B.3) are assigned a "Pass" quality designation; however, they will be denoted as "HI" for the quality reason.

Plate ID: Displays the plate name recorded in the data file.

5.B.3 Editing Peaks in the Electropherogram and Peak Table

You can edit peaks from the electropherogram and Peak Table. When peaks are edited, the corresponding cell in the 'No.' column of the Peak Table will be highlighted in a pink-orange color. Additionally, edited peaks can be labeled at the top of the peak when 'Mark Deleted/ Edited Peaks with Symbols' is selected in the 'Forensic' tab of the 'Preferences' window (Section 6.B).

Selecting Alleles

To select a single allele from an electropherogram, select the gray bar centered over the allele or the allele label. A selected allele will have a red triangle below the base of the peak and the gray bar will change to teal (Figure 78). In addition, the selected allele will be highlighted gray in the corresponding Peak Table. To select a single allele from the Peak Table, select the row in which the allele is represented. The selection will also be reflected in the corresponding electropherogram.



Figure 78. Electropherogram and Peak Table with selected alleles.

To select a **group of consecutive alleles** from an electropherogram, use Control + left mouse click to drag a highlighted area across the desired alleles. A teal box will display during the select and drag, indicating the area being selected. Repeat the select and drag across an area with selected alleles to deselect the alleles. To select alleles from within the Peak Table, use Shift + select.

To select a **group of nonconsecutive alleles** from an electropherogram or the Peak Table, use Control + left mouse click to select specific alleles.

With desired allele(s) selected, right click anywhere in the electropherogram or Peak Table to view a menu of options:

Set/Unset Reference Peak Height: Allows you to set a reference peak within the marker for calculating the height ratio. By default, the highest intensity peak in a marker is set as the reference peak.

Edit Allele: Opens the 'Edit Allele' window (Figure 79). You can change values in the 'Allele' and 'Size' field. The 'Allele' field will be blank if no panel was applied to the dataset. Select **Confirm the Allele** to automatically give the peak a quality designation of "Pass" (green).

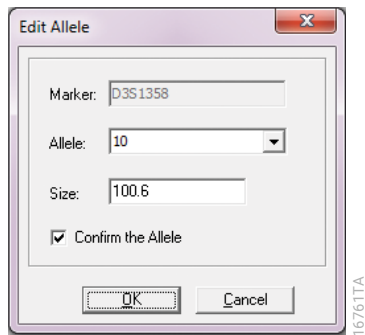


Figure 79. 'Edit Allele' window.

Edit Comments: Opens the 'Edit Allele Comments' window (Figure 80). Select a comment from the 'Comments Template' list, or enter a new comment in the 'Comments' field. Choose **OK** to assign the comment to the selected allele. Comments assigned to an allele are viewable in the 'Allele Comments' column of the Peak Table. Only one user comment can be added to a peak. Recent comments will be stored and can be subsequently selected from the displayed list for future samples. Stored comments can be managed and shared across software installations via the Sample Comments program template file (Section 2.C).

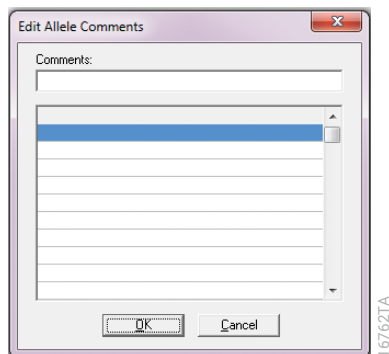


Figure 80. 'Edit Allele Comments' window.

Insert Allele: Inserts a peak label at the selected location by right-clicking within the electropherogram. This option is not available if an allele already exists at the chosen location. If the allele is to be inserted outside a marker range, a warning box will appear asking if you intend to add an allele outside of a marker. When selected, the 'Insert Allele Peak' window opens, displaying the allele designation and base pair size that will be assigned to the peak (Figure 81).

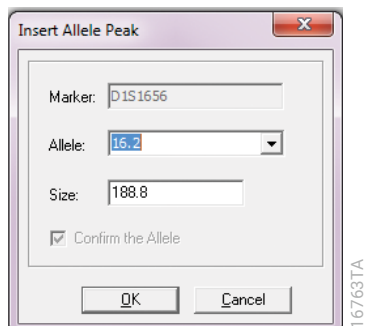


Figure 81. 'Insert Allele Peak' window.

You can choose a different allele designation from the drop-down list in the 'Allele' field and adjust the size in the 'Size' field. The 'Confirm the Allele' check box is automatically checked and cannot be unchecked. When the desired information is entered, select **OK** to add the allele to the profile. The peak specifications will be calculated and displayed in the Peak Table. "Confirmed" and "Inserted" will be displayed in the 'Allele Comments' column.

Delete/Undelete Allele: Deletes selected allele(s) (Shortcut = DEL). To undo a deletion, choose the peak(s), right-click and select **Undelete** (Shortcut = Shift + DEL).

Confirm/Unconfirm Allele: If a peak is marked with a yellow "Check" or red "Undetermined" quality flag, you can confirm the allele to give it a "Pass" (green) quality designation. To do this, right-click the center bar of the desired peaks and select **Confirm** (Shortcut = CTRL + M). The peak will be marked "Pass" (green) and receive a "Confirmed" comment in the 'Allele Comments' column of the Peak Table. To unconfirm the allele, select **Unconfirm** from the right-click menu (Shortcut = CTRL + ALT + M). This will revert the quality designation of the peak to its original state ("Check" or "Undetermined").

Confirm/Unconfirm All: 'Confirm All' and 'Unconfirm All' options perform the same actions as the 'Confirm/Unconfirm Allele' function for all peaks marked "Check" (yellow) or "Undetermined" (red) in the selected dye color for the selected sample.

Undo: Undoes the last edit made to the selected allele.

View History: Opens the 'Show Edit History' window, which displays a record of all manual edit(s) of the selected allele(s) (Figure 82). To view the details of a specific change, select the edit of interest from the 'Edit History' list. The 'Current/Old Values' field will populate with the recorded information for the allele for both before the edit was made (old value) and after the edit was made (current value). The cells that represent the edited element will be highlighted in red.

This record can be printed or saved as a jpeg, png, or pdf, by selecting **Print** at the bottom of the 'Show Edit History' window (Figure 82).

Note: The 'Show Edit History' window is only active when the **Help/User Management/Settings/Record Data Edit History** option is selected (Section 6.F).

Information recorded in the 'Current Value' row can be changed back to the previously recorded (old) value by right-clicking the edit in the 'Edit History' list and selecting **Recover Old Value**. A checkmark will appear next to the number of the edit in the list. Select **OK** at the bottom of the window, and a confirmation box will appear. Select **Yes** to revert the edit back to the original state, add the recover operation to the 'Edit History' list for the allele and return to the 'Main Analysis' window.

A version of this window displaying all edits to all samples in a project is accessible by selecting **View Project History** from the 'View' menu (Section 6.B).

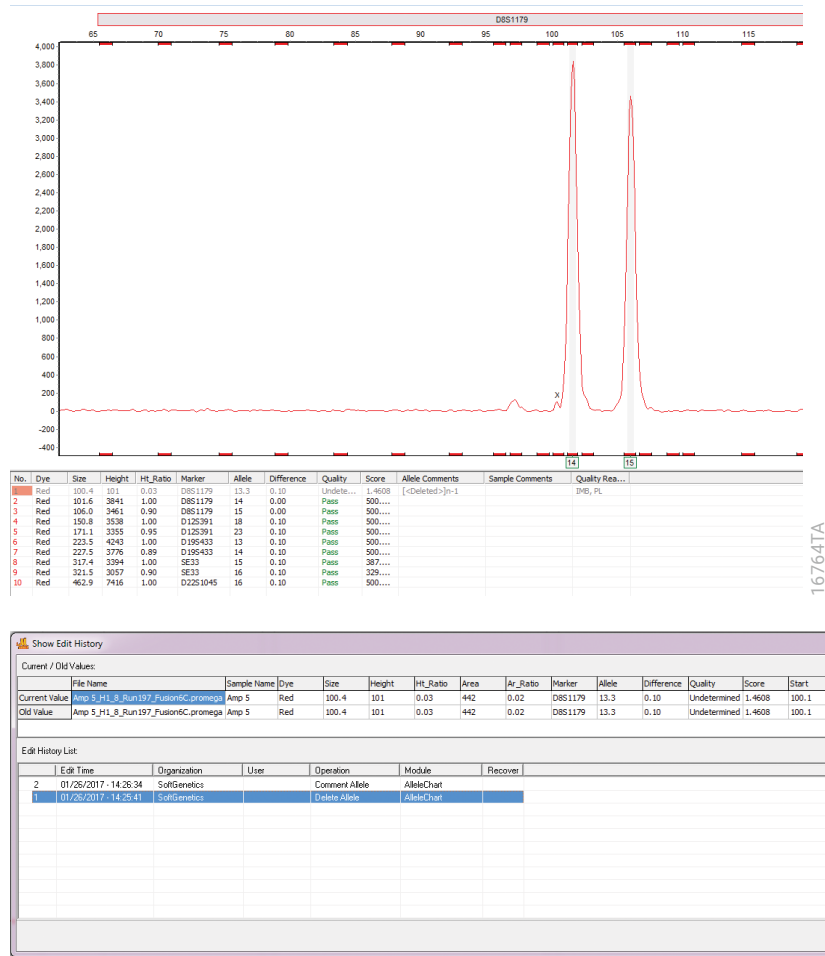



Figure 82. Edits recorded in the 'Show Edits History' window. Top Panel. A peak at 100.4 bases was deleted as indicated by the "x" in the electropherogram and in the allele comments in the Peak Table. **Bottom Panel.** The 'Show Edits History' window resulting from the peak deletion shown in the top panel.

Copy Table: Copies the displayed Peak Table information onto the Windows® clipboard (Shortcut = CTRL+C). This feature is only available in the right-click menu of the Peak Table. You can then paste the information into a spreadsheet or word processing program. Alternatively, you can save the Peak Table for all project samples for all displayed dye channels as an Excel® (.xlsx), text (.txt) or CSV (.csv) file by selecting the **Save Peak Table** icon  in the toolbar (Table 5). Save the file in the desired location with the desired file name from the 'Save Peak Table' window. A confirmation box will appear after the table is successfully saved.

5.C. Navigating the 'All Color Browser' Window

When reviewing processed data, select the **Browse by All Colors** icon in the toolbar to view the electropherograms with separate charts for each selected color (Figure 83). This functionality is similar to use of the 'Browse by All Colors' icon in the 'Main Analysis' window for raw data (Section 3.C.4) to view a separate electropherogram for each selected dye color.

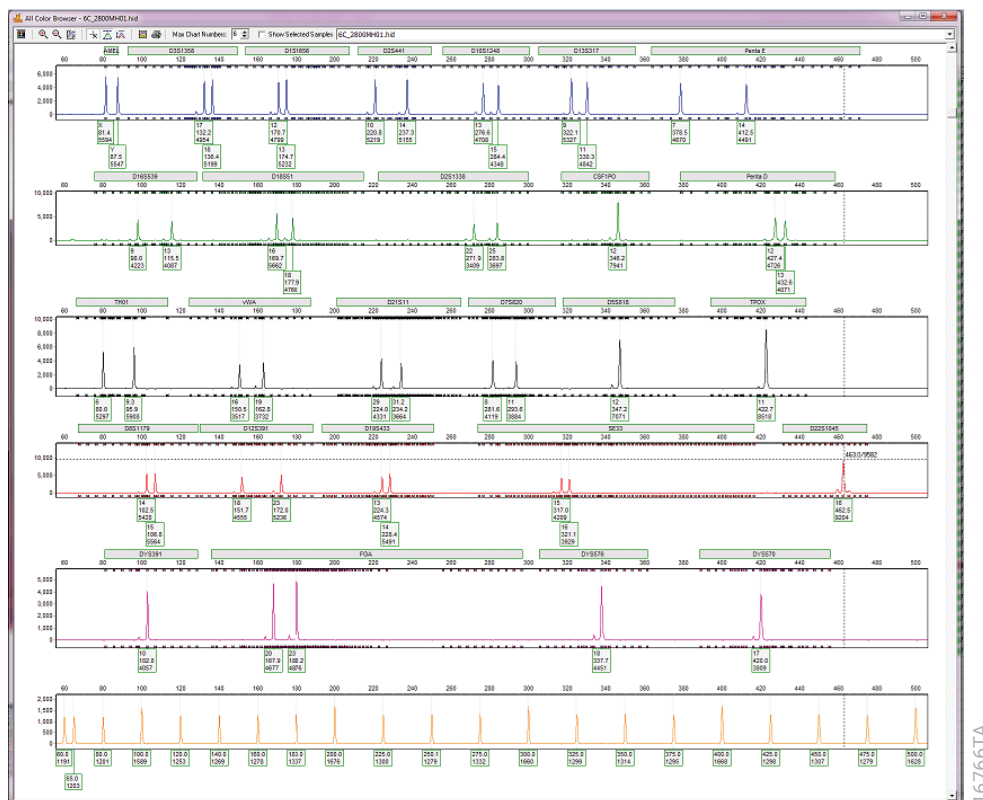





Figure 83. 'All Color Browser' window with processed data.

The same icons and functions used to view raw data in the 'Browse by All Colors' window (Table 4) can be used to view the processed data. Several additional icons are available when reviewing processed data (Table 7).

Table 7. Additional All Color Browser Icons and Functions Available for Processed Data.

	Show/Hide Bin Ranges: Toggles between a view where the bin brackets are displayed at the top and bottom of the electropherogram and a view where the bin brackets are not visible.
	Auto Scale Markers: When selected, adjusts the RFU intensities of low peaks to match the intensity of the highest peak in the dye color. When low peaks are increased, the intensity magnification factor is noted in the marker (2X–8X).
	Print: Opens the 'All Color Browser Settings' window to print or save the display of a single sample from the 'All Color Browser' window with the specified settings (Section 9).

The 'Show Chart/Table' is the same icon as in the 'Main Analysis' window (Table 5). The same right-click menu is available in the electropherogram chart of the 'Main Analysis' window and the 'All Color Browser' window, with the additional 'Set Axis' option. The 'Set Axis' option allows you to adjust the scale of the y axis.

To close the 'All Color Browser' window and return to the 'Main Analysis' window, select the **X** in the upper right-hand corner.

Note: The software will not close if the 'All Color Browser' window is open and minimized.

5.D. Navigating the Profile Comparison View

In addition to the standard electropherogram view (Section 5.B) and the 'All Color Browser' window (Section 5.C), you can view processed data using the 'Profile Comparison View' application. This application allows you to graphically display any combination of selected samples and dye colors in a 2-dimensional (2D) or 3-dimensional (3D) view. The Profile Comparison View can be accessed through the 'Applications' menu.

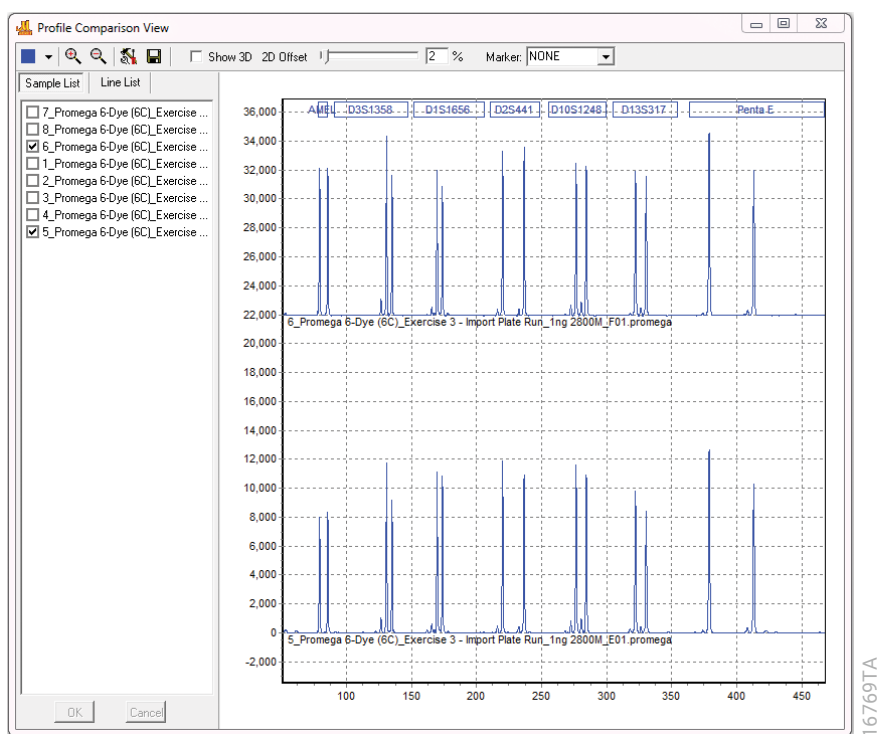


Figure 84. 'Profile Comparison View' window.

The toolbar within the 'Profile Comparison View' window (Figure 84) provides options for changing the display of the electropherograms. Many of these tools function the same or similarly to those found in the 'Main Analysis' window (Section C.3.2).

Table 8. Icons and Functions of the Profile Comparison View.






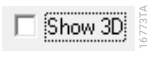


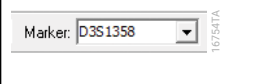
	<p>Show Dye: Allows you to select the displayed dye channels.</p>
	<p>Zoom In: Zooms in on the center point of the electropherogram</p>
	<p>Zoom Out: Zooms out from the center point of the electropherogram</p>
	<p>Option: Provides display options for the application. By default all viewing options are selected. Deselect options that are not needed or desired.</p>
	<p>Save: Opens a 'Save' window to save the 'Profile Comparison View' image as a bitmap (.bmp) file.</p>
	<p>Show 3D: When selected, displays the electropherograms of the selected samples in a 3D view.</p>
	<p>2D Offset: Changes the offset of displayed electropherograms. By default, the electropherograms are overlaid; sliding the bar to the right separates the plots to the desired degree, from 0–100%.</p>

Table 8. Icons and Functions of the Profile Comparison View (continued).

	<p>3D Percent: Indicates the depth of field when in 3D mode. The '2D Offset' slider bar becomes the '3D Percent' slider bar when the 'Show 3D' box is checked.</p>
	<p>Marker Drop-down Menu: Allows you to select a marker to view. This menu functions the same as in the 'Main Analysis' window (Table 5).</p>

Samples contained within a project are listed on the left of the 'Profile Comparison View' window. There are two display options: 'Sample List' and 'Line List'. 'Sample List' lists all samples in the project with check boxes next to the sample file names. 'Line List' displays only the samples selected from the 'Sample List'.

To compare electropherograms across samples, select the desired samples from the 'Sample List' by checking the box next to the sample file name and selecting **OK** at the bottom of the list. An overlay of the electropherograms for all selected samples will be displayed in the electropherogram area.

You can choose the dye channels displayed in the electropherogram using the **Show Dye** icon in the toolbar (Table 8).

Just as in the 'Main Analysis' window, users can zoom in or out from the electropherogram using the mouse or the 'Zoom In' and 'Zoom Out' icons in the toolbar.

By default, all the available display options are turned on. Specific options can be deselected using the 'Option' icon.

Auto Y: When selected, the y axis is set to auto scale based on the maximum peak intensity of the data within a given view.

Mouse Zoom: When selected, the mouse can be used to zoom in and out from the electropherogram.

Show Grid: When selected, grid lines for x- and y-axis labels appear.

Use Dye Color: When selected, electropherograms are displayed in their assigned dye channel colors. When not selected, electropherograms are displayed in black, regardless of assigned dye color channel.

Show Panel: When selected, the electropherogram displays the marker labels for all displayed dye channels.

Show File Names in 2D: When selected, the sample file names are displayed below the electropherograms of selected samples.

Note: When this option is used, set the 2D offset above 0% to ensure the sample file names do not obscure each other.

To close the 'Profile Comparison View' window and return to the 'Main Analysis' window, select **X** in the upper right-hand corner.




Note: The software will not close if the 'Profile Comparison View' window is open and minimized.

5.E. Navigating the Report Table

The Report Table displays peak information for each sample in a project based on defined settings for the table. Additional settings for the Report Table are accessed via the 'Report' tab of the 'Preferences' window (Section 6.B).

Note: Modifications to the report settings will automatically be saved.

Table 9. Report Table Toolbar Icons.

	Report Settings: Opens the 'Allele Report Settings' window where you can define the report style and other features displayed in the report .
	Save Report: Opens a 'Save Report' window to save the Report Table as an Excel (.xlsx), text (.txt) or CSV (.csv) file. The arrow allows you to choose between a standard report and a LIMS report. Additional settings options are available when the LIMS report is selected (Section 5.E.2).
	Help: Provides a list of Report Table actions as well as definitions for all quality reasons.

A peak selected in the Report Table will also be selected in the Peak Table and electropherogram. There are three options to select a peak in the Report Table:

1. Double-click on the desired peak.
2. Use the arrow keys to move to the cell of interest within the Report Table, and press the **Enter** key.
3. Use Alt + Arrow keys to move to different cells within the Report Table.

Note: A marker selected in the 'Marker Drop-down' menu in the main analysis toolbar will update to display information from the selected marker in the Report Table, Peak Table and electropherogram. To return to full display, select **None** from the 'Marker Drop-down' menu.

The rules by which the Report Table and other frames in the 'Main Analysis' window are linked can be changed in the 'Report' tab of the 'Preferences' window (Section 6.B).

5.E.1 Features of the Report Table

Display Options

You can change the display of the Report Table to suit your needs. To view or change the display, select the **Report Settings** icon in the Report toolbar. This will open the 'Allele Report Settings' window (Figure 85).

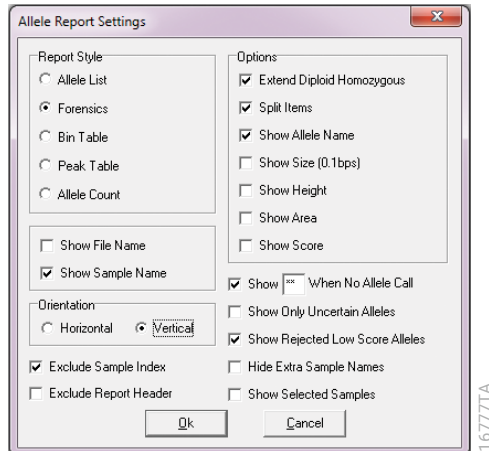


Figure 85. 'Allele Report Settings' window.

There are five options for report style (Figure 85) that affect the Report Table display: Allele List, Forensics, Bin Table, Peak Table and Allele Count. Each style has a different set of options for further customizing the information displayed. These styles and associated options are discussed in more detail below.

In addition to the 'Report Style' and 'Options' sections, the 'Allele Report Settings' window has several other options that affect display of samples in the Report Table. Some of these options are disabled for certain report styles, as noted below in the description of each report style.

Table 10. Options for Allele Report Settings.

Show File Name:	Displays the sample file name for each sample in the report.
Show Sample Name:	Displays the sample name for each sample in the report.
Orientation:	<p>Determines whether the report is displayed horizontally or vertically.</p> <ul style="list-style-type: none"> • "Horizontal": Displays sample names on the left in rows and markers at the top in columns. • "Vertical": Displays sample names in the far left column in rows with markers listed in the second column. Alleles, in order of base pair size, appear in subsequent columns.
Exclude Sample Index:	GMHID-Spectrum automatically assigns each line in the Report Table a consecutive number (sample index), displayed in the left-most column. Select the Exclude Sample Index box to exclude this column from the exported Report Table.
Exclude Report Header:	Excludes the report header from the exported table. This can be beneficial for import into some LIMS systems.
Show [] When No Allele Call:	Inserts a symbol or short word into the cells of the Report Table when no peaks are detected by the software at that marker. The default symbol is **. If this option is not selected, these cells will be blank.
Show Only Uncertain Alleles:	Displays only the peaks that did not pass the Analysis Quality metrics set in the Run Wizard and 'Preferences' window (Sections 4.C, 6.B and 10).
Show Rejected Low Score Alleles:	Displays peak scores below the settings found in the 'Peak Score Reject' setting in the 'Additional Settings - HID Settings' window of the Run Wizard.
Hide Extra Sample Names:	Displays the sample name once in the first row associated with that sample. If this option is not selected, the sample names are repeated for each row of data associated with that sample. This option is only available when "Vertical" is selected as the 'Orientation'.
Show Selected Samples:	Includes only samples selected in the Sample File Tree in the Report Table.

Each report style provides different advanced options.

'Allele List' Report Style

The most basic report style is 'Allele List' (Figure 86). This style displays the allele label (or just the base pair size, if a panel is not applied) of the called peaks in horizontal orientation. The 'Show [] When No Allele Call' and 'Hide Extra Sample Names' options are disabled. All other basic options can be changed (Figure 86).

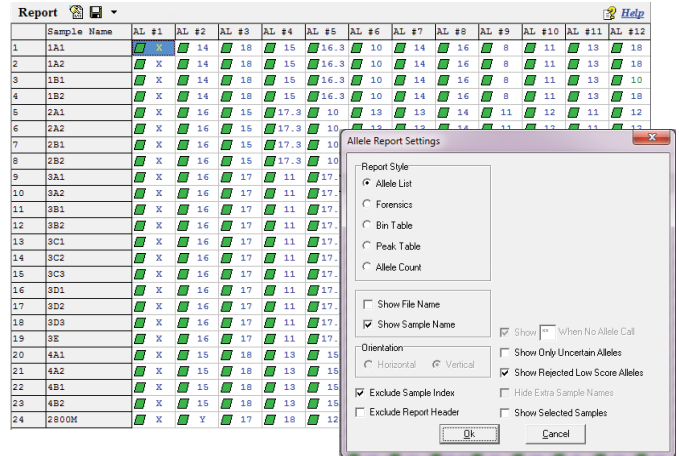





Figure 86. 'Allele List' Report Style.

'Quality' flags at the marker level (Table 11) are automatically displayed in the 'Allele List' report style. These flags provide a quick assessment of profile quality from the Report Table. Details of the parameter(s) not met, including quality reasons, are listed in the allele label and the Peak Table (Section 10).

The quality metrics in Table 11 are not viewable for samples listed in the 'Bin Table' or 'Allele Count' report styles.

Table 11. Quality Metrics Flags.

Flag	Quality
	Pass
	Check
	Undetermined

'Forensics' Report Style

The default report style of GMHID-Spectrum is 'Forensics' (Figure 87). This style displays the 'Quality' designation and 'Allele Label' of each called peak (Section 10.A.3). As in the Sample File Tree, samples that do not to meet the 'Analysis Quality' settings defined in the Run Wizard and 'Preferences' menu are flagged with a red question mark in the sample index column.

When the 'Forensics' report style is selected, additional display options are available in the 'Options' section of the window depending on the chosen orientation (i.e., "Horizontal" or "Vertical") of the table (Figure 87 and Figure 88).

Note: The 'Forensics' report style requires that a panel is applied to the data (Section 4.A.1).

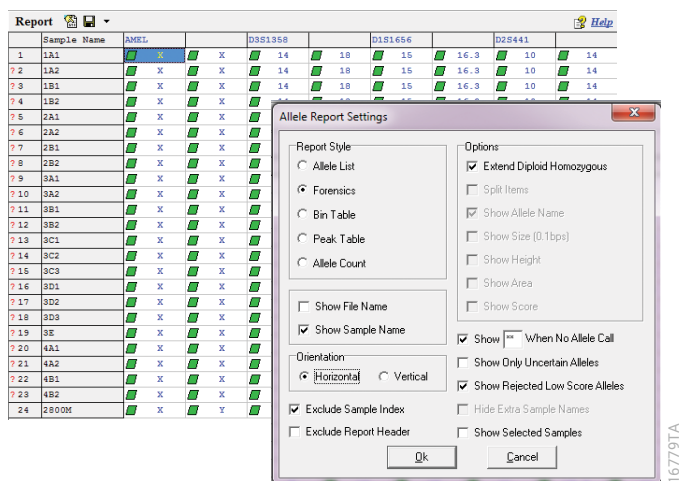


Figure 87. Horizontal orientation of the 'Forensics' report style.

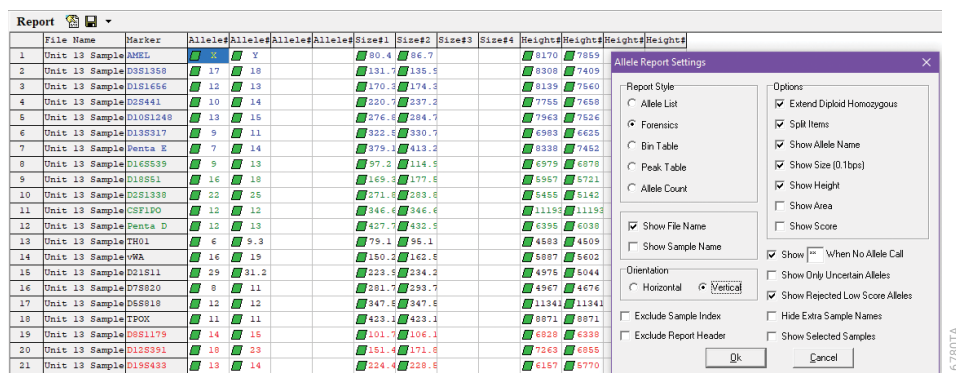


Figure 88. Vertical orientation of the 'Forensics' report style.

Options for 'Forensics' Report Style

Extend Diploid Homozygous: When selected, homozygous alleles are reported as a genotype (two alleles), not a phenotype (one allele). This option is only active when the 'Edit Panel Ploidy' option is set to 2-Diploid (Section 4.A.1 in the panel file).

Note: When the Orientation is set to horizontal, the 'Extend Diploid Homozygous' option is the only advanced option available and 'Show Allele Name' is on by default and cannot be disabled (Figure 87).

Split Items: When selected and when more than one of the **Show Allele Name, Show Size, Show Height, Show Area** or **Show Score** options are selected, the values are displayed in individual columns. When Split Items is not selected and the **Show Size, Show Height, Show Area** or **Show Score** options are selected, these values are displayed in parentheses in the Allele # columns of the report.

Note: When one or more of the **Show Allele Name, Show Size, Show Height, Show Area** or **Show Score** options are selected, these values are all displayed within the same cell of the 'Forensics' report. Parentheses separate the 'Allele Name' from any other peak attributes displayed in the same cell.

Show Allele Name: When selected, the Allele Name is displayed in the 'Forensics' report.

Show Size (0.1bp): When selected, the Size is displayed in the 'Forensics' report.

Show Height: When selected, the Peak Height is displayed in the 'Forensics' report.

Show Score: When selected, the Quality Score is displayed in the 'Forensics' report.

Quality flags at the marker level (Table 11) are automatically displayed in the 'Forensics' report style. These flags provide a quick assessment of profile quality from the Report Table. Details of the parameter(s) not met, including quality reasons, are listed in the allele label and the Peak Table (Section 10).

To export data for downstream use in STRMix™ and MaSTR™ software, ensure the 'Forensics' report style is used with the selected options shown below (Figure 89).

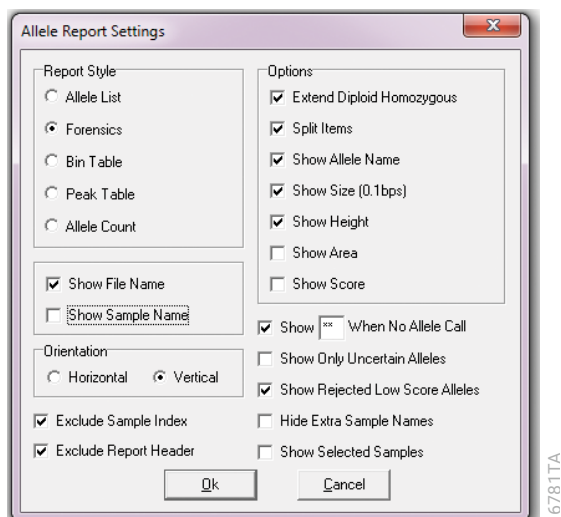


Figure 89. Configuration of 'Allele Report Settings' window for STRMix™ and MaSTR™ software compatibility.

'Bin Table' Report Style

The 'Bin Table' report style displays the presence or absence of a peak at every bin location for the samples in the dataset. The use of this style requires at least one peak in one sample to be detected. When the 'Bin Table' report style is selected, additional display options are available in the 'Options' section of the screen (Figure 90).

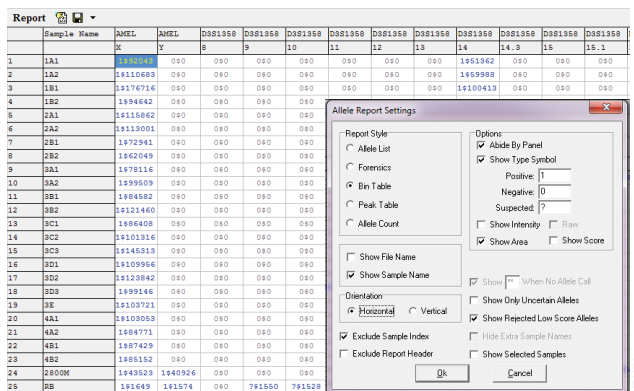


Figure 90. 'Bin Table' report style.

Options for 'Bin Table' Report Style

Abide By Panel: When selected, the Bin Table shows only called alleles within panel marker ranges. This option is only active when a panel is applied to the data.

Show Type Symbol: When selected, the specified symbols are displayed to indicate when a peak is present at the bin site (Positive), absent at the bin site (Negative) or did not pass a quality metric (Suspected) (Section 10.A.3).

Show Intensity (Raw): When selected, displays the peak intensity (RFU) value for all 'Positive' and 'Suspected' bin sites. A "0" value is given to 'Negative' bin sites. Selecting **Raw** will show the raw data peak intensity values for all bin sites including 'Negative' bin sites.

Show Area: When selected, displays the peak area value for all 'Positive' and 'Suspected' bin sites.

Show Score: When selected, displays the peak score for all 'Positive' and 'Suspected' bin sites.

Note: When more than one 'Bin Table' display option is selected, dollar signs (\$) separate these values in the 'Bin Table' report.

'Peak Table' Report Style

The 'Peak Table' report style displays user-defined peak statistics in horizontal orientation. All other options can be changed. When the 'Peak Table' report style is selected, additional display options are available in the 'Options' section of the window (Figure 91).

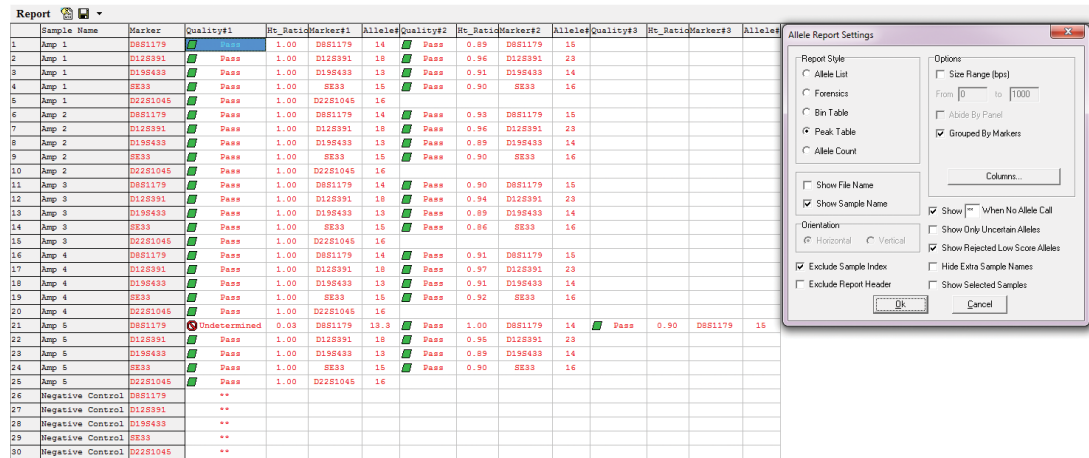


Figure 91. Peak Table report style.

Options for 'Peak Table' Report Style

Size Range (bps): When selected, allows you to define a specific base pair range from which the peak information will be displayed.

Abide By Panel: When selected, displays information for each bin in the panel regardless of whether a peak is detected in a bin or not. If no peak is detected in a marker, the symbol designated in the 'Show [] When No Allele Call' setting is displayed in the cell. This 'Abide By Panel' option is only active when a panel is applied to the data.

Grouped by Markers: When selected, displays each marker in its own row per sample and lists the information from all peaks within the marker consecutively across the columns at the top of the table. Under this setting, 'Abide By Panel' is locked and not applied. This option is only active when a panel is applied to the data.

When neither **Abide By Panel** nor **Grouped by Markers** is selected, each peak detected within a marker is represented in its own row.

Columns: Opens the 'Set Peak Table Columns' window (Figure 92) to select the columns to display in the Report Table. The columns displayed in the Peak Table report are similar to those available in the Peak Table of the 'Main Analysis' window (Section 5.B.2). The 'All Columns' field on the left lists the available columns not yet selected to be listed in the report. The columns currently being displayed in the Report Table are listed in the 'Selected Columns' field on the right.

Note: The order of the columns displayed in the 'Selected Columns' field is the order displayed in the Report Table.

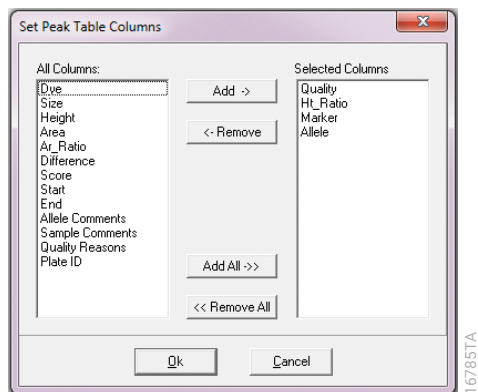


Figure 92. 'Set Peak Table Columns' window.

Adding Columns to the Report Table: Columns can be added to the Report Table individually or as a group. To add columns individually, select the desired column header in the 'All Columns' field and select the **Add** button. To add more than one column, hold down the **Shift** key and select the desired consecutive columns or hold down the **Ctrl** key and select the desired columns; select the **Add** button. To add all the columns listed in the 'All Columns' field to the Report Table, select the **Add All** button. Added columns will appear in the 'Selected Columns' field.

Removing Columns from the Report Table: Columns can be removed from the Report Table in the same manner as adding columns. Select the column(s) to be removed from the 'Selected Columns' field and select the **Remove** button to move the column(s) to the 'All Columns' field. To remove all columns listed in the 'Selected Columns' field from the Report Table, select the **Remove All** button. Removed columns will appear in the 'All Columns' field.

Quality flags at the marker level (Table 11) are automatically displayed in the 'Peak Table' report style. These flags provide a quick assessment of profile quality from the Report Table. Details of the parameter(s) not met, including quality reasons, are listed in the allele label and the 'Quality' and 'Quality Reason' columns of the Peak Table (Section 10).

'Allele Count' Report Style

The 'Allele Count' report style displays the number of called alleles marked with a 'Pass' quality designation in each marker. Peaks marked as 'OB' or with a 'Check' or 'Undetermined' quality designation will not be counted. A 'Total Number' column lists the number of peaks detected in the sample (Figure 93).

Note: Allele Count requires that a panel is applied to the data (Section 4.A.1).

The 'Show Only Uncertain Alleles', 'Show Rejected Low Score Alleles' and 'Hide Extra Sample Names' options are disabled in this report style. The 'Show Rejected Low Score Alleles' is on by default and cannot be changed. All other options are available for edit.

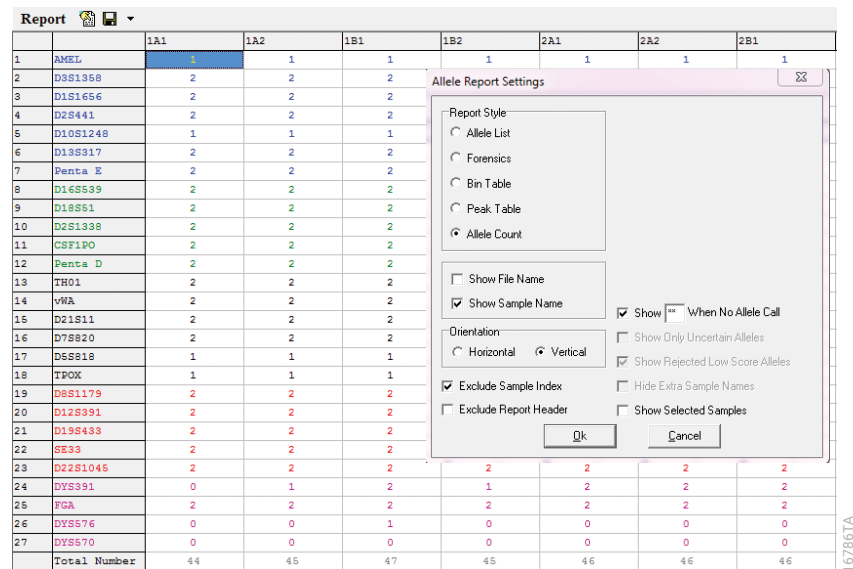


Figure 93. 'Allele Count' report style.

5.E.2 Sorting, Editing and Saving the Report Table

A variety of sorting and editing options are available depending on which report style is selected. The available options are accessible by right-clicking within the Report Table.

Table 12. Sort Option Availability by Report Style.

Sort Option	Report Style	Orientation
Sort by Marker	Forensics	Horizontal and Vertical
	Peak Table	
	Allele Count	
Sort by Column	Allele List	Horizontal
	Forensics	
	Bin Table	
	Peak Table	
	Allele Count	

Sort by Marker: Table is sorted based on the Peak Quality Score of the selected marker in ascending or descending order. Ascending order moves lower quality peaks to the top of the table; descending order moves lower quality peaks to the bottom of the table. This option is only available with 'Forensics', 'Peak Table' or 'Allele Count' report styles.

Sort by Column: Table is sorted based on the values in the selected column in ascending or descending order. If ascending is chosen, then lower values will be sorted to the top of the table and higher values to the bottom the table. If descending is chosen, higher values will be sorted to the top of the table and lower values to the bottom the table. This option is available with all report styles when viewed in the horizontal orientation.

Editing Peaks

Peaks can be edited from the Report Table. To edit a peak, first select the peak of interest by clicking on the cell that contains the peak; this selects the allele in the Report Table. Alternatively, select the peak by double-clicking the cell that contains the peak; this selects the allele in the Report Table, electropherogram and Peak Table. With the desired allele selected, right-click and select the appropriate editing function or use the shortcut described below. All changes made to peaks from the Report Table will be reflected in the electropherogram and Peak Table as described in Section 5.B.3.

Delete Peaks: Removes the peak from the profile (Shortcut = DEL).

Confirm Peaks: Acts in the same manner as the 'Confirm Allele' option within the electropherogram and Peak Table (Shortcut = CTRL+M). Confirmed alleles can be unconfirmed in the electropherogram or Peak Table as described in Section 5.B.3.

Peak Information

To view information about a peak, press the ALT key and select the peak cell of interest. The 'Allele Peak Info' window appears, displaying the 'Sample', 'Dye', 'Size', 'Marker', 'Allele', 'Score' and 'Comments' information (Figure 94). The information in these fields cannot be edited. This option is available in all report styles except 'Allele Count'.

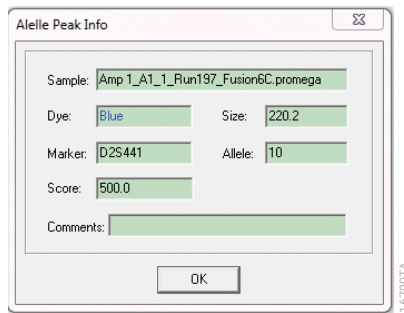


Figure 94. 'Allele Peak Info' window.

The 'Bin Table' report style offers additional options when a cell in the table is right-clicked.

Insert a Peak at this Bin Site

The 'Insert a Peak at this Bin Site' option is available only at bin sites where a peak is not present (i.e., Negative bin sites). To use this option, right-click the Negative bin site and select **Insert a Peak at this Bin Site**. A 'Confirmation' window will appear describing the location of the new peak. Select **Yes** to insert a peak at the selected bin site. The 'Negative Type Symbol' will change to the 'Positive Type Symbol'. The peak specifications will be calculated and displayed in the Peak Table. "Confirmed and Inserted" will be displayed in the 'Allele Comments' column of the Peak Table. (Shortcut = INS).

Delete Bin Columns

When the orientation is set as "Horizontal" in the 'Allele Report Settings' window (Figure 87), selecting the **Delete Bin Columns** option deletes the selected bin column in the Report Table. When the orientation is set as "Vertical", selecting this command deletes the bin row in the Report Table rows (not the columns that contain the sample information).

Saving the Report Table

The Report Table can be saved in the displayed format or as a LIMS Report.

Save Report Table

You can save changes to the Report Table as an Excel (.xlsx), text (.txt) or CSV (.csv) file by selecting the **Save Report** icon in the 'Report Table' toolbar.

In the 'Save Report Table' window, browse to the desired file location and save the file with the desired file name. When the report has been successfully saved to the location, an information box will appear to notify the user. Select **OK** to close the box.

To export only selected cells in the Report Table, highlight the desired cells, then right-click on the highlighted cells and select **Copy** (Shortcut = CTRL + C). The information is saved to the Windows® clipboard and can be pasted into any common word processing or spreadsheet program. The row and column headers for those cells will be copied along with the highlighted cell information.

In addition to saving the Report Table in the displayed format, you also can save the table as a LIMS Report.

LIMS Report

The LIMS Report is a specialized function designed for LIMS exporting compatibility. To access the LIMS Report settings, first ensure that the "Show LIMS Report Settings Box" in the 'Report' tab of the 'Preferences' window is selected (Section 6.B). Then select the **arrow** to the right of the **Save** icon, and select **Save LIMS Report** (Figure 95).

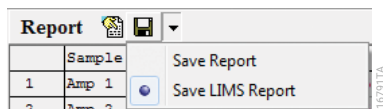


Figure 95. Saving a LIMS Report.

The 'LIMS Report Settings' window will be displayed (Figure 96). Some of the same report options available in the Report Table are available in the LIMS Report: 'Show File Name', 'Show Sample Name', 'Show Deleted Peaks', 'Show [] when no allele call', 'Show Only Uncertain Alleles', 'Show Rejected Low Score Alleles', 'Exclude Report Header' and 'Hide Extra Sample Names'. The advanced options available in the 'Peak Table' report style (Section 5.E.1) are also available in the LIMS Report. In addition, you can select which dyes are exported to the LIMS Report using the check boxes in the 'Dyes' section of the window.

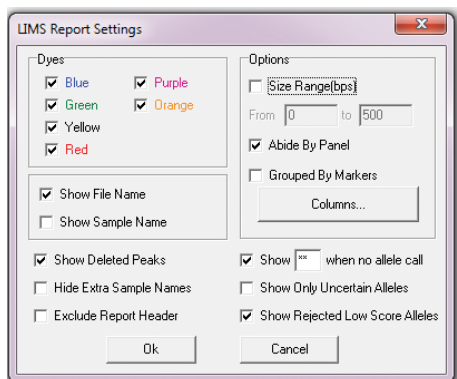


Figure 96. 'LIMS Report Settings' window.

5.F. Re-Analyzing Processed Data

A project can be re-analyzed after the initial run template is applied by using one of three options.

Note: Manual edits will be lost when data is re-analyzed.

1. **Re-analyze with Run Wizard:** To re-analyze with the 'Run Wizard' tool, select the **Run Project** icon in the 'Main Analysis' window toolbar. The Run Wizard will open and display the most recently selected parameters. Adjust parameters as necessary, and select **OK** in the 'Additional Settings – HID Analysis' window. The 'Use Old Calibration' window will appear with the option to 'Call size again' (Figure 97). Only select **Call size again** if the size standard template listed in the 'Template Selection' window of the Run Wizard was changed or any of the parameters in the 'Data Process – HID Analysis' window were changed. Select the **Apply to All** button to apply the chosen 'Use Old Calibration' window setting to all samples. Alternatively, select **Apply to Current** to choose and apply the setting on a sample by sample basis. The 'Data Processing Event Log' window will appear again, and the data will be re-analyzed with the new parameters.

Note: The 'Use Old Calibration' window has a countdown timer of 30 seconds after which the window will automatically close and re-analysis will continue.

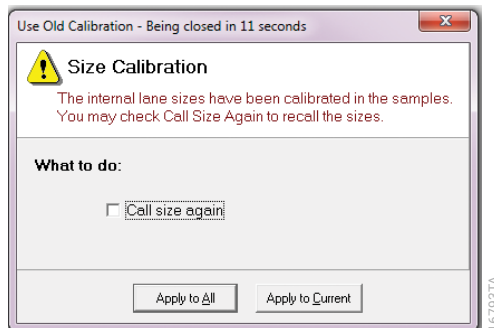



Figure 97. 'Use Old Calibration' window.

2. **Re-analyze with Auto Run:** To re-analyze with Auto Run, select the **Auto Run** option in the 'Project' menu. This will apply the most recently used 'Run Wizard' template to the project data.
3. **Re-analyze Individual Samples:** To re-analyze an individual sample, dye color or marker, select the **Call Allele** icon  in the 'Main Analysis' window toolbar to open the 'Recall Allele' window.

The arrow next to the 'Call Allele' icon opens a pop-out menu, which allows you to choose the samples to re-analyze (Figure 98).

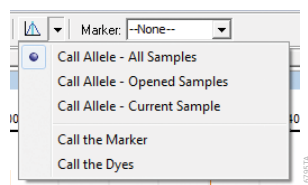


Figure 98. 'Call Allele' menu options.

Call Allele-All Samples: Applies the new parameter settings to all samples in the project.

Call Allele-Opened Samples: Applies the new parameter settings only to samples selected in the Sample File Tree.

Call Allele-Current Sample: Applies the new parameter settings only to the sample highlighted in the Sample File Tree.

Call the Marker: Applies the new parameter settings to the marker selected in the 'By Marker' field of the 'Call Allele By' section of the 'Recall Allele' window (Figure 99).

Call the Dyes: Applies the new parameter settings to all markers in the selected dye channels in the 'By Dyes' field of the 'Call Allele By' section of the 'Recall Allele' window (Figure 99).

The 'Recall Allele' window consolidates the main analysis parameters of the Run Wizard into one screen and allows the user to make adjustments that will be applied to the selected sample(s), dye color, or marker, serving as a helpful research tool when evaluating parameter settings (Figure 99). Once the desired changes to the 'Allele Call', 'Ladder', 'Auto Select Best Ladder', 'Auto Panel Adjustment', 'Positive Control Template', 'Allele Evaluation' or 'Mixture Evaluation' sections are made, select **OK** to apply the changes and re-analyze the selected data. The 'Data Processing Event Log' window will appear again and the data will be re-analyzed with the new parameters.

Note: When **Call Allele-Opened Samples**, **Call Allele-Current Sample**, **Call the Dyes** or **Call the Marker** is selected, the 'Panel', 'Ladder' and 'Positive Control Template' fields become inactive.

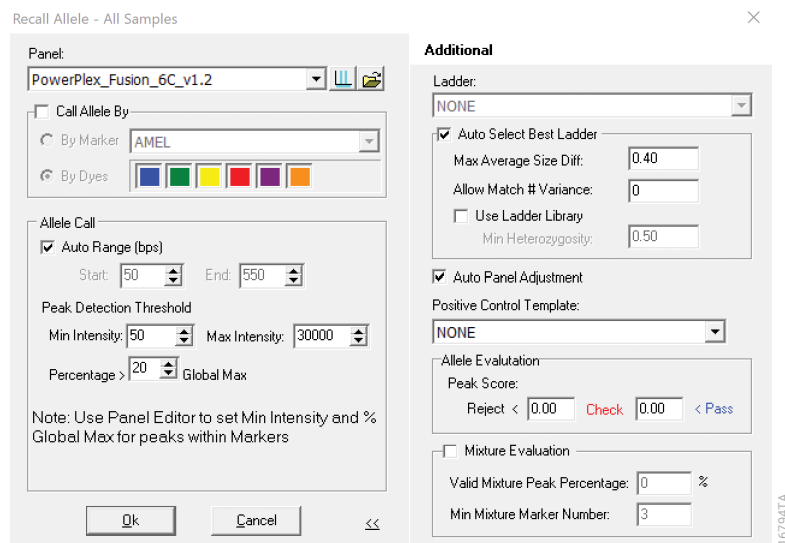


Figure 99. 'Recall Allele' window.

6 Main Menu Options

The following options can be found in the menu bar of the 'Main Analysis' window.

6.A. 'File' Menu

The 'File' menu contains functions for opening, saving and closing raw and processed data as well as exiting the program (Figure 100).

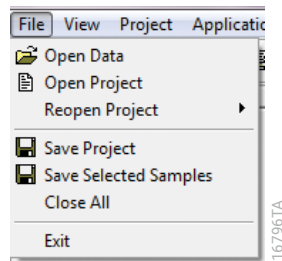


Figure 100. 'File' menu.

Open Data: Opens the 'Open Data Files' window, where you can select raw data files for uploading into the software.

Open Project: Opens the 'Open Project' window, where you can select previously saved project files (.sgf).

Reopen Project: Lists the last four projects that were opened by GMHID-Spectrum and allows the user to select any one of those four projects to open.

Save Project: Saves a project (.sgf) to a specified directory. Raw data files and analyzed data files with edits are saved within a project file. Once a project is saved, the name of the folder in the Sample File Tree will change from "Untitled" to the project name.

Save Selected Samples: Saves a project (.sgf) for the samples selected in the Sample File Tree to a specified directory. Raw data files and analyzed data files with edits for the selected samples are saved within the project file.

Close All: Closes a project without exiting the program.

Exit: Closes the GMHID-Spectrum software.

6.B. 'View' Menu

The 'View' menu contains options for controlling how data are displayed in the 'Main Analysis' window (Figure 101).

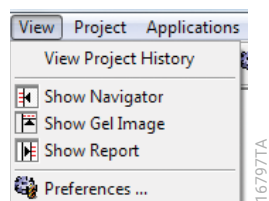


Figure 101. 'View' menu.

View Project History: Opens the 'Show Edit History' window for the entire project. This functions the same as the 'Show Edit History' window accessed via the 'View History' option within the Peak Table (Section 5.B.3). The project history displays all edits made to all samples in a project, including edits automatically applied by the software based on user preferences (e.g., auto-deleting variant alleles in allelic ladder samples; see Preferences below).

Show Navigator/Gel Image/Report: Opens and closes the 'File Navigator', 'Gel Image' or 'Report Table' frames in the 'Main Analysis' window.

Preferences: Opens the six-tab 'Preferences' window (Figure 102). Changes made to any of these tabs are automatically saved to the Preferences program template file (Section 2.C). Selecting **Import/Export** in the bottom left corner of the window provides two options. The user can export the current 'Preferences' settings as a .ini file using **Export to File** or import a 'Preferences' .ini file using **Import from File**.

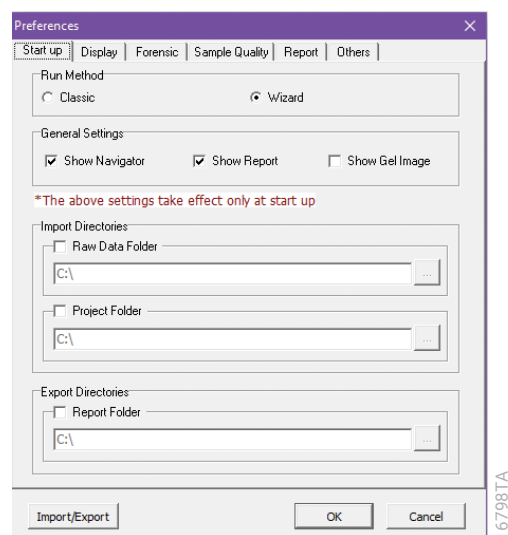


Figure 102. 'Start Up' tab of the 'Preferences' window.

The six tabs of the 'Preferences' window (Figure 102) are described as follows:

6.B.1 The 'Start up' Tab

The 'Start up' tab of the 'Preferences' window (Figure 102) allows you to select the **Run Method** and **General Settings** that will be applied when the software is launched. The 'Start up' tab also allows the user to set the file path directories for project file management.

Run Method: There are two options for run method operation within GMHID-Spectrum: Classic and Wizard.

1. **Classic:** Disables the 'Magic Wizard' upon launch of the software. This allows the user to move through data importing, activating the 'Run Wizard' and saving a project without prompts.
2. **Wizard:** Activates the 'Magic Wizard' (Section 6.E) when the software is launched to guide the user through the Data Import, Run Wizard and Save Project functionalities. The **Wizard** radio button is checked by default.

General Settings: Determines which sections of the 'Main Analysis' window ('Navigator', 'Gel Image' and 'Report Table') are displayed upon launch of the software.

Note: When **Show Report** is checked, the Report Table will automatically be displayed after the raw data are processed.

Import Directories: Defines the default folder location for importing raw data and saved project files to decrease the amount of navigation required.

Export Directories: Defines the default folder location for exporting reports to decrease the amount of navigation required.

6.B.2 The 'Display' Tab

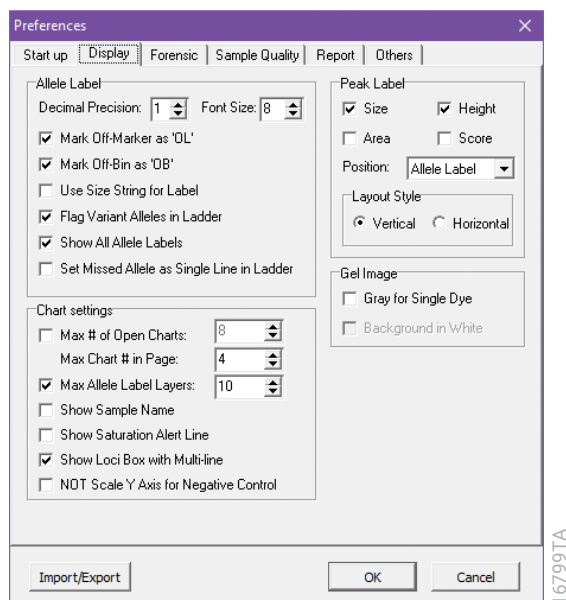


Figure 103. 'Display' tab of the 'Preferences' window.

The 'Display' tab of the 'Preferences' window defines how data are displayed in the Electropherograms, Gel Image and Sample File Tree (Figure 103).

The 'Allele Label' section defines how information is displayed in the Allele Label under peaks in the electropherogram.

Decimal Precision: Determines decimal places (0 to 2) to be displayed in peak size label.

Font Size: Sets the font size of the displayed text (also refers to size of text for print display).

Mark Off-Marker as "OL": Labels peaks that are outside of the marker ranges as "OL".

Mark Off-Bin as "OB": Labels peaks within a marker range, but outside of a bin, as "OB".

Use Size String for Label: Labels peaks according to size instead of allele call. To display the size string as an integer, set the 'Decimal Precision' to 0.

Flag Variant Alleles in Ladder: Marks peaks in virtual bins with higher than expected peak heights with yellow vertical bars and green allele labels in allelic ladder samples (Section 10).

Show All Allele Labels: Displays all allele labels for all selected dye channels. If more than one dye channel is displayed in the electropherogram and this check box is not checked, allele labels will be displayed only for the dye channel at the top of the electropherogram.

Set Missed Allele as Single Line in Ladder: Places allele labels for missing ladder peaks one row above allele labels for ladder peaks that were called.

The 'Chart Settings' section defines how data are displayed in the electropherograms of the 'Main Analysis' window. The 'Max Allele Label Layers', 'Show Saturation Alert Line' and 'NOT Scale Y Axis for Negative Control' settings will also apply to the electropherogram display in the 'All Color Browser' (Section 5.C).

Max # of Open Charts: Sets the maximum number of samples to be displayed in the Electropherogram and Peak Table area at one time (maximum = 96). To open the maximum number of samples specified here, right-click within the Sample File Tree and choose **Select Max**.

Max Chart # in Page: Sets the maximum number of electropherograms and Peak Tables to be displayed in the 'Main Analysis' window at one time (maximum = 8). To view other groups of samples, use the 'Page Up' or 'Page Down' links at the bottom of the Sample File Tree. If the 'Max Chart # in Page' is lower than the 'Max # of Open Charts', and the maximum number of open charts is open, a scroll bar will appear to view additional electropherograms and Peak Tables.

Max Allele Label Layers: Sets the maximum number of layers (i.e., rows) for allele labels (maximum = 10). For example, if "1" is entered, all allele labels will be displayed as one row. This may lead to overlap of allele labels in samples with multiple alleles (such as allelic ladders and mixtures). If the maximum of "10" is entered, the allele labels will be staggered across 10 rows below the electropherogram, minimizing the amount of label overlap.

Show Sample Name: When selected, displays the sample name as read from the data file above the electropherogram, in parentheses after the file name.

Show Saturation Alert Line: When selected, displays a purple vertical line through the regions of raw data where saturation was detected by the collection software.

Show Loci Box with Multi-line: When selected, displays the marker labels in a staggered format, rather than an overlaid format, when multiple dye channels are displayed in the 'Main Analysis' window. When this setting is applied, the marker labels are outlined in the color of the dye channel in which they're located.

Note: When **Show Loci Box with Multi-line** is selected, the marker labels in the red dye channel will mask allelic ladder quality flags for errors in bin shifting and peaks in virtual bins with higher-than-expected peak heights (Section 10).

NOT Scale Y Axis for Negative Control: When selected, zooming in and out on an electropherogram will not affect y-axis scaling for samples marked as negative controls.

The 'Peak Label' section defines information included in a peak label (if chosen) as well as layout options for the label (vertical or horizontal). Up to four labels (size, height, area and score) can be displayed for each peak in the electropherogram. If no labels are chosen, peaks are labeled with only allele calls as prescribed in the 'Allele Label' section.

Position: Sets the position of the peak label in relation to the peak: at the top, to the right side, or in the allele label in the electropherogram.

Layout Style: Determines whether peak labels are displayed vertically or horizontally.

The 'Gel Image' section defines the display of the synthetic gel image.

Gray for Single Dye: When selected, displays and prints the gel image with a black background and white bands instead of colored bands (depending on which dye channels are displayed).

Note: When all dye colors are selected, the bands in the gel image are displayed in color regardless of whether this option is selected.

Background in White: Inverts the gel image so that the background is white and band fragments are black. This setting is available only when **Gray for Single Dye** is selected.

6.B.3 The 'Forensic' Tab

The 'Forensic' tab of the 'Preferences' window is used to set additional Report Table and electropherogram settings, as well as the file naming conventions used by the laboratory for ladders and controls (Figure 104).

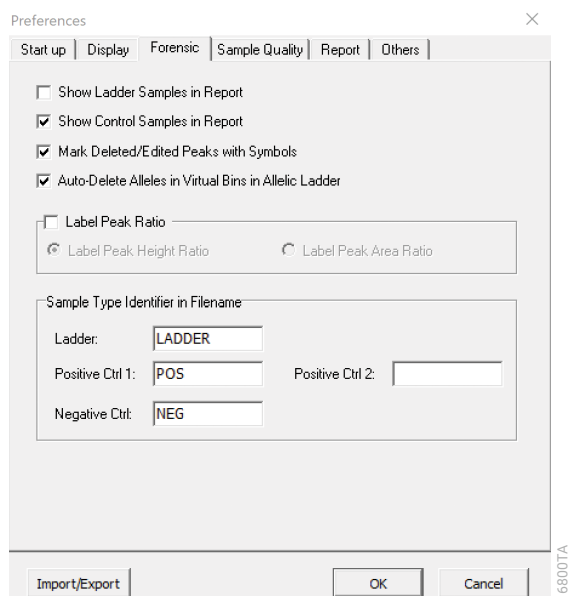


Figure 104. 'Forensic' tab of the 'Preferences' window.

Show Ladder Samples in Report: Includes samples designated as 'Ladders' by the 'Ladder' identifier field in the Report Table. Select this option if data will be exported for use in probabilistic software programs, including STRMix™ and MaSTR™ software.

Show Control Samples in Report: Includes samples designated as 'Positive' and 'Negative' controls by the 'Positive Ctrl 1', 'Positive Ctrl 2' and 'Negative Ctrl' identifier fields in the Report Table.

Mark Deleted/Edited Peaks with Symbols: Marks deleted peaks with an "X" at the top of the peak. Edited peaks are marked with an "E" at the top of the peak.

Auto-Delete Alleles in Virtual Bins in Allelic Ladder: Automatically deletes peaks in virtual bins with higher-than-expected peak heights in allelic ladder samples.

Label Peak Ratio: Allows you to display the peak ratio, calculated using the height or area, in peak labels. This option is only applicable when the peak label setting is set to peak top or peak right (Section 6.B.2).

Sample Type Identifier in Filename: Allows the software to automatically identify allelic ladders, positive controls and negative controls using the laboratory's file naming conventions. Entering the laboratory file naming convention in the fields provided allows the software to auto-identify these sample types. If the specified sequence of characters defined for a sample type is found anywhere in the sample name, the software automatically identifies the sample type. For example, if 'Ladder' is used as the identifier for the ladder sample type, samples named 'Ladder', 'Allelic Ladder' and 'Ladder 1' are all identified as ladders. Changes made to the identifiers are applied and samples containing these identifiers will be automatically identified when **OK** is selected in the 'Preferences' window.

Note: The 'Identifier' fields are not case-sensitive. To implement a change in the 'Identifier' fields without re-analyzing data with the Run Wizard, right-click any sample in the Sample File Tree and select **Set Sample Type/Auto Identify** (Figure 105).

Ladder: Labels samples identified as ladders with the prefix "LD" before the sample name and changes the font color of the file name to blue in the Sample File Tree. Samples identified as ladders will be checked against the expected allelic ladder profile for the selected panel. This setting is required to use the 'Auto Select Best Ladder' and 'Auto Panel Adjustment' features of the Run Wizard (Section 4.C.1). The default setting is "LADDER".

Note: The 'Ladder' option affects the operation of the 'Auto Select Best Ladder' and 'Auto Panel Adjustment' features in the 'Additional Settings - HID Analysis' window of the Run Wizard. After modifying the 'Ladder' field, re-activate the Run Wizard and proceed through the 'Additional Settings - HID Analysis' window (Section 4.C.1).

Positive Ctrl 1 and Positive Ctrl 2: Defines up to two positive control identifiers, 'Positive Ctrl 1' and 'Positive Ctrl 2'. Labels samples identified as Positive Ctrl 1 with the prefix "PC1". Labels samples identified as Positive Ctrl 2 with the prefix "PC2". The font color of the file name will change to green in the Sample File Tree. Samples identified as positive controls will be checked against the expected genotype of the corresponding **Positive Control Template** selected in the Run Wizard. A 'Positive Ctrl' identifier is required to use the 'Positive Control Template' feature of the Run Wizard. If only one Positive Control Template is used, the 'Positive Ctrl 2' field can be left blank. The default setting for Positive Ctrl 1 is "POS".

Note: When using both positive control identifiers, each identifier must be mutually exclusive from the other. Identifiers cannot contain the same string of letters or characters. For example, if Positive Ctrl 1 is "POS", Positive Ctrl 2 cannot be "POS2", "Positive" or any variation that contains the letters POS as part of the string.

Negative Ctrl: Labels samples identified as negative controls with the prefix "NC" and changes the font color of the file name to red in the Sample File Tree. A 'Negative Ctrl' identifier is required for Negative Control samples to be automatically identified and checked for absence of called peaks. The default setting is "NEG".

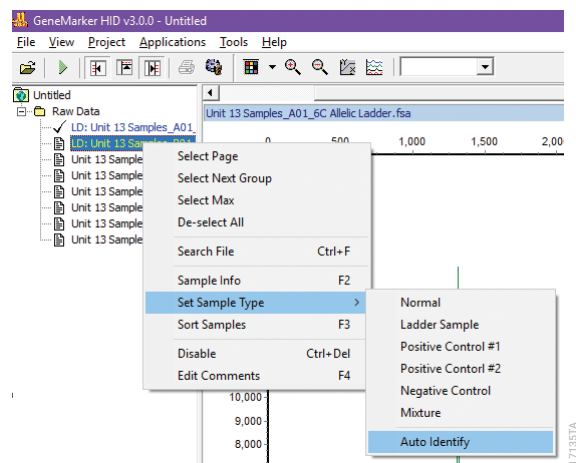


Figure 105. Selecting Auto Identify as the 'Set Sample Type' method.

Note: Allelic ladder, positive controls and negative controls for .promega files are identified automatically based on their assigned sample type in the plate record of the Spectrum Control Software. If .promega files do not contain assigned sample type information, GMHID-Spectrum identifiers are applied on file import. To apply identifiers from GMHID-Spectrum to these file types (.promega files), open the 'Preferences' window after data import, then select **OK** to refresh the software auto-identify feature.

6.B.4 The 'Sample Quality' Tab

The 'Sample Quality' tab of the 'Preferences' window is used to define additional criteria by which a sample is flagged for potential quality issues (Figure 106).

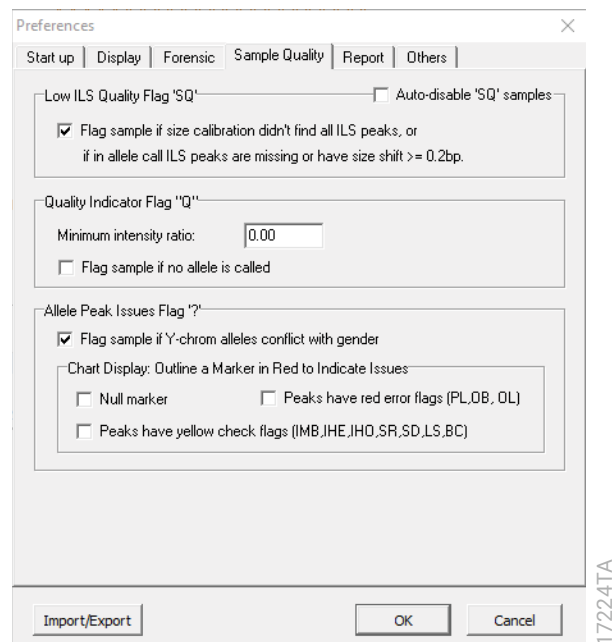


Figure 106. 'Sample Quality' tab of the 'Preferences' window.

Low ILS Quality Flag 'SQ': Selecting **Flag sample if size calibration didn't find all ILS peaks, or if in allele call ILS peaks are missing or have size shift \geq 0.2bp.** will flag samples with low-quality size standard peaks with 'SQ' in the Sample File Tree. When this setting is selected, you can choose to automatically disable samples flagged as 'SQ' by selecting **Auto-disable 'SQ' samples**.

Note: 'Auto-disable 'SQ' samples' is not a selectable option if **Flag sample if size calibration...** is not selected. See Section 10.A.2 for addition information on size calibration.

Quality Indicator Flag 'Q': Flags samples that do not meet the laboratory's Large Allele to Small Allele quality indicator peak height ratio when the "Sample Quality Peak(s)" box is checked in the 'Additional Settings - HID Analysis' window of the Run Wizard. The peak height of the Large Allele quality indicator is compared to the peak height of the Small Allele quality indicator and a Quality Intensity Ratio is calculated. Samples with ratios below the "Minimum intensity ratio" are marked with a red Q over the Size Match Score in the Sample File Tree. Selecting "Flag sample if no allele is called" marks samples with the Q flag if a quality indicator allele is not called.

Note: The "Sample Quality Peak(s)" option is available in GMHID-Spectrum software version 3.2.0 or later.

Allele Peak Issues Flag '?': Selecting "Flag sample if Y-chrom alleles conflict with gender" marks samples that have Amelogenin and Y-STR marker conflict(s) with a question mark in the Sample File Tree and the Report Table.

Chart Display: Outline a Marker in Red to Indicate Issues: Markers containing peaks which do not meet specific analysis parameters can be outlined in red to further aid in profile quality assessment. Select any or all of the following three criteria to trigger the red outline rule:

Null marker: Marker does not contain a called peak.

Peaks have red error flags (PL, OB, OL): Marker contains at least one peak with red Undetermined Quality flag (Section 10).

Peaks have yellow check flags (IMB, IHE, IHO, SR, SD, LS, BC): Marker contains at least one peak with yellow Check Quality flag.

6.B.5 The 'Report' Tab

The 'Report' tab of the 'Preferences' window allows you to select additional display preferences for the Report Table (Figure 107).

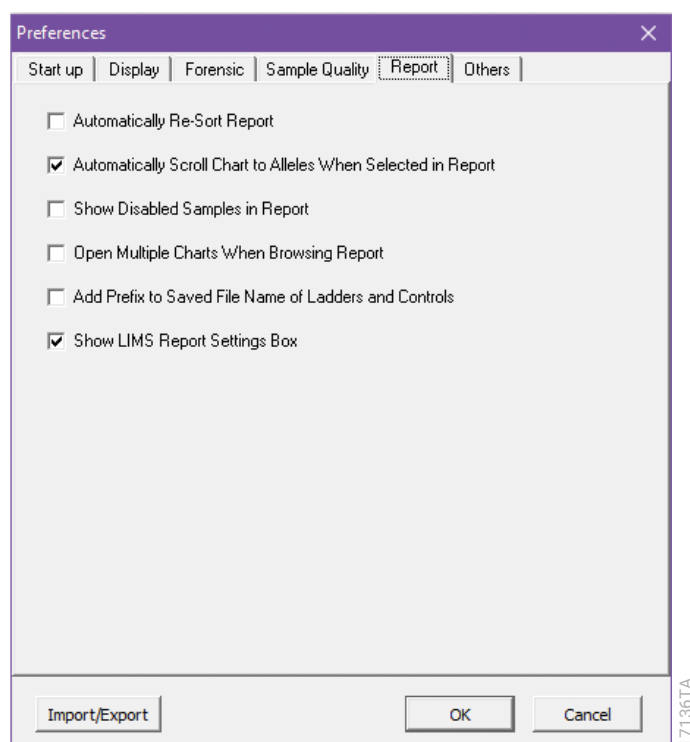


Figure 107. 'Report' tab of the 'Preferences' window.

Automatically Re-Sort Report: Sorts the report after edits are made to alleles. When unselected, you must select the right-click menu to sort the table. See Section 5.E.2 for report sorting options.

Automatically Scroll Chart to Alleles When Selected in Report: Allows the electropherogram display to automatically zoom in on a 60bp window around the peak selected in the Report Table.

Show Disabled Samples in Report: When selected, samples that are marked disabled, either by the user or automatically by the software, appear in the Report Table.

Open Multiple Charts When Browsing Report: Allows simultaneous viewing of multiple electropherograms, one for each sample selected in the Report Table. To add an additional sample to the electropherogram chart area, select that sample by double-clicking that entry in the Report Table.

Add Prefix to Saved File Name of Ladders and Controls: Adds the prefix “Ladders_” to the allelic ladder file names when saving a report, and the prefix “Controls_” to any samples designated as a positive or negative control (set in the ‘Forensic’ tab of the ‘Preferences’ window; Figure 104).

Show LIMS Report Settings Box: Opens the ‘LIMS Report’ settings window when the **LIMS Report** option is selected from the ‘Save Report’ icon. When not selected, the ‘LIMS Report’ settings window does not appear, you are taken directly to a navigation window to save the report. This allows you to save the LIMS Report but not modify the report content (Section 5.E.2).

6.B.6 The ‘Others’ Tab

The ‘Others’ tab of the ‘Preferences’ window is used to select additional project preferences, including CODIS export settings and folder settings (Figure 108).

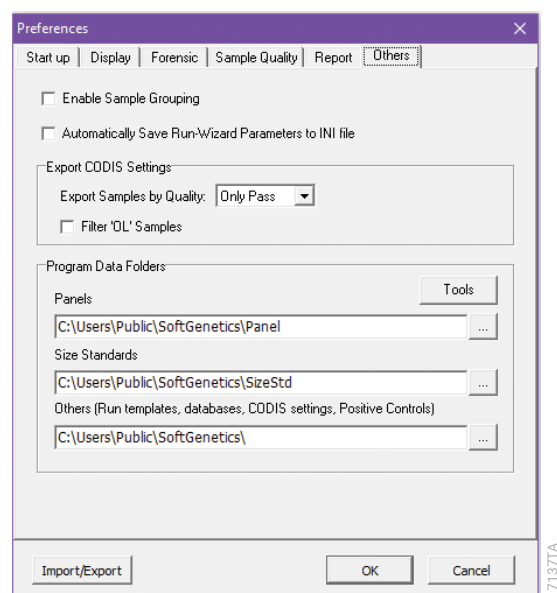


Figure 108. ‘Others’ tab of the ‘Preferences’ window.

Enable Sample Grouping: Activates **Apply Sample Grouping** in the ‘Project’ menu. Deselect **Enable Sample Grouping** to inactivate the ‘Apply Sample Grouping’ option. Information saved while ‘Apply Sample Grouping’ is active can be recalled by selecting **Enable Sample Grouping** (Section 12.A.1).

Automatically Save Run Wizard Parameters to INI file: Saves the project Run Wizard parameters as an .ini file when the project is saved. The .ini file is saved to the same location as the saved project and given the same file name as the saved project, with the “_RunWizardParameters.ini” suffix (e.g., SavedProjectName_RunWizardParameters.ini).

Export CODIS Settings: Allows you to select which samples, based on quality assignment ('Only Pass', 'Check & Pass' or 'All Sample'; Section 10.A.3), are exported to a CODIS report. Users can further filter exported results by checking **Filter 'OL' Samples**. When this option is selected, samples containing 'OL' peaks are not exported. For more information on CODIS reports, see Section 6.D.

Program Data Folders: Default folders can be defined individually by selecting the ... **browse** button next to each folder setting field and navigating to the desired location. Alternatively, a root folder for these settings can be defined. A root folder is the top-most folder in a specified directory. The default root folder for these settings is **C:\Users\Public\SoftGenetics**. Within that root folder, the default subfolder for panel templates is Panel, and the default subfolder for size standard templates is SizeStd.

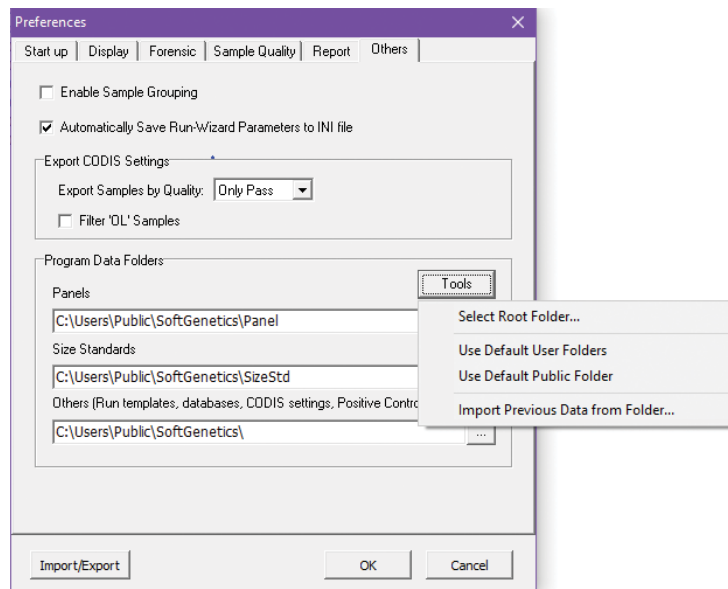


Figure 109. Selecting Tools in the 'Others' tab of the 'Preferences' window.

The **Tools** button provides three options for defining the root folder: 'Select Root Folder', 'Use Default User Folders' and 'Use Default Public Folder' (Figure 109):

Select Root Folder: Opens a 'Browse For Folder' window. Browse to the desired folder location, select the desired folder and select **OK** to close the window. Alternatively, to create a new folder, browse to the desired folder location and select the **Make New Folder** button.

Use Default User Folders: Uses the default SoftGenetics folder for the login user directory as the root folder: **C:\Users\[User Name]\AppData\Roaming\SoftGenetics\GeneMarker_HID**

Use Default Public Folder: Uses the default SoftGenetics folder in the public user directory as the root folder: **C:\Users\Public\SoftGenetics**

A fourth option under the 'Tools' button is 'Import Previous Data from Folder':

Import Previous Data from Folder: Opens a 'Browse For Folder' window. Browse to the desired folder location, select the desired folder and select **OK** to close the window.

Note: File paths must be entered exactly and should not include any spaces (unless a file name has a space in it). Network locations are indicated by two back slashes (\\).

6.C. 'Project' Menu

The 'Project' menu contains options for data processing and printing (Figure 110).

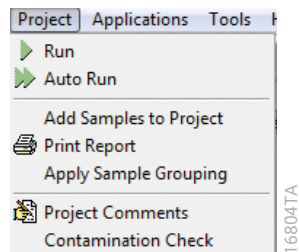


Figure 110. 'Project' menu.

Run: Activates the Run Wizard and begins the data processing setup. This allows you to select or adjust analysis settings in a sequential manner (Section 4).

Auto Run: Automatically analyzes data based on the last set of parameters selected in the Run Wizard. To change one or more of the run parameters, select **Run** from the 'Project' menu, change the desired setting(s) and re-analyze the samples.

Add Samples to Project: Allows you to add samples to a project that has already been processed. When selected, the 'Open Data Files' window will appear (Figure 46). To add individual files to the project, select the files, and select **Add** and then **OK**. The raw data file is processed with the same settings as the other files in the project and then added to the bottom of the Sample File Tree.

Print Report: Opens the 'Print Report' window, which allows you to define display settings in the Print Report (Section 9).

Apply Sample Grouping: Opens the 'File Name Group Editor' window (Section 12), which is used to group samples by specific identifiers and is needed to use the Project Comparison Tool (Section 12.B) and Replicate Comparison Tool (Section 12.C). The settings defined in the File Name Group Editor are used to print and save a Print Report (Section 9.A.) for individual samples or groups of samples when 'Print Samples with Grouping' is applied. This tool can also be used to simplify sample review (Section 12.A). By default, this option is disabled; to enable it, check the **Enable Sample Grouping** box in the 'Others' tab of the 'Preferences' window.

Project Comments: Allows you to write comments regarding the analysis. These comments are saved with the project file and can be displayed in the Print Report (Section 9).

Contamination Check: Opens the 'Contamination Check' window to allow you to define parameters for checking contamination within a project as well as comparing profiles in the current project to saved profiles in the Contamination Database (Section 10.B).

6.D. 'Applications' Menu

The 'Applications' menu contains individual modules for specific data and analysis types. These modules present advanced features and reporting options necessary for the particular application (Figure 111).

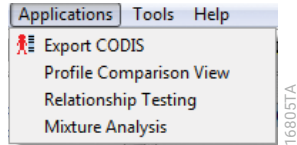


Figure 111. 'Applications' menu.

Export CODIS: Opens the 'CODIS Export' window, which allows export of the CMF 3.2 (.xml), CMF 3.0 (.xml) and CMF 1.0 (.dat) files for upload into the FBI's CODIS database. 'Export CODIS' settings can be managed and shared across software installations via the CODIS program template file (Section 2.C).

Note: Null Y-STR loci in samples with only an X allele at Amelogenin will not be marked null for CODIS export.

The 'CODIS Export' window is separated into three main sections: Export Format, Header and CODIS Marker. These areas allow you to specify the file export type, the information contained within the header and sample details (Figure 112).

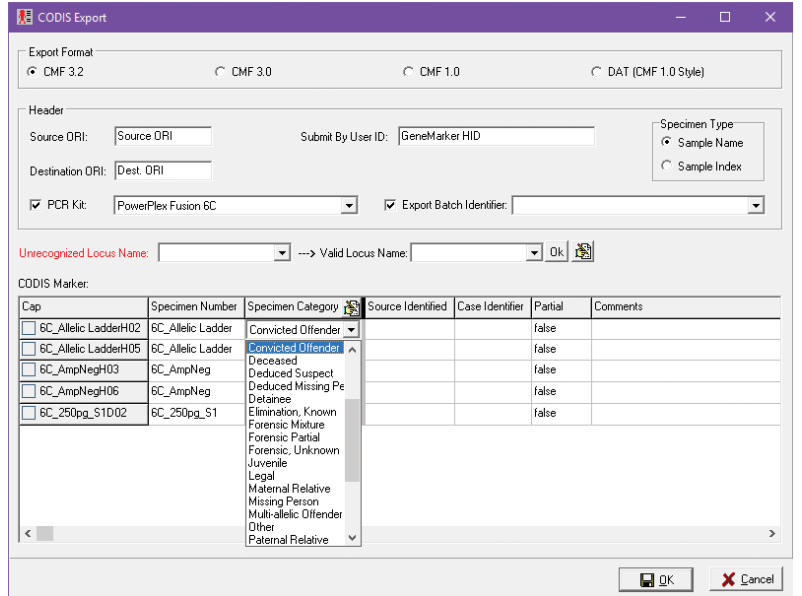



Figure 112. 'CODIS Export' window.

Export Format: You can select among the three different CODIS styles: CMF 3.2, CMF 3.0 and CMF 1.0. An additional style, DAT (CMF 1.0 Style), is available and allows 36 loci per sample. CODIS CMF formats are saved in the CODIS CMF program template file (Section 2.C).

Header: Each field within this section can be edited to reflect the laboratory's information. The 'PCR Kit' and 'Export Batch Identifier' boxes have a drop-down menu for selections and each can be excluded from the header by deselecting the respective check box. The 'Specimen Type' area allows you to specify the source of information in the 'Specimen Number' column—either the Sample Name or the Sample Index.

Note: The software will list locus names not recognized as CODIS-formatted names in the 'Unrecognized Locus Name' drop-down menu. You can select the unrecognized name from the list, select the correct format from the 'Valid Locus Name' drop-down menu and then select **OK** next to the 'Valid Locus Name' drop-down menu.

The 'Edit Recognized Locus Name Category' icon  allows you to enter lab-specific names for certain loci and convert them to the appropriate CODIS format (Figure 113). In the example below, the software will recognize 'PentaD', 'Penta_D', 'PentaE', 'Penta_E' and 'AM' and convert them to 'Penta D', 'Penta E' and 'Amelogenin', respectively, for CODIS export.

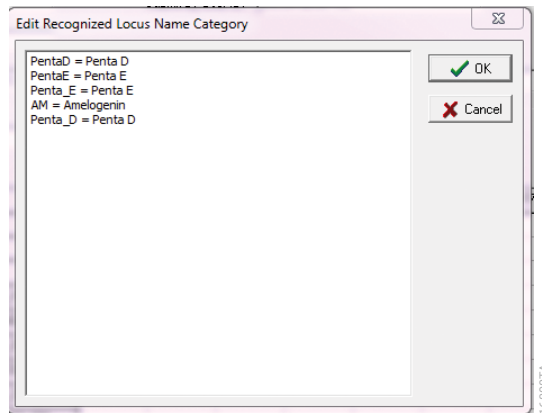



Figure 113. 'Edit Recognized Locus Name Category' window.

CODIS Marker: For each row, you can change the selection in each column, except for the 'Specimen Number' column. You can change the selections in the 'Specimen Category' and 'Partial' columns as well as the column for each marker, using the drop-down menus. The available options for 'Specimen Category' can be edited by selecting the **Edit Specimen Category** icon  in the column header. This will open the 'Edit Specimen Category' window where you can type in desired specimen categories (Figure 114). Categories listed in the 'Edit Specimen Category' window will appear in the drop-down menu in the 'Specimen Category' cells.

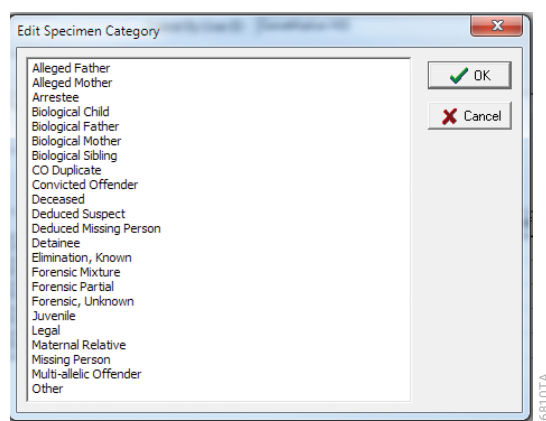


Figure 114. 'Edit Specimen Category' window.

The 'Case Identifier' and 'Comments' columns are editable by selecting and typing within a cell. Samples can be selected for export using the check box next to the Sample File Name in the 'Cap' column.

Once all information is updated in the 'CODIS Export' window, select **OK** and the option to save the CODIS file will appear. Select a location and save as either a CMF1.0 (.dat) , CMF3.0 (.xml) or CMF3.2 (.xml) file (as per US DOJ CODIS Interface Specification (CMF 3.0) CONTRACT NO. ITOP 97-0087 Sub Task Order 26CODIS and Interface Specification (CMF 3.2) Revision 14 DOJ-FBI-2005-C-2431). Select **Save**.

Profile Comparison View: Allows you to graphically display any combination of samples and dye colors. This feature includes a 2-dimensional and a 3-dimensional view of the selected samples (Section 5.D).

Relationship Testing: Allows you to perform relationship calculations, build pedigree diagrams and search the relationship database for potential familial matches. The 'Relationship Testing' application calculates probabilities and likelihood ratios for the following relationship levels: parent-child, siblings, half-siblings, aunt-niece/uncle-nephew, grandparent-grandchild and cousins. For further information on using the 'Relationship Testing' application, contact Promega Technical Services: genetic@promega.com

Mixture Analysis: Allows you to perform further analysis of mixture samples, including calculating statistics, deducing contributors and searching for potential matches within the software. For all mixture samples, the mixture analysis application automatically determines the number of contributors to a mixed sample and calculates PE, CPE, PI, CPI and RMNE. For two-contributor samples, the application reports all single-source files that are potential mixture contributors, automates repetitive calculations for all possible allele combinations, identifies major and minor contributor genotypes based on mixture ratios and calculates likelihood ratios for each locus as well as the combined likelihood ratio. For further information on using the 'Mixture Analysis' application, contact Promega Technical Services at: genetic@promega.com

6.E. 'Tools' Menu

The 'Tools' menu contains several functions including those used for managing templates, comparing data and exporting information (Figure 115).

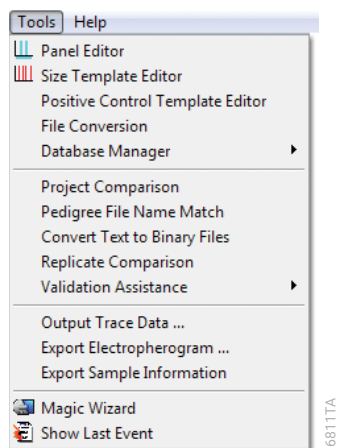


Figure 115. 'Tools' menu.

Panel Editor: Manages all panel templates for data analysis. In addition to setting the analysis parameters as described in Section 4.A.1, the Panel Editor provides tools to adjust, edit and create panels. See Section 8 for advanced use of the Panel Editor.

Size Template Editor: Manages all size standard templates. Use to create new templates, modify existing templates and delete templates from this editor as well as to compare sample size standards to templates. See Section 7.

Positive Control Template Editor: Manages all positive control templates. Use this editor to create new templates, modify existing templates and delete templates. Several positive control templates are preloaded with the software. A list of available templates is accessible via the drop-down menu in the 'Positive Control Standards' field (Figure 116). Positive control templates listed here are available for selection in the 'Additional Settings - HID Analysis' window of the Run Wizard (Section 4.C.3).

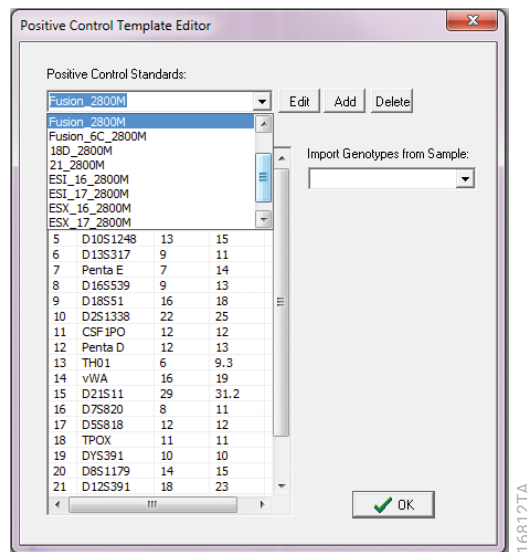


Figure 116. 'Positive Control Template Editor' window.

Listed templates can be edited by selecting the desired template from the drop-down menu, then selecting the **Edit** button to the right of the 'Positive Control Standards' field. This opens a second 'Positive Control Template Editor' window, where you can make the necessary changes (Figure 117). When all changes are made, select **OK** to return to the primary 'Positive Control Template Editor' window.

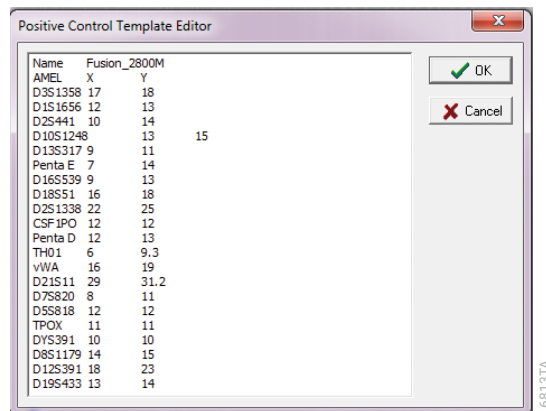


Figure 117. 'Positive Control Template Editor' window for edits.

New positive control templates can be added by selecting the **Add** button to the right of the 'Positive Control Standards' field (Figure 118). This opens the 'New Positive Control Standard' window where you enter the name of the new Positive Control Standard (Figure 118). When you select **OK**, the primary 'Positive Control Template Editor' window displays the new name in the 'Positive Control Standards' field and the corresponding genotypes field are blank.

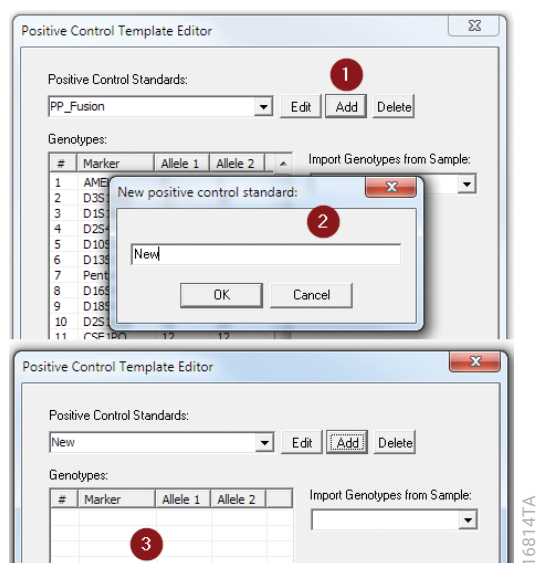


Figure 118. Creating a 'New Positive Control Standard' window.

Use one of two methods to populate the 'Genotypes' field with the correct genotype:

1. Select the **Edit** button and enter the genotype by typing the Marker name, Allele 1 and Allele 2, using the **Tab** key to navigate from one column to the next. Alternatively, you can copy and paste the genotype information from a text (.txt) file, formatted in the same way as described above: Marker name, **Tab**, Allele 1, **Tab**, Allele 2.
2. Use the 'Import Genotypes from Sample' function. The drop-down menu in this field lists all samples in the current dataset. Select the sample that contains the profile to be saved as a positive control template. A 'Confirm' window will appear. Select **Yes** to copy the genotype for the selected sample into this new positive control template. After selecting **Yes**, the genotype field will contain the sample genotype.

Positive Control Standards can be deleted by selecting the appropriate template from the drop-down menu, then selecting the **Delete** button on the right. A 'Confirm' window will appear to confirm the deletion.

File Conversion: Allows import of time and distance files from custom genetic analyzers for use with files formatted by the 'Convert Text to Binary File' tool (Figure 119).

Database Manager: Allows you to manage and submit profiles to the two databases contained within the software: Contamination DB and Relationship DB (Section 11).

Project Comparison: Allows you to compare the same dataset, analyzed in two different projects, and detect differences based on a number of parameters, including peak size and height, quality score and alleles with comments (Section 12.B).

Pedigree File Name Match: Allows you to automatically add additional files to a previously created pedigree diagram. This results in the export of a .smp file, which can be used in the 'Relationship Testing' application. For further information on using the 'Relationship Testing' application, contact Promega Technical Services at: genetic@promega.com

Convert Text to Binary Files: Allows you to upload raw data information in text (.txt) file format for conversion into a four-color SCF (.scf) or a five-color SG1 (.sg1) file. The SCF and SG1 files can then be read by GMHID-Spectrum and translated into electropherograms. This tool is useful for institutions developing their own fragment analysis instruments.

To upload files for conversion, select the **Load Text File** button in the 'Convert Text to Binary File' window (Figure 119), then select the desired text (.txt) file.

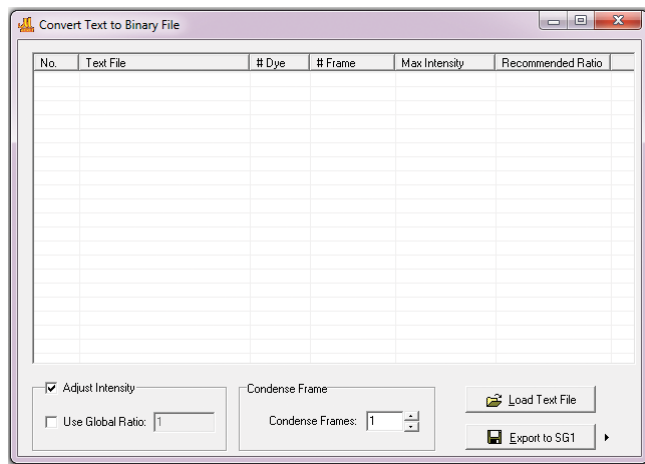


Figure 119. 'Convert Text to Binary File' window.

The software will automatically calculate a Recommended Ratio to condense the number of frames into a single electropherogram. Enter this value (or another desired value) in the 'Condense Frames' field, then select **Export to SG1** if exporting five-color data or **Export to SCF** if exporting four-color data.

Replicate Comparison: Compares replicate samples within a project. This can be used to detect contamination, allelic dropout or a failed injection or amplification. This tool also assesses concordance among these replicates when reporting consensus profiles. See Section 12.C for details on using the 'Replicate Comparison' tool.

Validation Assistance: Provides three calculations related to internal validation of STR kits: 'LOD', 'Height Ratio' and 'Migration Precision' (Section 13).

Output Trace Data: Allows you to export the electropherogram plot information for raw data or sized data (i.e., processed data) for selected sample files without instrument information (e.g., instrument name, run conditions; Figure 120).

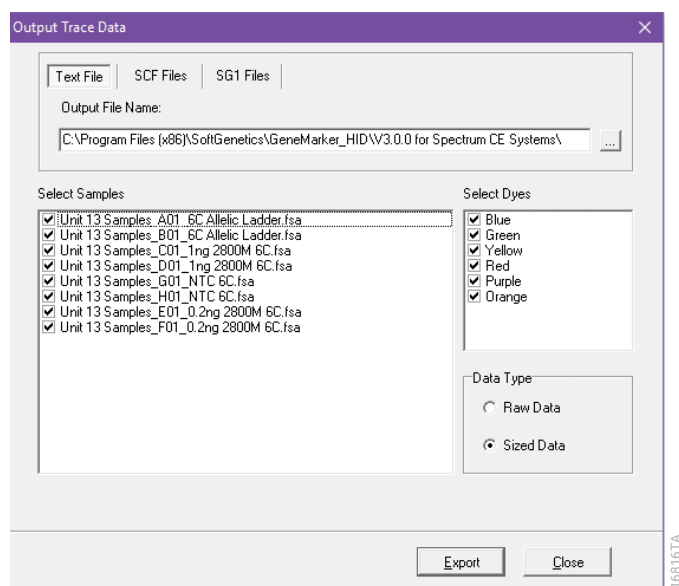


Figure 120. 'Output Trace Data' window.

There are three file format options available at the top of the 'Output Trace Data' window: text (.txt), SCF (.scf) or SG1 (.sg1). The location and file name of this file are indicated in the 'Output File Name' field. Data saved in .txt format can be saved in raw or sized form. Both the SCF and SG1 file formats will export an individual raw data file for each sample, which can be saved to the directory specified in the 'Output Folder' field.

Note: The SCF format can only export data with four dye channels.

The 'Output Trace Data' window is separated into four sections: 'Output File Name' (or 'Output Folder'), 'Select Samples', 'Select Dyes' and 'Data Type'. These areas allow you to specify which samples and dye channels are exported and, for .txt files, whether the export data are in raw or sized form. Select the desired file format by selecting the 'Text File', 'SCF Files' or 'SG1 Files' tab. A default file location (and file name for text file exports) is displayed in the 'Output File Name' field. To edit the location and file name, select the **Change directory** button to the right of the 'Output File Name' field. Select the desired samples from the 'Select Samples' list. Select the desired dye channels to export from the 'Select Dyes' section. Select the desired data (for .txt files only), raw or sized from the 'Data Type' section. When all fields are marked appropriately, select **Export**. A 'Confirmation' window will appear when the export is complete. When all desired files are exported, select the **Close** button to return to the 'Main Analysis' window.

Export Electropherogram: Allows you to export electropherograms as image files to a specified folder (Figure 121).

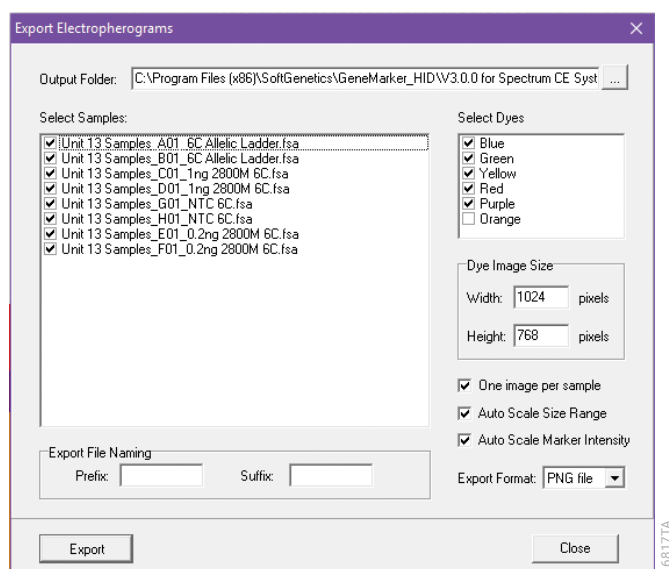


Figure 121. 'Export Electropherograms' window.

The 'Export Electropherograms' window (Figure 121) is separated into six sections: 'Output Folder', 'Select Samples', 'Select Dyes', 'Dye Image Size', 'Export File Naming' and 'Export Format'. These areas allow you to specify the file export type, which samples and dye channels will be exported and file prefix and suffix. Select the appropriate file location by selecting the **Change directory** button to the right of the 'Output Folder' field. Select the desired samples from the 'Select Samples' list. Select the desired dye channels to be exported from the 'Select Dyes' section. Enter the desired image size in the 'Dye Image Size' fields. You also have the option to select whether or not to save 'One image per sample', apply 'Auto Scale Size Range' and apply 'Auto Scale Marker Intensity' by selecting the appropriate check boxes. Specify the prefix and suffix for the exported file name(s) in the 'Export File Naming' fields. The full file name will be PrefixSamplename_DyenameSuffix.file extension. Select the desired file format from the 'Export Format' drop-down menu: .png, .jpeg, .bmp or .pdf. When all fields are marked appropriately, select **Export**. A confirmation window will appear when the export is complete. When all desired files are exported, select the **Close** button to return to the 'Main Analysis' window.

Export Sample Information: Allows you to export the information displayed in the 'Sample Information' window of the Sample File Tree to a specified location.

The 'Export Sample Info' window contains a list of all samples in the current project. Select desired samples by checking the box next to the sample file name, then select **Export** to open a browser window and choose the appropriate file location. Give the file an appropriate name, select the desired file format: Excel (.xlsx) or text (.txt), then select **Save**. A confirmation window will appear, indicating the file was exported successfully. Select **OK** to return to the 'Export Sample Info' window. To exit the 'Export Sample Info' window, select the **Close** button to return to the 'Main Analysis' window.

Magic Wizard: Provides three prompts to guide you through the main phases of data processing: opening files, analyzing data, and saving and printing projects. Each time the Magic Wizard is activated the software will display the most appropriate prompt for that point in the data processing procedure. The three prompts of the 'Magic Wizard' window are: 'Start your project', 'Run' and 'Report'. Use the **Previous** and **Next** buttons on the bottom of the window to toggle between the available prompts (Figure 122).

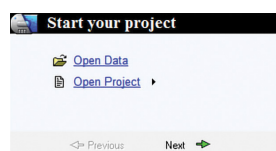


Figure 122. 'Start your project' prompt of the 'Magic Wizard' window.

Start your project (Figure 122): Provides links to the two methods for opening files: 'Open Data' and 'Open Project' (Section 6.A). The arrow next to 'Open Project' allows you to open the four most recently opened projects.

Run (Figure 123): Provides links to the two methods of processing data: 'Run' and 'Auto Run' (Section 6.C).

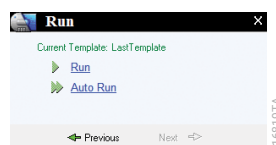


Figure 123. 'Run' prompt of the 'Magic Wizard' window.

Report (Figure 124): Provides links to save a project and print a report (Sections 6.A and 9.A).



Figure 124. 'Report' prompt of the 'Magic Wizard' window.

You can manually activate the Magic Wizard at any time using the option in the 'Tools' menu or the icon in the toolbar of the 'Main Analysis' window. The user may also have the Magic Wizard prompts display automatically throughout the data processing procedure by selecting **Wizard** as the Run Method from the 'Start up' tab of the 'Preferences' window (Section 6.B).

Show Last Event: Opens the 'Data Processing' window and displays the last 'Data Process' action performed by the software. The 'Show Last Event' tool can be opened via the 'Tools' menu or the icon in the toolbar of the 'Main Analysis' window.

6.F. 'Help' Menu

The 'Help' menu contains a searchable user manual, a 'User Management' feature and information on the installed software version (Figure 125).

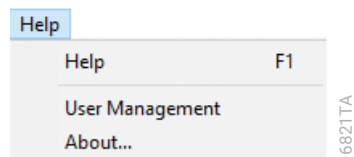


Figure 125. 'Help' menu.

Help: Opens a searchable version of this manual.

User Management: Opens the 'User Manager' window, which allows an administrator to assign access rights to different users and set up the password protection feature (Figure 126).

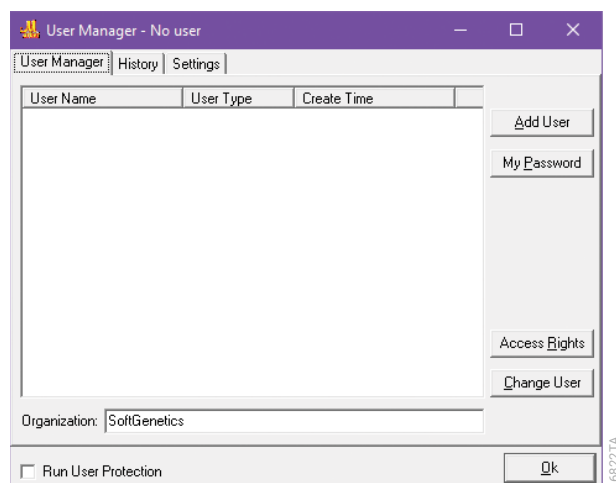


Figure 126. 'User Manager' window.

There are three tabs to the 'User Manager' window: 'User Manager', 'History' and 'Settings'.

User Manager: When an Administrator user is set up, this tab allows administrators to manage their password, add specific users and assign access rights to users. To set up the Administrator user, select the **Add User** button on the right side of the 'User Manager' tab. This opens the 'Setup Administrator' window where the administrator's Organization, User Name and Password can be defined. The administrator can now add users to the software and manage their access rights.

Note: Only the Administrator can add and delete users and change their access rights.

To add a user, select the **Add User** button, enter the desired user name and password and select the desired User Type: Reviewer, Analyst, Lab Manager or Administrator (Figure 127). Each User Type comes with pre-assigned access rights that can be modified by the Administrator. Users can be deleted or their User Type modified by right-clicking on the **User Name** in the list and selecting the appropriate function.

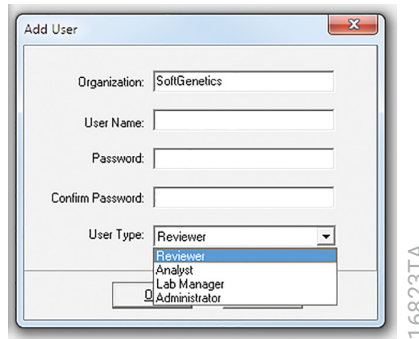


Figure 127. 'Add User' window.

To manage the access rights of specific User Types, select the **Access Rights** button. The 'Access Rights of User Types' window will open (Figure 128). Select a **User Type** from the 'User Type' field to see the default rights assigned to that type. Each user name assigned that User Type will appear in the 'Users' field. To modify the rights of the selected User Type, select or deselect the rights in the 'Access Rights' field. To reset rights to the default setting, select **Set Default**. When all access rights are set, select **OK** to return to the 'User Manager' window. To require users to log into the software, check the **Run User Protection** box in the 'User Manager' window.

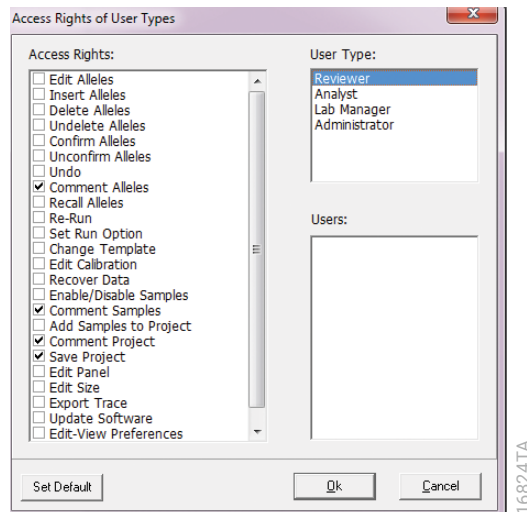


Figure 128. 'Access Rights of User Types' window.

You can manage your password by clicking on **My Password** in the 'User Manager' window (Figure 126). This will open the 'Change Password' window. Changes made here apply to the user currently logged into the software.

To change the logged-in user, select the **Change User** button. This will open a 'Confirm' window to verify that the current user will be logged out. Selecting **Yes** will log the user out and open a new login window.

History: This tab lists user activity associated with the 'User Manager' function (Figure 129). There are four columns of information within the 'History' tab.

1. **Date Time:** Records the computer's date and time for the activity.
2. **User:** Identifies the User Name of the individual that performed the activity.
3. **Events:** Records the User Manager activity that was performed.
4. **Comments:** Gives additional information for the event that was performed. For example, if a user is added, then the User Name of the person that was added is recorded under Comments.

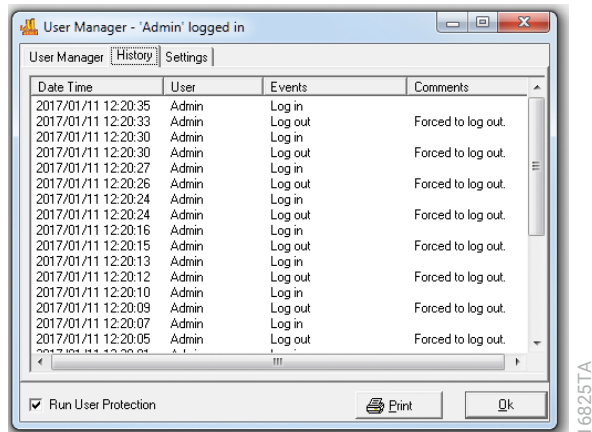


Figure 129. 'History' tab of the 'User Manager' window.

Settings: This tab contains additional options for the User Management function (Figure 130).

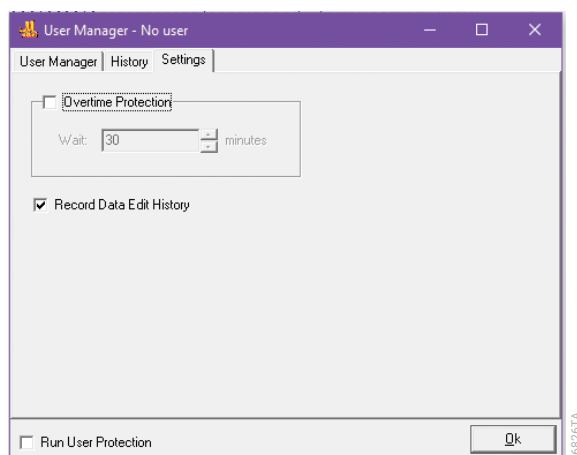


Figure 130. 'Settings' tab in the 'User Manager' window.

Overtime Protection: Logs the user out of GMHID-Spectrum after the specified inactive time entered in the 'Wait' field. When the user is logged out, the status of the analysis remains unchanged until the user logs back in (with user name and password).

Record Data Edit History: When selected, any changes made to allele calls within the project will be saved in the 'Project History' log (Section 6.B).

About...: Displays information specific to the version of GMHID-Spectrum running on the computer. Also contains links to the Promega web site and Promega Technical Services.

7

Fragment Sizing Standards

As described in Section 4.A.2, a size standard template is needed for each internal size standard used for data analysis. Each size standard template defines the fragment sizes for a specific internal size standard. The Size Template Editor allows you to manage the size standard templates.

After a size standard template is applied to a dataset, the quality of each sample's size standard can be assessed by comparing to the appropriate size standard template via the 'Calibration Charts' window (Section 7.B).

7.A. Navigating the Size Template Editor

In addition to reviewing and importing size standard templates (Section 4.A.2), the Size Template Editor allows you to create, edit and delete size standard templates. The Size Template Editor can be accessed through the 'Tools' menu (Section 6.E) or through the icon on the 'Template Selection' screen of the Run Wizard (Section 4.A).

The 'Size Template Editor' window (Figure 131) has a Menu Bar, Toolbar Ribbon, Size Standards File Navigator, Expected Size Standard Chart, Sample Size Standard Chart, Sample File Tree and Expected Size Standard Table.

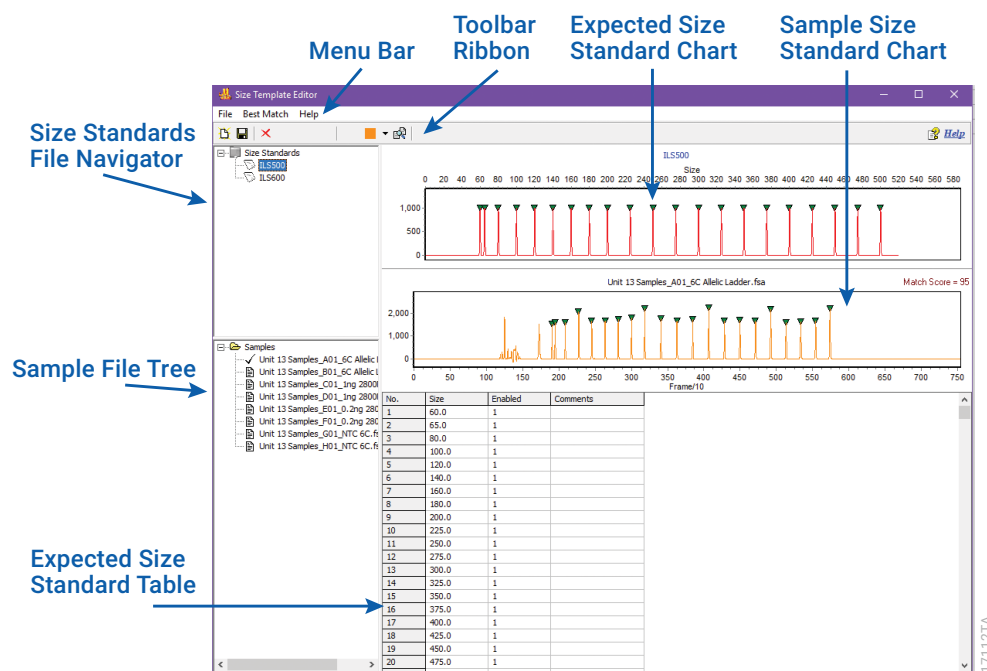








Figure 131. 'Size Template Editor' window.

Table 13. Toolbar Ribbon Icons of the Size Template Editor.

	<p>Create New Size Standard: Opens the 'Input Dialog' window with a field to enter a 'New Size Standard Name'. Allows creation of a new size standard template.</p>
	<p>Save Changes: Saves modifications to the 'Size Standards' directory (Section 6.B.6).</p>
	<p>Delete: Deletes the size standard template that is highlighted in the Size Standards File Navigator. Note: This action is irreversible.</p>
	<p>Show Dye: Allows you to select a single dye channel to view in the Sample Size Standard Chart by clicking on the down arrow. To cycle through individual dye channels, click on the icon.</p>
	<p>Size Match: Automatically matches the selected Sample Size Standard with the chosen size standard template, and displays the green size marker triangles atop size standard peaks in the electropherogram.</p>
	<p>Help: Provides shortcuts to the 'Size Editor Action Help' window descriptions of the display, editing and search functions of the Size Template Editor.</p>

7.A.1 Features of the Size Template Editor

The menu bar of the Size Template Editor contains three options: File, Best Match and Help. Each is described in detail in Section 7.A.5.

The Size Standards File Navigator lists all size standard templates saved to the software directory as indicated in the 'Others' tab of the 'Preferences' window (Section 6.B.6).

The Sample File Tree lists all samples within the open project. If the Size Template Editor is opened before sample files are imported, the Sample File Tree will be blank.

The Expected Size Standard Chart represents the expected pattern of size fragments for the selected size standard template. The peaks in this chart are always represented in **red** regardless of the dye color in which the actual size standard is represented in the data. Peaks that are used for both pattern recognition and sizing of sample peaks, enabled peaks, are indicated with green triangles at their apex.

When the Size Template Editor is opened after sample files are imported, the Sample Size Standard Chart displays the electropherogram chart of the selected sample. To select a sample from the Sample File Tree, double-click the sample file name. By default, the dye channel assigned to the Standard Color in the 'Template Selection' window of the Run Wizard (Section 4.A.2) is displayed. Peaks in the Sample Size Standard Chart are compared to the peaks in the Expected Size Standard Chart for pattern recognition. Peaks in the Sample Size Standard that match the enabled peaks of the Expected Size Standard are marked with green triangles and are used to size sample peaks. A total score for the match between the expected pattern of size standard fragments and the observed pattern in the Sample Size Standard Chart is displayed in the upper right corner of the Sample Size Standard Chart. Perfect matches receive a score of 100; no correlation receives a score of 0. Refer to Sections 7.B and 10.A.1 for further description of the Size Match Score.

The Expected Size Standard Chart and Sample Size Standard Chart are displayed with intensity in RFU along the y axis. The Expected Size Standard Chart is displayed with base pairs along the x axis, whereas the Sample Size Standard Chart is displayed with frames along the x axis. Use the same mouse functions to zoom or move within these charts as you do to zoom or move within the electropherograms of the 'Main Analysis' window.

The Expected Size Standard Table represents the expected size standard fragments in table format. There are four columns in the Expected Size Standard Table: 'No.', 'Size', 'Enabled' and 'Comments'. The fields of these columns are editable.

No.: Displays the sequential number given to each peak in the size standard template.

Size: Displays the expected size of each peak.

Enable: Displays the enabled status of the peak. Enabled peaks are used for both pattern recognition and sizing of sample peaks. A number 1 in this column indicates an enabled peak. A number 0 indicates a disabled peak. Disabled peaks are used for pattern recognition only.

Note: If you change the 'Enabled' value for a peak in the Expected Size Standard Table, you must select another cell in the table before saving the size standard or the change will not take effect.

Comments: Displays any comments that are made in the 'Comments' field of the 'Edit Size' window (Figure 134).

7.A.2 Creating a New Size Standard Template

You can create a new size standard template within the software using one of two methods: entering fragment details into the Expected Size Standard Table or using peak information from the Sample Size Standard Chart. To begin either of these processes, select the **Create New Size Standard** icon from the toolbar. This opens the 'Input Dialog' window (Figure 132).

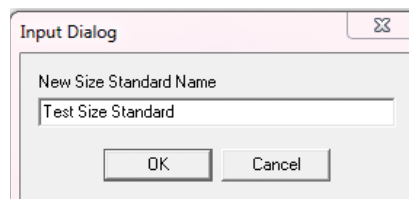


Figure 132. 'Input Dialog' window.

Enter a name for the new size standard template and select **OK**. The new template is listed and highlighted in the Size Standards File Navigator Tree. The Expected Size Standard Chart is blank.

Method 1: Creating a New Size Standard Template in the Expected Size Standard Table

1. To add expected size standard fragments to the size standard template from the Expected Size Standard Table, right-click anywhere in the table, and select **Insert Size** (Figure 133).

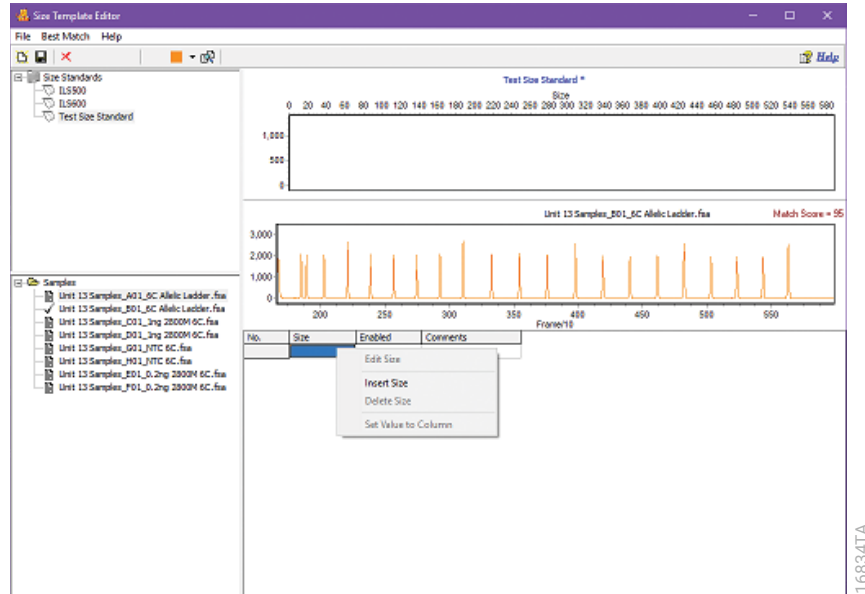


Figure 133. Inserting a fragment size in the Expected Size Standard Table.

2. The 'Edit Size' window appears (Figure 134). Enter the size of the expected peak in the 'Size' field.

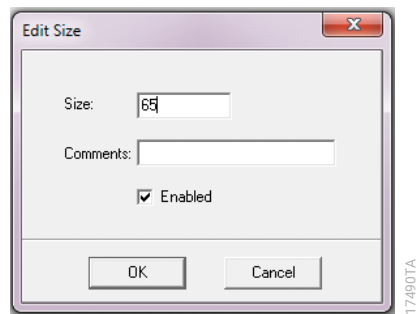


Figure 134. The 'Edit Size' window.

3. Ensure the 'Enabled' box is checked so the peak is used to size sample peaks. If the peak is only used for pattern recognition and not sizing, uncheck the "Enabled" box. Select **OK** to return to the 'Size Template Editor' window.

Note: All peaks used for data sizing will appear as a red peak with a green triangle in the Expected Size Standard Chart. Enabled peaks are indicated with a green triangle at their apex and disabled peaks are not displayed in the Expected Size Standard Chart.

4. Repeat Steps 1–3 to enter the remaining size standard fragments of the new size standard template.
5. Select the appropriate dye channel for the size standard using the **Show Dye** icon.
6. Select the **Save Changes** icon.

Method 2: Creating a New Size standard template from a Sample Size Standard Chart

If sample data files have been imported into the project, a new size standard template can be created using the Sample Size Standard Chart of one of the samples.

1. Select the sample from the Sample File Tree.
2. Select the appropriate dye color for the new size standard template using the **Show Dye** icon.
3. To add the size standard fragments displayed in the Sample Size Standard Chart to the new size standard template, right-click on a peak and select **Insert Size** (Figure 135).

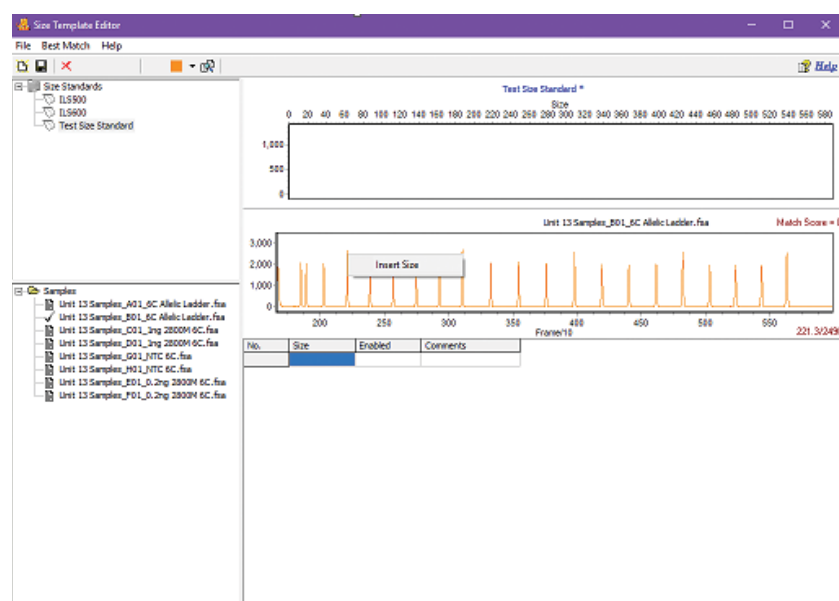


Figure 135. Inserting a size in the Sample Size Standard Chart.

4. The 'Edit Size' window appears (Figure 134). Enter the appropriate fragment size and select the appropriate Enabled status, as described in Method 1, above.
5. Repeat Steps 3 and 4 to continue entering the remaining size standard peaks.
6. When all peaks are entered, select the **Save Changes** icon.

Note: Using this method, after one peak is inserted, the software will estimate, by interpolation, the size values for the other peaks in the Sample Size Standard Chart. Interpolated size values are displayed in the 'Edit Size' window when **Insert Size** is selected from the Sample Size Standard Chart. Edit the size value and Enabled status as needed before selecting **OK**.

7.A.3 Editing an Existing Size Standard Template

You can edit or delete fragments within a size standard template and insert new fragments into an existing size standard template.

1. Select the size standard template to be edited from the Size Standard File Navigator.
2. To select a peak, click on the peak in the Expected Size Standard Chart or on the row for the peak in the Expected Size Standard Table. When a peak is selected, the triangle at the apex of the peak is yellow.

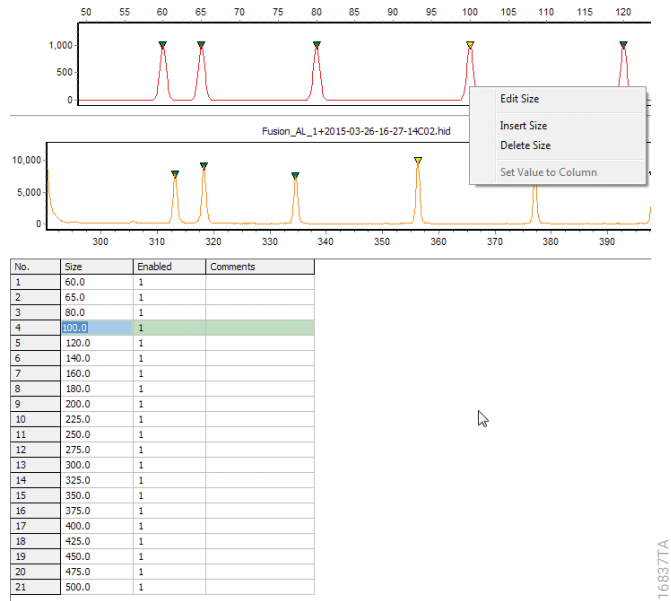


Figure 136. Editing an existing fragment in the Expected Size Standard Chart.

- To edit the selected peak, right-click and select **Edit Size** (Figure 136). The 'Edit Size' window (Figure 134) will appear allowing you to edit the size and Enabled state of the peak as well as to add a comment. Select **OK** to apply the change and close the 'Edit Size' window. To apply the Enabled status of one peak to all peaks in a size standard template, select the **Enabled** cell of the peak in the Expected Size Standard Table, then right-click the cell and select **Set Value to Column** (Figure 136).

The screenshot shows a table with 21 rows and 3 columns: 'No.', 'Size', and 'Enabled'. The 'Enabled' column contains the value '1' for all rows. A context menu is open over the 'Enabled' cell of row 1, with options: 'Edit Size', 'Insert Size', 'Delete Size', and 'Set Value to Column'. The 'Set Value to Column' option is highlighted.

No.	Size	Enabled
1	60.0	1
2	65.0	1
3	80.0	1
4	100.0	1
5	120.0	1
6	140.0	1
7	160.0	1
8	180.0	1
9	200.0	1
10	225.0	1
11	250.0	1
12	275.0	1
13	300.0	1
14	325.0	1
15	350.0	1
16	375.0	1
17	400.0	1
18	425.0	1
19	450.0	1
20	475.0	1
21	500.0	1

Figure 137. Editing an existing fragment in the Expected Size Standard Table.

- To delete a fragment within a size standard template, select the desired peak in either the Expected Size Standard Chart or Expected Size Standard Table, right-click and select **Delete Size** (Figure 136 and Figure 137).
- To insert a fragment within a size standard template, select the desired peak in either the Expected Size Standard Chart or Expected Size Standard Table, right-click and select **Insert Size** (Figure 136 and Figure 137). The 'Edit Size' window will appear with the interpolated size listed in the 'Size' field and the "Enable"d box checked. If this is the intended size and Enabled state for the inserted size, select **OK** to the new size fragment, and close the 'Edit Size' window; otherwise, edit the 'Size' field and Enabled status to the desired settings before selecting **OK**.
- When all edits are completed, select the **Save Changes** icon. If you navigate away from an edited size standard template before saving, a 'Confirm' window will appear with the question, "Size Standard has been changed, save the changes?".

Note: If you select **No** in the 'Confirm' window, the changes will remain in both the Expected Size Standard Chart and Expected Size Standard Table until you choose **Reload** (Section 7.A.4) or the program is closed.

7.A.4 Managing Size Standard Template Files

Once you create a size standard template, a file is saved to the 'Size Standards' directory as defined in the 'Others' tab of the 'Preferences' window (Section 6.B.6). You can delete, export and reload these files from within the software. Each of these functions is available by right-clicking on the desired size standard template file from the Size Standards File Navigator.

1. To delete a size standard template file from the software and the 'Size Standards' directory, right-click on the **size standard**, then select **Delete** (Figure 138).

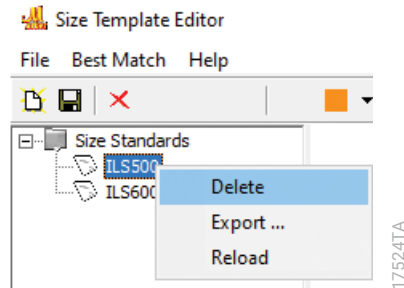


Figure 138. Deleting a size standard template file from GMHID-Spectrum and the 'Size Standards' directory.

A confirmation window will appear (Figure 139). Select **Yes** to delete the file and return to the main 'Size Template Editor' window.

Note: This action is irreversible.

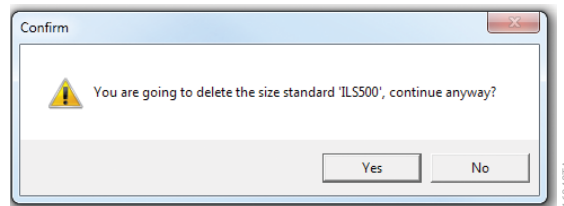


Figure 139. Confirmation window when deleting a 'size standard template' file.

2. To export a size standard template as an .xml file, right-click on the **size standard**, then select **Export** (Figure 138). This will open a 'Save As' window. Navigate to the desired location and save the exported file. A copy will still remain in the 'Size Standards' directory.
3. You can undo edits to a size standard template and restore the file to its previous state by right-clicking on the **size standard**, then selecting **Reload** (Figure 138).

7.A.5 Menu Options

The Size Template Editor contains three menu options: 'File', 'Best Match' and 'Help'. The 'File' menu allows the user to create, save and export size standards (Figure 140). The 'Best Match' menu contains options for matching a size standard template to the sample size standards in the open project (Figure 141). The 'Help' menu contains descriptions of the navigation and editing functions for the Size Template Editor (Figure 142).

'File' Menu

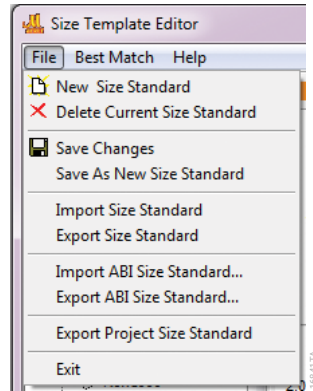


Figure 140. 'File' menu options in the Size Template Editor.

New Size Standard: Opens the 'Input Dialog' window with a field to enter a 'New Size Standard' name (Section 7.A.2).

Delete Current Size Standard: Deletes the size standard template that is highlighted in the Size Standards File Navigator. **Note:** This action is irreversible.

Save Changes: Saves edits and changes to the size standard template and in the size standard directory.

Save As New Size Standard: Allows you to save edits and changes to an existing size standard template as a new size standard template. When selected, the 'Input Dialog' window opens with a field to enter a 'New Size Standard' name. The new size standard template is added to the Size Standards File Navigator and saved in the 'Size Standards' directory.

Import Size Standard: Opens a browser window that allows you to navigate to a saved size standard (.xml) file. Use the 'Import Size Standard' option to find previously exported size template files (.xml) on local or network computers.

Export Size Standard: Exports the selected size standard template as an .xml file to the specified directory.

Import ABI Size Standard: Opens a browser window that allows you to navigate to a size standard file created for use with Applied Biosystems® GeneMapper® software.

Export ABI Size Standard: Exports the selected size standard template as an 'ABI Size Standard Files' .xml file to the specified directory.

Export Project Size Standard: Exports the size standard template used to process the opened project to the specified directory.

Exit: Closes the 'Size Template Editor' window.

'Best Match' Menu

The 'Best Match' menu provides options for matching the sample size standards to size standard templates based on calculated match scores (Section 7.B). This feature is used for research purposes only, when analyzing data where the size standard template is unknown.

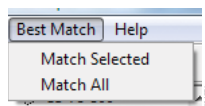


Figure 141. 'Best Match' menu options in the Size Template Editor.

Match Selected: Compares the size standard for each sample in the project to the currently selected size standard template in the Size Standards File Navigator. The 'Data processing...' window opens and the software makes the comparisons. The green triangles above the peaks in the Sample Size Standard Chart are adjusted to represent the best match to the selected size standard template.

Match All: Compares the size standard for each sample in the project to each size standard template in the Size Template Editor. The 'Data processing...' window opens and the software makes comparisons. The software calculates the average match score across all samples in the project for each size standard template. The size standard template with the highest average size match score is considered the Best Match and is highlighted in the Size Standards File Navigator.

'Help' Menu

The 'Help' Menu provides access to the 'Size Editor Action Help' window, which has descriptions of the display, editing and search shortcut functions (i.e., Hot Keys) of the Size Template Editor (Figure 142).

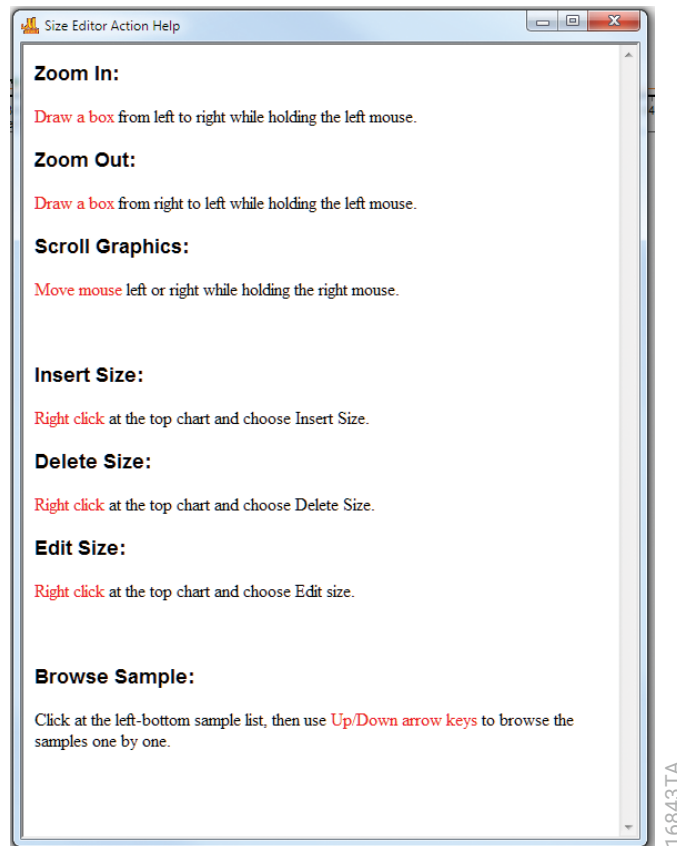


Figure 142. 'Size Editor Action Help' window.

7.B. Navigating the Calibration Charts

During data processing (Section 4.A), the size standard template is applied to the samples, a size calibration is performed and GMHID-Spectrum assigns a size match score to each sample based on how closely that size standard matches the pattern of the size standard template. Perfect matches receive a score of 100. No correlation receives a score of 0, and the sample size calling fails. A general quality assessment of each sample's size match score is available in the Sample File Tree of the 'Main Analysis' window (Section 10.A.1). A more detailed assessment of each sample's size match score is available in the 'Calibration Charts' window (Figure 144). To access the 'Calibration Charts' window, select the **Size Calibration** icon in the toolbar (Figure 143).

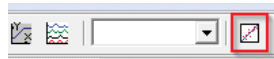


Figure 143. 'Size Calibration' icon.

The 'Calibration Charts' window has a toolbar, Sample List, size statistics for disabled peaks (when present), Expected Size Standard Chart, Sample Size Standard Chart and Calibration Plots (Figure 144).

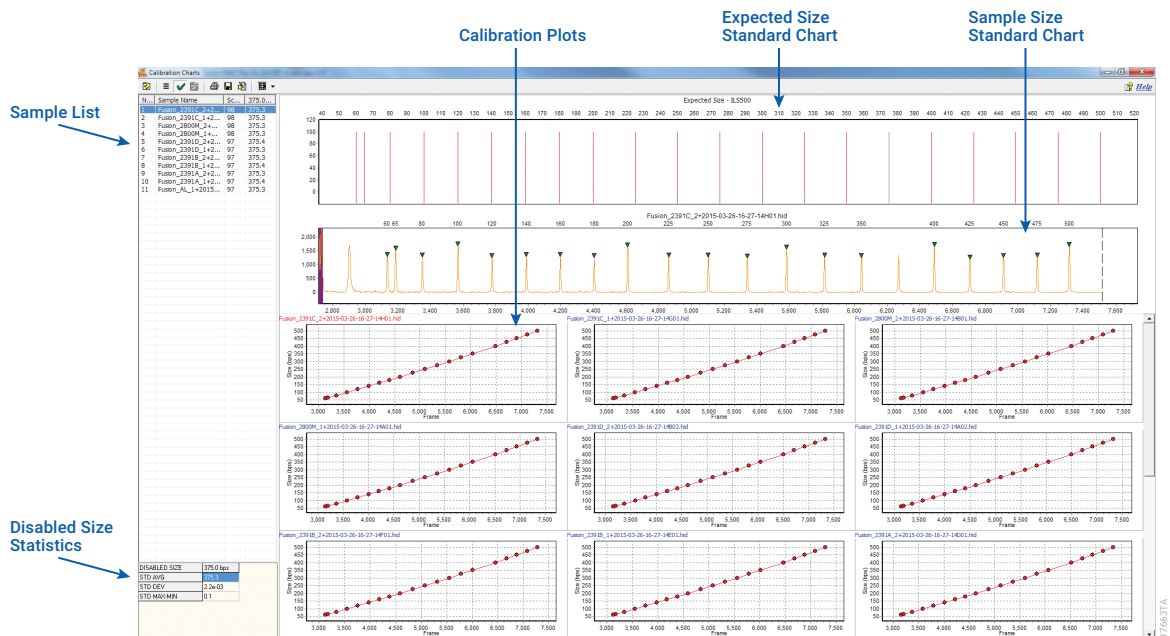


Figure 144. 'Calibration Charts' window.

Table 14. Toolbar Icons in the 'Calibration Charts' Window.




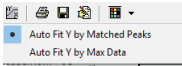




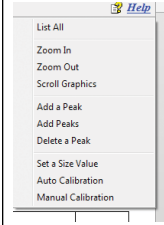
	View Mode: Opens the 'Options' window, which allows you to change the layout of the 'Calibration Plots' section. Users can set the maximum number of plots to display by adjusting the number of rows and columns to a maximum of 5. The 'Advanced Settings' section allows users to adjust the size of the file generated when charts are saved or printed. A smaller file size will generate more .png files per dataset compared to a larger file size; however, the larger file size may produce images with lower resolution.
	Chart Synchronize: Synchronizes the display settings of the Expected Size Standard and Sample Size Standard Chart. This option is selected by default.
	Preprocess Raw Data: Smooths the samples' raw data in the size standard dye channel. This affects data display in the 'Calibration Charts' window only.
	Auto Fit Y: Provides two options for scaling the y axis of the Sample Size Standard Chart. The default option automatically scales the y axis according to the maximum peak intensity of a peak that matches the Expected Size Standard Chart (Auto Fit Y by Matched Peaks). Alternatively, the y axis can be set to automatically scale to the maximum peak intensity of any peak in the Sample Size Standard Chart (Auto Fit Y by Max Data).
	Print: Opens the 'Calibration Print' window to print or save the calibration data using the specified settings (Section 9.C).
	Save: Opens a 'Save As' window to navigate to a location to save the Sample List and Calibration Plots as a .png file.
	Manual Calibration: Opens the 'Manual Calibration' window where size calibration information can be viewed and edited for the sample selected in the Sample List. The 'Manual Calibration' window displays three columns: (i) Standard Size (fragment sizes of the size standard template used for size calling), (ii) Peak position (in camera frames) of the selected sample size standard, and (iii) Size (in bp) of the selected sample size standard. You can edit the values listed in the 'Size' column. There are three buttons on the right side of the window that provide additional editing features: Restore, Clear and Auto. The 'Restore' button restores the values in the 'Size' column to their initial state. The 'Clear' button clears all the values in the 'Size' column. The 'Auto' button automatically populates the values in the 'Size' column with the sizes listed in the applied size standard template. Select Apply to apply changes. Select Close to close the 'Manual Calibration' window.

Table 14. Toolbar Icons in the 'Calibration Charts' Window (continued).

	<p>Show Other Color: Allows you to select all dye channels, hide all dye channels or choose a single dye channel to be overlaid with the size standard dye channel. To cycle through individual dye channels, select the Show Other Color icon.</p>
	<p>Help: Provides shortcuts to the 'Calibration Action Help' window descriptions of the display, editing and search functions of the 'Calibration Charts' window.</p>

7.B.1 Features of the Calibration Charts

Sample List

The Sample List includes all samples in the project numbered in order by size match score. There are three columns in the Sample List: sample number (No.), sample file name (Sample Name) and size match score (Score) for each sample in the project (Figure 144). An additional column for each disabled peak is displayed if the selected size standard template contains disabled peaks (Section 7.A.1). You can sort samples by sample name or score by selecting the appropriate column header. When a sample is selected in the Sample List, the electropherogram of the sample size standard is displayed in the Sample Size Standard Chart and the sample name in the Calibration Plot is red. Use the up and down arrows on the keyboard to scroll through the samples. To disable a sample, right-click the sample name and select **Mark As Failed**. Select **Unmark Failed** to reverse the action.

The names of samples disabled by the user (Section 3.C.1) or those automatically disabled by the software due to a poor size match score (Section 6.B.4) are displayed in light gray.

Disabled Size Statistics

If the selected size standard template contains disabled peaks (Section 7.A.1), the Disabled Size Statistics table is displayed in the bottom left corner of the 'Calibration Charts' window. This table lists the average base pair size, standard deviation and the difference between the maximum and minimum base pair size of each disabled size standard peak observed in the project. No statistics will be calculated for disabled peaks at the beginning or end of the size standard template.

Expected Size Standard Chart

The Expected Size Standard Chart displays the expected peak pattern for the selected size standard template. Enabled peaks are displayed in red; disabled peaks are displayed in gray.

Sample Size Standard Chart

The Sample Size Standard Chart displays the electropherogram of the size standard of the selected sample. Peaks that correspond to enabled peaks in the Expected Size Standard Chart have green triangle indicators at their apex with the peak size. Peaks with a question mark next to the green triangle indicate peaks that may be affected by spectral bleedthrough from other dye channels.

For both the Expected Size Standard Chart and the Sample Size Standard Chart, the peak size in base pairs is displayed along the x axis and intensity in RFU is displayed along the y axis. Use the same mouse functions to zoom or move within these charts as you do to zoom or move within the electropherogram in the 'Main Analysis' window.

Calibration Plots

The Calibration Plots chart the migration linearity of size standard peaks. A calibration plot (Figure 145) is displayed for each sample size standard as indicated by the sample name in the plot title. Use the scroll bar on the right to locate the calibration plot of a specific sample or select the sample in the Sample List. Each plot displays the peak size for each enabled peak in the sample size standard (in base pairs) on the y axis as a function of time (in camera frames) on the x axis. As linearity decreases so does the size match score for the sample. If the software misidentifies enabled peaks in the sample size standard, the sample size match score will be low. Samples with failed size match scores have blank calibration plots. Double-clicking a specific **Calibration Plot** selects that sample in the Sample List and displays the sample in the Sample Size Standard Chart.

Unit 13 Samples_B01_6C Allelic Ladder.fsa

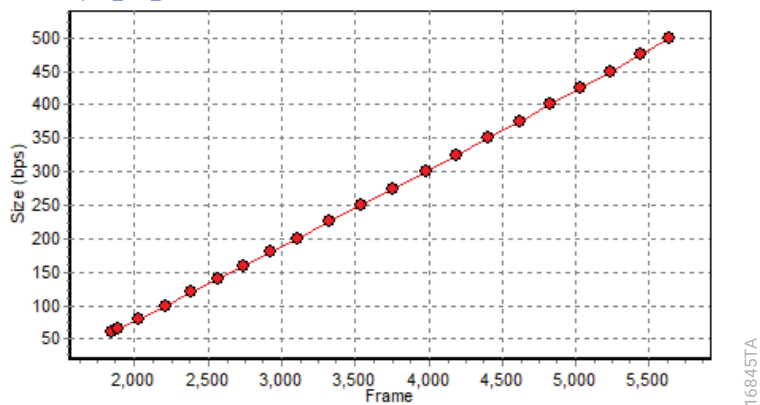


Figure 145. Calibration plot of the 'Calibration Charts' window.

7.B.2 Editing Size Calls

You can edit or delete peaks within the size standard for the selected sample. You can also add unlabeled peaks to the size standard. These edits will not affect the size standard template.

1. Select a sample in the Sample List to display the electropherogram for the sample size standard in the Sample Size Standard Chart and highlight the sample's calibration plot.
2. To select a peak within the size standard, click on the peak in the Sample Size Standard Chart. When a peak is selected, the triangle at the apex of the peak is yellow. Right-clicking on a specific peak in the electropherogram provides several editing options (Figure 146).

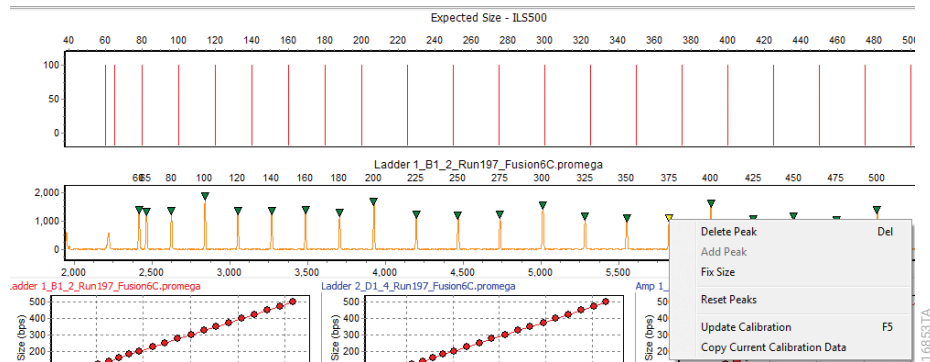


Figure 146. Editing size calls in the Sample Size Standard Chart of the 'Calibration Charts' window.

3. To edit the size of an existing peak, select the peak, hold down the Control key, left-click on the peak and drag the triangle to the desired position. Alternatively, edit a peak by right-clicking on the peak and selecting **Fix Size**. This opens the 'Calibration Editor' window (Figure 147), allowing you to edit the size and change the Fixed state of the peak. Peaks defined as enabled in the size standard template are fixed peaks. Fixed peaks are set to the specified positions and remaining enabled size standard peaks are adjusted to correctly align with the chosen size standard template.

The 'Fix Size' feature is useful when the selected size standard template has uniformly spaced peaks and the sample size standard has additional peaks or artifacts that influence the pattern recognition algorithm.

Note: Only size fragments that occur in the selected size standard template can be entered in the 'Calibration Editor' size field. The 'Fix Size' functionality is not active for manually added peaks or peaks outside the range of the size standard template.

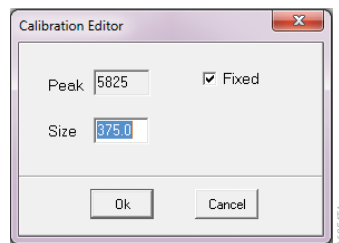


Figure 147. Editing selected peak size in the 'Calibration Editor' window.

- To delete an enabled size standard peak, right-click on the peak and select **Delete Peak** (Figure 146). The peak remains in the electropherogram; however, the green triangle peak indicator is removed and the peak is not used in the size match score calculation.

Note: Deleting an enabled size standard peak is irreversible. For those peaks to be recognized by the software again, you will need to re-analyze the data (Section 5.F).

- To add a peak to the sample size standard, right-click the desired peak and select **Add Peak** (Figure 148). A green triangle peak indicator will appear at the selected position and the peak will be included in the match score calculation.

Note: Add Peak is only available for peaks that do not already have a green triangle at their apex.

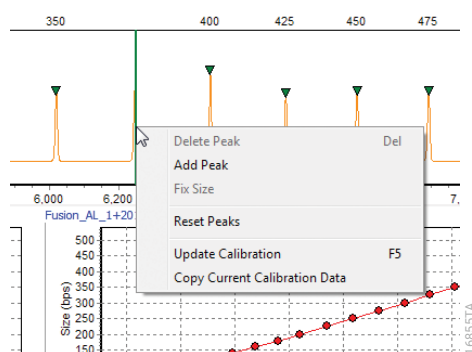


Figure 148. Adding a peak in the Sample Size Standard Chart of the 'Calibration Charts' window.

- To remove a manually added peak or peaks edited using the 'Fix Size' option, right-click anywhere in the Sample Size Standard Chart and select **Reset Peaks**. Deleted peaks or peaks edited using the Control + left click method are not reset by this feature.

When all desired edits are made, right-click anywhere in the Sample Size Standard Chart and select **Update Calibration** (Figure 146). The software will recalculate the size match score for the sample and update the calibration plots. If you navigate away from an edited size standard before updating the calibration, a 'Warning' window will open asking if the sample size standard calibration should be updated. Select **Yes** to update the calibration. Select **No** to undo all edits to the sample size standard. When you close the 'Calibration Charts' window, the Size Match Score indicators next to the file names in the Sample File Tree in the 'Main Analysis' window are updated.

Note: If the size standard of an allelic ladder sample is edited, a 'Warning' window will open asking if the 'Auto Panel Adjustment' and 'Auto Select Best Ladder' functions should be performed again. Select **Yes** to perform these functions again. Select **No** to keep the panel adjustment and best ladder selections the same.

You can copy the camera frame and base pair size of the calibration data for analysis in a spreadsheet or word processing program. To copy data from the selected Sample Size Standard Chart, right-click anywhere in the Sample Size Standard Chart and select **Copy Current Calibration Data** (Figure 146). The frame position and base pair size for each enabled peak will be copied.

8

Advanced Use of the Panel Editor

To assign an allele designation to a peak, the software uses a panel file, which defines the dye channel and expected size range for each marker in an STR kit. Within each marker, the panel defines the expected bin size range for each allele within that marker. Panels also define peak amplitude thresholds and stutter filters. Preloaded and user-defined panel files are known as panel templates. The Panel Editor is where all panel templates are managed. The Panel Editor can be accessed through the 'Tools' menu (Section 6.E) or through the icon on the 'Template Selection' window of the Run Wizard (Section 4.A).

In addition to viewing and editing analysis parameters prior to processing data using the Panel Editor (Section 4.A.1), you can also create, save, delete, import and export panel templates. The 'Panel Editor' window has a Menu Bar, Toolbar Ribbon, Panel File Navigator, Chart Overlay, Sample File Tree and Panel Table (Figure 149).

The Panel Table contains marker and bin information about the selected panel for the dye color displayed in the Chart Overlay.

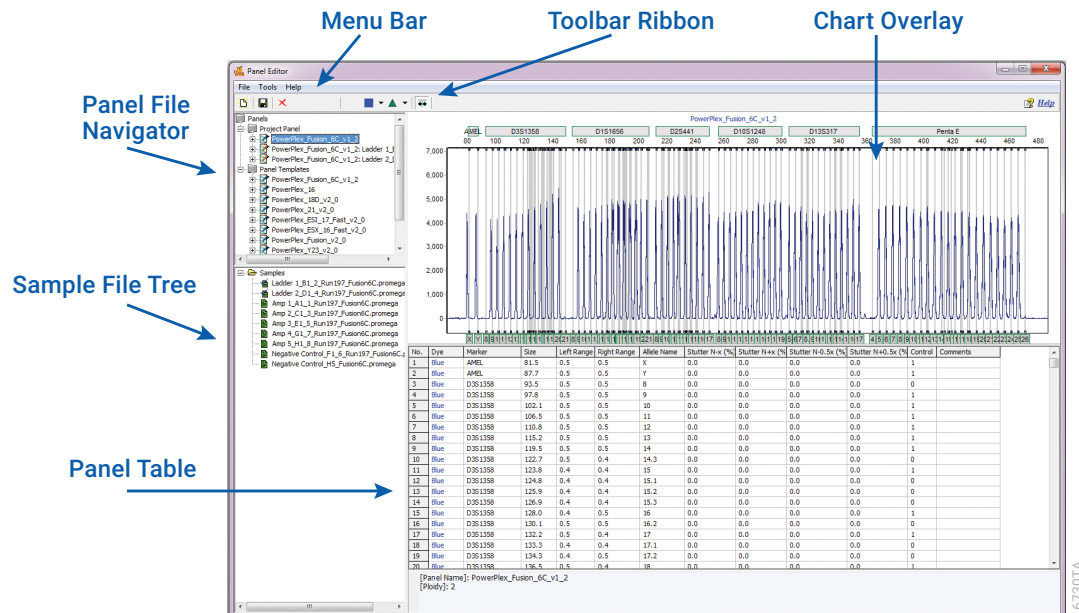





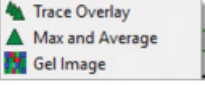





Figure 149. 'Panel Editor' window.

Table 15. Toolbar Icons in the 'Panel Editor' Window.

	Create New Panel: Opens the 'Create New Panel' window. Follow the steps in Section 8.D.
	Save Changes: Saves modifications to the panel template file saved in the 'Panel' directory (Section 6.B.6).
	Delete Current Panel/Marker: Deletes the panel or marker that is currently selected in the Panel File Navigator. This action is irreversible.
	Show Dye: Allows the user to select a single dye color to view in the Chart Overlay. Click the icon to cycle through the colors or use the drop-down menu to select a specific dye color.
 	<p>Trace Mode: Determines how electropherogram data are displayed in the Chart Overlay. Click the icon to cycle through the options or use the drop-down menu.</p> <p>Trace Overlay: Displays the electropherogram for all samples selected in the Sample File Tree. To select the electropherogram for a single sample in this mode, click on the electropherogram plot line (trace). The plot line is bolded, and the corresponding sample is highlighted in the Sample File Tree.</p> <p>Max and Average: Displays two plot lines in the Chart Overlay: the Max and the Average. The Max plot line is displayed in black and represents the maximum peak intensity observed at a base pair size for all selected samples. The Average plot line is displayed in the selected dye color and represents the average peak intensity observed at a base pair size for all selected samples.</p> <p>Gel Image: Displays selected samples as a synthetic gel image. Bin ranges in the Gel Image mode appear as white vertical lines and can be adjusted by holding the Shift key, left-clicking and dragging the bin boundary left or right.</p>
	Check Range in Edit: When selected, the software will warn the user if an edit to one bin range overlaps with another bin range. This feature will prevent the user from setting bin boundaries too close to neighboring bins. This option is selected by default.
 	Help: Provides shortcuts to the 'Panel Editor Action Help' window descriptions of the display, editing and navigation functions of the 'Panel Editor' window.

8.A. Navigating the Panel Editor

8.A.1 Panel File Navigator

The Panel File Navigator contains two expandable folders of files, one for Project Panel and one for Panel Templates (Figure 150).

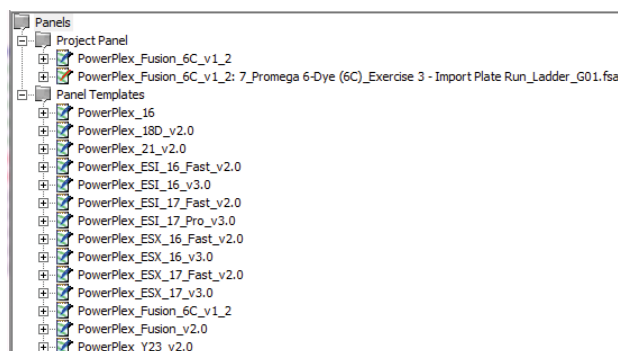


Figure 150. Expanded 'Project Panel' and 'Panel Templates' folders in the Panel File Navigator.

The 'Project Panel' folder includes the panel assigned for use with an open project. When **Auto Select Best Ladder** is selected in the 'Additional Settings - HID Analysis' window of the Run Wizard (Section 4.C), panels adjusted to each passing allelic ladder sample are also listed in the 'Project Panel' folder.

The 'Panel Templates' folder includes a list of all preloaded and user-defined panels saved to the 'Panel' directory as defined in the 'Others' tab of the 'Preferences' window (Section 6.B.6).

When a panel is selected from the Panel File Navigator, the marker and bin ranges for the selected dye color are displayed in the Chart Overlay and Panel Table. When a marker is selected from the Panel File Navigator, the marker range and bin ranges are displayed in the Chart Overlay and Panel Table (Figure 151). To select a marker within a panel, expand the panel in the navigator by selecting the **+** next to the panel name or by double-clicking on the panel name, and then selecting the marker name.

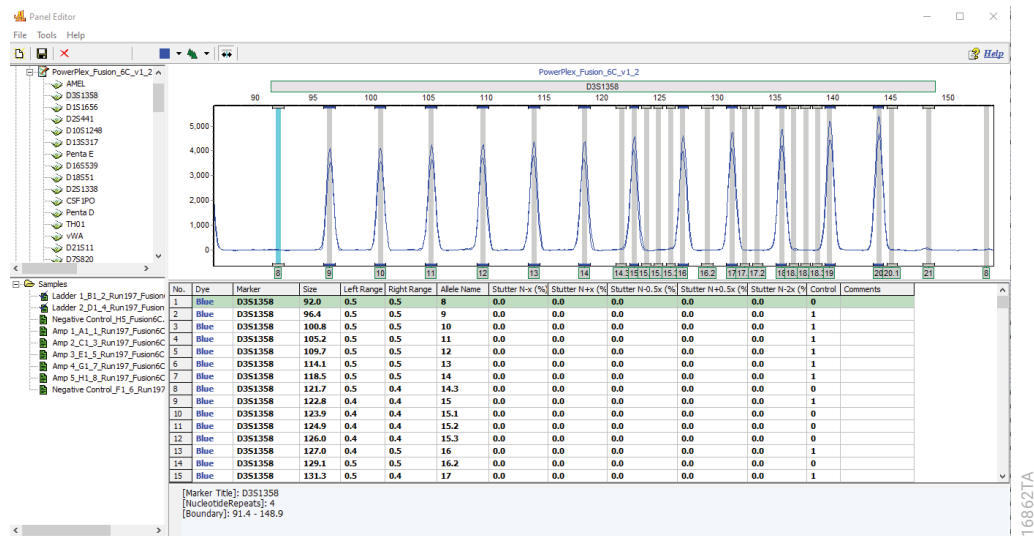


Figure 151. Selecting a marker in the Panel File Navigator to display in the Chart Overlay and Panel Table.

Panels can be edited from the Panel File Navigator (Section 8.B). Edits made to a panel listed in the 'Project Panel' folder will apply only to the panels in the open project. To make changes that will apply to all future uses of the selected panel, edit the corresponding panel in the 'Panel Templates' list. Several editing options are available by right-clicking a panel name (Figure 152, left panel).

Rename: Opens the 'Input Dialog' window where you can enter a new panel name in the provided field.

Edit: Opens the 'Edit Panel' window where you can change the panel name and ploidy setting (Figure 152, right panel). Ploidy can be set from Monoploid (1) to Decaploid (10) or Unlimited (0). If the number of peaks within a marker exceeds the ploidy setting, the additional peaks with the lowest peak heights will be flagged with the Quality Reason 'PL', designating the marker is 'Beyond Ploidy' (Section 10.A.3). When **Unlimited** is selected, peaks are not flagged.

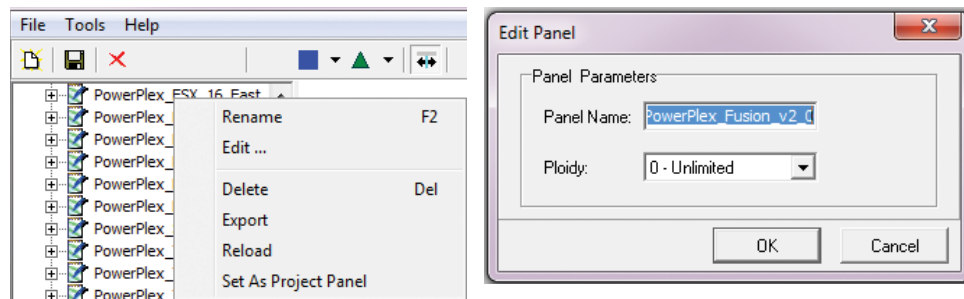


Figure 152. Accessing panel editing options from the Panel File Navigator (left) and the 'Edit Panel' window (right).

Delete: Deletes the panel file from the software and the 'Panel' directory. A confirmation window will appear. Select **Yes** to delete the file and return to the main window of the Panel Editor.

Note: This action is irreversible.

Export: Opens a 'Save As' window for users to navigate to the desired location to save the exported file as .xml. A copy will still remain in the 'Panel' directory. Exported panel files can be imported into other installations of the software.

Reload: Undoes edits to a panel file and restores the file to its previously saved state. If you make changes to the panel and then answer "No" to "The Panel has been changed, save changes to file?", the changes will remain visible in the Chart Overlay until you select **Reload** or the software is closed.

Set As Project Panel: Changes the panel applied to the project. The selected panel replaces the panel listed in the 'Project Panel' folder and is applied to the project samples when the Panel Editor is closed.

8.A.2 Sample File Tree

The Sample File Tree lists all samples within an open project. If the Panel Editor is opened before sample files are imported, this file tree will be blank. Following analysis, selected sample(s) in this list will be displayed in the Chart Overlay. To select or de-select individual samples, double-click the **Sample Name**. Alternatively, right-click the Sample Name and choose **Select** or **De-select** (Figure 153). To display all samples in the Chart Overlay, right-click anywhere in the Sample File Tree and choose **Select All**. To clear the Chart Overlay, right-click in the Sample File Tree and choose **De-select All**. To overlay the electropherograms of samples grouped by their associated reference ladder, right-click in the Sample File Tree, choose **Select All by Ladder**, then choose the desired ladder from the list.

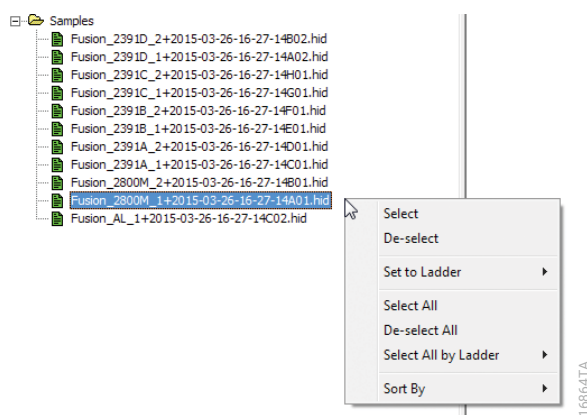


Figure 153. Right-click menu options for the Sample File Tree.

By default, samples are listed by sample file name, with designated control samples listed at the top followed by an alphabetical list of samples. To sort the samples in descending order by size match score (Section 7.B), right-click in the Sample File Tree, select **Sort By**, and then select **Size Score**.

After data processing, you can change which ladder is used for a specific sample from the Panel Editor. Right-click the **sample file name**, select **Set to Ladder** and then select the desired ladder. When you exit the Panel Editor the change will take effect. Alternatively, the 'Additional Settings - HID Analysis' window of the Run Wizard provides two options to select which allelic ladder is used to assign alleles to all samples in the project (Section 4.C.1).

8.A.3 Chart Overlay

The Chart Overlay displays the electropherograms of all selected samples of an analyzed project by dye channel. To select the electropherogram for a single sample in this mode, click on a peak in the **electropherogram plot line (trace)**. The plot line is bolded, and the corresponding sample file name is highlighted in the Sample File Tree. The marker ranges associated with the selected panel are displayed as a gray bar above the region for each marker. Bin ranges are displayed as dye-colored brackets at the top and bottom of the electropherogram. Allele labels are displayed below the electropherogram and correspond to the center of each called peak, which is also marked by a light gray vertical line in the electropherogram. In the Chart Overlay the size (in base pairs) is displayed along the x axis and peak intensity (in RFU) is displayed along the y axis. Use the same mouse functions to zoom or move within these charts as you do to zoom or move within the electropherogram of the 'Main Analysis' window.

Right-clicking on a peak or position within the Chart Overlay provides several editing options (Figure 154; see Section 8.B for more information on editing panels).

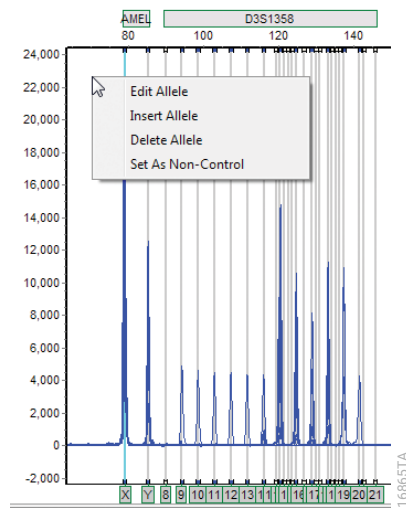


Figure 154. Right-click menu options in the Chart Overlay.

Edit Allele: Opens the 'Allele Editor' window with information prepopulated based on the selected allele (Figure 155).

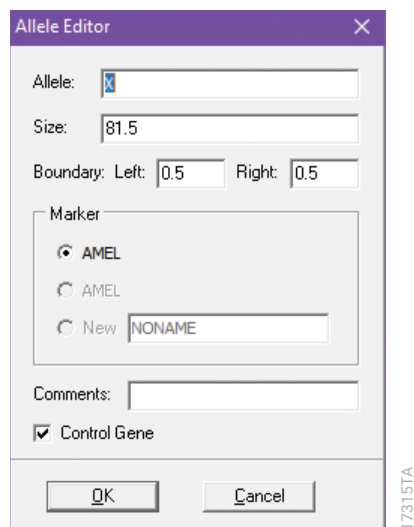


Figure 155. 'Allele Editor' window.

Insert Allele: Opens the 'Allele Editor' window (Figure 155) with information prepopulated based on the cursor position when **Insert Allele** was selected.

Note: This option is only available when the cursor is not on an already designated allele.

Delete Allele: Deletes the selected allele bin from the selected panel. A confirmation window will appear. Select **Yes** to delete the allele and return to the main window of the Panel Editor.

Set As Control or Set As Non-Control: Changes whether the allele bin is designated for a control allele in the 'Control' column of the Panel Table (Section 8.A.4). A "1" in this column indicates the bin is for a control allele; a "0" indicates the bin is for a virtual, non-control allele.

Right-clicking on the marker bar within the Chart Overlay provides several marker-editing options (Figure 156). See Section 8.B.1 for more information about editing markers.

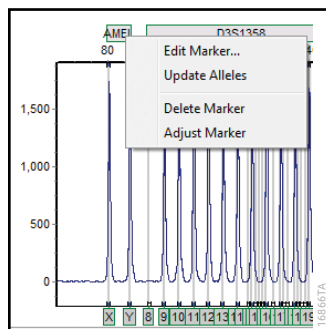


Figure 156. Right-click menu options from the marker bar in the Chart Overlay.

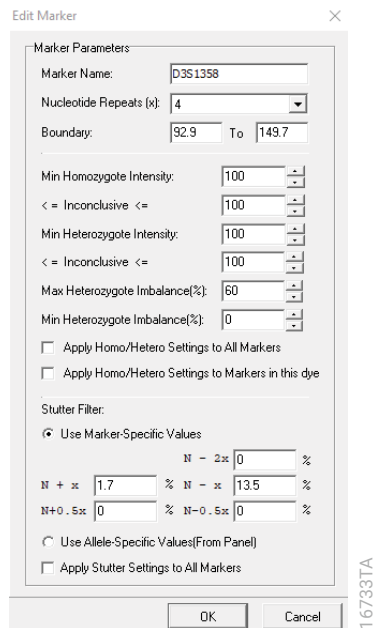


Figure 157. 'Edit Marker' window.

Edit Marker: Opens the 'Edit Marker' window (Figure 157; Section 4.A.1).

Update Alleles: Opens the 'Update Marker Alleles' window (Figure 158), allowing adjustment of the boundaries (in base pairs) of the marker. All other fields are uneditable in this view.

Note: Any alleles that fall within the updated marker will be renamed according to the allele size (in base pairs).

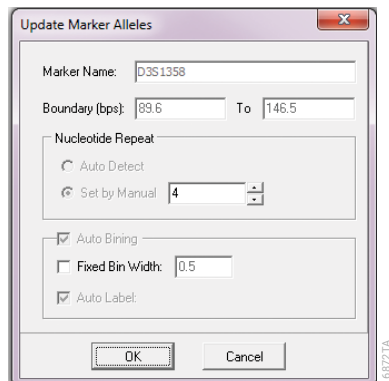


Figure 158. 'Update Marker Alleles' window.

Delete Marker: Deletes the selected marker from the panel. A confirmation window will appear. Select **Yes** to delete the marker and return to the main window of the Panel Editor.

Adjust Marker: Automatically moves the marker to align with the closest peaks.

Note: This function is recommended for research use only and in situations where the marker needs only slight adjustment to the right or left.

The default display of the Chart Overlay is the 'Trace Overlay' view described above. There are two other displays for the Chart Overlay that can be selected using the Trace Mode icon in the toolbar, Max and Average and Gel Image (Table 15).

Max and Average displays two plot lines in the Chart Overlay: the Max and the Average. The Max plot line is displayed in black and represents the maximum peak intensity observed at a base pair size for all selected samples. The Average plot line is displayed in the selected dye color and represents the average peak intensity observed at a base pair size for all selected samples.

Gel Image displays selected samples as a synthetic gel image. Bin ranges in the Gel Image mode appear as white vertical lines and can be adjusted by holding the Shift key, left-clicking and dragging the bin boundary left or right.

8.A.4 Panel Table

The Panel Table (Figure 159) displays detailed information about a panel based on the selections made in the Panel File Navigator and Chart Overlay. Each allele bin in a panel is given a number in sequential order, which is displayed in the No. column. There are 12 columns of information for each allele bin. The Panel Table is used to apply allele-specific stutter when **Use Allele-Specific Values (From Panel)** is selected in the 'Edit Marker' window (Section 4.A1).

No.	Dye	Marker	Size	Left Range	Right Range	Allele Name	Stutter N-x (%)	Stutter N+x (%)	Stutter N-0.5x (%)	Stutter N+0.5x (%)	Stutter N-2x (%)	Control	Comments
1	Blue	AMEL	81.5	0.5	0.5	X	0.0	0.0	0.0	0.0	0.0	1	
2	Blue	AMEL	87.7	0.5	0.5	Y	0.0	0.0	0.0	0.0	0.0	1	
3	Blue	D3S1358	93.5	0.5	0.5	8	0.0	0.0	0.0	0.0	0.0	0	
4	Blue	D3S1358	97.8	0.5	0.5	9	0.0	0.0	0.0	0.0	0.0	1	
5	Blue	D3S1358	102.1	0.5	0.5	10	0.0	0.0	0.0	0.0	0.0	1	
6	Blue	D3S1358	106.5	0.5	0.5	11	0.0	0.0	0.0	0.0	0.0	1	
7	Blue	D3S1358	110.8	0.5	0.5	12	0.0	0.0	0.0	0.0	0.0	1	
8	Blue	D3S1358	115.2	0.5	0.5	13	0.0	0.0	0.0	0.0	0.0	1	
9	Blue	D3S1358	119.5	0.5	0.5	14	0.0	0.0	0.0	0.0	0.0	1	
10	Blue	D3S1358	122.7	0.5	0.4	14.3	0.0	0.0	0.0	0.0	0.0	0	
11	Blue	D3S1358	123.8	0.4	0.4	15	0.0	0.0	0.0	0.0	0.0	1	
12	Blue	D3S1358	124.8	0.4	0.4	15.1	0.0	0.0	0.0	0.0	0.0	0	
13	Blue	D3S1358	125.9	0.4	0.4	15.2	0.0	0.0	0.0	0.0	0.0	0	
14	Blue	D3S1358	126.9	0.4	0.4	15.3	0.0	0.0	0.0	0.0	0.0	0	
15	Blue	D3S1358	128.0	0.4	0.5	16	0.0	0.0	0.0	0.0	0.0	1	
16	Blue	D3S1358	130.1	0.5	0.5	16.2	0.0	0.0	0.0	0.0	0.0	0	
17	Blue	D3S1358	132.2	0.5	0.4	17	0.0	0.0	0.0	0.0	0.0	1	
18	Blue	D3S1358	133.3	0.4	0.4	17.1	0.0	0.0	0.0	0.0	0.0	0	
19	Blue	D3S1358	134.3	0.4	0.5	17.2	0.0	0.0	0.0	0.0	0.0	0	
20	Blue	D3S1358	136.5	0.5	0.4	18	0.0	0.0	0.0	0.0	0.0	1	
21	Blue	D3S1358	137.5	0.4	0.4	18.1	0.0	0.0	0.0	0.0	0.0	0	
22	Blue	D3S1358	138.6	0.4	0.4	18.2	0.0	0.0	0.0	0.0	0.0	0	
23	Blue	D3S1358	139.6	0.4	0.4	18.3	0.0	0.0	0.0	0.0	0.0	0	
24	Blue	D3S1358	140.6	0.4	0.5	19	0.0	0.0	0.0	0.0	0.0	1	
25	Blue	D3S1358	144.8	0.5	0.4	20	0.0	0.0	0.0	0.0	0.0	1	
26	Blue	D3S1358	145.9	0.4	0.5	20.1	0.0	0.0	0.0	0.0	0.0	0	
27	Blue	D3S1358	149.1	0.5	0.5	21	0.0	0.0	0.0	0.0	0.0	0	

Figure 159. 'Panel Table' of the Panel Editor.

Dye: Indicates the bin dye color.

Marker: Indicates the marker that contains the bin.

Size: Indicates the size at the bin center in base pairs.

Left Range: Indicates the left boundary of the bin from the center in tenths of a base pair.

Right Range: Indicates the right boundary of the bin from the center in tenths of a base pair.

Allele Name: Indicates the allele label for peak size within the designated allele bin range.

Stutter N-x (%): Indicates the allele-specific N-x stutter filter (if applied) for the allele bin.

Stutter N+x (%): Indicates the allele-specific N+x stutter filter (if applied) for the allele bin.

Stutter N-0.5x (%): Indicates the allele-specific N-0.5x stutter filter (if applied) for the allele bin.

Stutter N+0.5x (%): Indicates the allele-specific N+0.5x stutter filter (if applied) for the allele bin.

Control: Indicates whether the bin is a control (1) or non-control (0). Control bins correspond to size ranges that include a peak in the allelic ladder. The brackets for control bins are displayed in the selected dye color. Non-control, or virtual bins correspond to size ranges that do not have a peak in the allelic ladder. The bracket for virtual bins are displayed in white.

Note: The designation of 0 and 1 in the Control column is used to assist with pattern recognition in the Auto Panel Adjustment algorithm.

Comments: Provides a free-form text field to enter a comment regarding the bin.

All columns except Dye and Marker can be edited in the Panel Table by selecting a cell and typing a new value in the cell (Section 8.C.1 for further description of editing panels). Right-clicking a highlighted cell provides several editing options, most of which are the same as those available in the right-click menu of the Chart Overlay (Figure 160).

No.	Dye	Marker	Size	Left Range	Right Range	Allele Name	Stutter N-x (%)	Stutter N+x (%)	Stutt
1	Blue	AMEL	79.3	0.5	0.5	X	0.0	0.0	0.0
2	Blue	AMEL	85.5	0.5	0.5	Y	0.0	0.0	0.0
3	Blue	D3S1358	90.2	0.5	0.5	8	0.0	0.0	0.0
4	Blue	D3S1358	94.5	0.5	0.5	9	0.0	0.0	0.0
5	Blue	D3S1358	98.8	0.5	0.5	10			
6	Blue	D3S1358	103.1	0.5	0.5	11			
7	Blue	D3S1358	107.5	0.5	0.5	12			
8	Blue	D3S1358	111.9	0.5	0.5	13			
9	Blue	D3S1358	116.2	0.5	0.5	14			
10	Blue	D3S1358	119.5	0.5	0.5	14.3			

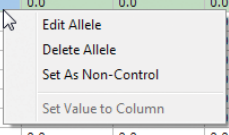


Figure 160. Right-click menu options in the Panel Table.

Edit Allele: Opens the 'Allele Editor' window with information prepopulated based on the selected allele.

Delete Allele: Deletes the chosen allele bin from the selected panel. A confirmation window will appear. Select **Yes** to delete the allele and return to the main window of the Panel Editor.

Set As Control or Set As Non-Control: Changes the state listed in the 'Control' column for the selected allele bin.

Set Value to Column: Changes the values in the selected column to the value in the highlighted cell. This option is not available for the 'Dye', 'Marker', 'Size' or 'Allele Name' columns.

8.B. Editing Existing Panels

Any panel listed in the Panel File Navigator can be edited at either the marker or bin level. To edit a panel, select the panel file from the Panel File Navigator.

Notes:

- Changes made to panels listed in the 'Project Panel' folder will apply to the current project. To make changes to a template that will be applied to all future uses of the selected panel, select the appropriate panel from the 'Panel Templates' folder.
- The use of processed data, particularly allelic ladder samples, aid in the editing of panels. We recommend having at least one sample imported into the project and processed through the Run Wizard before editing a panel.

8.B.1 Editing Markers

Markers can be added to, edited or deleted from a panel.

Adding Markers to a Panel

To add a marker to an existing panel, hold down the control key, left-click and drag across the area in the Chart Overlay where the new marker should be added. A light-blue box will appear (Figure 161). Right-click in the light-blue box and select **Create Marker**.

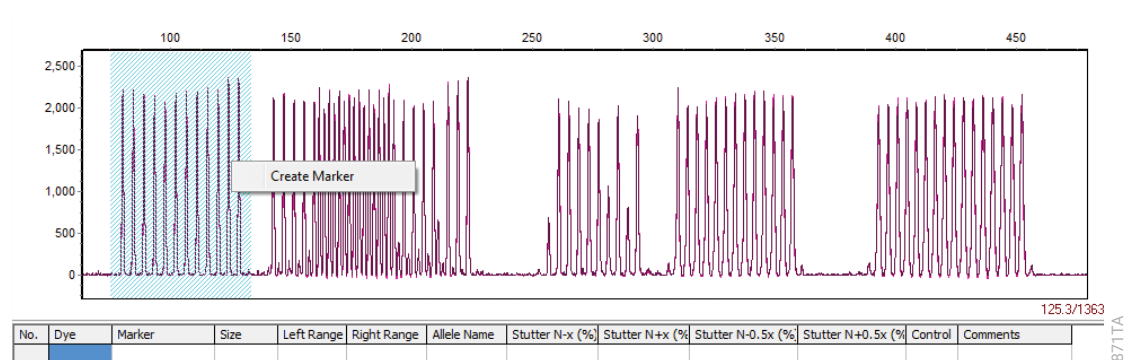


Figure 161. Right-click menu from the light-blue box in the Chart Overlay.

This opens the 'Create Marker' window (Figure 162) allowing you to adjust certain marker parameters.

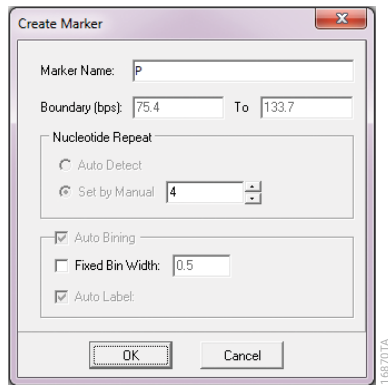


Figure 162. 'Create Marker' window.

Note: When the highlighted area overlaps with an existing marker, right-clicking in the light-blue box will provide a 'Change Marker' option, which opens the 'Edit Group Allele' window (Figure 163).

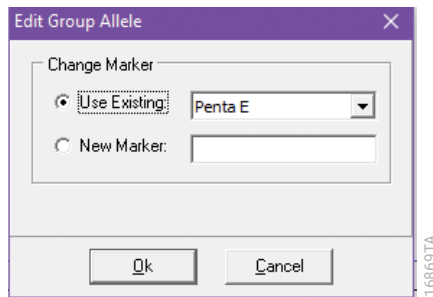


Figure 163. 'Edit Group Allele' window.

Marker Name: Defines the marker name displayed in the panel and marker bars.

Boundary (bps): Defines the marker size range, which is displayed as the light-blue box. This option is inactive in the 'Create Marker' window.

To edit the Boundary, see Edit Marker in Section 4.A.1.

Nucleotide Repeat: The nucleotide repeat of the marker is automatically set in GMHID-Spectrum; therefore, this field is inactive in the 'Create Marker' window. To edit the Nucleotide Repeat for a marker, see Edit Marker in Section 4.A.1.

Auto Binning

Fixed Bin Width: Check this option to enter the number of base pairs to the right and left of the bin center. If 0.5 is selected as the bin width, then the total bin range will be 1.0 base pair.

Auto Label: This feature is inactive in GMHID-Spectrum.

When the parameters are set for the new marker, select **OK** to add the marker to the panel.

Editing a Marker within a Panel

To edit a marker within the selected panel, double-click or right-click the **marker bar** in the Chart Overlay and select **Edit Marker**. Alternatively, you can expand the panel in the Panel File Navigator by selecting the **+** next to the panel name to reveal the list of all kit markers. Select a **marker name**, then right-click and select **Edit**. The 'Edit Marker' window appears (Figure 157). You can change marker-specific settings from this window, as described in Section 4.A.1.

Deleting Markers from a Panel

To remove a marker from a panel, either right-click the **marker bar** within the Chart Overlay and select **Delete Marker**, or highlight the marker in the Panel File Navigator and select **Delete Current Panel/Marker** (Shortcut = delete) in the toolbar (Table 15). A confirmation window will appear to confirm or cancel the deletion.

8.B.2 Editing Marker Bins

Bins can be added to, edited or deleted from a marker within a panel.

Adding Bins to a Marker

To create a bin within a marker, right-click in the electropherogram at the exact position to place the new bin. Select **Insert Allele**. This opens the 'Allele Editor' window with parameters prepopulated based on the area selected in the electropherogram (Figure 164). Adjust these parameters as needed. The information entered in the 'Allele Editor' window is displayed in the Panel Table (Section 8.A.4).

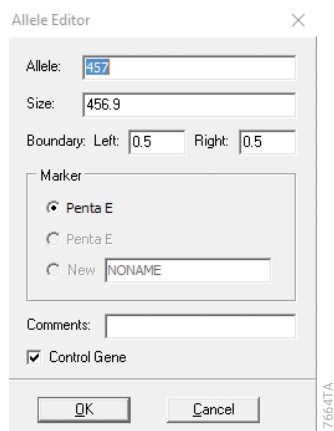


Figure 164. 'Allele Editor' window.

Allele: Indicates the label for peaks that migrate within the bin parameters. The default is the size in base pairs rounded to the nearest integer.

Size: Indicates the base pair position of the bin center.

Boundary: Indicates the bin width in base pairs. The 'Left' and 'Right' fields indicate how far the lower and upper boundaries of the bin are offset from the center of the bin, respectively.

Marker: Indicates the marker associated with the bin. When a bin is inserted within an existing marker, the marker section is set to that marker and cannot be changed. When a bin is inserted between two existing markers, the markers to the left and right of the bin position are displayed as well as the option to create a new marker for the bin. All bins must be associated with a marker.

Comments: Provides a free-form text field to associate a comment with the bin.

Control Gene: Indicates the control status of the bin (Section 8.A.4). When the box is selected, the bin is marked as a control bin and a "1" is listed in the 'Control' column of the Panel Table (Section 8.A.4). When the box is not selected, the bin will be marked as a non-control, virtual bin and a "0" is listed in the 'Control' column of the Panel Table.

When parameters are set for the new bin, select **OK** to return to the main window of the Panel Editor.

Editing Bins within a Marker

To edit all parameters of an existing bin within a marker, right-click the vertical grey bar in the center of the bin in the Chart Overlay and select **Edit Allele**. This opens the 'Allele Editor' window (Figure 164) and allows the user to adjust the bin parameters (see Adding Bins to a Marker, above).

You can move an existing bin to a new location by holding down the **Shift** key, left-clicking the vertical gray bar in the center of the bin and dragging to the left or right to the desired position.

You can edit a bin boundary by selecting **Gel Image** from the 'Trace Mode' icon in the toolbar. Then hold down the **Shift** key and place the cursor over the vertical white line of the left or right bin boundary. When a double-headed arrow appears, continue to hold down the **Shift** key, left-click on the bin boundary and drag the bin boundary to the desired position. Repeat with the other bin boundary as necessary.

To assign bins at the edge of a marker to a different marker, hold down the **Control** key, left-click and drag across the desired bins. A light-blue box will appear. Right-click in the light-blue box and select **Change Marker**. This will open the 'Edit Group Allele' window and allow you to keep the bins within the existing marker or create a new marker (Figure 165). You can keep the predefined name or create a new name. Select **OK** to place the highlighted bins into the newly created marker.

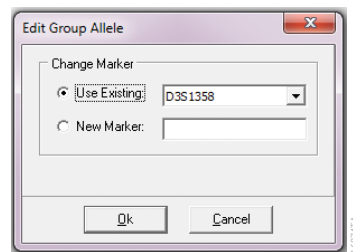


Figure 165. 'Edit Group Allele' window.

Deleting Bins from a Marker

To delete an existing bin, right-click the vertical grey bar in the center of the bin in the Chart Overlay and select **Delete Allele**. A confirmation window will appear to confirm or cancel the deletion. To delete multiple bins at once, hold down the **Control** key, left-click and drag across the desired bins in the Chart Overlay. A light-blue box will appear. Right-click in the light-blue box and select **Delete Alleles**. A confirmation window will appear to confirm or cancel the deletion.

8.C. Adjusting Panels to Data—Manual and Automated Panel Calibration

The panel alignment will shift due to variations in genetic analyzers or run conditions (such as temperature, polymer age or injection time). Panels can be automatically adjusted by selecting **Auto Select Best Ladder** and **Auto Panel Adjustment** from the 'Additional Settings - HID Analysis' window of the Run Wizard (Section 4.C). If the panel is shifted more than 5.0 base pairs from the defined parameters, the software cannot auto-adjust the panel, and the user may wish to manually adjust the panel to fit the ladder(s) of a project.

Note: Manual alignment is used for research purposes only.

If two or more bins within a marker are not aligned properly with alleles of the ladder, you can adjust the position and range of the marker. To move the marker position without changing the range, hold down the **Shift** key, then left-click on the marker bar. This will outline the marker bar in red to indicate the marker can be repositioned (Figure 166).

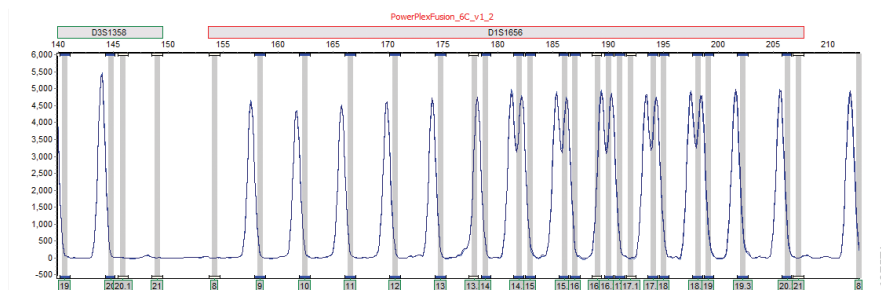


Figure 166. Selecting a marker for manual adjustment.

Drag the marker left or right to the desired new position to align with the displayed ladder(s). To extend or shorten the range of the marker, hold down the **Shift** key while hovering over the edge of the Marker bar. When the double-headed arrow appears, right-click on the edge of the marker and drag to the desired position.

Note: Extending the range of a marker increases the spacing between bins, while shortening the range decreases the spacing between bins. You can adjust both the marker position and range to achieve the best fit to the data. Manual adjustment is used for research purposes only.

If single bins are not aligned with alleles of the ladder, you can adjust the position of individual bins. To align a bin with a ladder peak, hold down the **Shift** key and then left-click the vertical gray bar of the bin. The bar will change to blue with a red outline (Figure 167). Drag the bar left or right to align the bin with the peak.

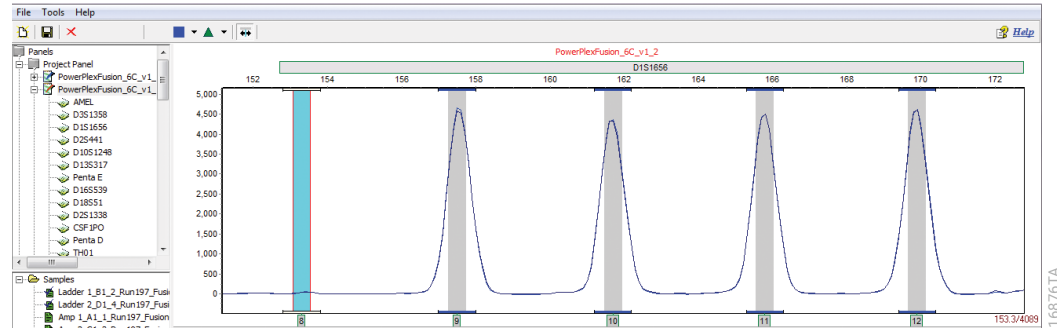


Figure 167. Selected bin outlined in red.

When all adjustments are made to the panel, select **Save Changes** from the File menu to save the changes to the selected panel file or select **Save As New Panel** to save as a new panel file.

8.D. Custom Panel Creation

Custom panels can be created from within the software. The use of sized data, particularly allelic ladder samples, aids in the creation of custom panels. To create a custom panel, first import the desired data and analyze using the Run Wizard with no panel applied (select **None** from the 'Panel' drop-down list of the 'Template Selection' screen). This will apply the size standard template to the data and assign sizes to the peaks in the project samples. Next, open the Panel Editor and select **Create New Panel** from the 'File' menu or toolbar. This opens the 'Create New Panel' window (Figure 168).

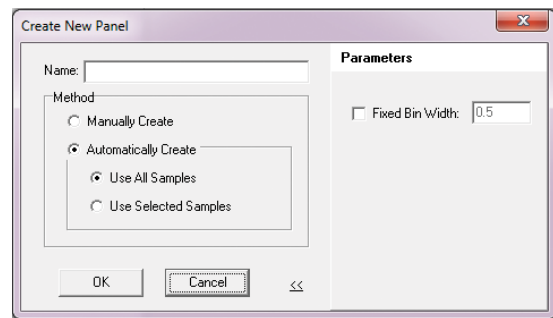


Figure 168. 'Create New Panel' window.

There are three fields in the 'Create New Panel' window:

Name: Allows you to name the new panel.

Method: Provides two options for panel creation: manual and automatic (Sections 8.D.1 and 8.D.2).

To access additional options from this window, select the >> button in the bottom right corner.

Parameters: Allows you to apply a fixed bin width by checking the **Fixed Bin Width** box and entering the desired width.

8.D.1 Manual Panel Creation

The manual panel creation method allows you to create a panel by manually entering information. Select **Manually Create** in the 'Create New Panel' window (Figure 168), then select **OK** to return to the main window of the Panel Editor. The newly created panel file will be listed in the Panel File Navigator. The Chart Overlay will display the electropherograms of all samples with no marker or bin labels. The Panel Table will be blank. Follow the instructions in Sections 8.B.1 and 8.B.2 to create markers and bins, respectively.

8.D.2 Automatic Panel Creation

The automatic panel creation method allows the software to automatically determine the position of markers and bins based on the imported data. Select **Automatically Create** in the 'Create New Panel' window (Figure 168). You can build the new panel from all samples in the project (Use All Samples) or from just those selected in the Sample File Tree (Use Selected Samples). Select **OK** to return to the main window of the Panel Editor (Figure 169).

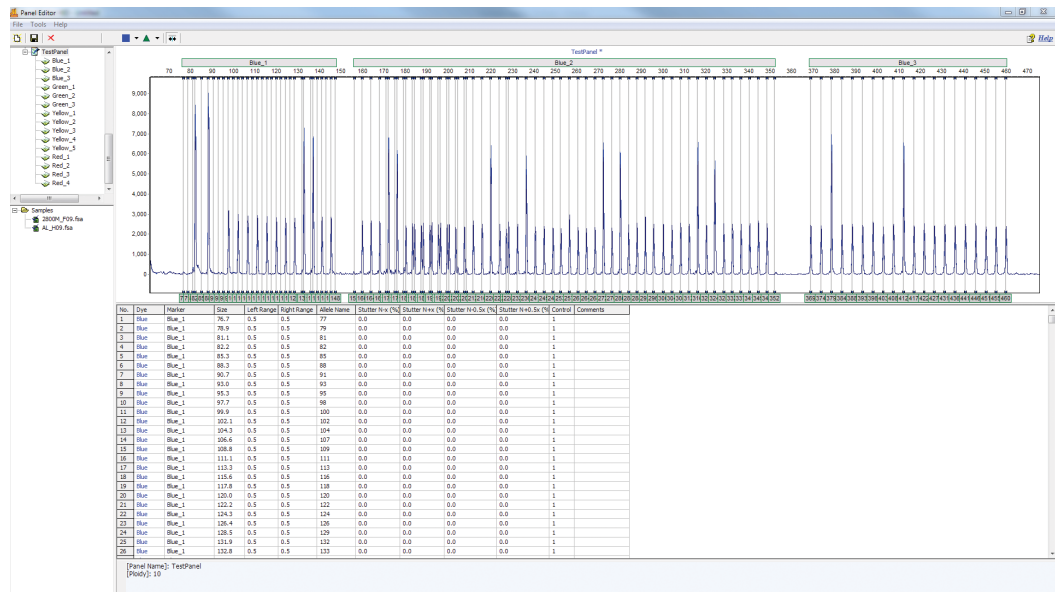


Figure 169. 'Panel Editor' window after automatic panel creation.

The newly created panel file is listed in the Panel File Navigator. The Chart Overlay displays the electropherograms of the selected samples, with the positions of the automatically created markers and bins labeled. The Peak Table contains the details for each automatically created bin position. By default, the markers are listed alphanumerically by dye channel (e.g., Blue_1, Blue_2, etc.), and every bin is designated as a control bin (1) in the 'Control' column. To make changes to any of these marker and bin positions, follow the instructions in Sections 8.B.1 and 8.B.2.

Note: When creating new panels, the software preferentially uses the more intense peaks to position bins when peaks do not overlap perfectly with bins.

8.D.3 Menu Options

The Panel Editor contains three menu options: File, Tools and Help. The 'File' menu allows you to create, delete, save, import and export panels (Figure 170). The 'Tools' menu contains options for projects with at least one allelic ladder and exporting a panel (Figure 172). The 'Help' menu contains descriptions of the navigation and editing functions of the Panel Editor (Figure 173).

'File' Menu

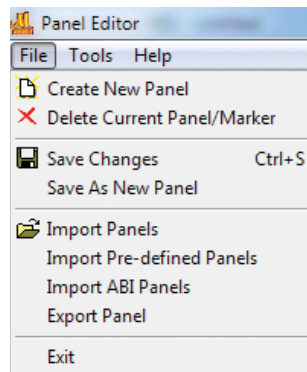


Figure 170. 'File' menu of the Panel Editor.

Create New Panel: Opens the 'Create New Panel' window with the options to create a new panel automatically or manually.

Delete Current Panel/Marker: Deletes the panel or marker that is currently highlighted in the Panel File Navigator.

Save Changes: Saves edits and changes to the panel file in the 'Panel' directory (Shortcut = Control + S).

Save As New Panel: Saves edits and changes to the panel as a new panel template. When selected, the 'Input Dialog' box opens with a field to enter a new panel name. The panel is added to the Panel File Navigator and saved in the 'Panel' directory.

Import Panels: Opens a browser window that allows you to navigate to a saved panel file (.xml). Use the 'Import Panels' option to find previously exported panel files (.xml) on local or network computers.

Import Pre-defined Panels: Opens a browser window to the 'Panel' directory to allow you to import a saved panel file (.xml) from that location.

Import ABI Panels: Opens the 'Import Panels from GeneMapper' window (Figure 171). Select the **...(browse)** button in the 'Panel File' field to locate a panel file created for use in GeneMapper® software. If a GeneMapper® bin file is available for the selected panel file, choose **Load From File** in the 'Bin' section, then select the **...(browse)** button in that field to locate the file. If a GeneMapper® bin file is not available, choose **Auto Build** in the 'Bins' section. When this method is selected, GMHID-Spectrum creates the bin boundaries based on the selected panel file, the bins are named by their size, not their allele designation, and all bins are designated as controls.

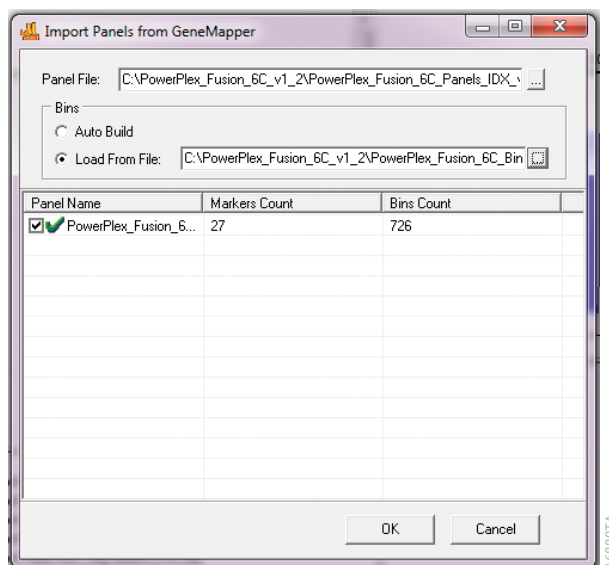


Figure 171. 'Import Panels from GeneMapper' window.

When the files are successfully loaded, their panel name, number of markers and number of bins will be displayed in the table of the 'Import Panels from GeneMapper' window. For panel files that contain panel information for multiple chemistries, each chemistry will be listed in the table and can be selected or deselected for import into GMHID-Spectrum. Select **OK** to convert the GeneMapper® files into GMHID-Spectrum files and return to the main window of the Panel Editor. The imported GeneMarker® panels will be listed in the Panel File Navigator.

Export Panel: Exports the selected panel in the Panel File Navigator as an .xml file to a specified directory on a local or network computer.

Exit: Closes the 'Panel Editor' tool.

'Tools' Menu

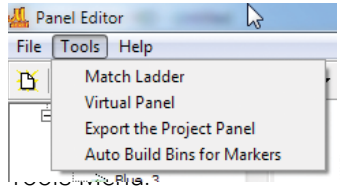


Figure 172. 'Tools' menu of the Panel Editor.

Match Ladder: Adjusts panels with no virtual bins to match the allelic ladder in a project.

Note: This feature should only be used for research purposes. Large differences between peak and bin positions cannot be resolved with the Match Ladder function.

Virtual Panel: Allows you to create a new panel based on the selected panel. When **Virtual Panel** is selected, the 'Create virtual panel' process window opens, and the software scans the dataset to identify the best ladder. A confirmation window will appear. Select **Yes** to create a virtual panel that matches the allelic ladder identified as the best allelic ladder. The new virtual panel will be added to the Panel File Navigator and labeled **VPanel_PanelName** to reflect that this is a virtual copy of the original panel. Select **No** in the confirmation window, and a virtual panel is created but is not adjusted to match the best allelic ladder.

Note: We recommend using the Virtual Panel only when small adjustments to the marker and bin placements are required. This feature functions similarly to selecting an allelic ladder from the drop-down list in the 'Additional Settings - HID Analysis' screen of the Run Wizard (Section 4.C.1).

Export the Project Panel: Exports the selected panel in the Panel File Navigator as an .xml file to a specified directory on a local or network computer.

Auto Build Bins for Markers: Redefines the bins within each marker. A series of 1.0 base pair bins is created across the entire marker.

Note: The Auto Build Bins for Markers should only be used for research purposes. Selecting this tool will overwrite existing bin designations; therefore, be sure to save the original panel as a new panel before using this tool.

'Help' Menu

Hot Keys: Opens the 'Panel Editor Action Help' window, which describes the display, editing and navigation shortcut functions (i.e., Hot Keys) of the Panel Editor.

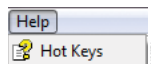


Figure 173. 'Help' menu of the Panel Editor.

9 Printing

GMHID-Spectrum software provides several options to print project-related information. Print menus are available from the following areas in the software:

- 'Main Analysis' window (Section 9.A)
- All Color Browser (Section 9.B)
- 'Calibration Chart' window (Section 9.C)
- 'Show Edit History' window (Section 9.D)
- 'User Management History' window (Section 9.E)

Each of these print menus also provides the option of saving a file instead of printing.

9.A. Printing from the 'Main Analysis' Window

The main print feature of the software is the 'Print Report' window accessible from the 'Main Analysis' window. This feature prints an Allele Report containing electropherograms, Peak Table information or both for all or selected samples in a project. To access the 'Print Report' window, select **Print Report** from the 'Project' menu (Section 6.C) or select the **Print Report** icon in the toolbar. If the Magic Wizard is selected as the Run Method (Section 6.B.1) or if you activate the Magic Wizard from the 'Tools' menu or the toolbar, the software will prompt you to save, print or both after the Run Wizard is complete by providing a 'Print Report' link. Clicking on this link will open the 'Print Report' window, which allows you to select the information in the report.

9.A.1 Navigating the 'Print Report' Window

The 'Print Report' window is divided into two sides (Figure 174). The left side of the window allows the user to create a new or select an existing print template. The default setting is to use the most recent template. The right side of the window contains two settings tabs: Standard and Advanced.

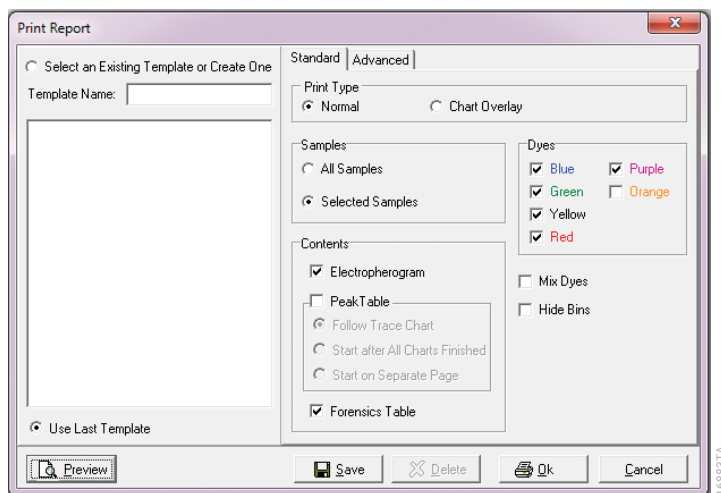


Figure 174. 'Standard' tab of the 'Print Report' window.

The 'Standard' Tab

In the 'Standard' tab, there are four sections to specify your print preferences (Figure 174).

Print Type

Normal: All options of the 'Print Report' window are available when **Normal** is selected as the Print Type.

Chart Overlay: Displays an overlay of the electropherograms for all selected dyes.

Samples

All Samples: Displays all the samples in the project.

Selected Samples: Displays only those sample files that are selected in the Sample File Tree of the 'Main Analysis' window.

Contents

Electropherogram: Displays the electropherogram chart for each dye color and sample selected.

Note: The zoom setting of the electropherogram in the 'Main Analysis' window will be reflected in the Allele Report. Zoom out fully to include all peaks in the Allele Report.

Peak Table: Prints the Peak Table for each dye color and sample selected. The following 'Peak Table' options are available:

Follow Trace Chart: The Peak Table for each dye channel will immediately follow that channel electropherogram.

Start after All Charts Finished: The Peak Table for all samples will begin after the last electropherogram.

Start on Separate Page: The Peak Table for all samples will begin after the last electropherogram, but will start on a new page.

Forensics Table: Prints genotype tables for the selected samples. This option is not active when **Peak Table** and **Follow Trace Chart** are selected.

Note: If neither **Electropherogram** or **Peak Table** is selected, the Allele Report will contain a list of each dye color for each selected sample and the allele count within each dye color.

Dyes

Dyes: Allows you to select the dye colors included in the Allele Report.

Mix Dyes: Displays all selected dye colors overlaid on one electropherogram.

Hide Bins: Does not display bin markers on the x axis of electropherograms in the Allele Report.

The 'Advanced' Tab

The 'Advanced' tab contains four sections to further define how information is displayed in the Allele Report (Figure 175).

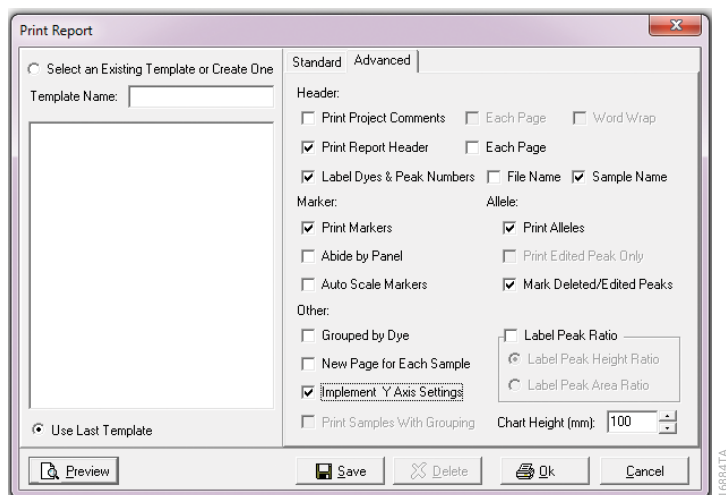


Figure 175. 'Advanced' tab of the 'Print Report' window.

Header

The checkboxes included in the 'Header' section show the information present in the header of the Allele Report.

Print Project Comments: Includes information recorded in the Project Comments (Section 6.C). This option is selected by default. Select **Each Page** to display the Project Comments on every page of the Allele Report. When the 'Each Page' option is not selected, the Project Comments are only displayed on the first page of the Allele Report. Select **Word Wrap** to display Project Comments that exceed one line in their entirety as multiple lines in the Allele Report.

Print Report Header: Includes the Institution and User ID as defined in the 'User Manager' window (Section 6.F) as well as the Template Name and Panel, as defined in the Run Wizard Template (Section 4.A). Select **Each Page** to display the Report Header on every page of the Allele Report.

Label Dyes & Peak Numbers: Displays the dye color name and number of peaks observed in the dye channel above the electropherogram for each dye channel. This feature is inactive when the **Chart Overlay** Print Type is selected from the 'Standard' tab.

File Name: Includes the file name in the header of the respective electropherogram chart for each dye channel.

Sample Name: Includes the sample name in the header of the respective electropherogram chart for each dye channel.

Marker

The checkboxes included in the 'Marker' section define how markers are displayed in the Allele Report.

Print Markers: Displays the marker bars above the electropherogram. This option is selected by default.

Abide by Panel: Displays only the alleles found within the range of the applied panel. Alleles that are outside of the panel are not included in the report.

Auto Scale Markers: The software adjusts the peak heights of the lower peaks to match the peak height of the highest peak in the dye color. The scaling factor (2X–8X) is noted in the electropherogram below the marker bar. This option is only active when **Print Markers** is selected.

Allele

Print Alleles: Displays the allele labels below the peaks in the electropherogram. This option is selected by default.

Print Edited Peak Only: Includes only edited peaks in the Peak Table, if the Peak Table is being printed. This option is only active when both **Print Alleles** and **Peak Table** are selected in the 'Standard' tab.

Mark Deleted/Edited Peaks: Displays an "X" above a deleted peak and an "E" above an edited peak in the electropherogram. This option is only active when **Print Alleles** is selected.

Other

Grouped by Dye: Lists the electropherograms in the Allele Report by dye color selected (i.e., lists all samples in blue first, then all samples in green, etc.).

Note: If this option is selected, New Page for Each Sample should not be selected.

New Page for Each Sample: Begins each sample on a new page instead of continuing on the same page as the previous sample.

Note: If this option is selected, Grouped by Dye should not be selected.

Implement Y Axis Settings: Applies the y-axis settings selected using the **Set Axis** icon in the 'Main Analysis' window to the electropherogram in the report.

Print Samples With Grouping: Groups samples in the report following the rules defined using the File Name Group Editor (Section 12.A). Use this setting to save a separate .pdf file for each sample by selecting **Apply Sample Grouping** in the 'Main Analysis' window and making each sample its own group (Section 6.C), then selecting **Print Samples With Grouping** in the 'Print Report' window. This setting is only active if sample grouping has been applied.

Label Peak Ratio: Labels peak ratios on the electropherogram in the report. This selection is only available when the peak label position is set to top in the 'Display' tab of the 'Preferences' window (Section 6.B).

Chart Height (mm): Use to adjust the height of each dye channel of the electropherogram in the report. The range of possible heights is 30–300mm.

To save the selections made in the 'Standard' and 'Advanced' tabs as a template for future use, type a name in the 'Template Name' field on the left side of the 'Print Report' window, then select **Save**. The newly created print report template is now included in the list of templates. Changes made to an existing template can be saved in the same manner. To delete a print report template from the list, select the template, then select **Delete**.

9.A.2 Navigating the Print Preview Window

When the desired report settings are selected, print the report by selecting **OK**. A 'Print' window will open, allowing you to select a printer, the page range and the number of copies. Alternatively, preview the report by selecting the **Preview** button. This will open a separate 'Preview' window displaying a print preview of the Allele Report. The 'Preview' window has a toolbar ribbon that provides shortcuts to adjust report settings as well as printing and saving.

Table 16. Icons and Functions of the Print 'Preview' Window Toolbar.


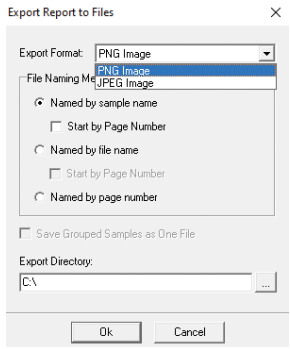
	<p>Print: Opens the 'Print' window. Select a printer, the page range and the number of copies.</p>
	<p>Export to Files: Opens the 'Export Report to Files' window. The Export Format drop-down menu provides two options for file format: .png or .jpg. You can name files by sample name, file name or page number, by selecting the appropriate radio button in the 'File Naming Methods' section. When naming files by sample or file name, you also can include the page number for the selected sample in the file name. To do so, select either Named by sample name or Named by file name and then Start by Page Number.</p> <div data-bbox="841 972 1128 1318" style="text-align: center;">  </div> <p>When Print Samples With Grouping in the 'Advanced' tab is selected, the option to 'Save Grouped Samples as One File' will be active. To select a location for the exported file, choose the ... (browse) button in the 'Export Directory' field and navigate to the desired directory. The 'Export Report to Images' process box will appear. When the export is complete, select OK to return to the 'Preview' window.</p> <p>(continued next page)</p>

Table 16. Icons and Functions of the Print 'Preview' Window Toolbar (continued).


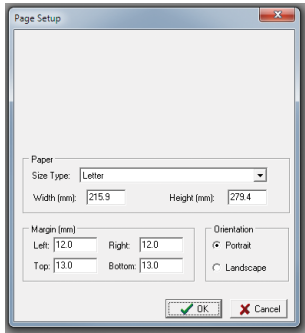





	<p>Export to File (continued):</p> <p>Examples of File Naming Methods:</p> <p>Named by Sample Name</p> <table border="1"> <thead> <tr> <th>Name</th> <th>Type</th> </tr> </thead> <tbody> <tr> <td> Amp 1_01</td> <td>PNG image</td> </tr> <tr> <td> Amp 2</td> <td>PNG image</td> </tr> <tr> <td> Amp 2_01</td> <td>PNG image</td> </tr> </tbody> </table> <p>Named by File Name</p> <table border="1"> <thead> <tr> <th>Name</th> <th>Type</th> </tr> </thead> <tbody> <tr> <td> Amp 1_A1_1_Run197_Fusion6C_01</td> <td>PNG image</td> </tr> <tr> <td> Amp 2_C1_3_Run197_Fusion6C</td> <td>PNG image</td> </tr> <tr> <td> Amp 2_C1_3_Run197_Fusion6C_01</td> <td>PNG image</td> </tr> <tr> <td> Amp 3_E1_5_Run197_Fusion6C_01</td> <td>PNG image</td> </tr> </tbody> </table> <p>Named by Page Number</p> <table border="1"> <thead> <tr> <th>Name</th> <th>Type</th> </tr> </thead> <tbody> <tr> <td> _Pg1</td> <td>PNG image</td> </tr> <tr> <td> _Pg2</td> <td>PNG image</td> </tr> <tr> <td> _Pg3</td> <td>PNG image</td> </tr> <tr> <td> _Pg4</td> <td>PNG image</td> </tr> </tbody> </table>	Name	Type	Amp 1_01	PNG image	Amp 2	PNG image	Amp 2_01	PNG image	Name	Type	Amp 1_A1_1_Run197_Fusion6C_01	PNG image	Amp 2_C1_3_Run197_Fusion6C	PNG image	Amp 2_C1_3_Run197_Fusion6C_01	PNG image	Amp 3_E1_5_Run197_Fusion6C_01	PNG image	Name	Type	_Pg1	PNG image	_Pg2	PNG image	_Pg3	PNG image	_Pg4	PNG image
Name	Type																												
Amp 1_01	PNG image																												
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Amp 2_01	PNG image																												
Name	Type																												
Amp 1_A1_1_Run197_Fusion6C_01	PNG image																												
Amp 2_C1_3_Run197_Fusion6C	PNG image																												
Amp 2_C1_3_Run197_Fusion6C_01	PNG image																												
Amp 3_E1_5_Run197_Fusion6C_01	PNG image																												
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_Pg2	PNG image																												
_Pg3	PNG image																												
_Pg4	PNG image																												
 <p>168887A</p>	<p>Page Setup: Opens the 'Page Setup' window. Choose the paper size, margins and orientation (Portrait or Landscape).</p>  <p>168887A</p>																												

Table 16. Icons and Functions of the Print 'Preview' Window Toolbar (continued).

	<p>Content Options: Opens the 'Print Report' window. See Section 9.A.1.</p>
	<p>Zoom to Fit: Zooms out to view the entire print preview page.</p>
	<p>Zoom to Width: Zooms in to view the print preview page at maximum width while still displaying the entire page.</p>
	<p>Zoom Ratio: Allows you to enter percentage numbers to increase or decrease the zoom aspect of the print preview page.</p>
	<p>Close: Closes the 'Preview' window.</p>

Note: Selections in the 'Display' tab of the 'Preferences' window (Section 6.B) will affect how the Allele Report is displayed.

An example of an Allele Report is shown in Figure 176. The standard page header displays the SoftGenetics logo, software version, date and time the report was generated and page number. To add a customized logo to the page header, see Section 9.A.3. A Print Report Header is displayed listing the institution's name and project information. For each sample, the sample number (based on order in the project) is displayed followed by the reference ladder (best match) used for the sample, then the run date and time.

9.A.3 Including a Customizable Logo in the Allele Report

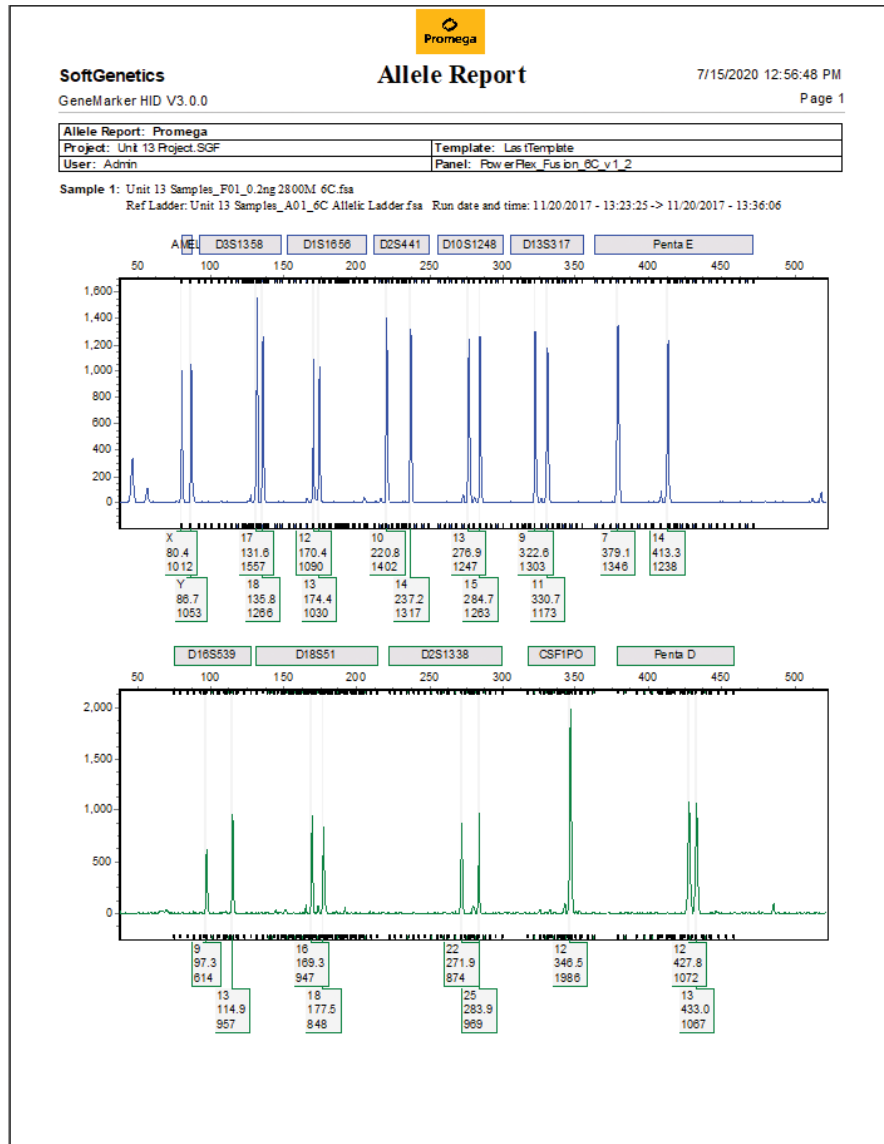


Figure 176. Example of an Allele Report with a customized logo.

GMHID-Spectrum allows you to include a customized logo (Figure 176) in an Allele Report generated from the 'Main Analysis' window or the All Color Browser (Section 9.B). To add a logo to the page header, save the desired logo as a .jpg, .jpeg, .bmp, .ico, .emf or .wmf file (**Note:** .png is **not** permitted) and rename the file "PartnerLogo" (no space).

Move the file to the following directory:

C:\Program Files(x86)\SoftGenetics\GeneMarker_HID\[your version number]

Once the file is placed in this directory, the logo will appear at the top of all Allele Reports generated from the 'Main Analysis' window or the All Color Browser. For best results, use images that are rectangular. To disable the logo, simply delete the PartnerLogo file, or name the file something other than "PartnerLogo".

9.B. Printing from the All Color Browser

The print options in the All Color Browser are similar to those provided through the 'Print Report' window but are more limited. Selecting the **Print** icon in the toolbar opens the 'All Color Browser Settings' window (Figure 177).

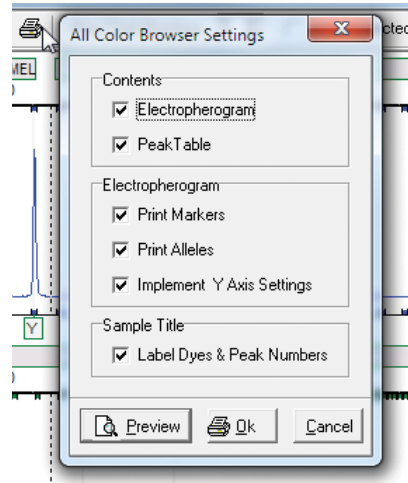


Figure 177. 'All Color Browser Settings' window.

There are three sections to the 'All Color Browser Settings' window. By default, all options are selected.

Contents

Electropherogram: Displays the electropherogram for the selected dye channels in the report.

Peak Table: Displays the Peak Table for each selected dye channel below the associated electropherogram in the report.

Electropherogram

Print Markers: Displays the marker bars above the electropherogram in the report.

Print Alleles: Displays the allele labels below the peaks in the electropherogram in the report.

Implement Y Axis Settings: Applies the y-axis settings selected using the **Set Axis** icon in the 'All Color Browser' window to the electropherogram in the report.

Note: The zoom setting of the electropherogram in the 'All Color Browser' window will be reflected in the Allele Report. Zoom out fully to include all peaks in the Allele Report.









Sample Title

Label Dyes & Peak Numbers: Displays the dye color name and number of peaks observed in the dye channel in a header above each electropherogram.

When the desired settings are selected, select **OK** to print. A 'Print' window will open, allowing the user to select a printer, the page range and the number of copies. Alternatively, preview the printed report by selecting the **Preview** button. This will open a separate 'Preview' window displaying a print preview of the Allele Report. This 'Preview' window has the same toolbar ribbon as the 'Preview' window of the 'Print Report' window (Section 9.A.2). Each toolbar icon functions in the same manner as described above, except the 'Content Options' icon will open the 'All Color Browser Settings' window.

The following icons are available in the print 'Preview' window of the All Color Browser. Refer to Section 9.A.2 for detailed descriptions.

Table 17. Icons and Functions of the Print 'Preview' Window of the All Color Browser.

	Print: Opens the 'Print' window. Select a printer, the page range and the number of copies.
	Export to File: Opens the 'Export Report to Files' window.
	Page Setup: Opens the 'Page Setup' window. Choose the paper size, margins and orientation (Portrait or Landscape).
	Content Options: Opens the 'All Color Browser Settings' window (Figure 159).
	Zoom to Fit: Zooms out to view the entire print preview page.
	Zoom to Width: Zooms in to view the print preview page at maximum width while still displaying the entire page.
	Zoom Ratio: Allows you to enter percentage numbers to increase or decrease the zoom aspect of the print preview page.
	Close: Closes the 'Preview' window.

9.C. Printing from the 'Calibration Charts' Window

The print options from the 'Calibration Charts' window allow you to define the layout and order of the calibration charts in the printed or exported file. To print or export the calibration charts, select the **Print** icon in the toolbar. This opens the 'Calibration Print' window, which has two sections to define print preferences (Figure 178).

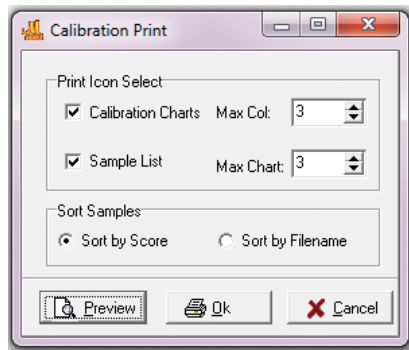


Figure 178. 'Calibration Print' window.

Print Icon Select

Calibration Charts: Allows you to enter a number in the 'Max Col' field to define the maximum number of calibration charts displayed across a printed page.

Sample List: Adds information from the 'Sample List' section of the 'Calibration Charts' window to the printed pages.

Max Chart: Defines the maximum number of sample calibration charts displayed per printed page.

Sort Samples









Sort by Score: Sorts calibration charts by score from highest to lowest

Sort by Filename: Sorts calibration charts alphanumerically by sample file name.

When the desired settings are selected, select **OK** to print the calibration charts. A 'Print' window will open, allowing you to select a printer, the page range and the number of copies. Alternatively, preview the report by selecting the **Preview** button. This 'Preview' window has the same toolbar ribbon as the 'Preview' window of the 'Print Report' window (Section 9.A.2). Each toolbar icon functions in the same manner as described above, except the Content Options icon will open the 'Calibration Print' window.

The following icons are available in the print 'Preview' window of the 'Calibration Charts' window.

Table 18. Icons and Functions for the Print 'Preview' Window of Calibration Charts.








	Print: Opens the 'Print' window. Select a printer, the page range and the number of copies.
	Export to File: Opens the 'Export Report to Files' window.
	Page Setup: Opens the 'Page Setup' window. Choose the paper size, margins and orientation (Portrait or Landscape).
	Content Options: Opens the 'Calibration Print' window (Figure 160).
	Zoom to Fit: Zooms out to view the entire print preview page.
	Zoom to Width: Zooms in to view the print preview page at maximum width while still displaying the entire page.
	Zoom Ratio: Allows you to enter percentage numbers to increase or decrease the zoom aspect of the print preview page.
	Close: Closes the 'Preview' window.

9.D. Printing from the 'Show Edit History' Window

An Edit History Report can be printed or exported from the 'Show Edit History' window of the Peak Table (Section 5.B.3). To print or export the report, select the **Print** button at the bottom of the window. This opens a 'Preview' window.

The following icons are available in the print 'Preview' window of the 'Show Edit History' window.

Table 19. Icons and Functions in the Print 'Preview' Window of Show Edit History.

	Print: Opens the 'Print' window. Select a printer, the page range and the number of copies.
	Export to File: Opens the 'Export Report to Files' window.
	Page Setup: Opens the 'Page Setup' window. Choose the paper size, margins and orientation (Portrait or Landscape).
	Zoom to Fit: Zooms out to view the entire print preview page.
	Zoom to Width: Zooms in to view the print preview page at maximum width while still displaying the entire page.
	Zoom Ratio: Allows you to enter percentage numbers to increase or decrease the zoom aspect of the print preview page.
	Close: Closes the 'Preview' window.

The 'Edit History' report contains the standard page header as well as a project header with organization, user, run and data analysis information. The report provides a record of each Edit Time, Organization, User and Operation (Figure 179).

SoftGenetics		Edit History				12/16/2020 4:26:37 PM							
GeneMarker HID V3.0.0						Page 1							
Allele Report: Promega		Template: LastTemplate											
Software: GeneMarker HID V3.0.0		Panel: PowerFlex_Fusion_8C_v1.2											
Project: Unit 13 Project SGF		Run date and time: 11/20/2017 - 13:23:25 -> 11/20/2017 - 13:36:06											
User: Admin													
File: Unit 13 Samples_C01_1ng2800M8C.fsa													
No.	Edit Time			Organization			User		Operation		Module		Recover
1	12/16/2020 - 16:26:19			Promega			Admin		Delete Allele		AllelsChart		
	Dye	Size	Height	Ht_Ratio	FWHM	Marker	Allele	Difference	Quality	Score	Allele Comments	Sample Comments	
Current Value	Blue	85.4	177	0.02		AMEL	CB	1.20	Undetermined	3.1147	[<Deleted>]		
Old Value	Blue	85.4	177	0.02		AMEL	CB	1.20	Undetermined	3.1147			
	Quality Reasons		Plate ID										
Current Value	CB, INE												
Old Value	CB, INE												








Figure 179. Example of an 'Edit History' report.

9.E. Printing from the 'User Manager' Window

When user management is enabled, a 'User Management History' report can be printed or exported from the User Manager (Section 6.F).

To print or export the report, select the **Print** button at the bottom of the 'History' tab of the 'User Manager' window. This opens a print 'Preview' window for the report. The following icons are available in the print 'Preview' window of the 'User Manager' window. See Section 9.A.2 for details.

Table 20. Icons and Functions in the Print 'Preview' Window of the User Management History Report.

	Print: Opens the 'Print' window. Select a printer, the page range and the number of copies.
	Export to File: Opens the 'Export Report to Files' window.
	Page Setup: Opens the 'Page Setup' window. Choose the paper size, margins and orientation (Portrait or Landscape).
	Zoom to Fit: Zooms out to view the entire print preview page.
	Zoom to Width: Zooms in to view the print preview page at maximum width while displaying the entire page.
	Zoom Ratio: Allows you to enter percentage numbers to increase or decrease the zoom aspect of the print preview page.
	Close: Closes the 'Preview' window.

The 'User Management History' report contains the standard page header. The report provides a record of each event that occurred from login including: Date, Time, User, Events and Comments (Figure 180).

SoftGenetics		User Management History		12/16/2020 4:31:51 PM
GeneMarker HID V3.0.0				Page 1
DataTime	User	Events	Comments	
12/16/2020 10:31:43 PM	Admin	Add new user	GI TSTM	
12/16/2020 10:30:10 PM	Admin	Log in		
12/16/2020 10:30:04 PM	Admin	Log out	Forced to log out.	
12/16/2020 10:25:47 PM	Admin	Log in		
12/16/2020 10:25:47 PM	System	Add administrator		

Figure 180. Example of a 'User Management History' report.

17533TA

10 Quality Assessment

GMHID-Spectrum provides several options to assess the quality of STR data within a project. Users can define the parameters by which the software will process the data and flag samples that do not meet the quality criteria as well as set up conditions for contamination checks.

10.A. Profile Quality

Profile quality can be assessed at three levels within GMHID-Spectrum: size match (as defined by the chosen size standard template, Section 7.B), size standard quality (Section 10.A.2) and analysis quality (Section 10.A.3). A fourth level of assessment is available for STR kits containing two quality indicator peaks in GMHID-Spectrum software version 3.2.0 or later (Section 10.A.4). Samples designated as positive controls, negative controls or ladders are assessed by the additional metric of concordance to an expected result.

A summary of the quality assessment is displayed in the Project Summary Bar at the bottom of the 'Main Analysis' window. Controls or ladders that do not meet the concordance criteria will be counted as an error in their respective boxes (Figure 181).

Note: Positive control profiles are defined in the Positive Control Template Editor (Section 6.E); negative controls are expected to be void of labeled peaks, and allelic ladders are defined by parameters of the applied panel (Section 4.A.1). Samples with failed size standards or failed injections will be tallied in the '#Failed' box as will failed controls (Figure 181). Samples, including controls and ladders that do not meet defined analytical measures, will be tallied in the '#Flagged' box (Figure 181).

The Sample File Tree also provides a visual display of quality measures as described in Section 5.A.

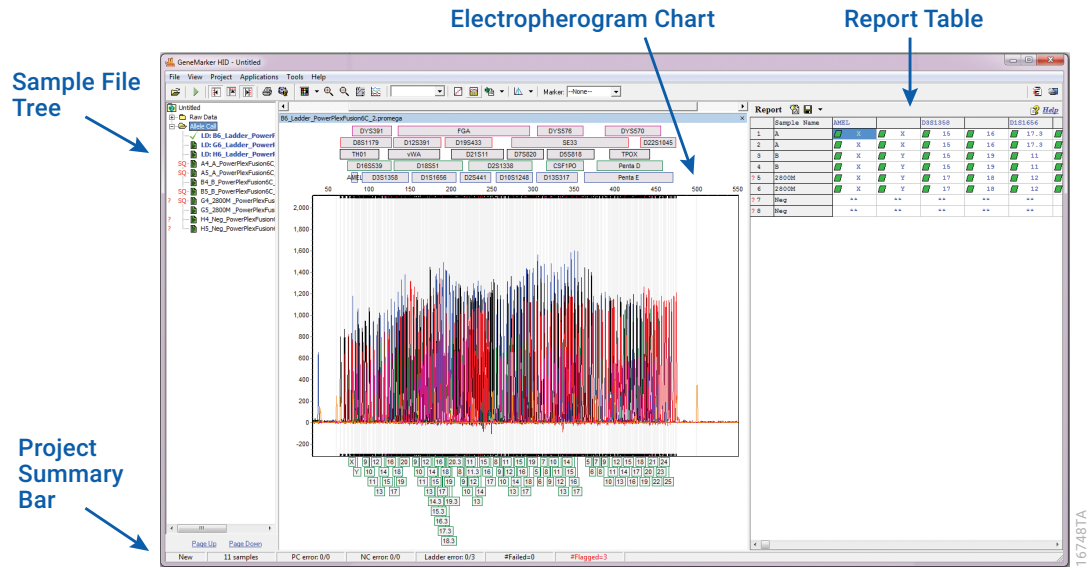


Figure 181. Assessing STR data quality.

10.A.1 Size Match Score

After a size standard template is applied to a dataset, a size match score is assigned to each sample based on how closely the sample size standard matched the size standard template. Perfect matches receive a score of 100. Samples with no correlation receive a score of 0 and fail size calling. Each sample's size match score is available in the Sample File Tree of the 'Main Analysis' window. Samples with a high match score (greater than 80) are indicated by a green sheet (Figure 182). Those with a low match score (1–80) will have a yellow sheet. Samples for which size calling failed (size match score of 0) receive a red strike-through. A more detailed assessment of each sample's size match score is available in the 'Calibration Charts' window (Section 7.B).

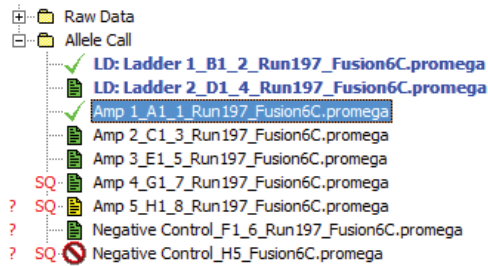


Figure 182. Size match score displayed in the Sample File Tree.

10.A.2 Size Standard Quality

In addition to assessing the match between a sample's size standard and the size standard template, the software can also assess the quality of size standard peaks. To choose this option, known as SQ (Size Quality) flagging, select the flagging option in the Low ILS Quality Flag 'SQ' section in the 'Sample Quality' tab of the 'Preferences' window (Section 6.B). When this option is selected, the software will evaluate the three criteria listed below to determine whether a sample size standard meets the size quality requirements. If the sample size standard does not meet all three criteria, the sample is marked with a red SQ flag in the Sample File Tree of the 'Main Analysis' window and in the upper left corner of the sample electropherogram.

1. The sample size standard contains all peaks that are marked as "Enabled" in the chosen size standard template.
2. All of the expected size standard peaks are called in the 'Main Analysis' window.
3. All of the expected size standard peaks are of high quality such that the absolute difference in size between the actual size standard peaks and the expected, Enabled size standard peaks is less than 0.2bp.

To display the reason for the SQ flag, hover over the SQ flag in the electropherogram. Samples that will receive an SQ indicator include:

- Samples with one or more size standard fragments outside of the expected linear range for size calibration (Figure 183).

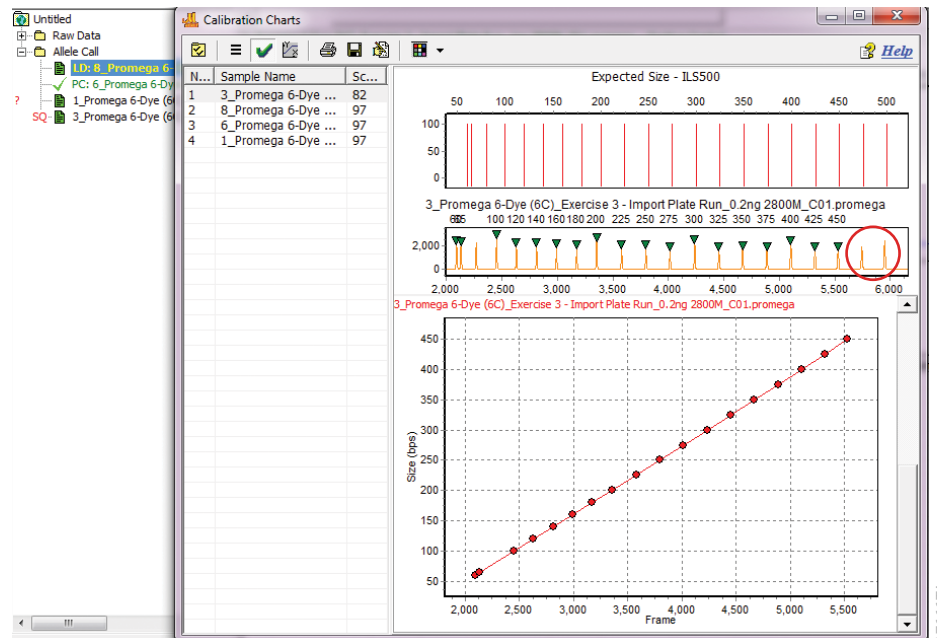


Figure 183. Sample size standard with two peaks (red circle) outside of the expected size calibration linear range.

- Samples in which all ILS fragments are detected for pattern recognition and are in the linear range (Figure 184), but one or more peaks are of low quality and not called in the 'Main Analysis' window (Figure 185).

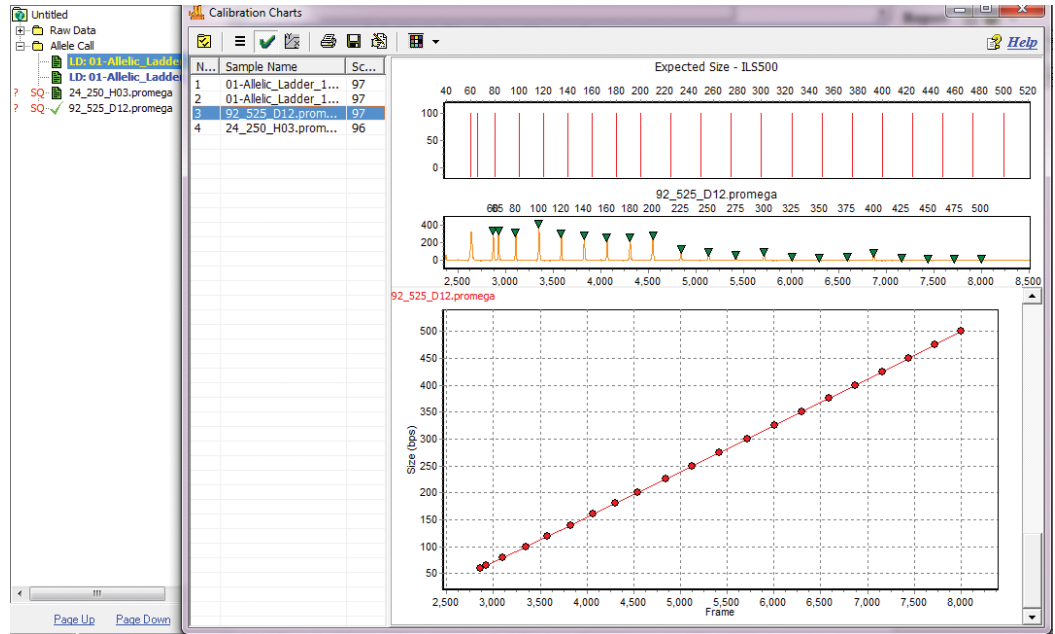


Figure 184. Example where all of the ILS fragments are detected in the 'Calibration Charts' window.

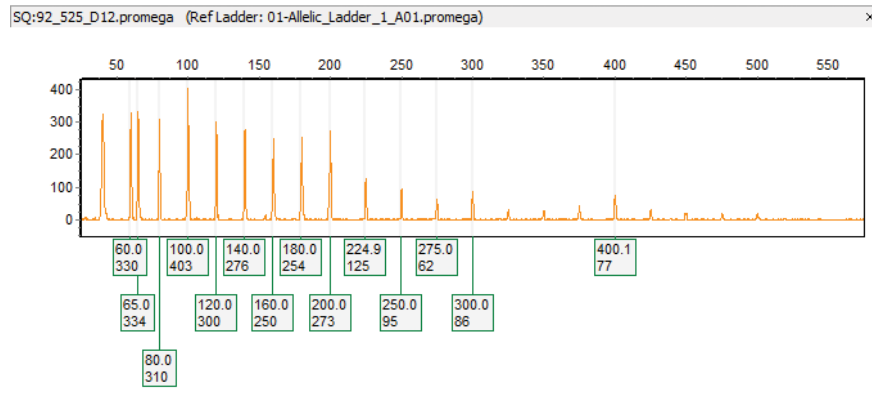


Figure 185. 'Main Analysis' window of the sample in Figure 166 showing one or more low-quality peaks not called by the software.

10.A.3 Analysis Quality

Samples, including controls and ladders, have defined analysis parameters that are applied during data processing. When one or more parameters are not met, a sample is flagged for Analysis Quality with a red question mark (Section 5.A) before the file name in the Sample File Tree and the Report Table of the 'Main Analysis' window. The parameter(s) not met, including the peaks that triggered the Analysis Quality flag, and the corresponding quality reasons are indicated in the allele label within the electropherogram and listed in the associated Peak Table (Section 5.B.2). If all peaks triggering the Analysis Quality flag are addressed through allele editing (Section 5.B.3), the red question mark will be replaced with an "E" in the Sample File Tree.

Also flagged are samples with null marker(s) and samples with Amelogenin and Y-STR marker conflict(s) for STR multiplexes that amplify Amelogenin and Y-STR markers. Samples are evaluated for Amelogenin and Y-STR marker conflicts only when "Allele Peak Issues Flag '?'" is applied in the 'Sample Quality' tab of the 'Preferences' window (Section 6.B).

The following situations will cause an 'Analysis Quality' flag.

Controls and Ladders

Ladders: The software compares all allelic ladder peaks to the expected bin positions as defined by the applied panel. Allelic ladders are flagged for Analysis Quality when there is an error in bin shifting (Section 4.C.2; Figure 186), when peaks in virtual bins have greater than expected peak heights (i.e., resemble real peaks, Figure 187) or if the expected peaks cannot be identified in a marker. When expected peaks cannot be identified in a marker, the marker bar will be shaded dark green, indicating the marker failed analysis.

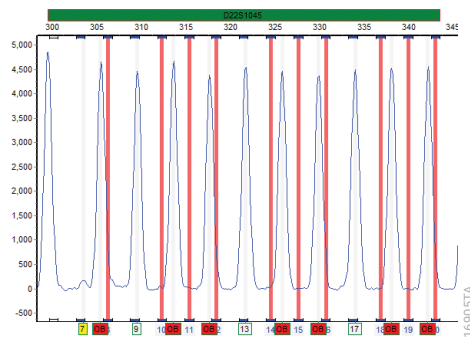


Figure 186. Example of a ladder that failed analysis.

When errors in bin shifting occur or when peaks in virtual bins have higher than expected peak heights, the marker bars will be outlined in red (Figure 187).

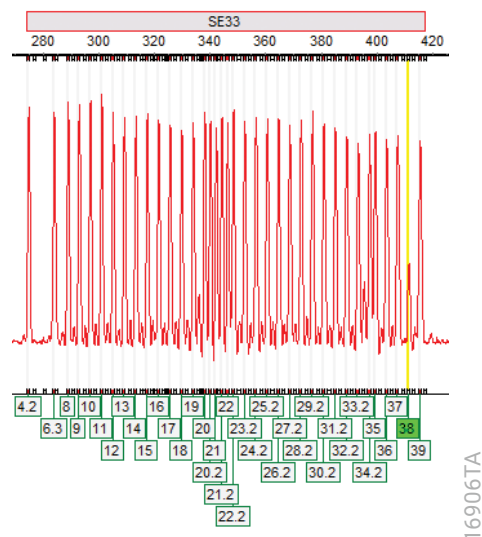


Figure 187. Example of higher than expected peak height for a ladder peak in a virtual bin.

Any peaks that cause an Analysis Quality flag are indicated in the electropherogram and Peak Table. File names for allelic ladders that pass all quality metrics and are available for use as best ladders for the Auto Select Best Ladder function (Section 4.C.1) are shown in bold.

When **Flag Variant Alleles in Ladder** is selected in the 'Display' tab of the 'Preferences' window (Section 6.B), peaks in virtual bins with higher than expected peak heights are indicated by yellow vertical bars and green allele labels (Figure 187). You can set your preferences to automatically delete these peaks by selecting **Auto-Delete Alleles in Virtual Bins in Allelic Ladder** in the 'Forensics' tab of the 'Preferences' window.

Peaks that are not present in the expected bins are marked with red vertical bars and red allele labels (Figure 188).

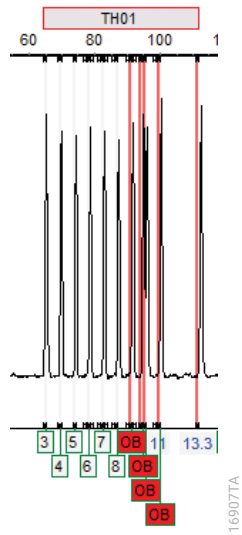


Figure 188. Example ladder peaks not in the expected bins, highlighted in red.

Positive controls: Positive control samples are compared to the positive control template(s) designated in the 'Additional Settings - HID Analysis' window of the Run Wizard (Section 4.C). When peaks in a positive control sample match the peaks in the positive control template, the positive control is concordant (Figure 190). Positive controls are flagged for Analysis Quality if expected peaks migrate outside of the expected bins, extraneous peaks are detected or any sample quality criteria are not met (see below). If extraneous peaks are detected or an expected peak does not fall within the appropriate bin, the marker bar will be outlined in red. Peaks that migrate outside of the expected bin are marked with red vertical bars (Figure 189).

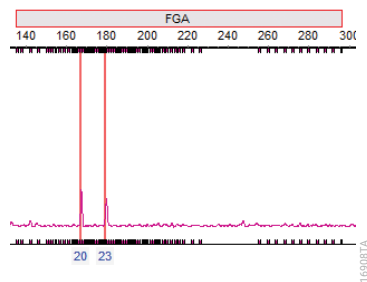


Figure 189. Example of positive control peaks outside of the expected bins marked with red vertical bars.

Extraneous peaks detected in the positive controls are marked with yellow vertical bars and green allele labels (Figure 191). Positive controls that do not meet Analysis Quality criteria are flagged with a red question mark before the file name in the Sample File Tree and Report Table. The parameter(s) not met, including which peaks triggered the Analysis Quality flag and the corresponding quality reasons (Table 21), are indicated in the allele label within the electropherogram and listed in the associated Peak Table (Section 5.B.2).

In Figure 190, the negative controls and one of the positive controls do not exhibit Analysis Quality flags. One of the positive controls is discordant and exhibits an Analysis Quality flag in the Sample File Tree.

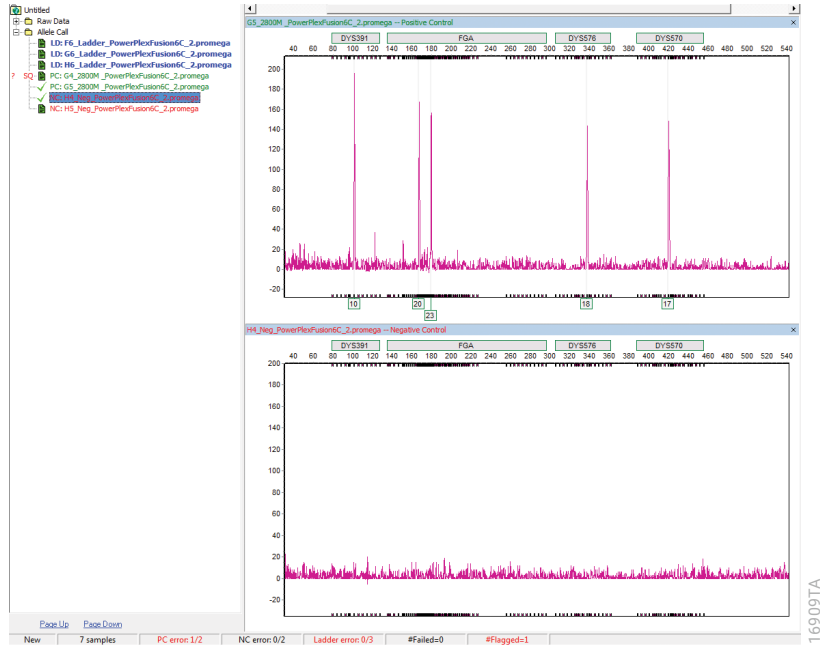


Figure 190. Examples of a concordant positive control (top) and negative control (bottom) that do not exhibit 'Analysis Quality' flags.

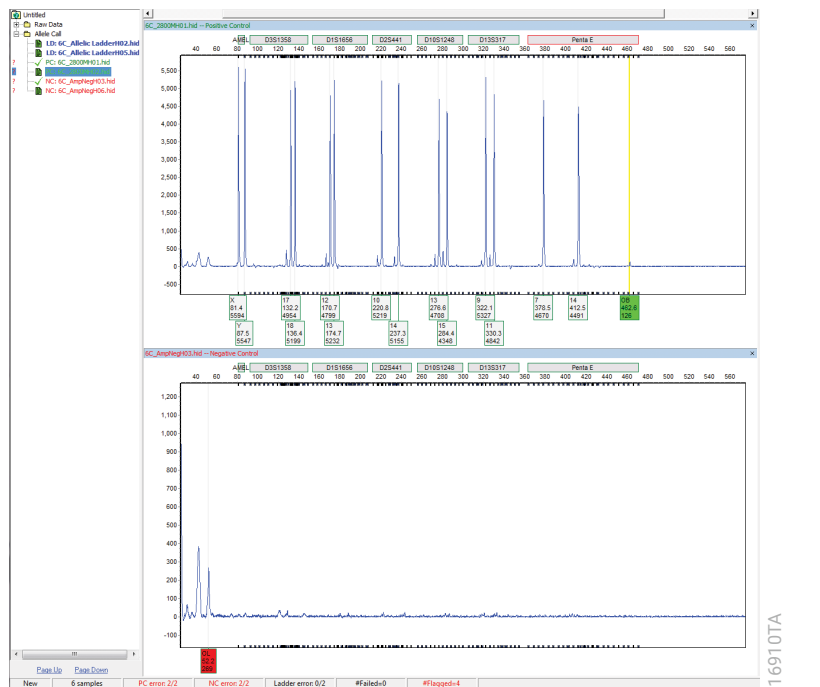


Figure 191. Discordant positive control (top) and negative control (bottom) that exhibit flags for extraneous peaks.

Negative controls: To be considered concordant, negatives must be devoid of called peaks. Negative controls will be flagged if peaks are present in the sample (Figure 191).

Samples

Sample quality is assessed using the settings established in the Run Wizard (Section 4), including the applied panel (Section 4.A.1). Each sample peak that does not meet a quality criterion is flagged by a colored allele label in the electropherogram, by a colored allele symbol in the Report Table (Section 5.E) and by colored font in the 'Quality' column of the Peak Table (Section 5.B.2). Yellow flags indicate peaks with a quality designation of "Check". Red flags indicate a quality designation of "Undetermined". Peaks with quality designations of "Check" or "Undetermined" should be evaluated.

Each peak with a "Check" or "Undetermined" designation is assigned a quality reason (Table 21). The "PL", "OB" and "OL" quality reasons are associated with a quality designation of "Undetermined" (red flags). All other quality reasons are associated with a quality designation of "Check" (yellow flags).

Note: Peaks that exceed the maximum peak intensity threshold set in the 'Data Process - HID Analysis' window of the Run Wizard (Section 4.B.3) are assigned a Pass quality designation; however, they will be denoted as "HI" for the quality reason.

You can view the quality reason by hovering over the allele label of the flagged peak in the electropherogram or in the 'Quality Reason' column of the Peak Table. Table 21 lists quality reasons in the software. Definitions of these abbreviations are available by selecting the **Help** icon in the Report Table.

Table 21. Quality Reason Acronyms in GMHID-Spectrum.

Acronym	Name	Comments
BC	Bin Conflict	Peak overlaps two or more bin ranges.
HI	High Intensity	Peak intensity approaches or exceeds the maximum peak intensity threshold set in the 'Data Process - HID Analysis' window (Section 4.B.3).
IHE	Inconclusive Heterozygous	Peak intensity is within the heterozygous inconclusive range for the marker set in the 'Edit Panel' window of the Panel Editor (Section 4.A.1).
IHO	Inconclusive Homozygous	Peak intensity is within the homozygous inconclusive range for the marker set in the 'Edit Panel' window of the Panel Editor (Section 4.A.1).
IMB	Heterozygote Imbalance	The balance of heterozygous peaks is below the maximum value set in the 'Edit Marker' window of the Panel Editor (Section 4.A.1).
LO	Low Intensity	Intensity of a single peak is below the 'Minimum Homozygote Intensity' threshold set in the 'Edit Marker' window of the Panel Editor because a second peak was detected above the N-X stutter filter percentage value (Section 4.A.1).
LS	Low Score	Peak has a peak score below the threshold for a passing peak score (Section 4.C.4).
OB	Out of Bin	Peak is within the marker range but outside of a bin.
OL	Off Ladder	Peak is outside of the marker range.
PL	Beyond Ploidy	The number of peaks identified within a marker exceeds the maximum number of peaks expected as set in the 'Edit Panel' window of the Panel Editor (Section 4.A.1).
SD	Saturation Detection	Peak intensity exceeds the linear range of the CE instrument. Peak exhibits a morphology characteristic of a saturated peak or exhibits pull-up peaks associated with saturated peaks.
SP	Saturated (Pull-up)	Pull-up peak associated with a Saturated (Repaired), SR, peak (see below).
SR	Saturated (Repaired)	Peak has a peak morphology characteristic of saturated peaks but was "repaired" for allele calling (Section 4.B.1).

10.A.4 Quality Indicators

Analysis parameters can be set for assessing the quality indicator peaks, in STR kits containing them. To choose this option, known as Q (Quality Indicator) flagging, select the "Sample Quality Peak(s)" box in the 'Additional Settings - HID Analysis' window of the Run Wizard, then select the appropriate peaks for the Small Allele and Large Allele quality indicators. (Section 4.C.4). Set the threshold for quality indicator flagging in the 'Preferences' window (Section 6.B).

Samples with quality indicator peak ratios below the 'Minimum intensity ratio' are marked with a red Q over the Size Match Score in the Sample File Tree of the 'Main Analysis' window and in the upper left corner of the sample electropherogram (Figure 192).

Hover over the Q flag in the electropherogram to display the reason ('Q- Peak imbalance, e.g. PCR inhibition'). Select the Q flag in the electropherogram, to display the 'Sample Quality Detail' window with the calculated 'Quality Intensity Ratio' (Figure 192).



Figure 192. Example of quality indicator flagging in the 'Main Analysis' window.

10.B. Contamination Check

You can compare analyzed profiles within a project against each other and against the laboratory's established Contamination Database (Section 11) using the Contamination Check feature. Before using the Contamination Check feature, process data through the Run Wizard and review the data, editing as necessary. To access this feature, select **Contamination Check** from the 'Project' menu of the 'Main Analysis' window. This opens the 'Contamination Check' window, which allows you to define search and display parameters as well as to save the results of the contamination check (Figure 193).

10.B.1 Navigating the 'Contamination Check' Window

The 'Contamination Check' window contains five sections to define search and display options, a toolbar and a list of search results.

The screenshot shows the 'Contamination Check' window with the following settings:

- Exclude Sample Setting:** Ladder (checked), NC (checked), PC (checked), With more than 0 null Markers, With more than 3 Markers that have Allele F.
- Other Settings:** Export Similarity Ratio >= 0 %, Specified Sample as Composition: 3_Promega 6 Dye (ISCL_Exercise 3).
- Match Type:** Within Project (checked), With Database (checked), DB as Composition (unchecked).
- Highlight Groups:** By Order (5), By Similarity Range (5 %).
- Display Name:** File Name, Sample Name.

The results table is as follows:

Group No.	Comparison Sample	Reference Sample	Matched/Total Reference	Similarity Ratio	Matched Markers(Aleles)
1	3_Promega 6 Dye (ISCL_Exercise 3 - Import Plate...	3_Promega 6 Dye (ISCL_Exercise 3 - Import Plate...	48/48	100.00%	AMELKY.Y, D3S1358(17.18), D1S1656(12.13), D2S...
2	3_Promega 6 Dye (ISCL_Exercise 3 - Import Plate...	3_Promega 6 Dye (ISCL_Exercise 3 - Import Plate...	48/48	100.00%	AMELKY.Y, D3S1358(17.18), D1S1656(12.13), D2S...
3	4_Promega 6 Dye (ISCL_Exercise 3 - Import Plate...	4_Promega 6 Dye (ISCL_Exercise 3 - Import Plate...	48/48	100.00%	AMELKY.Y, D3S1358(17.18), D1S1656(12.13), D2S...
4	4_Promega 6 Dye (ISCL_Exercise 3 - Import Plate...	6_Promega 6 Dye (ISCL_Exercise 3 - Import Plate...	48/48	100.00%	AMELKY.Y, D3S1358(17.18), D1S1656(12.13), D2S...
5	4_Promega 6 Dye (ISCL_Exercise 3 - Import Plate...	6_Promega 6 Dye (ISCL_Exercise 3 - Import Plate...	48/48	100.00%	AMELKY.Y, D3S1358(17.18), D1S1656(12.13), D2S...
6	5_Promega 6 Dye (ISCL_Exercise 3 - Import Plate...	5_Promega 6 Dye (ISCL_Exercise 3 - Import Plate...	47/47	100.00%	AMELKY.Y, D3S1358(17.18), D1S1656(12.13), D2S441...
7	6_Promega 6 Dye (ISCL_Exercise 3 - Import Plate...	4_Promega 6 Dye (ISCL_Exercise 3 - Import Plate...	48/48	100.00%	AMELKY.Y, D3S1358(17.18), D1S1656(12.13), D2S...
8	3_Promega 6 Dye (ISCL_Exercise 3 - Import Plate...	6_Promega 6 Dye (ISCL_Exercise 3 - Import Plate...	47/48	97.92%	AMELKY.Y, D3S1358(17.18), D1S1656(12.13), D2S...
9	3_Promega 6 Dye (ISCL_Exercise 3 - Import Plate...	5_Promega 6 Dye (ISCL_Exercise 3 - Import Plate...	47/48	97.92%	AMELKY.Y, D3S1358(17.18), D1S1656(12.13), D2S...
10	4_Promega 6 Dye (ISCL_Exercise 3 - Import Plate...	5_Promega 6 Dye (ISCL_Exercise 3 - Import Plate...	47/48	97.92%	AMELKY.Y, D3S1358(17.18), D1S1656(12.13), D2S...
11	3_Promega 6 Dye (ISCL_Exercise 3 - Import Plate...	6_Promega 6 Dye (ISCL_Exercise 3 - Import Plate...	47/48	97.92%	AMELKY.Y, D3S1358(17.18), D1S1656(12.13), D2S...
12	4_Promega 6 Dye (ISCL_Exercise 3 - Import Plate...	5_Promega 6 Dye (ISCL_Exercise 3 - Import Plate...	47/48	97.92%	AMELKY.Y, D3S1358(17.18), D1S1656(12.13), D2S...
13	3_Promega 6 Dye (ISCL_Exercise 3 - Import Plate...	4_Promega 6 Dye (ISCL_Exercise 3 - Import Plate...	47/48	97.92%	AMELKY.Y, D3S1358(17.18), D1S1656(12.13), D2S...
14	5_Promega 6 Dye (ISCL_Exercise 3 - Import Plate...	5_Promega 6 Dye (ISCL_Exercise 3 - Import Plate...	47/48	97.92%	AMELKY.Y, D3S1358(17.18), D1S1656(12.13), D2S441...
15	5_Promega 6 Dye (ISCL_Exercise 3 - Import Plate...	4_Promega 6 Dye (ISCL_Exercise 3 - Import Plate...	47/48	97.92%	AMELKY.Y, D3S1358(17.18), D1S1656(12.13), D2S...
16	3_Promega 6 Dye (ISCL_Exercise 3 - Import Plate...	4_Promega 6 Dye (ISCL_Exercise 3 - Import Plate...	47/48	97.92%	AMELKY.Y, D3S1358(17.18), D1S1656(12.13), D2S...
17	5_Promega 6 Dye (ISCL_Exercise 3 - Import Plate...	6_Promega 6 Dye (ISCL_Exercise 3 - Import Plate...	47/48	97.92%	AMELKY.Y, D3S1358(17.18), D1S1656(12.13), D2S...
18	3_Promega 6 Dye (ISCL_Exercise 3 - Import Plate...	5_Promega 6 Dye (ISCL_Exercise 3 - Import Plate...	47/48	97.92%	AMELKY.Y, D3S1358(17.18), D1S1656(12.13), D2S...
19	6_Promega 6 Dye (ISCL_Exercise 3 - Import Plate...	4_Promega 6 Dye (ISCL_Exercise 3 - Import Plate...	47/48	97.92%	AMELKY.Y, D3S1358(17.18), D1S1656(12.13), D2S...
20	4_Promega 6 Dye (ISCL_Exercise 3 - Import Plate...	5_Promega 6 Dye (ISCL_Exercise 3 - Import Plate...	46/47	97.87%	AMELKY.Y, D3S1358(17.18), D1S1656(12.13), D2S441...
21	3_Promega 6 Dye (ISCL_Exercise 3 - Import Plate...	5_Promega 6 Dye (ISCL_Exercise 3 - Import Plate...	46/47	97.87%	AMELKY.Y, D3S1358(17.18), D1S1656(12.13), D2S441...
22	3_Promega 6 Dye (ISCL_Exercise 3 - Import Plate...	6_Promega 6 Dye (ISCL_Exercise 3 - Import Plate...	46/47	97.87%	AMELKY.Y, D3S1358(17.18), D1S1656(12.13), D2S441...
23	6_Promega 6 Dye (ISCL_Exercise 3 - Import Plate...	5_Promega 6 Dye (ISCL_Exercise 3 - Import Plate...	46/47	97.87%	AMELKY.Y, D3S1358(17.18), D1S1656(12.13), D2S441...
24	4_Promega 6 Dye (ISCL_Exercise 3 - Import Plate...	5_Promega 6 Dye (ISCL_Exercise 3 - Import Plate...	46/47	97.87%	AMELKY.Y, D3S1358(17.18), D1S1656(12.13), D2S441...
25	4_Promega 6 Dye (ISCL_Exercise 3 - Import Plate...	3_Promega 6 Dye (ISCL_Exercise 3 - Import Plate...	47/49	95.82%	AMELKY.Y, D3S1358(17.18), D1S1656(12.13), D2S...
26	4_Promega 6 Dye (ISCL_Exercise 3 - Import Plate...	3_Promega 6 Dye (ISCL_Exercise 3 - Import Plate...	47/49	95.82%	AMELKY.Y, D3S1358(17.18), D1S1656(12.13), D2S...
27	4_Promega 6 Dye (ISCL_Exercise 3 - Import Plate...	3_Promega 6 Dye (ISCL_Exercise 3 - Import Plate...	47/49	95.82%	AMELKY.Y, D3S1358(17.18), D1S1656(12.13), D2S...
28	5_Promega 6 Dye (ISCL_Exercise 3 - Import Plate...	3_Promega 6 Dye (ISCL_Exercise 3 - Import Plate...	47/49	95.82%	AMELKY.Y, D3S1358(17.18), D1S1656(12.13), D2S...
29	6_Promega 6 Dye (ISCL_Exercise 3 - Import Plate...	3_Promega 6 Dye (ISCL_Exercise 3 - Import Plate...	47/49	95.82%	AMELKY.Y, D3S1358(17.18), D1S1656(12.13), D2S...
30	4_Promega 6 Dye (ISCL_Exercise 3 - Import Plate...	3_Promega 6 Dye (ISCL_Exercise 3 - Import Plate...	47/49	95.82%	AMELKY.Y, D3S1358(17.18), D1S1656(12.13), D2S...
31	5_Promega 6 Dye (ISCL_Exercise 3 - Import Plate...	3_Promega 6 Dye (ISCL_Exercise 3 - Import Plate...	47/49	95.82%	AMELKY.Y, D3S1358(17.18), D1S1656(12.13), D2S...
32	5_Promega 6 Dye (ISCL_Exercise 3 - Import Plate...	3_Promega 6 Dye (ISCL_Exercise 3 - Import Plate...	47/49	95.82%	AMELKY.Y, D3S1358(17.18), D1S1656(12.13), D2S...
33	5_Promega 6 Dye (ISCL_Exercise 3 - Import Plate...	4_Promega 6 Dye (ISCL_Exercise 3 - Import Plate...	46/48	95.83%	AMELKY.Y, D3S1358(17.18), D1S1656(12.13), D2S441...
34	5_Promega 6 Dye (ISCL_Exercise 3 - Import Plate...	6_Promega 6 Dye (ISCL_Exercise 3 - Import Plate...	46/48	95.83%	AMELKY.Y, D3S1358(17.18), D1S1656(12.13), D2S441...
35	6_Promega 6 Dye (ISCL_Exercise 3 - Import Plate...	4_Promega 6 Dye (ISCL_Exercise 3 - Import Plate...	48/51	94.12%	AMELKY.Y, D3S1358(17.18), D1S1656(12.13), D2S...
36	4_Promega 6 Dye (ISCL_Exercise 3 - Import Plate...	4_Promega 6 Dye (ISCL_Exercise 3 - Import Plate...	48/51	94.12%	AMELKY.Y, D3S1358(17.18), D1S1656(12.13), D2S...
37	5_Promega 6 Dye (ISCL_Exercise 3 - Import Plate...	3_Promega 6 Dye (ISCL_Exercise 3 - Import Plate...	46/49	93.88%	AMELKY.Y, D3S1358(17.18), D1S1656(12.13), D2S441...
38	5_Promega 6 Dye (ISCL_Exercise 3 - Import Plate...	3_Promega 6 Dye (ISCL_Exercise 3 - Import Plate...	46/49	93.88%	AMELKY.Y, D3S1358(17.18), D1S1656(12.13), D2S441...
39	3_Promega 6 Dye (ISCL_Exercise 3 - Import Plate...	4_Promega 6 Dye (ISCL_Exercise 3 - Import Plate...	47/51	92.16%	AMFI BX.Y, D3S1358(17.18), D1S1656(12.13), D2S...

Figure 193. 'Contamination Check' window.

Exclude Sample Setting: Excludes certain samples from the search: ladders, negative controls (NC), positive controls (PC), samples with greater than the specified number of null markers or samples with greater than the specified number of markers with greater than the specified number of alleles.

Other Settings: Refines the comparison parameters.

Export Similarity Ratio Greater Than: Defines the percent similarity required for the comparison sample and reference sample to be considered a match. Setting this to zero will display all possible matches.

Similarity Ratio is calculated as:
$$\frac{\text{Number of reference sample alleles included in the comparison sample}}{\text{Total number of reference sample alleles}} \times 100$$

Percent similarity is calculated using only the called alleles observed in each sample.

Specified Sample as Comparison: You can select a specific sample from the project as the comparison sample. To activate this option, check the box in the field and select the desired sample from the drop-down list. To compare all samples in the project, leave this box unchecked.

Match Type: Specifies the type of search to perform: “Within Project” or “With Database”. When the **With Database** type is selected, use the drop-down menu to select the Contamination Database.

Note: It is also possible to select and perform a search against the Relationship database. Genotypes must be submitted to the respective database (Section 11.A) before a contamination check can be performed.

By default, samples in the project are the comparison samples and the database samples (i.e., samples with “DB” in the sample name) are the reference samples for a With Database search. To run the reverse search (database samples as the comparison samples and project samples as the reference samples), check the “DB as Comparison” box. In a Within Project search, samples within a project are compared to each other. Each project sample is both a comparison sample and a reference sample.

Highlight Groups: Highlights groups of matches based on one of two criteria.

By Order: Samples are displayed in groups based on the number entered into this field, and groups have alternate shading of white and gray. In the example shown in Figure 194, samples are highlighted in groups of 2.

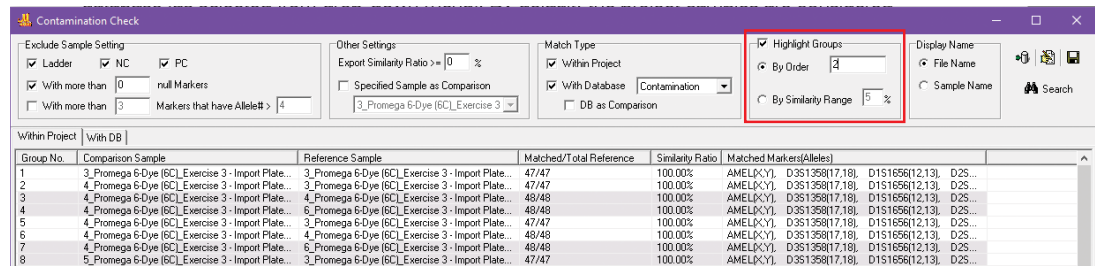


Figure 194. Highlight Groups By Order.

By Similarity Range: Samples are displayed as groups based on their similarity ratios. For example, if the user enters 5 in this field (Figure 195), groups include samples with similarity ratios in increments of 5%. All matches with 100% similarity ratios are grouped together, followed by all matches with 95–99% similarity ratios, matches with 90–94% similarity ratios, etc. Groups are indicated by alternate shading.

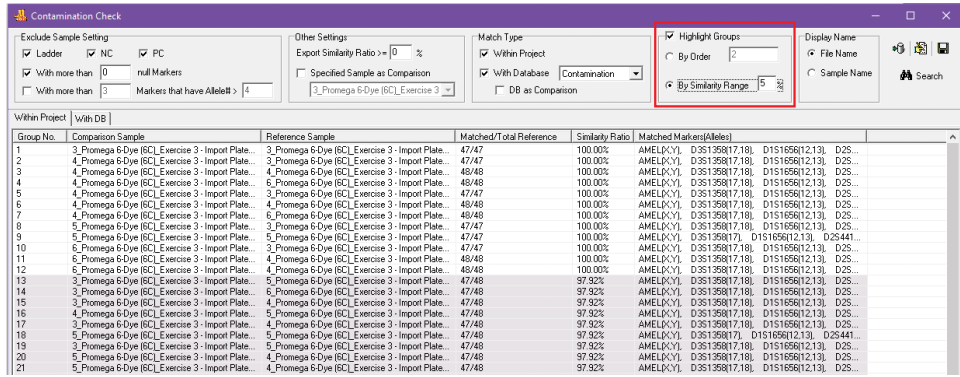






Figure 195. Highlight Groups by Similarity Range.

Display Name: You can choose to display samples by File Name or Sample Name.

Search: After customizing these search and display options, be sure to select the **Search** icon to initiate the contamination check. If you change any of these options, be sure to select the **Search** icon to refresh the search results.

The 'Contamination Check' window also includes a toolbar, which has the following icons and functions.

Table 22. Icons and Functions of the 'Contamination Check' Window.

	Save to Database: Opens the 'Submit Genotypes' window, which enables saving of genotypes to the Contamination Database. Upload genotypes from samples in the current project or samples saved in a .txt file or .cmf file (Section 11.A).
	Edit Database: Opens the 'Contamination Database Editor' window from which to manage, edit and delete genotypes in the Contamination Database (Section 11.B).
	Search: Initiates the contamination check and refreshes search results if search or display options are changed.
	Save Report: Opens the 'Save Report' window. You can export the results of the contamination check as an Excel (.xlsx), text (.txt) or CSV (.csv) file.

The 'Contamination Check' window includes a list of search results. The name of the tab indicates the type of match search ('Within Project' or 'With DB'). Each list contains six columns of information. You can sort the list by each column, except 'Group No.', by clicking on the column header.

Group No.: Displays the sequential number assigned to each profile comparison. Samples are displayed numerically by group number.

Comparison Sample: Lists the sample being used for comparison.

Reference Sample: Lists the reference sample being used for comparison.

Matched/Total Reference: Lists the number of matched markers and number of total markers in the reference sample. For example, a “47/47” in this column indicates that 47 of the markers in the comparison sample matched the 47 markers in the reference sample.

Similarity Ratio: Lists the calculated similarity ratio (see equation above).

Matched Markers (Alleles): Lists the markers that matched between the comparison and reference samples, with the matching allele(s) listed in parentheses.

10.B.2 Performing a Contamination Check

You can perform a contamination check between samples within a project once the raw data is processed and quality flags are addressed. The example below details the process using two samples: a two-person mixture (Mix01_1ng_M-F_19-1) and a single-source sample (Mix01_1ng_M-F_1-0).

The search and display parameters were set as follows (Figure 196):

Exclude Sample Setting: Exclude ladders and controls as well as samples with 3 null markers.

Export Similarity Ratio Greater Than: Display all samples with similarities greater than 0%.

Match Type within Project: Perform all sample-to-sample comparisons within the project.

Match Type with Database: Check for matches within the Contamination Database.

Highlight Groups: Not selected.

Display Name: Display the samples by file name.

The example in Figure 196 shows the matches within the project. In Group No. 1, 100% of the reference sample alleles (i.e., the single-source sample alleles) are included in the comparison sample (i.e., the mixture sample). In Group No. 2, 65.33% of the reference sample alleles (i.e., the mixture sample alleles) are included in the comparison sample (i.e., the single-source sample alleles). The last column of each row contains the shared genotype.

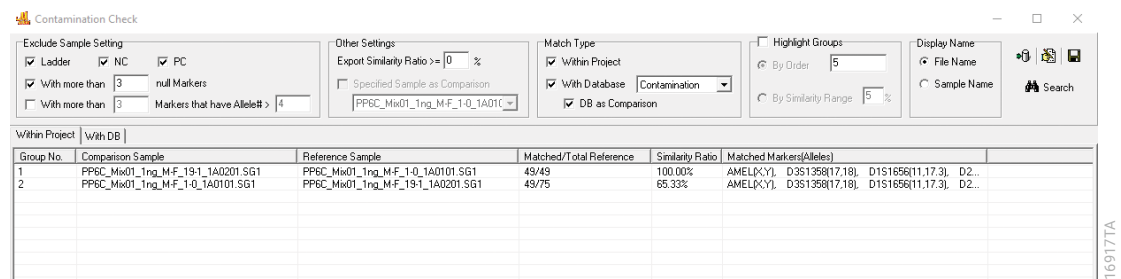


Figure 196. Example of the 'Contamination Check' window showing matches within the project.

The example in Figure 197 shows matches with samples in the selected Contamination Database. For the purposes of this demonstration, the single-source file in the above example and several other single-source files were previously saved to the Contamination Database to provide matches to the two samples within this project. The results indicate that 100% of alleles in the Mix01_1ng_M-F_1-0 sample are included in the single-source sample (comparison sample for Group No. 1) and in the mixture sample (comparison sample for Group No. 2). The comparison samples for group numbers 3–6 share less than 50% of the alleles present in two reference samples in the Contamination Database (NIST 2391 C-C and NIST 2391 C-A).

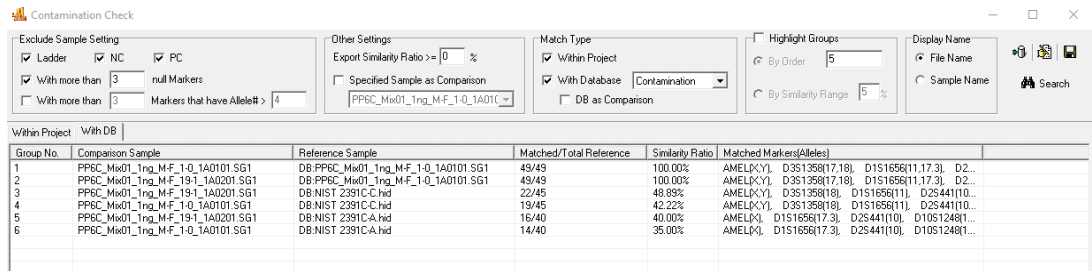


Figure 197. Example of the 'Contamination Check' window showing matches with the Contamination Database.

10.B.3 Submitting Genotypes to the Contamination Database from the 'Contamination Check' Window

You can save profiles to the Contamination Database from within the 'Contamination Check' window. To add samples to the database, select the **Save to Database** icon (Table 22). This opens the 'Submit Genotypes' window, with a list of all project samples (Figure 198). By default all samples are marked for submission. To remove samples from the list, deselect the check box in that sample row. The 'Name', 'Department' and 'Comments' columns can be edited. Information added to these columns will be saved with the sample(s) in the database. When all samples are marked appropriately, select the **Submit** button. The selected samples are now included in the Contamination Database. Samples that are not part of the current project also can be added to the Contamination Database from this window following instructions in Section 11.A.

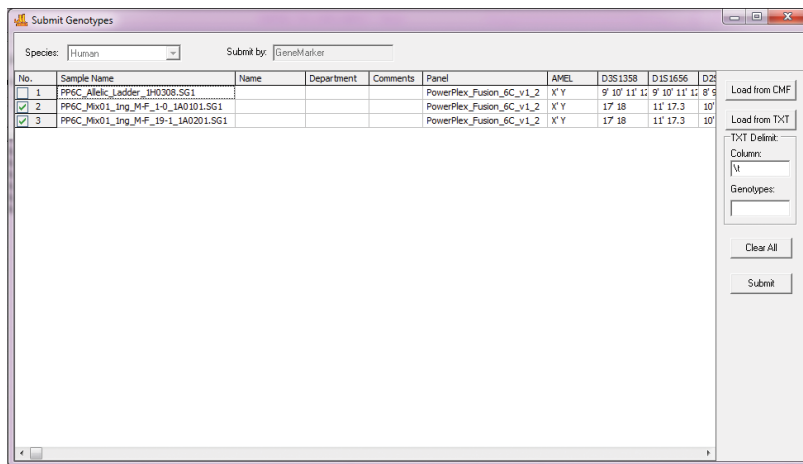


Figure 198. 'Submit Genotypes' window.

11

Database Management

GMHID-Spectrum contains two databases for profile searching: contamination and relationship. The Contamination Database allows you to store profiles to be searched using the 'Contamination Check' feature (Section 10.B). The Relationship Database allows you to store profiles to be searched using the Relationship Testing application. For further information on using the Relationship Testing application, contact Promega Technical Services at: **genetic@promega.com**. You can manage the information contained within each database through the Database Manager, which is accessible via the 'Tools' menu in the 'Main Analysis' window (Figure 199). There are three options for database management: Submit Genotypes to DB, Contamination DB and Relationship DB.

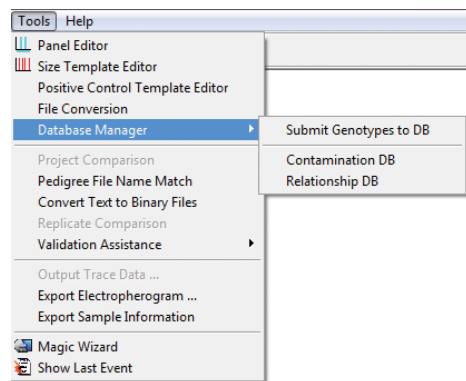


Figure 199. 'Database Manager' menu.

11.A. Submitting Genotypes to a Database

You can submit genotypes to a database by selecting **Submit Genotypes to DB** from the Database Manager in the 'Tools' menu. This opens the 'Submit Genotypes' window (Figure 200). There are three fields shown at the top of the window:

Species: Displays "Human" by default and cannot be changed in GMHID-Spectrum.

Submit by: Displays "GeneMarker" by default and cannot be changed in GMHID-Spectrum.

Submit to: Provides a drop-down menu to select the appropriate database for the sample(s).

Note: Genotypes also can be submitted directly to the Contamination Database from the 'Contamination Check' window (Section 10.B.3).

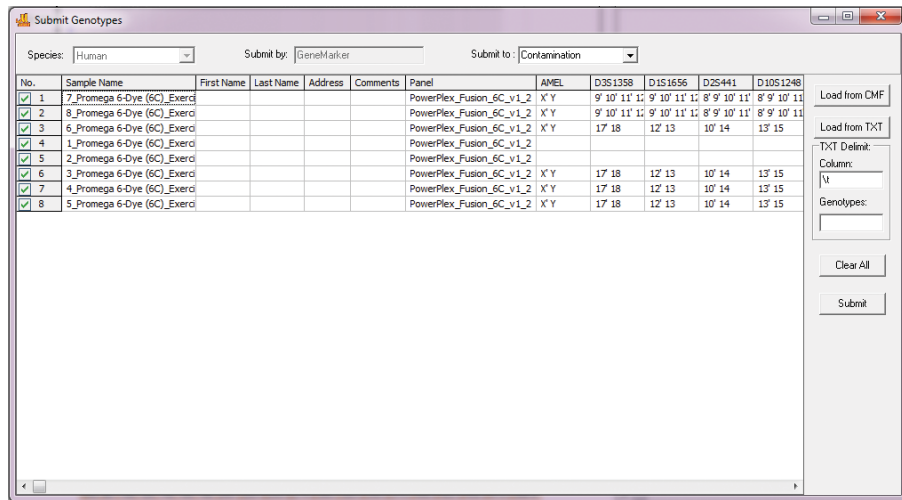


Figure 200. 'Submit Genotypes' window.

To submit a sample to the Contamination Database, you must first import samples into the 'Submit Genotypes' window. Samples can be imported from either .cmf or .txt files.

Importing genotypes from a .cmf file:

1. Select **Load from CMF** on the right side of the window.
2. This opens the 'Open CMF Files' window. Select **Add...** to open a navigation window to locate the desired file(s).
3. Select **OK** to return to the 'Submit Genotypes' window.
4. The genotypes from the selected file(s) are listed in the window. Select **Submit** to add the genotypes to the selected database.

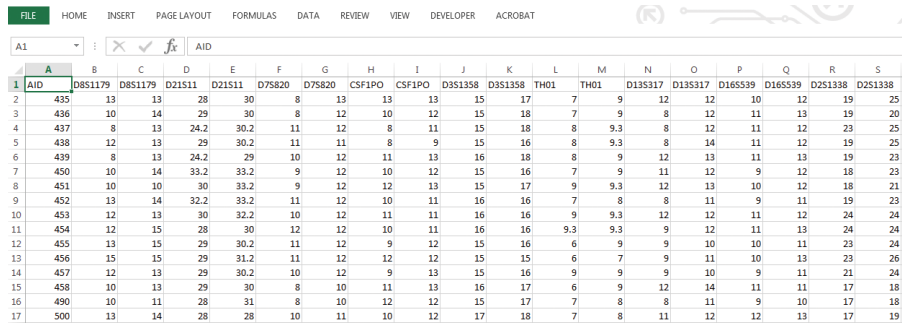


Figure 201. Required spreadsheet format for importing genotypes as .txt files.

Importing Genotypes from a .txt File

Genotypes imported as a text (.txt) file must first be entered into a Microsoft Excel® spreadsheet or another spreadsheet program. Samples must have the exact format shown in Figure 201.

1. The term "AID" (accession ID number) must be written in the upper left corner of the spreadsheet.
2. List each marker name twice (once for each allele).
3. For samples that have no data at certain markers, type ****** into those marker cells. Blank cells are not allowed.
4. When all genotypes are entered, save the file as a tab-delimited text file.
5. In the 'Submit Genotypes' window, ensure the appropriate text file delimiters are represented in the Column and Genotypes field of the TXT Delimit section.
 - a. Ensure that **\\t** is entered in the Column field to indicate the columns are tab-delimited.
 - b. Leave the Genotypes field blank.
6. Select **Load from TXT** from the right side of the 'Submit Genotypes' window to open a navigation window.
7. Browse to and select the appropriate text file. Select **Open** to start the import process. A 'Load data from text file...' window will track the file import process and indicate how many samples were imported successfully.
8. Select **OK** to return to the 'Submit Genotypes' window (Figure 202). The genotypes from the selected file will be listed in the window.
9. Select **Submit** to add the genotypes to the selected database.

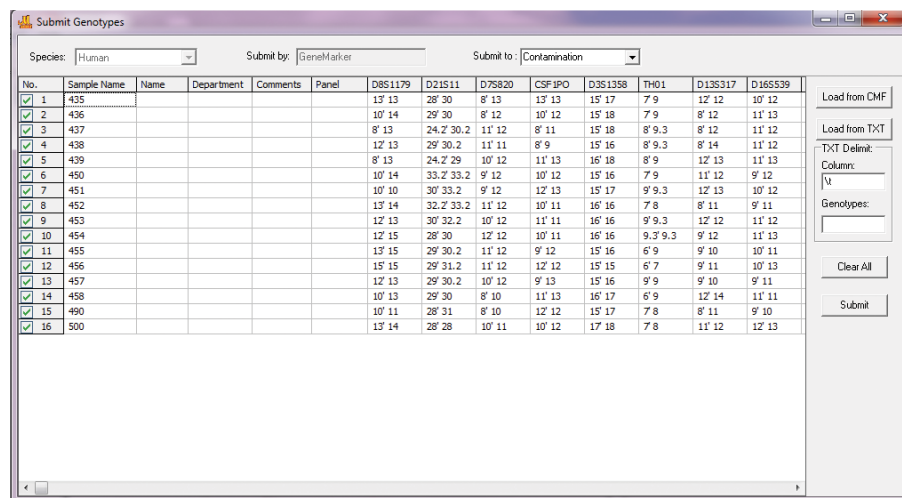


Figure 202. 'Submit Genotypes' window after the .txt file shown in Figure 200 was used to import genotypes.

Samples with the same name but genotyped using different STR chemistries can be submitted to (and combined in) the database. When you submit a sample with the same name as a sample already present in the database, a confirmation window opens to ask you if you want to overwrite the original sample (Figure 203). To accept the second sample into the database and combine the results of the two records, select **No**. To overwrite the existing sample with the new data, select **Yes**. Select **Cancel** to skip the sample(s) with the same names. Select **Abort** to cancel the genotype submission.

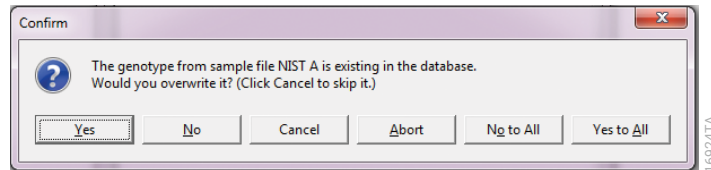


Figure 203. Choices presented if a genotype exists in the database when submitting.

11.B. Database Editors

Each GMHID-Spectrum database has its own database editor, which allows you to manage, edit or delete genotypes from the database. To edit a database, select the appropriate database from the list next to Database Manager in the 'Tools' menu (Figure 199). This opens the database editor for the selected database. The options for each database editor are the same (Table 23). The 'Contamination Database Editor' window is shown in Figure 204.

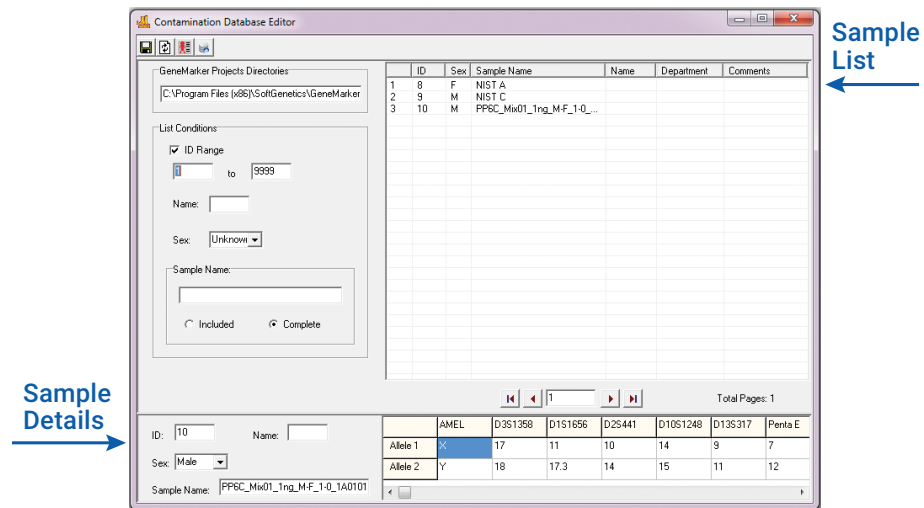


Figure 204. 'Contamination Database Editor' window.

The Contamination Database Editor contains a toolbar, sections to define directory and search display options, a Sample List section and a Sample Details section.

GeneMarker Projects Directories: Lists the file path for the directory of the chosen database.

List Conditions: Allows you to search for a specific sample or restrict the samples displayed in the list.





ID Range: Allows you to specify the samples displayed by defining the desired range of ID numbers. You can also limit samples in the list by Name, Sex or Sample Name. Choose the desired options, then select the **Refresh** icon in the toolbar to refresh the Sample List (Table 23).

Sample List (upper right corner of Contamination Database Editor): Displays all samples in the specified directory that meet the criteria specified in the 'List Conditions' section. Each sample is assigned a number, which is displayed in the first column. The remaining six columns contain sample information included when the sample was added to the database. Select the row of a specific sample to display its genotype in the 'Sample Details' section at the bottom of the screen.

If the list spans multiple pages, the total number of pages is listed at the bottom of the section and the arrows can be used to navigate through the pages. Use the < > buttons to move forward and backward one page, or use the |< >| buttons to move directly to the first and last page, respectively.

Sample Details (bottom of Contamination Database Editor): All fields in the Sample Details section are editable. To edit information on the left side of the section (ID, Name or Sample Name), click in the field and edit as needed. Use the drop-down list in the Sex field to select the appropriate designation. To edit information in the genotypes table on the right side, double-click on a cell and edit the value.

Table 23. Icons and Functions of the Contamination Database Editor.

	Save: Saves any changes that were made to a sample in the database. A confirmation window shows that the database was updated.
	Refresh: Updates the Sample List section. The search criteria specified in the 'List Conditions' section are not applied until you select the Refresh icon.
	Export CODIS: Opens the 'CODIS Export' window (Section 6.D), which can be used to export genotypes in CODIS format.
	Export Database (available only in the Contamination Database): Opens a 'Save As' window to create and export a report representing the sample information and genotypes displayed in the Sample List as either an Excel file (.xlsx) or text (.txt) file.

You can remove a sample from a database by right-clicking on the sample entry and selecting **Delete**. To select and delete multiple samples, hold down the **Control** key while selecting individual samples or hold down the **Shift** key while selecting groups of samples. A confirmation window will appear. Select **Yes** to remove the sample from the database and return to the Contamination Database Editor.

12 File Name Grouping and Profile Comparisons

12.A. File Name Group Editor

The File Name Group Editor (Figure 205) can be used to group samples in the dataset based on their file names for simplified reviewing of results and printing. The group designations defined in this editor are reflected in the Sample File Tree. This window is also used to modify file groups to allow profile comparison both between projects (Section 12.B) and within projects (replicate comparison, Section 12.C).

In addition to grouping samples by file name, you can define groups of samples using defined control match criteria (Figure 205). For example, samples can be grouped by their designation as controls or reference samples. In GMHID-Spectrum, control groups are typically defined only for use in the 'Relationship Testing' application (Section 6.D).

12.A.1 Navigating the File Name Group Editor

To use the File Name Group Editor, ensure "Enable Sample Grouping" is checked in the 'Others' tab of the 'Preferences' window, then select **Apply Sample Grouping** from the 'Project' menu (Section 6.B.6). This opens the 'File Name Group Editor' window. The 'File Name Group Editor' window contains a toolbar, two list sections, a section to define match criteria and a section to define control or reference samples (Figure 205). The File Name List section displays the file name for all samples loaded into the File Name Group Editor. The Matched Groups List section displays all groups that meet the specified match criteria.

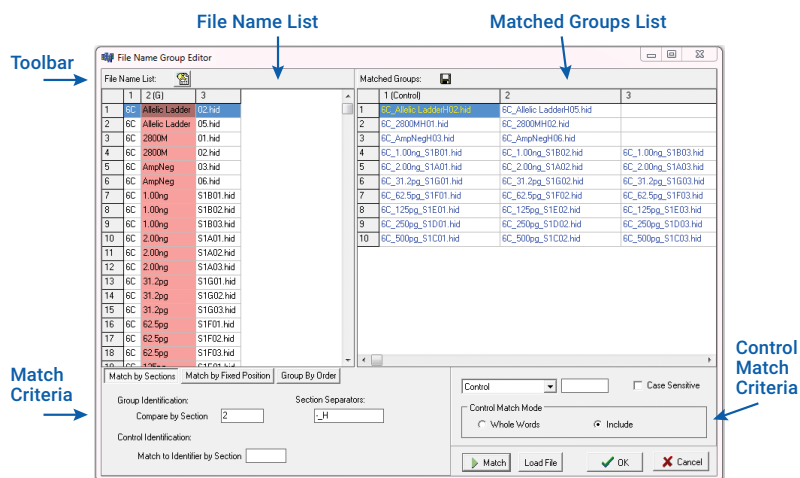




Figure 205. 'File Name Group Editor' window.

Table 24. Icons and Functions of the File Name Group Editor Toolbar.

	Edit File Name: Allows you to change file names to aid sorting if the file name from the instrument is not optimal for sorting. Any edits made to the file name are only applied in the File Name Group Editor. The file names remain unchanged outside of this window.
	Save Groups to File: Saves the file names of the samples grouped in the Matched Groups List section. Samples identified as controls are in the first column of the resulting Matched Groups text (.txt) file.

Samples can be grouped by sections of their file names (Match by Sections), fixed positions within the file names (Matched by Fixed Position) or group size (Group by Order) by selecting the corresponding tab in the match criteria section.

Match by Sections

The 'Match by Sections' tab allows you to group samples based on the designated section of their file names (Figure 205). File names are split into sections based on a Section Separator defined by the user. The user also must specify which section of the file name is used to group the samples.

Section Separators: Allows you to define which delimiter(s) (e.g., hyphens, underscores, periods and commas) define sections of a file name. For example, a sample with the file name A01_Sample 1_1ng.fsa would have three sections if an underscore was defined as the section separator.

	Section 1	Section 2	Section 3
1	A01	Sample 1	1ng.fsa

If both an underscore and period are defined as section separators, the same file name would have four sections:

	Section 1	Section 2	Section 3	Section 4
1	A01	Sample 1	1ng	fsa

Group Identification: Use the Compare by Section field to identify the section of the file names used to group the samples (i.e., first section, second section, third section, etc.). The specified section is highlighted in red in the File Name List, and the column heading includes "(G)" (Figure 206).

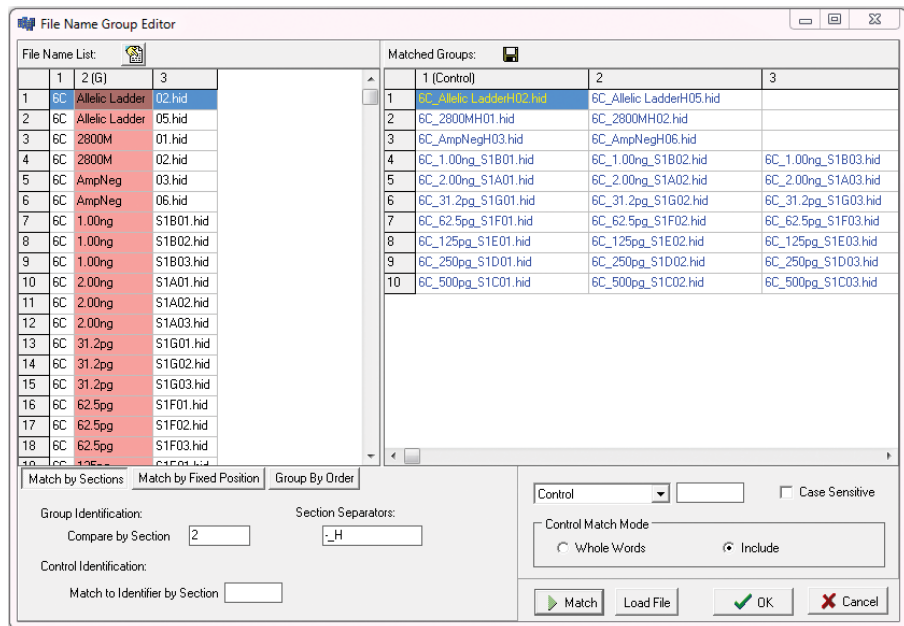


Figure 206. Example of samples grouped by the second file name section.

Control Identification: Use the 'Match to Identifier by Section' field to define the range of characters within a file name that identify a sample as a control or reference sample. The specified section of the file name is highlighted in green in the File Name List, and the column heading includes "(C)". Leave this field blank unless you are assessing paternity trios. Please contact Promega Technical Services at: genetic@promega.com for assistance in using the 'Relationship Testing' application for paternity trios.

Match by Fixed Position

The 'Match by Fixed Position' tab allows you to group samples by the character at the specified position within the file name (Figure 207). Each character, including section separators, is counted from left to right, starting at 1.

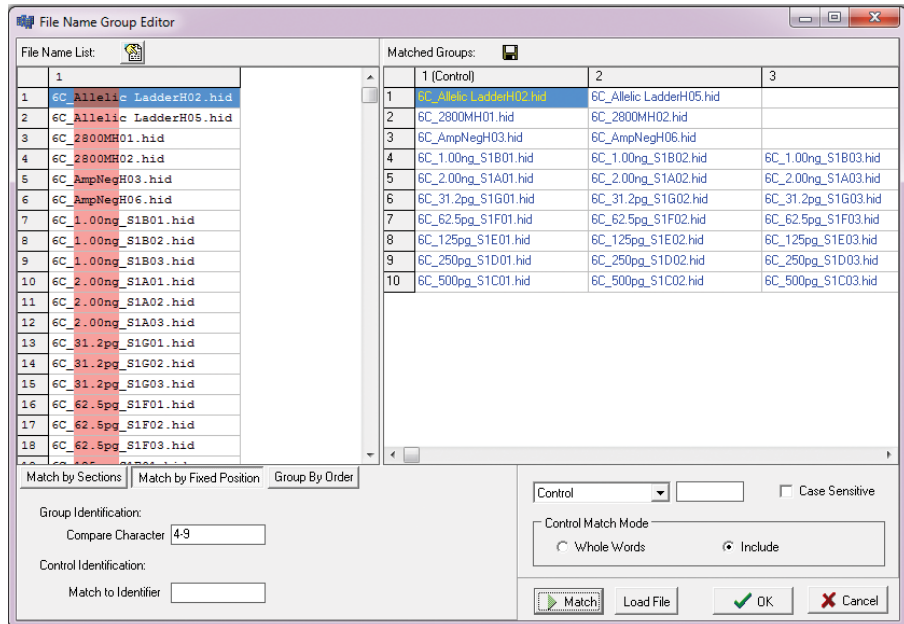


Figure 207. Samples grouped by characters at the specified position of the file name.

Group Identification: Use the Compare Character field to define the range of characters within a file name used to group the samples. The specified characters in the file name are highlighted in red in the File Name List (Figure 207).

Control Identification: Use the Match to Identifier field to define the range of characters within a file name that identify a sample as a control or reference sample. The specified characters within the file name are highlighted in green in the File Name List. Leave this field blank unless you are assessing paternity trios. Please contact Promega Technical Services at:

genetic@promega.com for assistance in using the 'Relationship Testing' application for paternity trios.

Group by Order

The 'Group by Order' tab groups samples by group size (Figure 208). Enter a number or use the arrows to adjust the number in the Group Size field to the desired group size. For example, if the dataset contains three replicates of each sample and the project is ordered so that replicates follow in succession, these three replicates will be grouped with a group size of 3. Because Group Size is set to 3, this group of replicates and any other groups of three, regardless if they are replicates or not, are displayed as groups in the File Name List and Matched Groups List sections. You can also group samples designated as positive and negative controls. You can define a control group using the fields in the Control Match Criteria section (Figure 205).

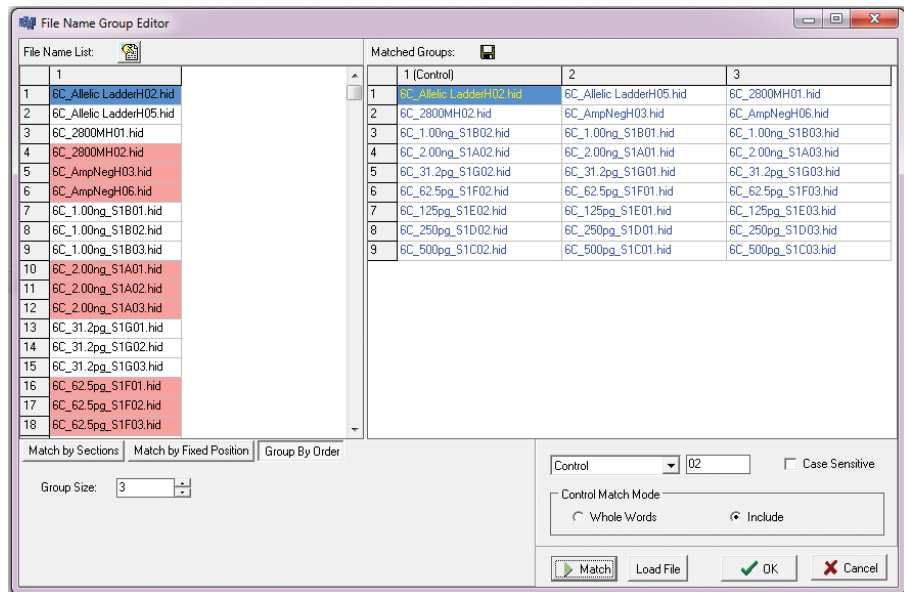


Figure 208. Samples arranged by group size.

Control Match Criteria

When using the Group by Order method to group control samples, the user completes the 'Control Match Criteria' section to define a control group. The software uses this information to group control samples based on characters within the file name. Enter the desired characters in the field to the right of the 'Control' drop-down field. Check the "Case Sensitive" box if the upper- or lowercase letters of the match criteria must match exactly.

Select **Whole Words** to look for file names that contain an exact match to the characters entered into the Control field.

Select **Include** to look for file names that include the characters in the Control field. An exact match is not required.

In the example shown in Figure 208, the user defined a control group as all samples that included "02" in the file name. The criteria are not case-sensitive.

12.A.2 Applying File Name Grouping

File name grouping can be applied to samples in the current open project. When the File Name Group Editor is opened, the software automatically loads file names for all project samples into the File Name List. You can add additional samples to the list by selecting the **Load File** button at the bottom of the editor (Figure 208).

Choose the desired match or group method, define the necessary identifiers and then select the **Match** button to apply the group matching criteria. The resulting groups will be displayed in the Matched Groups List. The first column in the Matched Groups List is labeled "Control" regardless of whether a control group was defined or not. When samples are grouped correctly, you can save the group matching criteria as a text (.txt) file by selecting the **Save Groups to File** icon (Table 24). You can apply previously saved match criteria to the same file names by selecting the **Load File** button at the bottom of the editor. To apply the match criteria to the project samples and return to the 'Main Analysis' window, select **OK**. The group designations are now displayed in the Sample File Tree.

12.B. Project Comparison Tool

The Project Comparison tool can be used to compare data analyzed in two different projects. Users may want to perform such a comparison to assess differences in: analyses between analysts, changes in run or analysis parameters or results generated from two different capillary electrophoresis instruments.

12.B.1 Navigating the Project Comparison Tool

To use the Project Comparison tool, open the project that will be used as the reference project, ensure file name grouping is applied such that reference samples are matched with their corresponding comparison samples (Section 12.A), then select **Project Comparison** from the 'Tools' menu of the 'Main Analysis' window (Section 6.E). This opens the 'Project Comparison' window, which contains a Toolbar, Electropherogram Charts (with optional Peak Table display), the Peak Comparison Table and the Comparison Report Table (Figure 209).

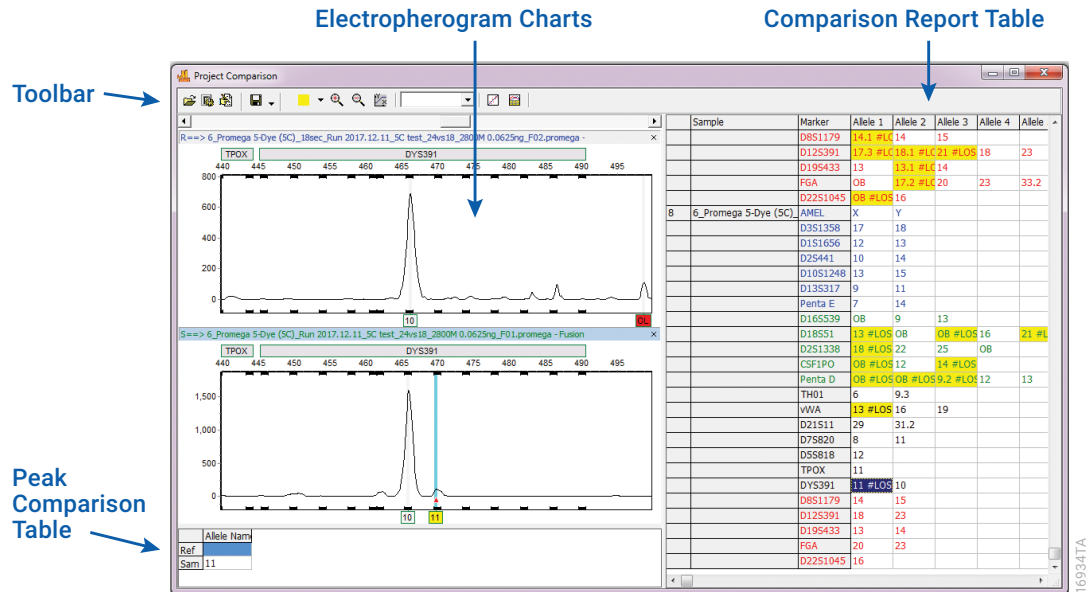


Figure 209. 'Project Comparison' window.

Select the **Open Project to Compare** icon (Table 25) in the toolbar to select the project to be compared to the reference project.

The Electropherogram Charts section displays the electropherogram for the selected reference profile (top) and the sample comparison profile (bottom). The display reflects the display settings chosen in the 'Preferences' window of the 'Main Analysis' window (Section 6.B) and from the toolbar of the 'Project Comparison' window (Table 25). The same zoom and navigation features available in the electropherogram of the 'Main Analysis' window are also available in this window. The same options are available by right-clicking within the electropherogram and Peak Table as are available in the 'Main Analysis' window.

Select the **Project Comparison Settings** icon (Table 25) to open the 'Project Comparison Settings' window (Figure 210). Information selected in this window is displayed in the Peak Comparison Table for the reference sample (ref) and comparison sample (sam) profiles.

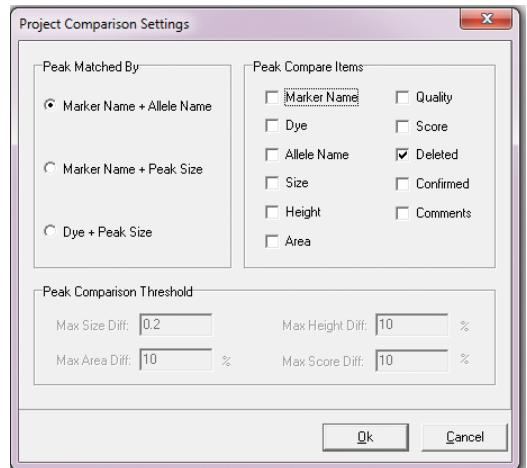


Figure 210. 'Project Comparison Settings' window.

The 'Project Comparison Settings' window (Figure 210) allows you to specify the parameters to be compared between the reference and comparison profiles. This window is split into three sections:

Peak Matched By: Allows you to define which peak identifiers are used during the comparison.

Marker Name + Allele Name: When selected, compares peaks with the same allele name within the same marker.

Marker Name + Peak Size: When selected, compares peaks with the same size within the same marker.

Dye + Peak Size: When selected, compares peaks with the same size in the same dye channel.












Peak Compare Items: Allows you to choose which parameters are compared. When these parameters are different between the reference and comparison profiles, these differences are indicated in the Comparison Report Table and Peak Comparison Table (Figure 209).

Peak Comparison Threshold: Allows you to specify the maximum acceptable differences in peak attributes, including peak size, peak area, peak height and peak score. Samples that exceed the specified Peak Comparison Thresholds will be highlighted in yellow in the Comparison Report Table.

Note: Peak Comparison Threshold fields will be inactive unless the corresponding identifier is selected in the Peak Compare Items section.

The Comparison Report Table displays the genotypes for all samples in both projects, with the reference and comparison profiles grouped together. Peaks that differ by more than the thresholds set in the 'Project Comparison Settings' window are marked with "#DIFF" followed by the threshold that was exceeded (Size, Area, Height or Score). Peaks detected in one profile but not the other are marked with "#LOSS". Cells that contain "#DIFF" or "#LOSS" are highlighted in yellow in the Comparison Report Table. Selecting a cell within the table displays that marker in the Electropherogram Charts, with the chosen peak highlighted in both the reference and comparison profiles.

Table 25. Icons and Functions of the 'Project Comparison' Window.

	Open Project to Compare: Opens a directory window to allow you to identify the project to be compared (the comparison project) to the project already open in GMHID-Spectrum (the reference project).
	Project Comparison Settings: Opens the 'Project Comparison Settings' window (Figure 191) allowing you to specify the parameters to be compared between the reference and comparison profiles.
	Edit File Groups: Opens the File Name Group Editor to change group settings as necessary to better reflect the project file names (Section 12.A).
	Save Results: Opens a 'Save As' window to save the Comparison Report Table as a .xlsx or .txt file. The arrow allows a choice of whether to save the whole comparison (Save whole result) or only the differences between the projects (Save difference result).
	Show Dye: Allows you to view all colors, hide all colors or choose any combination of dye layers by clicking on the down arrow. To cycle through individual dye channels, click on the icon.
	Zoom In: Zooms in on the center point of the electropherogram.
	Zoom Out: Zooms out from the center point of the electropherogram.
	Set Axis: Provides options for setting the x and y axes of the electropherogram charts. The default setting automatically sets the y axis according to the maximum peak intensity of the samples (Auto Fit Y) and the x-axis analysis range (Auto Fit X). You can also set specific ranges for the x and y axes by selecting Fixed X and Fixed Y .
	Open Charts Group: Opens the Electropherogram Charts of the first sample in each comparison group.
	Size Calibration: Opens the 'Calibration Charts' window, allowing you to review and edit size standard calibrations for the dataset (Section 7.B).
	Show Chart/Table: Displays the Peak Table below each electropherogram, which includes information such as peak size, height and user comments. Right-click on the table to modify the contents.

12.B.2 Comparing Projects with the Same File Names

Projects that contain samples with the exact same file names are compared automatically without adjusting file name groups. The software will automatically match the reference profile to the sample comparison profile based on identical file names and display the results in the 'Project Comparison' window. The profile from the first project opened in GMHID-Spectrum is the reference profile (marked as R==> next to the sample name in the electropherogram), and the profile from the second project opened in the 'Project Comparison' window is the sample comparison profile (marked as S==>).

12.B.3 Comparing Projects with Different File Names

Projects that contain samples with different file names must be grouped using the File Name Group Editor tool prior to comparison. The software cannot automatically compare profiles that do not have identical file names. To access the File Name Group Editor tool and group files appropriately, select the **Edit File Groups** icon from the toolbar (Table 25). Set the grouping criteria (Section 12.A) to appropriately compare the samples across the two projects, then select **OK** to return to the 'Project Comparison' window. The samples can now be compared, with the profile from the first project opened in GMHID-Spectrum as the reference profile (labeled "Reference" and marked R==>), and the profile from the second project opened as the sample comparison profile (labeled "Sample" and marked S==>).

12.C. Replicate Comparison Tool

The Replicate Comparison tool allows the user to compare the results of replicate samples within a single project to check for concordance, contamination and reaction failure. Before using the Replicate Comparison tool, process data through the Run Wizard and review the data, editing as needed. Peaks cannot be edited from the 'Replicate Comparison' window; however, they can be edited using the shortcut to the 'All Color Browser' window from the Replicate Comparison tool (Table 26).

Disabling Allelic Ladders

Because the Comparison Report Table expands to include every allele call for the selected replicate group, we recommend disabling all allelic ladders prior to using this tool.

To disable an allelic ladder sample, right click on an allelic ladder sample in the Sample File Tree of the 'Main Analysis' window and select **Disable** (Figure 211 and Section 3.C.1).

Note: If the disabled ladder was used during the analysis you may be prompted to reprocess your samples. Select **No**.

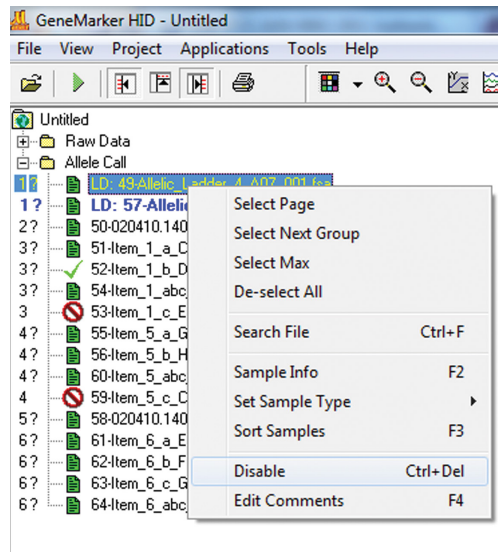


Figure 211. Disabling an allelic ladder from the Sample File Tree.

12.C.1 Navigating the Replicate Comparison Tool

To use the Replicate Comparison tool, open the project that contains the replicate samples, ensure file name grouping is applied such that replicate samples are grouped (Section 12.A), then select **Replicate Comparison** from the 'Tools' menu of the 'Main Analysis' window (Section 6.E).

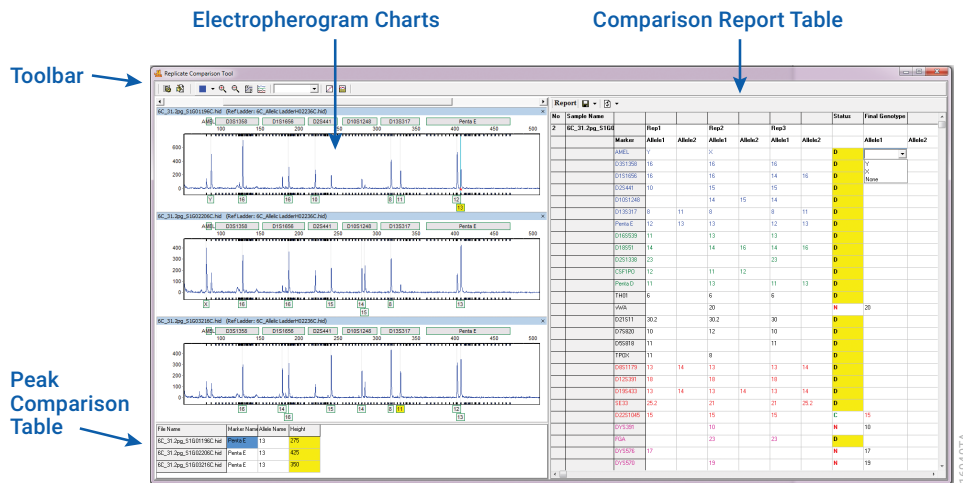


Figure 212. 'Replicate Comparison' window.

This opens the 'Replicate Comparison' window (Figure 212), which contains a Toolbar, Electropherogram Charts (with optional Peak Table display), Peak Comparison Table and a Comparison Report Table. The Electropherogram Charts section displays the electropherogram for each replicate group. The display reflects the display settings chosen in the 'Preferences' window of the 'Main Analysis' window (Section 6.B) and from the toolbar of the 'Replicate Comparison' window (Table 26). The same zoom and navigation features available in the electropherogram of the 'Main Analysis' window are also available in this window. Some, but not all, of the same options available by right-clicking within the electropherogram and Peak Table of the 'Main Analysis' window are available in this window.

The 'Replicate Comparison Setting' window (Figure 213) allows you to specify the parameters compared between replicate samples. These same settings are available in the 'Project Comparison Settings' window (Section 12.B). This window is divided into three sections:

Peak Matched By: Allows you to define which peak identifiers are used during the comparison.

Note: The data must be analyzed with a panel file before the replicate comparisons are made.

Marker Name + Allele Name: When selected, compares peaks with the same allele name within the same marker.

Marker Name + Peak Size: When selected, compares peaks with the same size within the same marker.

Dye + Peak Size: When selected, compares peaks with the same size in the same dye channel.

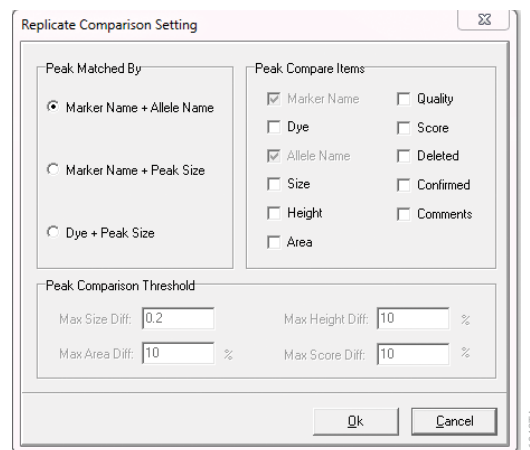


Figure 213. 'Replicate Comparison Setting' window.

Peak Compare Items: Allows you to choose which parameters are compared. When these parameters are different between replicate samples, these differences are indicated in the Comparison Report Table.

Peak Comparison Threshold: Allows the user to specify the maximum acceptable differences in peak attributes, including peak size, peak area, peak height and peak score. Samples that exceed the specified Peak Comparison Threshold will be highlighted in yellow in the Comparison Report Table.

Note: 'Peak Comparison Threshold' fields will be inactive unless the corresponding identifier is selected in the 'Peak Compare Items' section.

The Comparison Report Table displays the genotype report for each replicate, grouped by matched samples, as well as a final "consensus" genotype for each replicate group. Peaks that differ by more than the peak comparison thresholds set in the 'Replicate Comparison Settings' window are highlighted in yellow in the Comparison Report Table. Loci with peak identifiers that differ between replicates are marked with a **D** (discordant) and highlighted yellow in the Status column.

Double-clicking on a cell within the table displays that marker in the electropherograms. Likewise, selecting a peak within the electropherogram will highlight the corresponding cell in the Report Table. Right-clicking on an allele in the electropherogram provides an abbreviated list of the same options available in the electropherogram of the 'Main Analysis' window (Section 5.B.3).

Table 26. Icons and Functions of the 'Replicate Comparison' Window.













	Replicate Comparison Settings: Opens the 'Replicate Comparison Settings' window where you can determine which parameters are used for comparison. These are the same settings available in the 'Project Comparison' tool (Section 12.B).
	Edit File Groups: Opens the File Name Group Editor to change group settings as necessary to better reflect the project file names (Section 12.A).
	Show Dye: Allows you to view all colors, hide all colors or choose any combination of dye layers by clicking on the down arrow. To cycle through individual dye channels, click on the icon.
	Zoom In: Zooms in on the center point of the electropherogram.
	Zoom Out: Zooms out from the center point of the electropherogram.
	Set Axis: Provides options for setting the x and y axes of the electropherogram charts. The default setting automatically sets the y axis according to the maximum peak intensity of the samples (Auto Fit Y) and the x-axis analysis range (Auto Fit X). You can also set specific ranges for the x and y axes by selecting Fixed X and Fixed Y .
	Browse by All Color: Opens the All Color Browser, which allows you to see the alignment of each separate dye trace for a given sample. Use the sample drop-down menu in the upper right corner to change samples.
	Open Charts Group: Opens the Electropherogram Charts of the first sample in each replicate group.

Table 26. Icons and Functions of the 'Replicate Comparison' Window (continued).**Comparison Report Table Icons**

	Size Calibration: Opens the 'Calibration Charts' window, where you can review and edit size standard calibrations for the dataset (Section 7.B).
	Show Chart/Table: Displays the Peak Table below each electropherogram, which includes information such as peak size, height and user comments. Right-click on the table to modify the contents.
	<p>Save Report: Allows you to save the Comparison Report Table as a text (.txt) file. Select the arrow to switch between export formats. All exports contain a header with project and analysis information.</p> <p>Whole Report: When selected, exports the entire Comparison Report Table.</p> <p>Final Report: Exports a header, the Status column and the Final Genotype column only.</p> <p>Final Report With Only Valid Allele: Exports the final report but excludes markers with "None" selected as the final genotype (see below) from the export.</p> <p>Note: You will receive a warning if you attempt to export the Comparison Report Table if discordant (D) allele calls are present. Select Yes to export the Report Table with discordant allele calls. Select No to return to the 'Replicate Comparison' window to resolve discordant allele calls before exporting the Report Table.</p>
	<p>Sort Report: Allows sorting of replicate groups in the Comparison Report Table. Select the preferred sorting option from the drop-down, and then choose the Sort Report icon to apply the sorting. There are two sorting options:</p> <p>Sort by Group Sequence: Sorts replicate groups according to their order in the Sample File Tree of the 'Main Analysis' window.</p> <p>Sort by Status: Sorts replicate pairs with discordant allele calls (D) or null allele calls (N) to the top of the Comparison Report Table. Concordant (C) replicate pairs appear at the bottom of the Comparison Report Table.</p>

12.C.2 Performing a Replicate Comparison

Sample replicates within the same analyzed project file can be compared. Be sure to analyze and edit all samples to be compared before making replicate comparisons. Samples are grouped in the Comparison Report Table based on the file name grouping applied (Section 12.A). Each group is assigned the number displayed in the 'No.' column. The genotype for each replicate is displayed in two columns of the Comparison Report Table, one column for each allele at a locus. Results for the first replicate (Rep 1) are in the rightmost two columns, followed by the second replicate, etc. The final consensus genotype is displayed in the 'Final Genotype' column.

The 'Status' column in the Comparison Report Table displays the concordance status for each locus across all replicates within a group (Figure 214). The replicates can be designated as discordant (D), concordant (C) or null (N).

Status

C (Concordant): All match criteria are met across all replicates. The concordant genotype is displayed in the Final Genotype column.

D (Discordant): The software is unable to determine a consensus genotype, and the 'Final Genotype' column is left blank. The user can manually populate the final genotype at a locus by right-clicking on a cell and choosing an allele from the drop-down menu. The drop-down menu includes all alleles that were detected at that marker in all sample replicates. Alternatively, the user can choose **None**. If the user chooses one of the alleles, the status for the locus changes to E (Edit) to indicate the locus was manually changed. When exporting the results to a final report, markers designated as "None" are not exported if the **Final Report with Valid Alleles Only** option is selected (Table 26).

N (Null): One or more replicates have no allele calls at that locus. In cases where only one replicate has an allele call at a locus, the Final Genotype column is populated with the results from that one replicate.

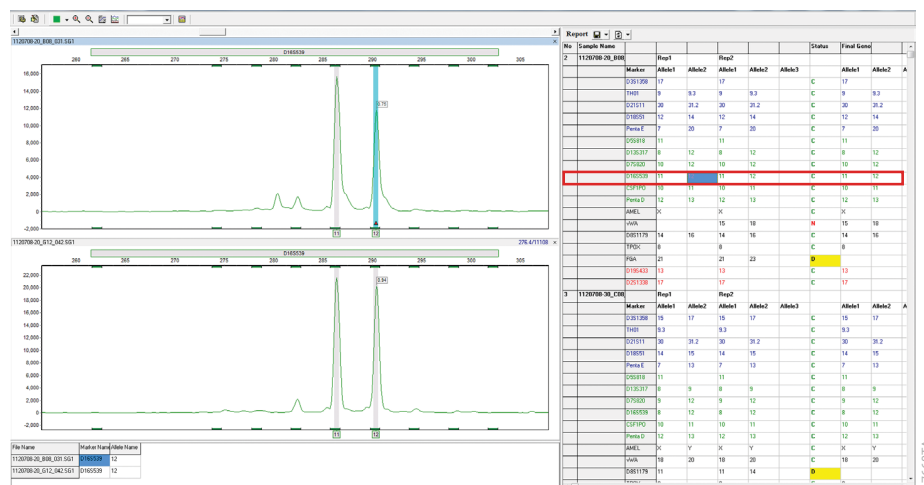


Figure 214. Example of a concordant locus, D16S539, within a replicate group.

Disabling Entire Replicates

The 'Replicate Comparison' tool allows you to remove entire replicate groups from the comparison. Right-click the 'Sample Name' column of a replicate group and select **Disable**. Allele calls and the status of disabled replicates are colored dark gray (Figure 215). This process can be reversed by right-clicking the 'Sample Name' column and selecting **Enable**.

Disabled replicates are never exported, regardless of the reporting option selected.

No	Sample Name	Rep1	Rep2	Rep3	Status	Final Genotype	
2	6C_31.2pg_S16011						
	Marker	Allele1	Allele2	Allele1	Allele2	Allele1	Allele2
	D3S1368	16	16	16		D	
	D151656	16	16	14	16	D	
	D2S441	10	15	15		D	
	D10S1248		14	15	14	D	
	D13S317	8	11	8	11	D	
	Penta E	12	13	13	12	13	D
	D16S539	11	13	13		D	
	D18S51	14	14	16	14	16	D
	D2S1338	23		23		D	
	CSF1PO	12	11	12		D	
	Penta D	11	13	11	13	D	
	TH01	6	6	6		D	
	vWA	30.2	20			N	20
	D21S11	30.2	30.2	30		D	
	D7S820	10	12	10		D	
	D5S818	11		11		D	
	TPDX	11	8			D	
	D8S1179	13	14	13	14	D	
	D12S391	18	18	18		D	
	D19S433	13	14	13	14	D	
	SE33	25.2	21	21	25.2	D	
	D22S1045	15	15	15		C	15
	DYS391	10				N	10
	FGA	23		23		D	
	DYS576	17				N	17
	DYS570	19				N	19

169457A

No	Sample Name	Rep1	Rep2	Rep3	Status	Final Genotype	
2	6C_31.2pg_S16011						
	Marker	Allele1	Allele2	Allele1	Allele2	Allele1	Allele2
	AMEL					D	
	D3S1368	16	16	16		D	
	D151656	16	16	14	16	D	
	D2S441	10	15	15		D	
	D10S1248		14	15	14	D	
	D13S317	8	11	8	11	D	
	Penta E	12	13	13	12	13	D
	D16S539	11	13	13		D	
	D18S51	14	14	16	14	16	D
	D2S1338	23		23		D	
	CSF1PO	12	11	12		D	
	Penta D	11	13	11	13	D	
	TH01	6	6	6		D	
	vWA	30.2	20			N	20
	D21S11	30.2	30.2	30		D	
	D7S820	10	12	10		D	
	D5S818	11		11		D	
	TPDX	11	8			D	
	D8S1179	13	14	13	14	D	
	D12S391	18	18	18		D	
	D19S433	13	14	13	14	D	
	SE33	25.2	21	21	25.2	D	
	D22S1045	15	15	15		C	15
	DYS391	10				N	10
	FGA	23		23		D	
	DYS576	17				N	17
	DYS570	19				N	19

169467A

Figure 215. Example of disabling a replicate group (top). Allele calls and the status of all disabled replicates are colored dark gray in the 'Replicate Comparison' window (bottom).

13 Validation Assistance Tools

The Validation Assistance tools allow you to perform calculations pertinent to internal validation of an STR chemistry. There are four calculations available in the Validation Assistance tool menu: LOD (limit of detection), stutter ratio, height ratio and migration precision, which are accessible via the 'Tools' menu of the 'Main Analysis' window (Section 6.E). Process data with the Run Wizard before using the Validation Assistance tools. Peaks cannot be edited from within the Validation Assistance tools.

13.A. Performing an LOD Calculation

The LOD tool analyzes each sample in the dataset for the presence of noise peaks. Peaks recognized as alleles and/or stutter are excluded from this calculation. The software determines the LOD by calculating the average peak height (AVE.PH) and standard deviation (SD.PH) of observed noise peaks, and then adding a user defined number of standard deviations to the average. The number of standard deviations to apply to the calculation is selected using the **LOD Equation** icon in the toolbar (Table 27).

To use the LOD tool, import and analyze the samples to be used for the LOD calculation. Select **Validation Assistance** then **LOD** from the 'Tools' menu of the 'Main Analysis' window. This opens the 'LOD Settings' window (Figure 216), which allows you to select the raw data analysis parameters (Section 4.B.1) to apply to the dataset when calculating the LOD. Select the same settings used to process the samples through the Run Wizard.

Note: All Enabled samples in a project are used in the LOD calculation. To omit samples, such as ladders, from the LOD calculation, disable the sample(s) in the Sample File Tree (Section 3.C.1) before initiating the LOD tool. If a disabled ladder was used during the analysis of the dataset, you may be prompted to reprocess your samples. Select **No**.

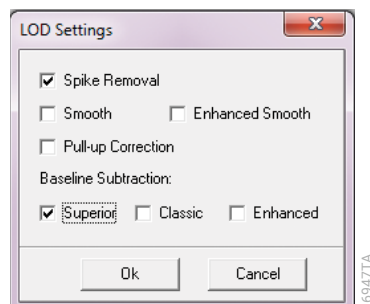


Figure 216. 'LOD Settings' window.

An 'LOD Process' window opens and displays the progress of the calculation. When calculations are complete, select **OK** to display the 'LOD' window (Figure 217). The 'LOD' window contains a toolbar, electropherogram, Sample File Tree and LOD Report Table section.

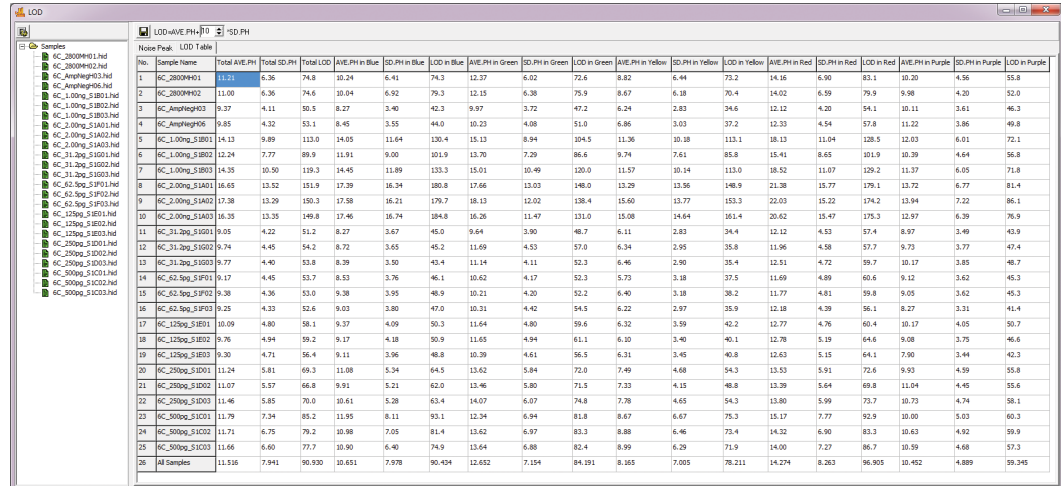


Figure 217. 'LOD' window.

The Sample File Tree and electropherogram function in the same manner as those in the 'Main Analysis' window (Sections 5.A and 5.B). When a sample is selected in the Sample File Tree, its electropherogram opens within the 'LOD' window (Figure 218).

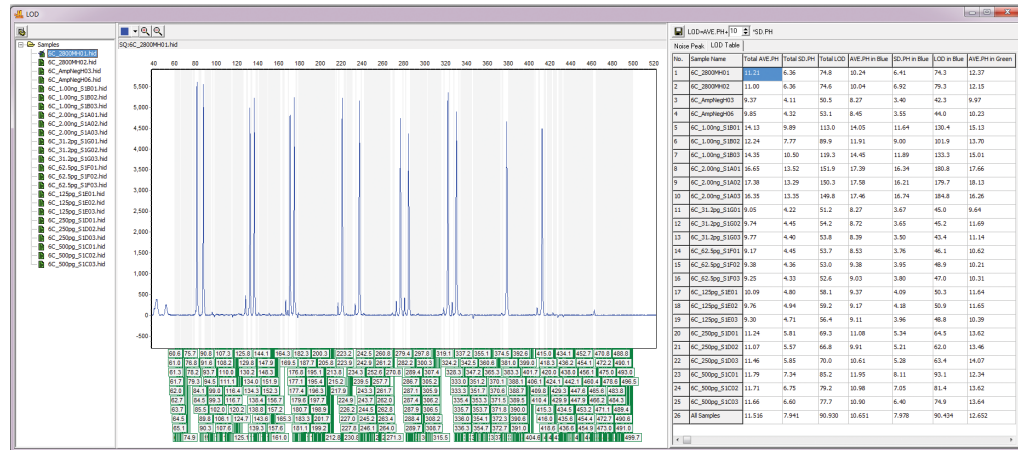


Figure 218. Example of an electropherogram opened in the 'LOD' window.

The zoom and navigation features available in the electropherogram of the 'Main Analysis' window are also available in this field. There are two tabs to the LOD Report Table: Noise Peak and LOD Table (Figure 219).

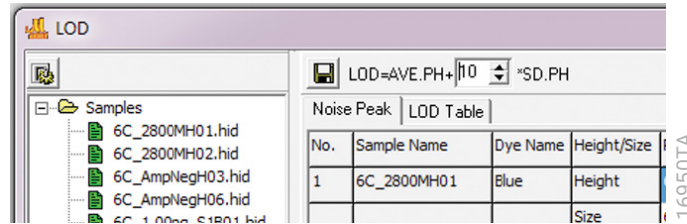





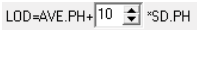


Figure 219. 'Noise Peak' and 'LOD Table' tabs of the LOD Report Table.

The 'Noise Peak' tab displays the peak height for each observed noise peak within each sample. The 'LOD Table' tab displays the average peak height and standard deviation for noise peaks, and the resulting LOD (based on the value selected in the toolbar), for each sample across all dye channels and the average peak height, standard deviation and LOD for each dye channel within each sample. The total average peak height, standard deviation and LOD across all samples for all dye channels as well as for each dye channel is displayed at the bottom of the 'LOD Table' tab.

Table 27. Icons and Functions of the 'LOD' Window.

Sample File Tree Icon	
	LOD Settings: Opens the 'LOD Settings' window where you can specify which raw data analysis parameters are applied to the dataset when calculating LOD.
Electropherogram Chart Icons	
	Show Dye: Allows you to view all colors, hide all colors or choose any combination of dye layers by clicking on the down arrow. To cycle through individual dye channels, click on the icon.
	Zoom In: Zooms in on the center point of the electropherogram.
	Zoom Out: Zooms out from the center point of the electropherogram.
LOD Report Table Icons	
	Save: Allows you to save the LOD Report Table as a text (.txt) file.
	LOD Equation: Displays the equation used in the LOD calculation. Select the desired number of standard deviations (SD) used to calculate the LOD using the up or down arrow.

13.B. Performing Stutter Ratio Calculations

The Stutter Ratio tool analyzes the stutter for each allele at each marker for every sample in the data set for the $N-x$, $N+x$, $N-0.5x$, and $N+0.5x$ positions. The number of stutter peaks detected at each position is displayed (Count). The maximum observed stutter ratio at each position at each marker in a sample is reported (Max). The average stutter ratio (Mean) and standard deviation (SD) for each position at each marker across the data set is also calculated.

Note: Partial stutter positions ($N+0.5x$ and $N-0.5x$) are not calculated for trinucleotide and penta-nucleotide repeat markers.

To use the Stutter Ratio tool, import and analyze the samples to be used for the stutter ratio calculation. Note: Samples should be analyzed with all stutter filters set to zero in the applied panel editor. In addition, the peak detection thresholds may need to be lowered (e.g. 10RFU) to capture sufficient stutter data. Select **Validation Assistance** then **Stutter Ratio** from the Tools menu of the 'Main Analysis' window. This opens a 'Stutter Ratio Process' window that displays the progress of the calculation (Figure 220).

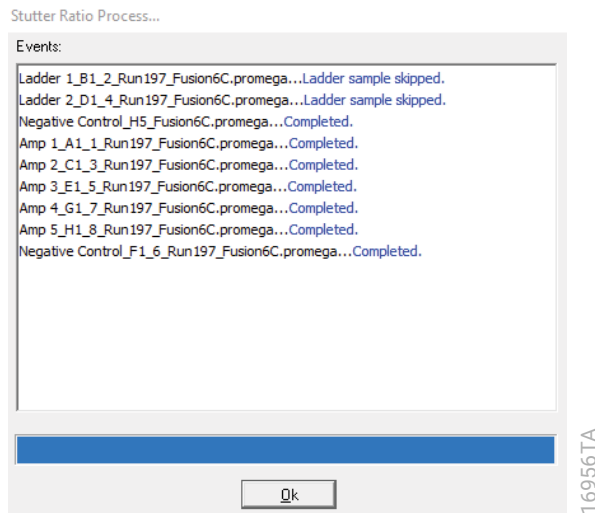


Figure 220. 'Stutter Ratio Process...' window.

When the calculations are complete, select **OK** to display the 'Stutter Ratio' window.

The 'Stutter Ratio' window displays a table of the calculated results (Figure 221). Cells with ** recorded indicate no stutter peak was detected at that position []. You can save this table as a tab-delimited text (.txt) file by selecting **Save** at the bottom of the window. To return to the main analysis window, select **OK**.

Stutter Ratio		D3S1338				D151656				D25441				D1051248				D13S317				Penta E								
No.	Sample Name	N-x	N+x	N+0.5x	N-x	N+x	N+0.5x	N-x	N+x	N+0.5x	N-x	N+x	N+0.5x	N-x	N+x	N+0.5x	N-x	N+x	N+0.5x	N-x	N+x	N+0.5x	N-x	N+x	N+0.5x	N-x	N+x	N+0.5x		
12	6C_500pg_S1C02	**	**	**	0.0039	0.0890	0.0076	**	**	0.0883	**	0.0191	**	0.0278	0.0046	**	0.0022	0.0793	**	**	**	0.0228	0.0105	**	**	0.0300	**	**	**	
13	6C_500pg_S1C03	**	**	**	0.0032	0.0805	0.0061	**	0.0011	0.1016	**	0.0235	**	0.0307	0.0041	**	0.0027	0.0828	**	**	**	0.0249	0.0043	**	**	0.0025	**	0.0035	**	
14	6C_250pg_S1D01	**	**	**	0.0062	0.0822	0.0070	**	**	0.0890	**	0.0277	**	0.0250	0.0055	**	**	0.0870	**	**	**	0.0240	0.0080	**	**	0.0337	**	**	**	
15	6C_250pg_S1D02	**	**	**	0.0070	0.0924	0.0080	**	**	0.0879	**	0.0256	**	0.0334	0.0047	**	0.0043	0.0890	**	**	0.0044	**	**	0.0226	0.0092	**	**	0.0322	**	
16	6C_250pg_S1D03	**	**	**	0.0046	0.0891	0.0084	**	**	0.0924	**	0.0169	**	0.0276	**	**	0.0928	**	**	**	**	0.0215	0.0098	0.0074	**	0.0307	**	**	**	
17	6C_125pg_S1E01	**	**	**	0.0055	**	**	**	**	**	0.1158	0.0243	0.0054	0.0533	0.0184	0.0130	**	**	**	**	**	0.0527	0.0084	0.0073	**	**	0.0059	**	**	
18	6C_125pg_S1E02	**	**	**	0.0776	0.0072	**	**	0.0859	**	0.0294	**	0.0316	**	0.0150	**	0.0887	**	**	**	**	0.0214	0.0124	0.0107	**	0.0466	**	**	**	
19	6C_125pg_S1E03	**	**	**	0.0910	0.0059	**	0.0054	0.0954	**	0.0217	0.0141	0.0411	**	0.0124	**	0.0944	**	**	**	**	0.0198	0.0062	0.0105	**	0.0807	**	**	**	
20	6C_62.5pg_S1F01	**	**	**	0.0830	0.0103	**	0.0077	0.0911	**	0.0173	**	0.0316	**	0.0128	0.0148	0.0887	**	**	**	**	0.0229	0.0046	**	**	0.0327	**	**	**	
21	6C_62.5pg_S1F02	**	**	**	0.0790	**	**	**	0.1111	**	0.0489	0.0533	0.0287	**	**	0.0239	0.0876	**	**	**	**	**	**	**	0.0474	0.0309	**	**	**	
22	6C_62.5pg_S1F03	**	**	**	0.1026	0.1018	0.0207	**	**	0.0836	**	0.0293	**	0.0404	0.0239	**	**	0.0800	**	**	**	0.0191	**	**	0.0318	0.0372	**	**	**	
23	6C_31.2pg_S1G01	**	**	**	0.0212	0.0850	0.0266	**	**	0.1052	**	**	0.0420	**	0.0178	0.1037	**	**	**	**	**	0.0255	**	**	0.0645	0.0132	**	**	0.0415	**
24	6C_31.2pg_S1G02	**	**	**	0.0903	0.1289	0.0510	**	**	0.1322	**	**	0.0632	0.0662	**	0.0368	0.0368	0.1241	**	**	**	0.0791	**	**	**	0.0282	**	**	**	
25	6C_31.2pg_S1G03	**	**	**	0.0301	0.0923	0.0655	**	**	0.1335	0.0354	0.0436	**	**	**	0.1010	0.0791	**	**	**	**	0.0456	**	**	0.0254	**	**	**	**	
Count		0	5	18	23	23	3	5	23	21	39	16	44	34	18	15	23	15	2	6	41	35	12	10	23	18	0	0		
Mean		0.0000	0.0000	0.0293	0.0113	0.0904	0.0938	0.0018	0.7129	0.0979	0.0202	0.0531	0.0126	0.0469	0.0113	0.1290	0.2118	0.0877	0.0113	0.5005	0.7524	0.0445	0.0097	0.0770	0.2915	0.0347	0.0477	0.0000	0.0000	
SD		0.0000	0.0000	0.0556	0.0210	0.0105	0.3723	0.0003	1.3401	0.0151	0.0098	0.1722	0.0187	0.0137	0.0062	0.3359	0.4966	0.0100	0.0187	0.7064	0.6866	0.0209	0.0040	0.2317	0.5186	0.0126	0.1790	0.0000	0.0000	
Max		0.0000	0.0000	0.1288	0.0903	0.1289	1.8000	0.0021	3.0833	0.1335	0.0510	1.1000	0.0632	0.0731	0.0257	1.1667	1.6875	0.1241	0.0690	1.0000	1.6364	0.0817	0.0237	0.8125	1.3750	0.0807	0.7647	0.0000	0.0000	

Figure 221. 'Stutter Ratio' window.

13.C. Performing Height Ratio Calculations

The Height Ratio tool calculates the peak height ratio by comparing the peak heights of the two sister alleles at each marker. The ratio is expressed as the lower peak height of the two alleles divided by the higher peak height of the two alleles. The software then calculates the average peak height ratio (Ave HR), standard deviation (Std Dev) and minimum and maximum peak height ratios (Min HR, Max HR) observed at each marker across the dataset. The values are reported in the 'Height Ratio' window (Figure 222).

No.	Sample Name	AMEL	D3S1358	D151656	D2S441	D10S1248	D13S317	Penta E	D16S539	D18S51	D2S1338	CSF1PO	Penta D	TH01	YWA	D1S11	D7S820	D5S818	TPOX	D6S1179	D12S391	D19S430	SE33	D2Z51045	D1S391	FGA	D1S576	D1S570
1	6C_AmpHeqH03	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**
2	6C_AmpHeqH06	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**
3	6C_1.00ng_S1E01	0.8954	**	0.8883	0.9512	0.9965	0.6924	0.9184	0.8670	0.9795	0.9149	0.8708	0.9704	**	0.9164	0.9373	0.9651	**	0.9878	0.9589	**	0.9011	0.9882	0.9844	**	**	**	**
4	6C_1.00ng_S1E02	0.9393	**	0.8672	0.9622	0.8556	0.9285	0.8253	0.9509	0.9535	0.9438	0.9467	0.9446	**	0.9361	0.8059	0.9490	**	0.7460	0.9290	**	0.8740	0.9762	0.9560	**	**	**	**
5	6C_1.00ng_S1E03	0.9441	**	0.9051	0.9979	0.9451	0.9560	0.9812	0.9328	0.9300	0.9042	0.7088	0.8804	**	0.8966	0.9076	0.8220	**	0.8174	0.8456	**	0.9665	0.9933	0.9551	**	**	**	**
6	6C_1.25ng_S1E01	0.9034	**	0.6089	0.7443	0.9840	0.9628	0.9440	0.7251	0.6552	0.6321	0.6465	0.9578	**	0.8528	0.6595	0.9855	**	0.9254	0.9009	**	0.9136	0.7984	0.7537	**	**	**	**
7	6C_1.25ng_S1E02	0.4713	**	0.6194	0.8977	0.6419	0.9639	0.4388	0.5458	0.9427	0.7203	0.8474	0.7947	**	0.6634	0.8184	0.3688	**	0.9083	0.9546	**	0.7652	0.6672	0.9883	**	**	**	**
8	6C_1.25ng_S1E03	0.9639	**	0.7447	0.9239	0.9848	0.5027	0.9228	0.7895	0.7152	0.9980	0.5679	0.8636	**	0.7351	0.6833	0.7256	**	0.7843	0.6194	**	0.9690	0.9242	0.8119	**	**	**	**
9	6C_2.00ng_S1A01	0.9025	**	0.9561	0.9503	0.8749	0.9798	0.8723	0.9893	0.9176	0.9056	0.6761	0.9938	**	0.9252	0.9810	0.9451	**	0.8232	0.9855	**	0.8798	0.8941	0.9790	**	**	**	**
10	6C_2.00ng_S1A02	0.9432	**	0.9476	0.9437	0.8308	0.9779	0.9939	0.9323	0.9505	0.9468	0.9794	0.8849	**	0.9083	0.9692	0.9663	**	0.9826	0.9531	**	0.8605	0.9863	0.9490	**	**	**	**
11	6C_2.00ng_S1A03	0.9027	**	0.9917	0.9282	0.9363	0.8916	0.9785	0.8368	0.8898	0.9179	0.9404	0.8446	**	0.9922	0.9724	0.9742	**	0.9418	0.9499	**	0.9681	0.9803	0.9190	**	**	**	**
12	6C_2.50ng_S1D01	0.8038	**	0.8838	0.9254	0.9131	0.5547	0.9564	0.6249	0.8971	0.6869	0.8637	0.8422	**	0.8896	0.8481	0.6277	**	0.7860	0.8189	**	0.9078	0.9755	0.6260	**	**	**	**
13	6C_2.50ng_S1D02	0.8291	**	0.9125	0.8991	0.7267	0.8728	0.8364	0.6907	0.9723	0.6595	0.8179	0.8841	**	0.9151	0.9309	0.7899	**	0.9614	0.7193	**	0.7503	0.7564	0.9167	**	**	**	**
14	6C_2.50ng_S1D03	0.9144	**	0.8556	0.8457	0.7958	0.9213	0.9913	0.8414	0.9611	0.6446	0.8231	0.7544	**	0.9536	0.8963	0.9838	**	0.6582	0.8749	**	0.9440	0.9900	0.8807	**	**	**	**
15	6C_3.1.2ng_S1G01	0.2860	**	0.5494	0.7133	**	0.6206	0.5156	0.5245	0.5510	0.3802	**	0.5293	**	0.9005	0.7767	**	**	0.4240	0.9653	**	0.9542	**	0.5671	**	**	**	**
16	6C_3.1.2ng_S1G02	0.4510	**	**	0.8978	0.8907	**	0.4023	**	0.6548	**	0.9426	**	**	0.7353	0.8009	**	**	**	0.7389	**	0.8161	0.9564	**	**	**	**	**
17	6C_3.1.2ng_S1G03	0.8824	**	0.8447	**	0.5920	0.5516	0.9106	0.5107	0.6011	0.8370	0.7500	0.8035	**	**	0.7512	0.7915	**	**	0.6463	**	0.4274	0.8828	0.5724	**	**	**	**
18	6C_500ng_S1C01	0.9481	**	0.8201	0.8436	0.8312	0.8093	0.7445	0.9775	0.9965	0.9525	0.8218	0.9272	**	0.9563	0.9063	0.8512	**	0.9904	0.9108	**	0.8132	0.9667	0.7258	**	**	**	**
19	6C_500ng_S1C02	0.9460	**	0.7500	0.9360	0.8707	0.9730	0.7584	0.6635	0.7216	0.9582	0.7120	0.9306	**	0.9796	0.6218	0.9840	**	0.9288	0.6808	**	0.9173	0.9482	0.9431	**	**	**	**
20	6C_500ng_S1C03	0.9559	**	0.9015	0.9359	0.8544	0.9719	0.8863	0.8371	0.6832	0.9373	0.7658	0.8467	**	0.9862	0.8174	0.9791	**	0.8389	0.7621	**	0.9554	0.9459	0.9456	**	**	**	**
21	6C_62.5ng_S1F01	0.8985	**	0.4406	0.6767	0.6004	0.3679	0.4401	0.5115	0.6776	0.7729	0.9539	0.9598	**	0.6887	0.9469	0.7052	**	0.5197	0.7945	**	0.2777	0.7977	0.8010	**	**	**	**
22	6C_62.5ng_S1F02	0.8413	**	0.5351	0.6477	0.3721	0.9680	0.7092	0.8761	0.7763	0.7322	0.7744	0.8345	**	0.2914	0.8283	0.4021	**	0.9806	0.7655	**	0.9934	0.7414	0.9029	**	**	**	**
23	6C_62.5ng_S1F03	0.5475	**	0.8730	0.9357	0.7448	0.7738	0.6788	0.6639	0.8803	0.4626	0.7148	0.8201	**	0.8347	0.4444	0.6246	**	0.7941	0.7797	**	0.9869	0.6452	0.9361	**	**	**	**
24	6C_2809M-H01	0.9913	0.9529	0.9173	0.9875	0.9239	0.9089	0.9615	0.9679	0.8406	0.9218	**	0.8615	**	0.8965	0.9424	0.8460	0.9431	**	**	0.9742	0.8700	0.8330	0.9336	**	**	0.9591	**
25	6C_2809M-H02	0.8499	0.7858	0.9813	0.8022	0.8742	0.9153	0.9880	0.9336	0.8017	0.8384	**	0.9743	**	0.8407	0.9024	0.8101	0.9973	**	**	0.9852	0.9483	0.7454	0.8110	**	**	0.8433	**
Ave HR		0.8266	0.8693	0.8088	0.8793	0.8200	0.8211	0.8111	0.7815	0.8239	0.8031	0.8062	0.8683	0.8686	0.8546	0.8243	0.8277	**	0.8315	0.8484	0.9092	0.8443	0.8636	0.8557	**	0.9012	**	**
Std Dev		0.1917	0.1182	0.1587	0.1007	0.1539	0.1866	0.1937	0.1650	0.1386	0.1693	0.1146	0.1001	0.0295	0.1573	0.1293	0.1909	**	0.1589	0.1165	0.0554	0.1737	0.1510	0.1375	**	0.0819	**	**
Min HR		0.2860	0.7858	0.4406	0.6477	0.3721	0.3679	0.4023	0.5107	0.5510	0.3802	0.5679	0.5293	0.8407	0.2914	0.4444	0.3688	**	0.4240	0.6194	0.8700	0.2777	0.9664	0.5671	**	0.8433	**	**
Max HR		0.9913	0.9529	0.9917	0.9979	0.9965	0.9798	0.9939	0.9893	0.9965	0.9980	0.9794	0.9938	0.8965	0.9922	0.9810	0.9973	**	0.9904	0.9855	0.9483	0.9934	0.9933	0.9883	**	0.9591	**	**

Figure 222. 'Height Ratio' window.

To use the 'Height Ratio' tool, import and analyze the desired samples for the height ratio calculation. Select **Validation Assistance** then **Height Ratio** from the 'Tools' menu of the 'Main Analysis' window. This opens a 'Height Ratio Process' window (Figure 223), which displays the progress of the calculation.

Samples that are designated as allelic ladders (Sections 3.C.1 and 4.C.1) are not used in the calculations. When calculations are complete, select **OK** to display the 'Height Ratio' window (Figure 222). The 'Height Ratio' window displays a table of the calculated results. Cells with "*" indicate a marker with one or fewer called peaks. You can save this table as a text (.txt) file by selecting **Save** at the bottom of the window. To return to the 'Main Analysis' window, select **Close**.

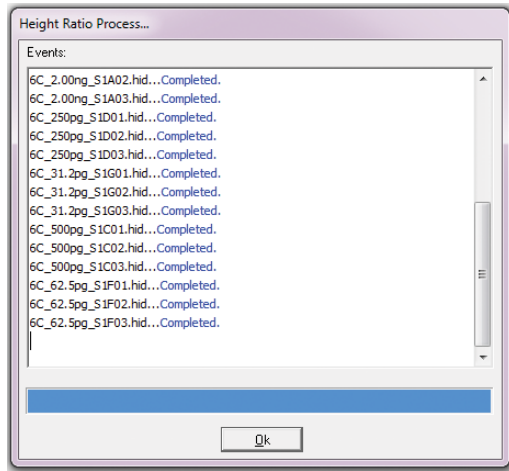


Figure 223. 'Height Ratio Process' window.

13.D. Performing Migration Precision Calculations

The 'Migration Precision' tool calculates sizing precision of alleles present in all allelic ladders within a dataset. The software reports the observed peak size (in base pairs) for each allelic ladder peak in the 'Migration Precision' window (Figure 224). The software then calculates the average size (Ave Migration) and standard deviation for each allelic ladder peak and marker (Std Dev Per Allele, Std Dev For The Marker) across the entire dataset.

The 'Migration Precision' window displays a table with the following columns: No., Sample Name, Y, Y', 8, 9, 10, 11, 12, 13, 14, 14.3, 15, 15.1, 15.2, 15.3, 16, 16.2, 17, 17.1, 17.2, 18, 18.1, 18.2, 18.3, 19, 20, 20.1, 21, 8, 9, 10, 11. The table contains 80 rows of data for various sample markers (e.g., PPF Ladder_20_002_03, PPF Ladder_01_001_04, etc.) and summary rows for 'Ave Migration', 'Std Dev Per Allele', and 'Std Dev For The Marker'. The 'Ave Migration' row shows values like 80.836, 87.015, 98.261, 102.370, 106.499, 110.624, 114.776, 118.911, 123.019, etc. The 'Std Dev For The Marker' row shows values like 3.921, 3.225, 0.049, 0.046, 0.011, 0.043, 0.043, 0.032, 0.039, etc. A 'Save' button is visible at the bottom right.

Figure 224. 'Migration Precision' window.

To use the 'Migration Precision' tool, import and analyze the desired samples for the migration precision calculation. Ensure the desired allelic ladders are designated as ladders in the Sample File Tree (Sections 3.C.1 and 4.C.1). Select **Validation Assistance** then **Migration Precision** from the 'Tools' menu of the 'Main Analysis' window. This opens a 'Migration Precision Process' window (Figure 225), which displays the progress of the calculation. Samples that are not designated as allelic ladders are not used in the calculations.

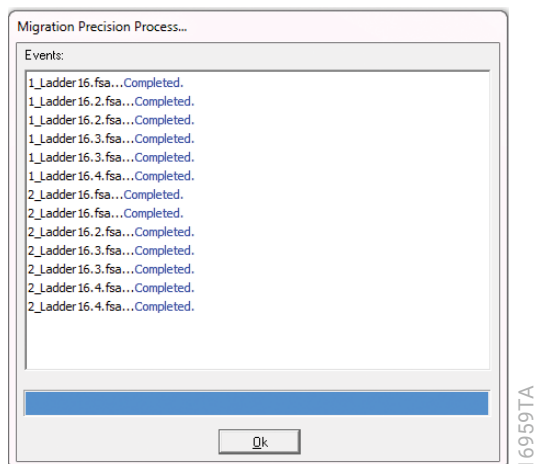


Figure 225. 'Migration Precision Process' window.

When calculations are complete, select **OK** to display the 'Migration Precision' window (Figure 224). The 'Migration Precision' window displays a table of the calculated results. Cells with “**” indicate a bin with no peaks detected, typically a virtual bin.

Note: Peaks in virtual bins with higher-than-expected peak heights will be used in the calculation unless “Auto-Delete Alleles in Virtual Bins in Allelic Ladder” is selected in the 'Forensic' tab of the 'Preferences' window (Section 6.B).

You can save this table as a text (.txt) file by selecting **Save** at the bottom of the window. To return to the 'Main Analysis' window, select **Close**.

Additional Information

For more information about GMHID-Spectrum, visit: www.promega.com/gmhid/

For information on the Spectrum CE Systems, visit: www.promega.com/spectrumsystem/

For more information on PowerPlex® Systems for amplification of STRs, visit: www.promega.com/products/forensic-dna-analysis-ce/str-amplification/

For Technical Services support, please contact your local Promega representative or: genetic@promega.com

14 Summary of Changes

The following changes were made to the 1/23 revision of this document:

1. Revised Sections 2.B and 2.C.
2. Updated images in Sections 4.A, 4.C and 8.A.
3. Inserted new Sections 4.C.6 Sample Quality Peak(s) and 10.A.4 Quality Indicators.
4. Updated Table 6 in Section 5.A.
5. Edited Sections 5.B.1, 5.E.2, 6.B.3, 6.B.4, 10.A and 13.
6. Inserted new Section 13.B Performing Stutter Ratio Calculations, and renumbered subsequent sections.
7. Moved document to a new template.

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