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User Manual

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Chapter 1 Installing ChimerMarker

Computer System Requirements and Recognized File Formats

ChimerMarker™ software is tested and validated for various computer systems. The minimum system requirements include the following:

Windows® PC

- OS: Windows® Windows®10.
- Processor: Pentium® III, 1 GHz.
- RAM: 512MB CPU.
- Available hard disk space: 20GB.

Intel® Powered Macintosh®

- OS: 10.4.6, with Parallels® desktop for MAC or VMware Fusion® or Apple™ Boot Camp.
- RAM: 2GB.
- Available hard disk space: 20GB.

Installation of ChimerMarker is not supported on Linux or UNIX-based operating systems.

ChimerMarker Recognized File Formats

ChimerMarker recognizes only PC file formats. To convert Macintosh file formats to PC file formats, download the ABI PRISM® 3100 Genetic Analyzer Conversion Utilities to convert Mac files to PC files from: http://www.appliedbiosystems.com/support/software/3100/conversion.cfm.

Installation and Registration

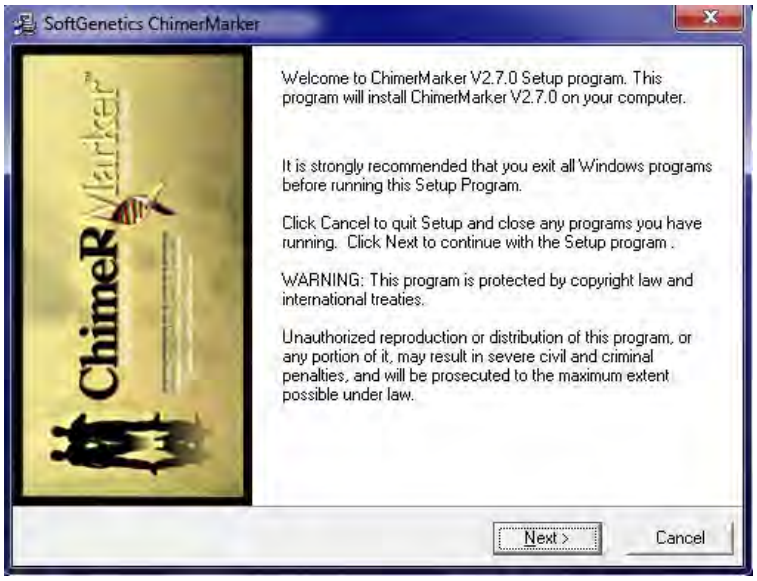
Local License Option

Installation

1. Insert the SoftGenetics CD into the optical or CD-ROM drive. For computers not set to automatically open a CD, navigate to its optical or CD-ROM drive and manually open the directory. For those that choose not to receive a disc, download the executable file from the emailed link.
2. Double-click the **ChimerMarker Setup** executable file (EXE) and the Installation Wizard launches.

   The **Welcome** dialog displays.

   Figure 1 Welcome to ChimerMarker Setup Program



3. Select **Next**.
4. Read the SoftGenetics End User License Agreement. Select the option *I accept the terms of the License Agreement*, and click **Next** in the **Read Me File** dialog.
5. In the **Select Program** dialog, select the **Install ChimerMarker (Recommended)** radial button and click **Next**.
6. In the **Destination Location** dialog, to install ChimerMarker in the default folder, click **Next**. To choose a different installation directory, Click **Browse**.

   Note: The default destination location for the ChimerMarker program is `C:\ProgramFiles\SoftGenetics\ChimerMarker\ver#`.
7. In the Select Program Manager Group dialog, accept the default Program Manager Group, and click **Next**.

Note: Changing the location from the default for the Program Manager Group may affect program operability. It is recommended to accept the default.

8. In the **Start Installation** dialog, to install ChimerMarker, click **Next**.
9. In the Installation Complete dialog, select **Finish**.

   The installation wizard closes.
10. Eject the SoftGenetics CD.
11. Launch ChimerMarker by either double-clicking the **ChimerMarker** desktop icon or by selecting **Start** and navigating to **SoftGenetics > ChimerMarker** > **ChimerMarker** program, which is the version that was just installed.

    The Choose Registration Method dialog displays.
12. Select **Register Local Text-based Key** and the Register Local Text-based Key dialog displays.

Registration

After the **Register Local Text-based Key** dialog displays, make a registration method decision. If ChimerMarker is installed on a computer that has an internet connection, select **Register Online**. If the computer does not have an internet connection or is connected to a proxy server, select **Register Offline**. Choose the correct registration method as required.

Select the appropriate registration option.

1. If installing ChimerMarker on a computer with an internet connection, select **Register Online**.
2. If installing on a computer without an internet connection or connected to a proxy server, select **Register Offline** using the **Offline Registration >>** link, as illustrated in Figure 3.

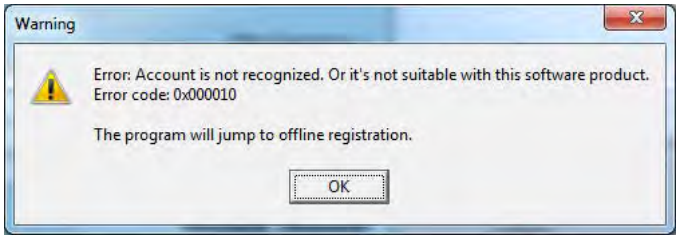
Online Registration

1. Locate the **Account** and **Password** on the SoftGenetics CD or in the email confirming the software purchase.
2. Enter your **Account**, **Password**, and e-mail address information in the appropriate fields. The Request Code information is automatically generated by ChimerMarker.
3. Click **Register**. Registration is automatic.

   A confirmation e-mail is sent once registration is complete.

   Note: If an error displays while trying to register, check for number “1” and lower case letter “L” or number “0” and upper case letter “O” confusion. Some keyboard characters can commonly be misread.

Figure 2 Online Registration



4. After registration, launch ChimerMarker and begin analysis.

Offline Registration

1. Select the **Register Offline** option **Offline Registration >>**.

   Figure 3 Offline Registration

   The license message displays the offline registration procedure.

2. Click **Copy** and then paste the **User ID**, **Account**, and **Password** into an email and send the credentials to tech_support@softgenetics.com. SoftGenetics replies with an appropriate Registration ID within one business day.
3. Paste the received ID into the **Registration ID** field.
4. Click **Register** to complete the registration process, then click **OK.**
5. Launch ChimerMarker and begin analysis.

Network-Licensing Option

The network-licensing option of ChimerMarker is installed on any computer in a network configuration. SoftGenetics uses the License Server Manager (LSM) to control the number of concurrent users accessing the network-licensing option of ChimerMarker v2.00 (and above). LSM uses text-based registration—no hardware is required. Both software components, ChimerMarker and LSM, are installed from the same EXE. The computer where License Server Manager program is installed is considered the *Server* computer. Computers on the network other than the server are called *Client* computers.

Installing License Server Manager requires restarting the system to complete installation. Save all work and close all applications before installing LSM.

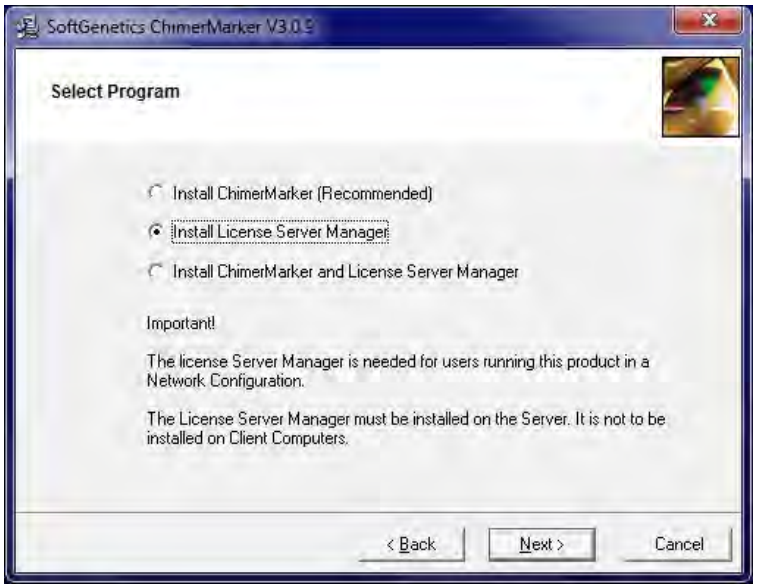
Installation

1. Insert the SoftGenetics CD into the optical or CD-ROM drive. If the computer does not automatically open a CD drive, navigate to the optical or CD-ROM drive on the computer and manually open the directory. For those that choose not to receive a disc, download the executable file from the emailed link.
2. Double-click the ChimerMarker Setup executable file (EXE).

   The installation wizard launches.
3. Click the **Next** button in the **Welcome** dialog.
4. Read the SoftGenetics End User License Agreement, and select the option *I accept the terms of the License Agreement*, and click **Next** in the **Read Me File** dialog.

   The **Select Program** dialog displays.

   Figure 4 Installing the License Server Manager (LSM)



5. Select Install License Server Manager and click Next.

   The **Destination Location** dialog displays. Click **Next**.
6. The Select Program Manager Group dialog displays. Click Next.
7. In the **Start Installation** dialog, to run the LSM installation wizard, click **Next**.

   The **Welcome** dialog displays.
8. Click **Next**.

   Read the **SoftGenetics End User License Agreement** and check the option *I accept the terms of the License Agreement*.
9. In the **Read Me File** dialog, click **Next**.

10. In the **Destination Location** dialog, to install LSM in the default folder, click **Next**. Select the **Browse** button to choose a different installation directory

    Note: The default Destination Location for the License Server Manager program is `C:\ProgramFiles\SoftGenetics\License Server`.

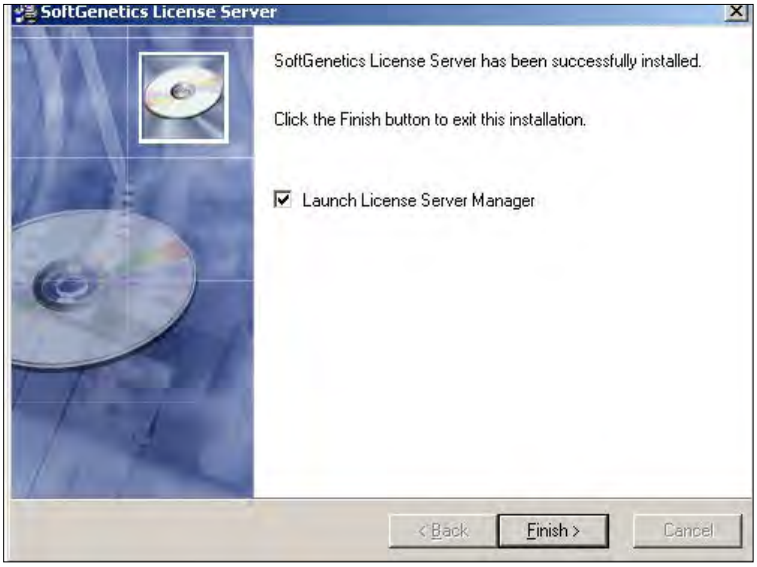
    The **SoftGenetics Licenses Server** dialog displays.

    Figure 5 SoftGenetics License Server

11. In the **SoftGenetics License Server** dialog, to install License Server Manager, click **Next**.

    The **Launch Licenses Server Manager** dialog displays.

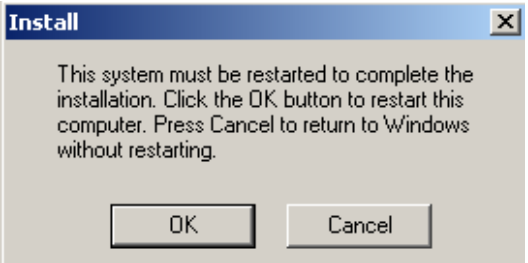
    Figure 6 Launch Licenses Server Manager



12. Select the **Launch License Server Manager** checkbox and click **Finish**.

    The **Install** dialog displays.

    Figure 7 Restart System to Complete the Installation



13. In the **Install** dialog, click **OK** to restart the system.

    The Installation Wizard closes and the system restarts.

14. Eject the SoftGenetics CD.

Registration

1. Open License Server from the system or icon tray by clicking the LSM icon.

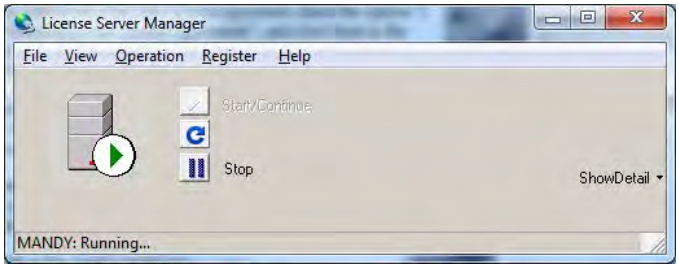
   Figure 8 SoftGenetics Licenser Server

   Note: The icon with a red star indicates the license server is not running.

   Note: The icon with a white star indicates that the License Server is running.

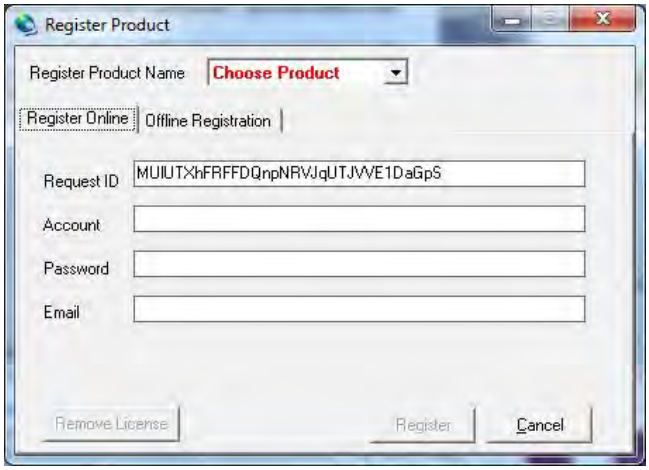
2. In the **SoftGenetics License Server** dialog in Figure 8, click **OK** to proceed with registering the License Server from the **License Server Manager** console.

Figure 9 License Server Manager



3. On the main toolbar, select **Register** to activate the **Register Product** dialog.

Figure 10 Register Product



4. From the **Register Product Name** drop-down menu, select **ChimerMarker**.
5. If the computer the License Server is installed on has an internet connection, select **Online Registration**. If the computer does not have an internet connection or is connected to a proxy server, select **Offline Registration**.

Online Registration

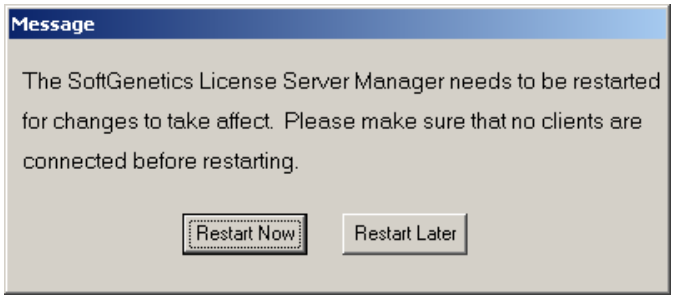
1. Locate the **Account** and **Password** on the SoftGenetics CD or in the email confirming the software purchase.
2. In the appropriate fields enter the **Account**, **Password**, and **E-mail** address information.

   The License Server automatically generates the Request Code information.
3. Click **Register**.

   The software is automatically registered. A confirmation e-mail is sent after registration is complete.

   Note: If an error appears while trying to register, check for number “1” and lower case letter “L” or number “0” and upper case letter “O” confusion. Some keyboard characters can commonly be misread.
4. The **Message** dialog displays.

Figure 11 Restart System to Complete Registration

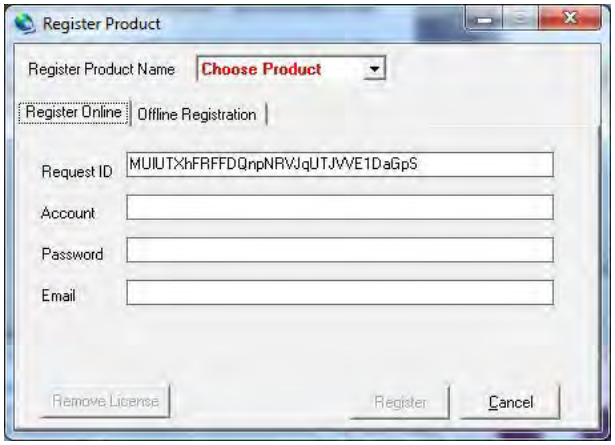


5. Select **Restart Now** to apply the registration information for the License Server.
6. Install the ChimerMarker software and configure the network client.

Offline Registration

1. From the **Register Product** dialog, select the **Register Online** tab, then copy and paste in the **Request ID** field the entire Request Code string.

   Figure 12 Register Product with Register Online Tab



2. From the SoftGenetics CD or in the email confirming the software purchase, locate the account and password and type the information into the body of an e-mail. Send the -email to tech_support@softgenetics.com.

   The Register ID character string is returned within one business day.

3. Once received, in the **Register Product** dialog, in the **Offline Registration** tab, copy and paste the ID from the e-mail into the **Register ID** field.

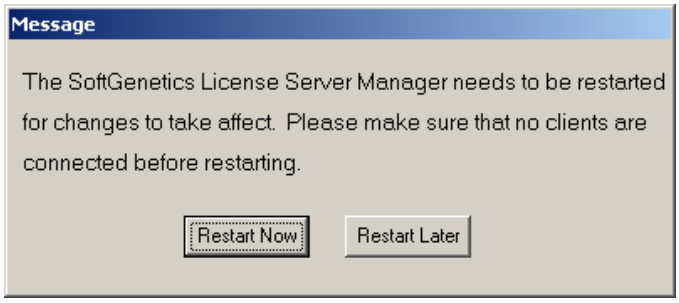
Figure 13 Register Product Dialog

Note: Be sure to copy the entire registration ID character string.

4. Click Register.

   The **Message Restart Now** dialog displays.

   Figure 14 Restart System to Complete Registration



5. Select **Restart Now** to restart the License Server and apply the registration information.
6. Install ChimerMarker software and configure network client.

Installing ChimerMarker Software

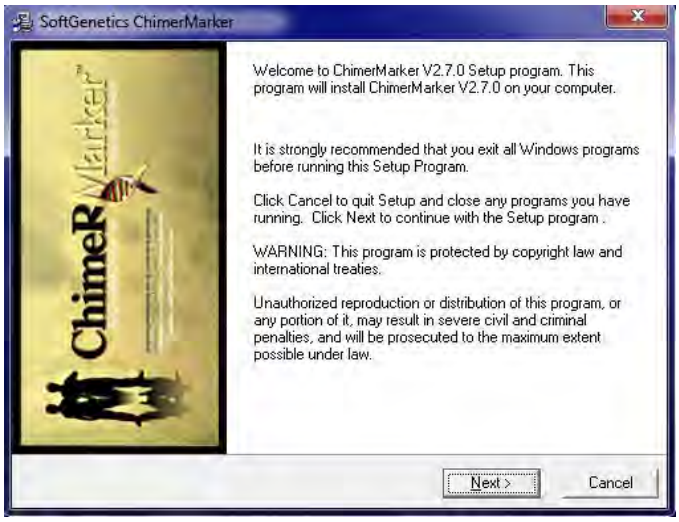
Install ChimerMarker software on client computers

1. Insert the **SoftGenetics CD** into the optical or CD-ROM drive, or download from the emailed link. If the computer does not automatically open a CD, navigate to the optical or CD-ROM drive on the computer and manually open the directory.

2. Double-click the ChimerMarker Setup executable file (EXE).

   The installation wizard launches.

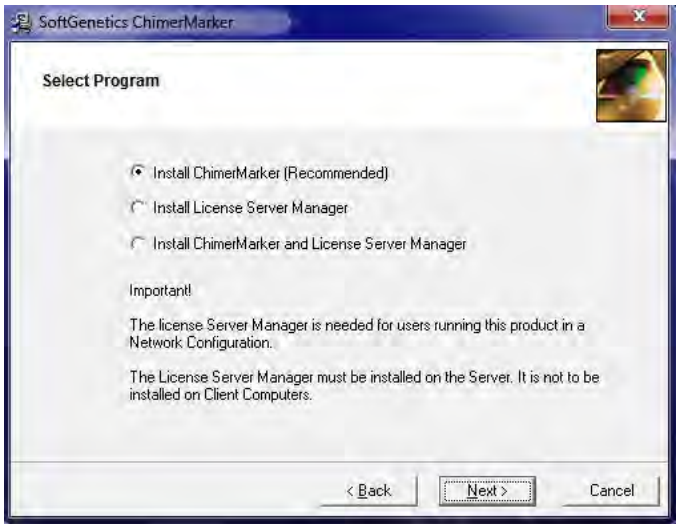
   Figure 15 SoftGenetics ChimerMarker Install



3. Select **Next**.
4. Read the **SoftGenetics End User License Agreement**, check the option *I accept the terms of the License Agreement*, and click **Next** in the **Read Me File** dialog.

   The **Select Program** dialog displays.

   Figure 16 Select Program



5. Select the **Install ChimerMarker** (Recommended) radial button and click **Next**.
6. In the **Destination Location** dialog, to install ChimerMarker in the default folder, click **Next**. If desired, click the **Browse** button to navigate to a different installation directory.

   Note: The default Destination Location for the ChimerMarker program is `C:\ProgramFiles\SoftGenetics\ChimerMarker\ver#`.

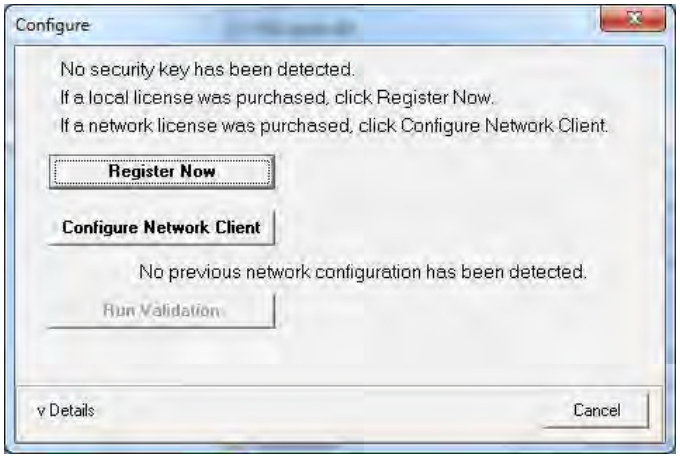
7. In the **Select Program Manager Group** dialog, click **Next** to accept the default location for the Program Manager Group.

   Note: Changing the Program Manager Group default may affect program operability. It is recommended to accept the default.

8. In the **Start Installation** dialog, to install ChimerMarker, click **Next**.
9. In the **Installation Complete** dialog, click **Finish**. The installation wizard closes.
10. Eject the SoftGenetics CD.
11. Launch ChimerMarker by either double-clicking the **ChimerMarker** desktop icon or by selecting the **Start** menu and navigating to **SoftGenetics** > **ChimerMarker** > **ChimerMarker** program, which is the version that was just installed.

    The **Configure** dialog displays.

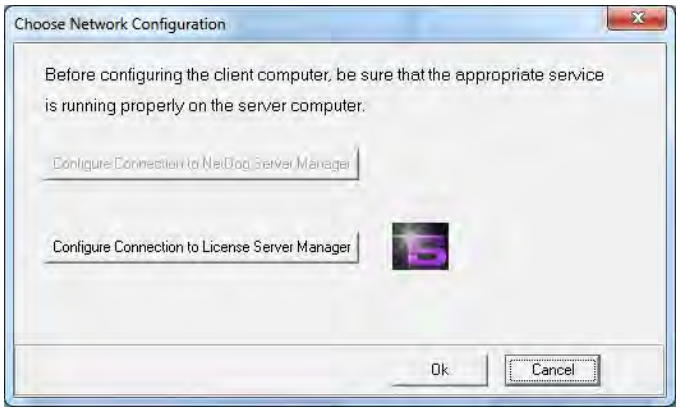
    Figure 17 Configure Dialog



12. Select **Configure Network Client** to configure the client software to contact License Server Manager.

    The **Choose Network Configuration** dialog displays.

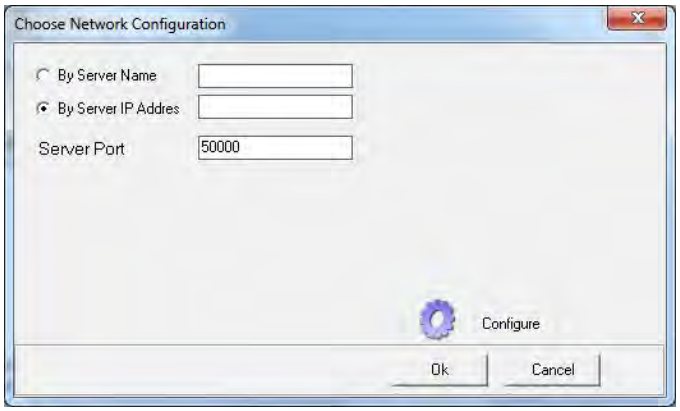
    Figure 18 Choose Network Configuration



13. From the Choose Network Configuration dialog, select **Configure Connection to License Server Manager** and click **OK**.

    The **Choose Network Configuration** dialog redisplays.

    Figure 19 Choose Network Configuration



14. In the **By Server Name** field, type the Server Name or in the **By Server IP Address** field, type the Server IP Address of the License Server Manager server.
15. Click **Configure** and ChimerMarker software automatically opens if the connection is properly established and a license is available.
16. Click **OK**.

Questions

If you have any questions during installation, setup, or program operation, please contact us at (814) 237-9340 *OR* (888) 791-1270 *OR* email us at tech_support@softgenetics.com

Chapter 2 Chimerism Analysis

This chapter provides a description of the Chimerism analysis the software provides.

Analysis Overview

ChimerMarker™ is an automated chimerism analysis software that integrates speed and accuracy with a biologist-friendly interface. The software is used to monitor chimerism levels in both allogeneic and autologous stem cells transplant (SCT) or in hematopoietic stem cells transplant (HSCT), bone marrow transplant (BMT), post-bone marrow engraftment) cord, and peripheral blood stem cells transplant (PBSCT) samples.

ChimerMarker provides accurate, rapid genotyping with chimerism analysis. It automatically identifies donor and recipient peaks in post-BMT samples, calculates percent chimerism and quality metrics for single donor or double donor cases. It also has multi-lineage capabilities for chimerism analysis of Tcells, Bcells, and other cell-type populations.

ChimerMarker includes functions for comparing samples at different time points to conduct longitudinal studies for monitoring each individual with the ability to generate a comprehensive chimerism analysis report. The chimerism analysis performs repetitive calculations using published methods.

ChimerMarker is compatible with the following software products:

- ABI® PRISM, Applied Biosystems® SeqStudio, Beckman-CEQ™, and MegaBACE® and Promega Spectrum Compact CE Systems genetic analyzers.
- Custom primers or commercially available human identification chemistries from ThermoFisher (AB), Promega, and Qiagen for Short Tandem Repeats (STR) genotyping, including the following:
    - GlobalFiler™ 6-Dye™
    - Identifiler®
    - Minifiler®
    - PowerPlex®16, 18D, and 21
    - PowerPlex®ESI/ESX
    - PowerPlex®Fusion
    - GenePrint®24
    - PowerPlex®Fusion 6C
    - Investigator®24Plex
    - Investigator®ESSplex Plus

A Chimerism analysis is linked to the main analysis screen, which removes the error-prone step of data transfer from genotyping software to chimerism analysis software.

This chapter provides step-by-step instructions for performing fragment analysis. For details, see the Frequently Asked Questions (FAQ) as posted on the company website.

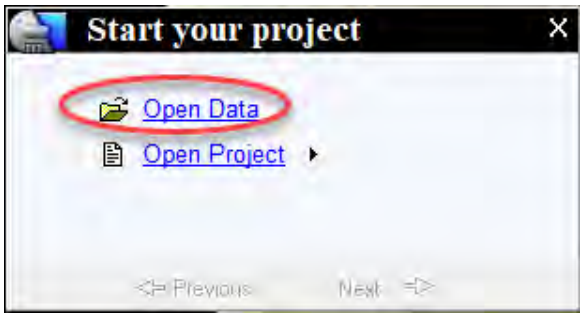
Step 1. Import Data Files

The first step when performing fragment analysis is to upload raw data files to ChimerMarker. ChimerMarker allows the import of the following file types:

- ABI (.fsa and .hid)
- Promega (.fsa)
- MegaBACE (.rsd)
- Beckman-Coulter (.esd)
- Generic (.scf and .sg1)

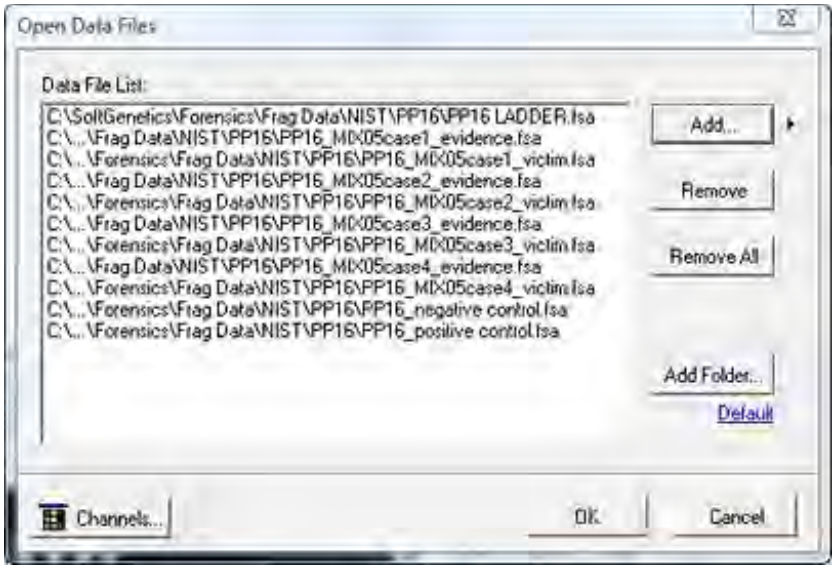
1. Launch ChimerMarker.
2. Enter username and password (if this has been previously established by the lab administrator, see Chapter 10 User Management).
3. In the **Start your project** dialog, either click **Open Data** or select **File** > **Open Data**.

Figure 20 Start Your Project Dialog



The **Open Data Files** dialog displays.

Figure 21 Open Data Files Dialog



4. Select **Add**.

   Note: The buttons on the dialog are described in Table 1 Features of the Open Data Files Dialog that follows this procedure.

5. Navigate to the directory in Figure 21 that contains the raw data files.
6. Type **CTRL+A** to select all files in the list or type **CTRL** and/or **SHIFT** keys to select individual samples.
7. In Figure 20, the **Start Your Project** dialog, select **Open Data**.
8. The files selected display in the **Data File List** field in Figure 21.
9. In the **Open Data Files** dialog, to upload the samples to ChimerMarker, click **OK**.

The next table describes the functions of the buttons in the **Open Data Files** dialog.

Table 1 Features of the Open Data Files Dialog

Feature	Description
Add	Finds and selects raw data files for upload. Select the arrow (**>**) next to the **Add** button to see the four (4) most recently accessed directories.
Remove	Removes samples from the **Data File List** field. In the **Data File List** field, select a sample and then click **Remove**.
Remove All	Removes all sample files from the **Data File List** field.
Add Folder	Upload raw data files from a specific folder in the file directory tree by selecting **Add Folder**. Select the **Default** hyperlink to choose a folder that becomes the default folder ChimerMarker opens when the **Add** or **Add Folder** button is selected.

Feature	Description
Channels (Dyes)	Select to open the **Set Channels** dialog that provides 4-, 5-, and 6-color options in tabs. This dialog also allows the selecting of standard dye color orders defaults from Universal, ABI, Promega, MegaBACE, and Beckman-Coulter or allows the manual entering of dye color and name. The default channel color setup is Universal (color rather than dye name). Set the dye color channels before clicking **OK** in the **Open Data Files** dialog.

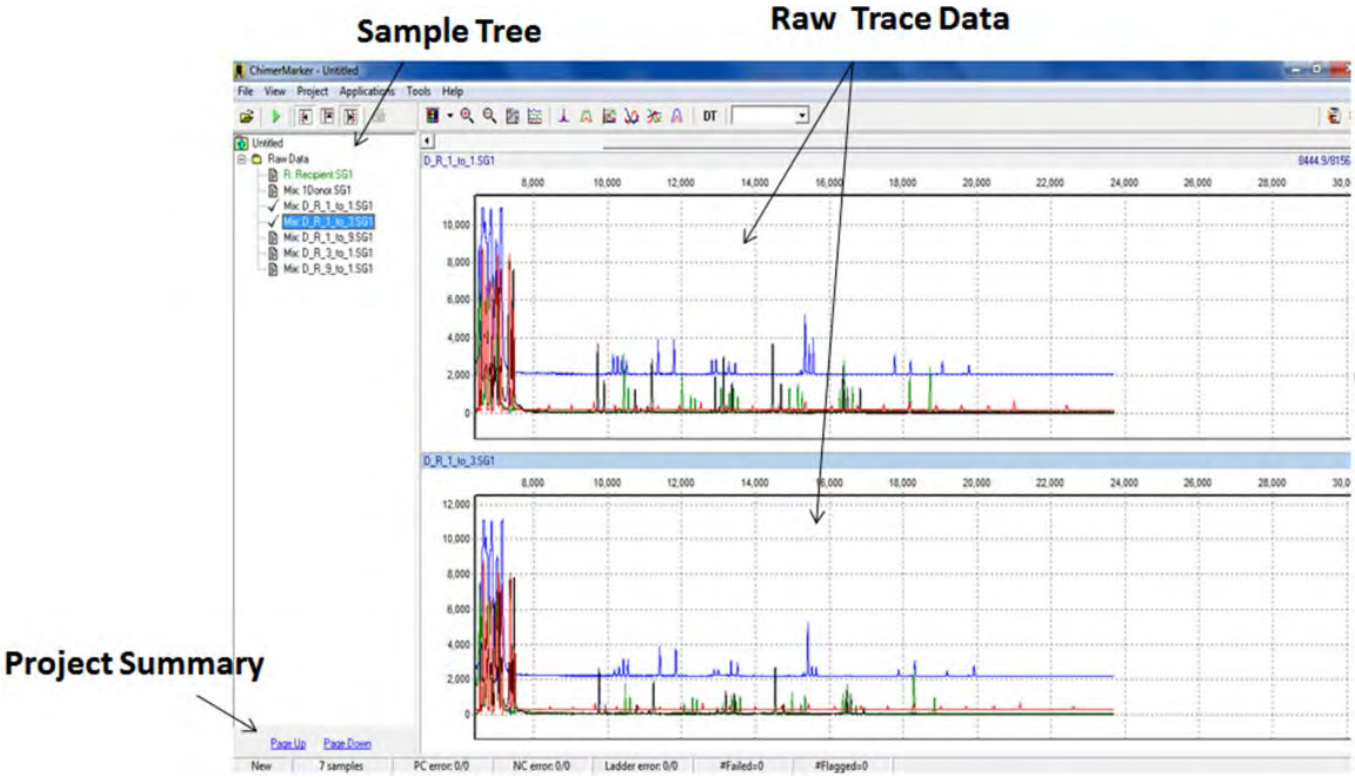
Step 2. Raw Data Analysis

After you upload the raw data files, the **Raw Data Main Analysis** dialog displays as illustrated in Figure 22.

Double-click the samples in the **Sample Tree** to open the individual **Raw Data Traces**. The following functions are available in this view:

- In the left pane, the sample tree lists the name of each sample brought into ChimerMarker. Select a name to display the raw trace data associated with the sample in the workspace to the right.
- The Synthetic Gel Image shows the unprocessed data in a traditional gel format, with larger fragments located on the right. The default settings provide this data display. If the gel is desired, select the center toggle button (fourth from the left in the main tool bar).
- Electropherograms display the fluorescent signal intensities as a single-line trace for each dye color.
- Signal intensities, recorded in Relative Fluorescent Units (RFUs), are plotted along a frame scale in the **Raw Data Analysis** pane with fragment mobility displayed from right to left.
- The largest size fragments appear on the far right of the trace.

Figure 22 Raw Data Main Analysis Screen



Step 3. Select Pre-transplant Sample Identifiers

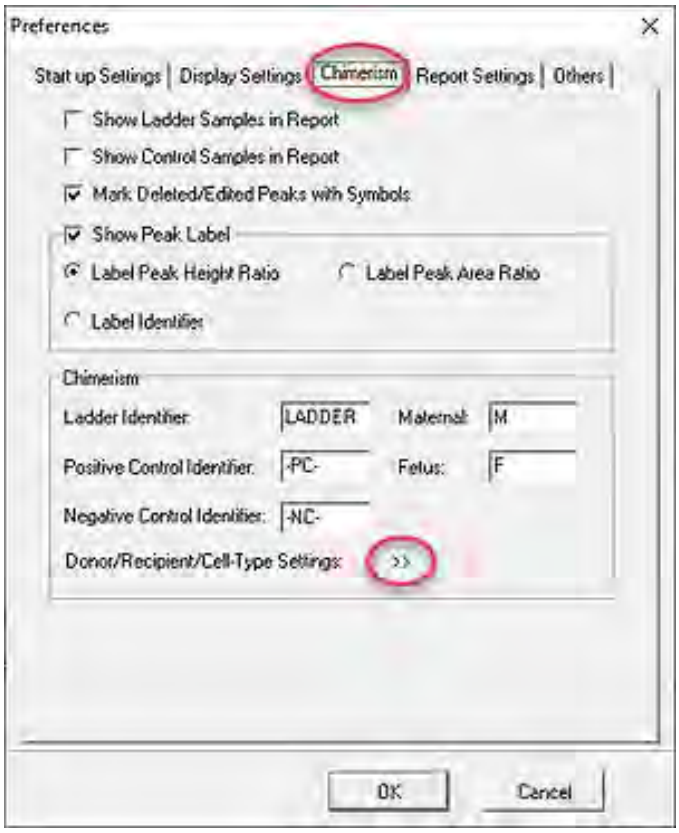
ChimerMarker uses the allele calls of the donor(s) and recipient pre-transplant samples to automatically detect and label peak origin in all post-transplant samples (donor, recipient, or shared). This information is set automatically when file-naming systems are used (see Figure 23 below) or by right-clicking a sample name and selecting the set sample type.

To configure ChimerMarker to automatically differentiate donor and recipient samples using filename recognition, follow the next procedure:

1. In **the Raw Data Main Analysis**, select **View > Preferences** drop-down.

   The **Preferences** dialog displays as illustrated next.

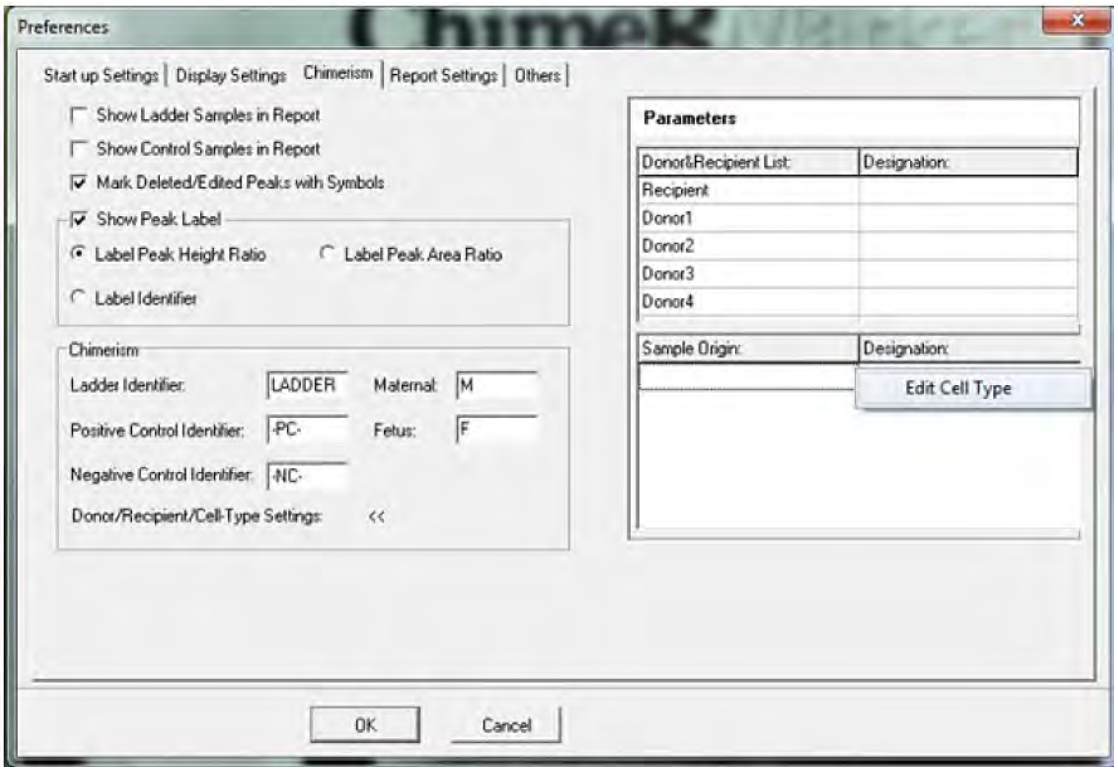
   Figure 23 Preferences Dialog



2. Select the **Chimerism** tab and next to **Donor/Recipient/Cell Type Settings**, click the double right-angle brackets (**>>**).

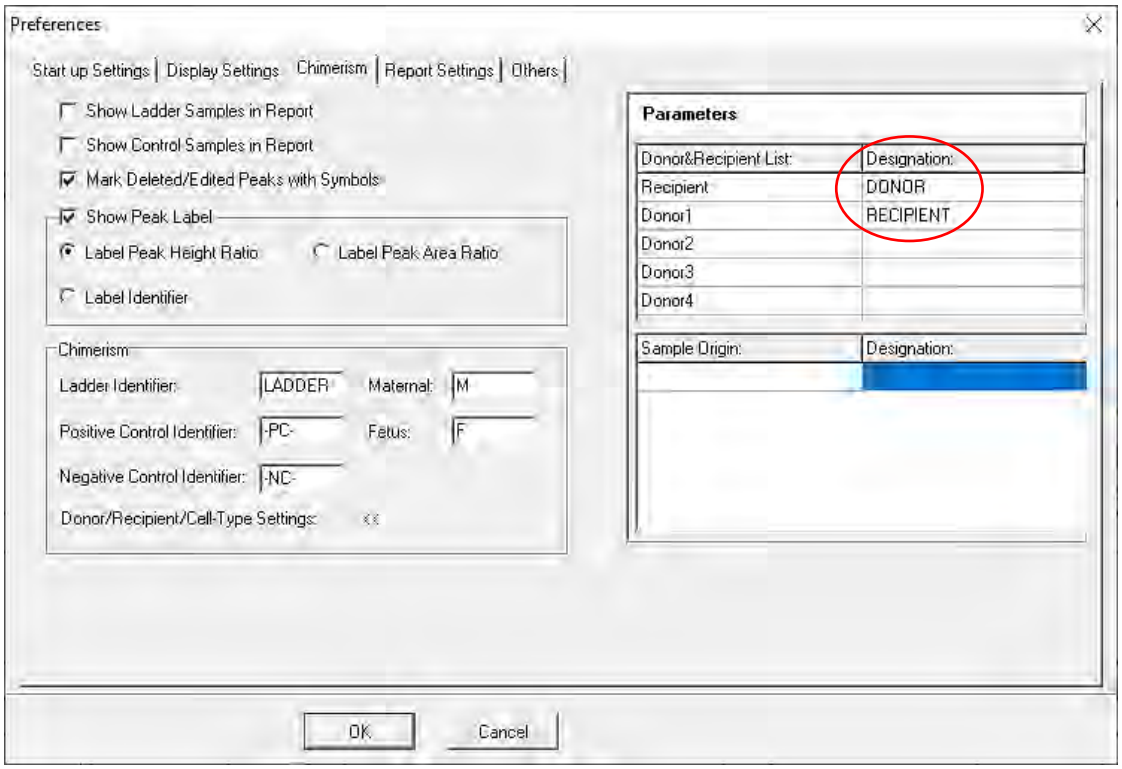
   The **Preferences** dialog displays.

   Figure 24 Preferences Dialog Parameters Pane



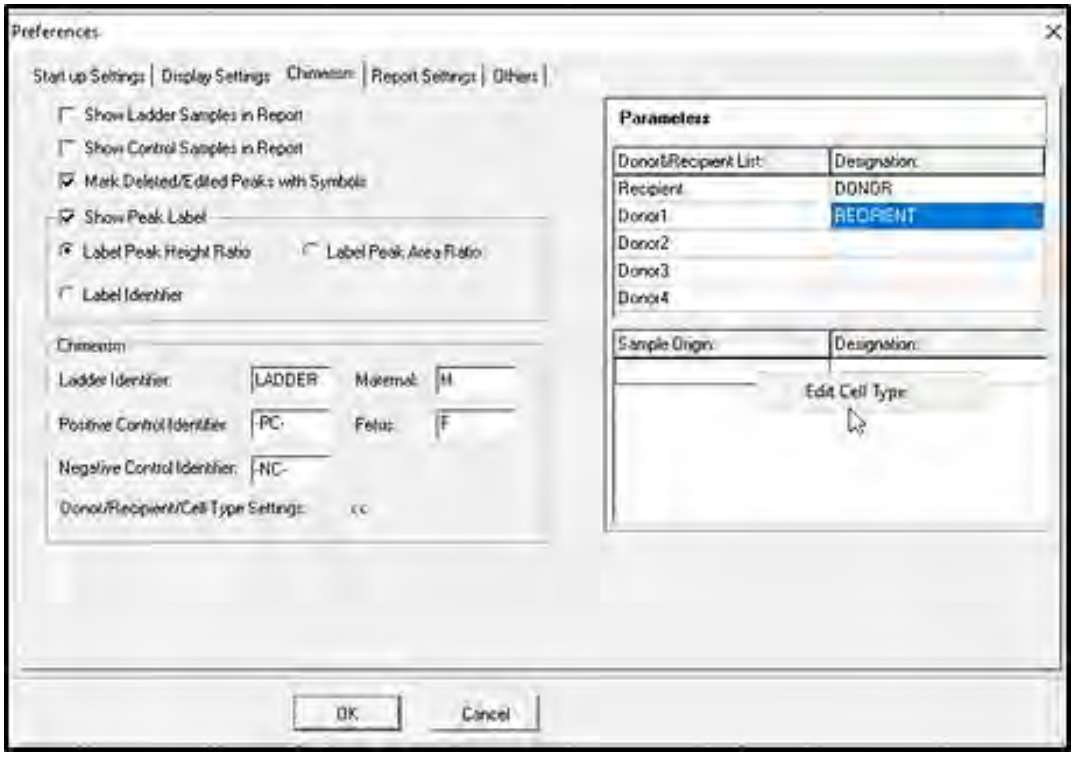
For example, as illustrated in the next graphic, the **Parameters** pane, **Recipient**, and **Donor1** fields are populated.

Figure 25 Preferences Dialog Parameters Populated



Each designation should be at least three (3) characters long and is not case-sensitive.

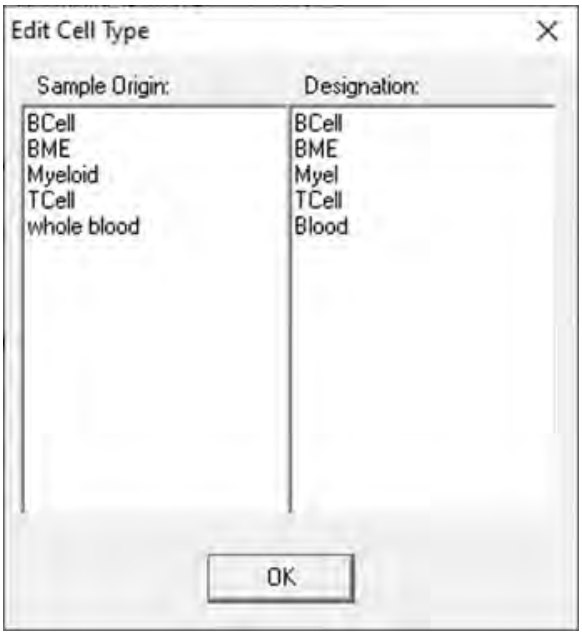
Figure 26 Preferences for Edit Cell Type



3. If there are different cell types, define them as detailed in the following steps:
    a. Right-click in the lower half of the **Parameters** area and select **Edit Cell Type**, as illustrated above.

       The **Edit Cell Type** dialog displays.

       Figure 27 Enter Cell Type Dialog



    b. Click the **Sample Origin** column and type your entries, then press the **Enter** key to move to the next line.
    c. Click the **Designation** column and type a corresponding entry. Press the **Enter** key to move to the next line.
    d. When finished, click **OK**.

Analysis Preferences, Multi-Lineage Analysis

ChimerMarker preferences include labeling peak ratios by height or area, with multi-lineage capabilities for chimerism analysis of T-cells, B-cells, and other cell-type populations. By conducting multi-lineage assessments, see valuable information about the host's current graft status.

ChimerMarker also has functions for comparing samples at different time points to conduct longitudinal studies and produce a comprehensive graph. Case-specific panels are constructed automatically using Donor and Recipient allele calls.

Step 4. Select Allele and Size Call Parameters

All genotyping panels performed in the following steps are **panels that have been calibrated by either the validation team or the analyst**. See the section on panels calibrated either by the validation team or the analyst, Chapter 4 Genotyping Main Analysis Overview and at the end of this chapter.

Perform a genotyping panels action by following the next procedure:

1. Click **Tools > Magic Wizard**.

   The **Run** dialog displays.

   Figure 28 Run Dialog

2. Select **Run**.

   The **Run Wizard** dialog displays.

   Figure 29 Analysis Template Selection Dialog

3. In the **Run Wizard** dialog take the following actions:

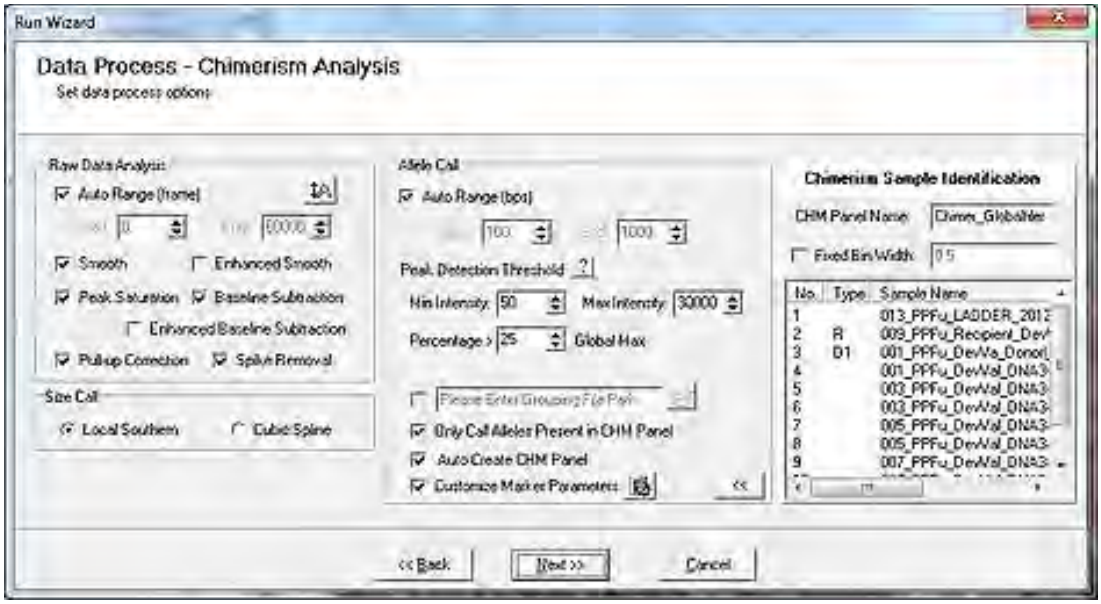
   a. Select from the pre-loaded run templates for many commercially available human identification kits or select custom parameters and save the template using the save icon, as illustrated in Figure 31.

   b. Make selections from the drop-down menus for the following fields: **Panel**, **Size Standard**, and **Standard Color**.

   c. Click **Next**.

The **Data Process Chimerism Analysis** dialog displays.

Figure 30 Data Process Chimerism Analysis Dialog

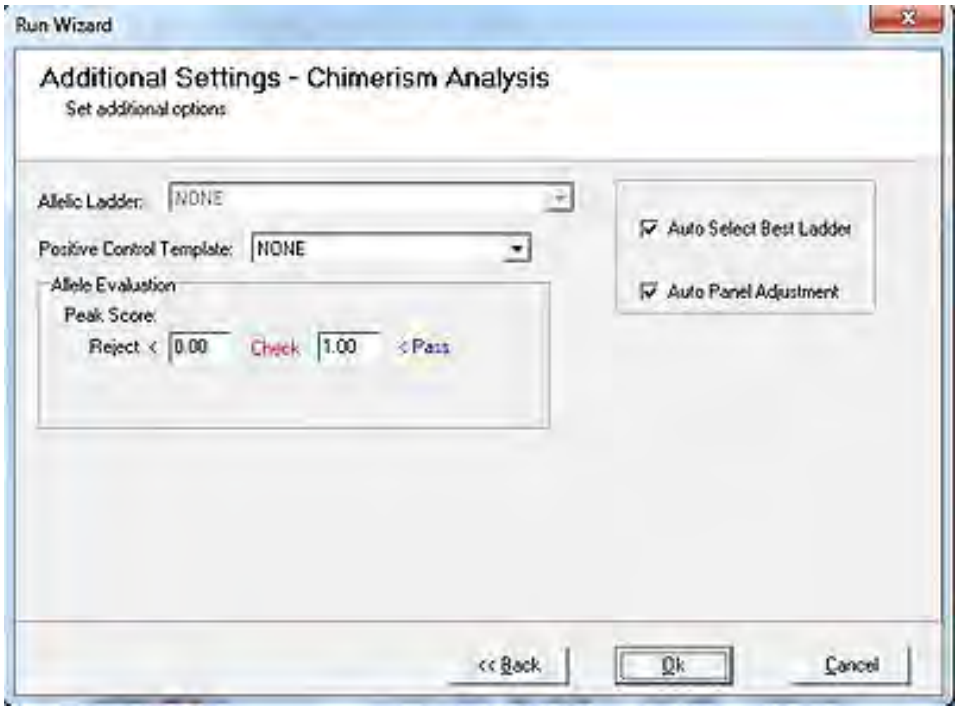


4. Make the following selections as listed next.
   a. In the **Allele Cell** pane, select the **Auto Create CHM Panel** checkbox to have ChimerMarker automatically create a **Chimertyping** panel and apply it (recommended).
   b. Deselect the **Auto Create CHM Panel** checkbox to manually create **Chimertyping** panel. See the **Alternative Chimerism Method** dialog (Figure 31) for additional information.
   c. In the **Raw Data Analysis** pane, the default settings for **Size Call** and **Allele Call** are enabled for most data sets.
   d. Select the **Customize Marker Parameters** checkbox. Default settings are typically used but click the parameter icon to specify different marker parameters for the **Chimertyping** panel.

e. Click **Next**.

The **Run Wizard Additional Analysis Settings** dialog displays.

Figure 31 Additional Analysis Settings



In the **Run Wizard**, the **Allelic ladder** drop-down list, the **Auto Select Best Ladder** and **Auto Panel Adjustment** checkboxes, and the **Positive Control Templates** drop-down list are not used during the chimerism workflow. They are described in Chapter 4 Genotyping Main Analysis Overview when calibrating the panel and checking positive control genotypes.

Note: If data has a high noise-to-signal ratio, see Chapter 4 for information about increasing the minimum heterozygous imbalance filter of the genotyping panel.

Important Note: If donor and recipient CE data files are not available, import the Donor and Recipient Genotypes as a text file, as described in the next section.

Importing Donor and Recipient Genotypes

Follow the procedure in this section if donor and recipient CE data files are not available.

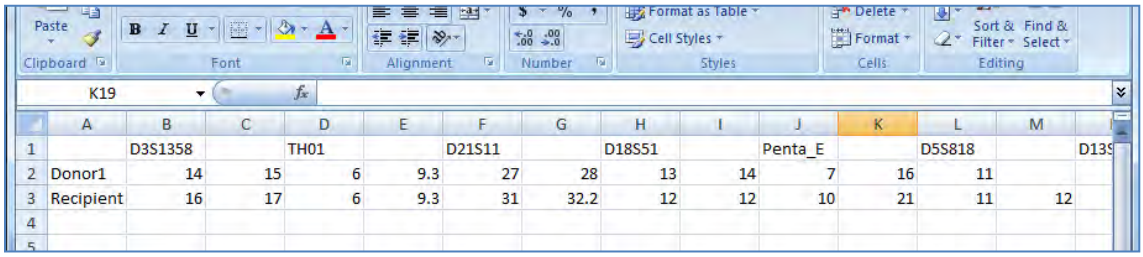
If sample files (.fsa, .hid, etc.) are not available for the recipient and donor(s), upload the genotypes in a tab-delimited text file. ChimerMarker supports uploads of allele calls or allele sizes. The text file must be formatted according to the following guidelines: build the file in Excel or another spreadsheet program and then save the file as a tab delimited text-file. Use the ChimerMarker text-input tool to upload the tab delimited text-file.

Use the Excel format displayed as detailed next.

- The file may contain Allele Sizes or Allele Calls, but not a mixture of both.
- Marker names should be written exactly as they appear in your panel. They are case sensitive.

- For homozygous markers, enter each allele only once. (See D5S818 below).
- Do not put a space between the word Donor and 1 or 2.
- Include every marker that is in your project panel. If no calls are available for a given marker, leave the donor and recipient cells blank, but keep the marker name.
- Save the Excel sheet as a tab-delimited text-file (.txt).

Figure 32 File Format for Cases That Do Not Have Donor / Recipient Raw Data

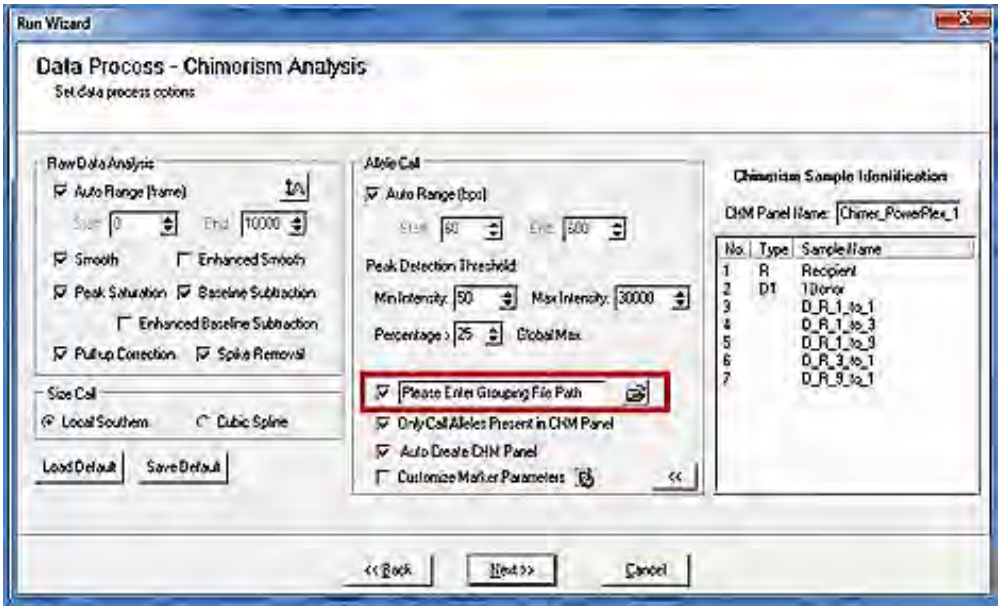


	A	B	C	D	E	F	G	H	I	J	K	L	M
1		D3S1358		TH01		D21S11		D18S51		Penta_E		D5S818	
2	Donor1	14	15	6	9.3	27	28	13	14	7	16	11	
3	Recipient	16	17	6	9.3	31	32.2	12	12	10	21	11	12
4													
5													

Note: Manually edit text files using the text input tool. After a text file is uploaded, select a cell and type to modify the file. When finished editing, select **Export** to save the update. When editing a file using the text input tool, save the changes before proceeding with data processing.

- After reviewing the guidelines above, use the following procedure to import text files into ChimerMarker:
    a. From the main analysis screen, import mixture data files (post-transplant files) following Step 1. Import Data Files and Step 4. Select Allele and Size Call Parameters.
    b. Select the appropriate genotyping panel and the size standard in the first pane of the **Run Wizard** dialog.
    c. Select the option to import a text file, as illustrated in Figure 33.

Figure 33 Using the .txt file of Donor and Recipient Genotypes

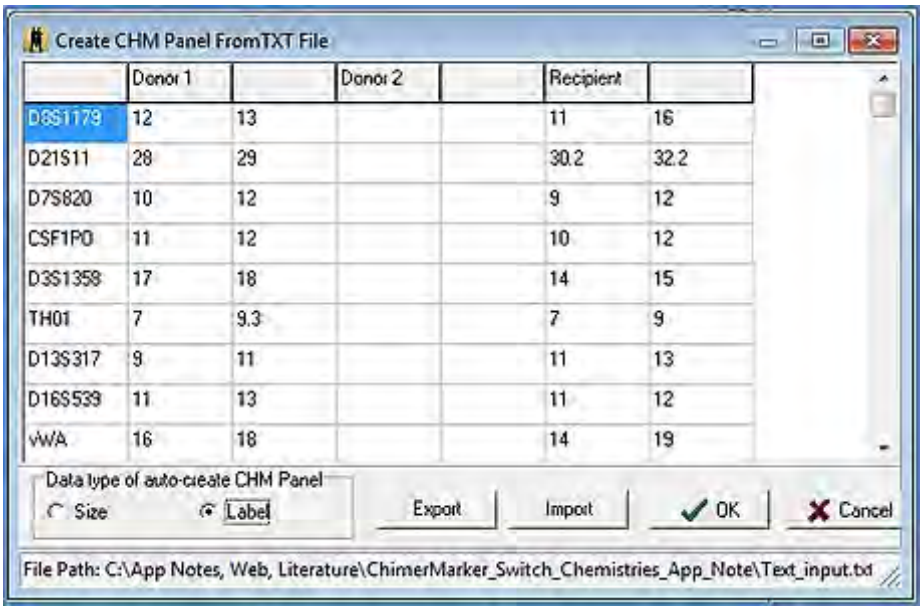


d. Click the **Folder** icon next to the **Please Enter Grouping File Path** checkbox (as displayed in the red box in Figure 33) to open the text-input tool.

e. Click **Next.**

The **Create CHM Panel from TXT File** dialog displays.

Figure 34 Create CHM Panel from TXT File



f. Select **Import** and navigate to the text file.

g. Select **Size** or **Label** to indicate whether the text file contains allele sizes or allele labels.

h. Click **OK** to proceed with the analysis.

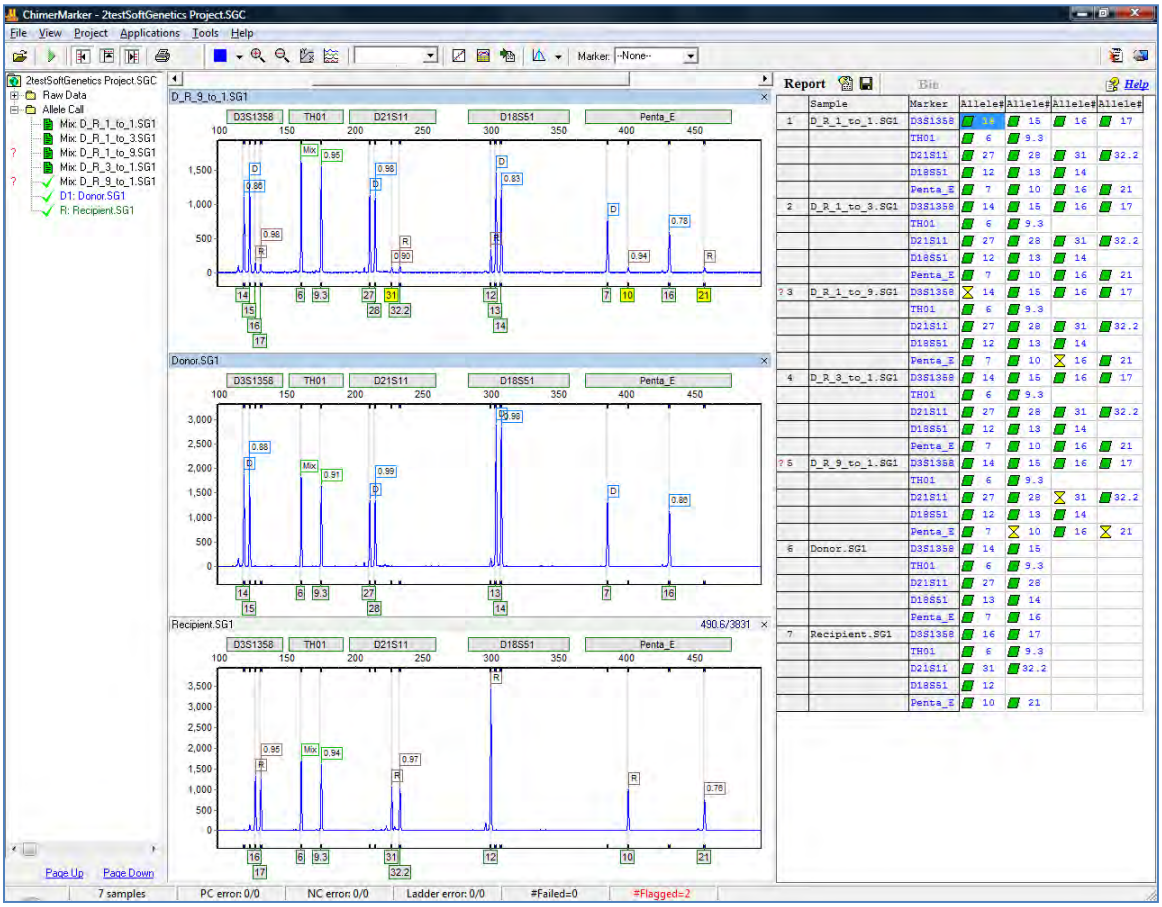
Review Main Analysis Screen Results

The peak origin of each post-transplant sample is displayed in a flag at the top of the peak: D = donor (D1 and D2 for double donor cases), R = recipient, and D1R or D2R for shared peaks.

Sample names with a green sheet have high-quality size calls. If the sample has yellow or red sheets by the file name, refer to the Section Size Calibration Charts. Allele calls are displayed below each peak. If there are any red off ladder(OL) allele peaks, see the Chapter 6 Panel Editor for information on panel alignment and calibration.

The following figure shows sample review results. The **Donor** and **Recipient** indicated in the **File Name Tree** are based on user-specified names in **View** > **Preferences** > **Chimerism** tab.

Figure 35 Review Analyzed Data with Automated Detection D, R, Shared Peaks



Note: Post-Transplant Sample: for shared alleles in each locus ChimerMarker differentiates and labels peaks for Donor (D), Recipient (R), or Mixture (Mix). Heterozygous imbalances are also calculated separately for sister alleles of the same locus for donor and recipient.

If there is excessive heterozygous imbalance in one or more single source samples for a given allele, it is recommended by several publications on Chimerism analysis that these are unsuitable for Chimerism calculations and long-term monitoring. The heterozygous imbalance is displayed in the peak flags to assist researchers in the rapid evaluation of peak

height balance in heterozygous, single source samples. The heterozygous imbalance in the mixture sample is the peak ratio of sister peaks.

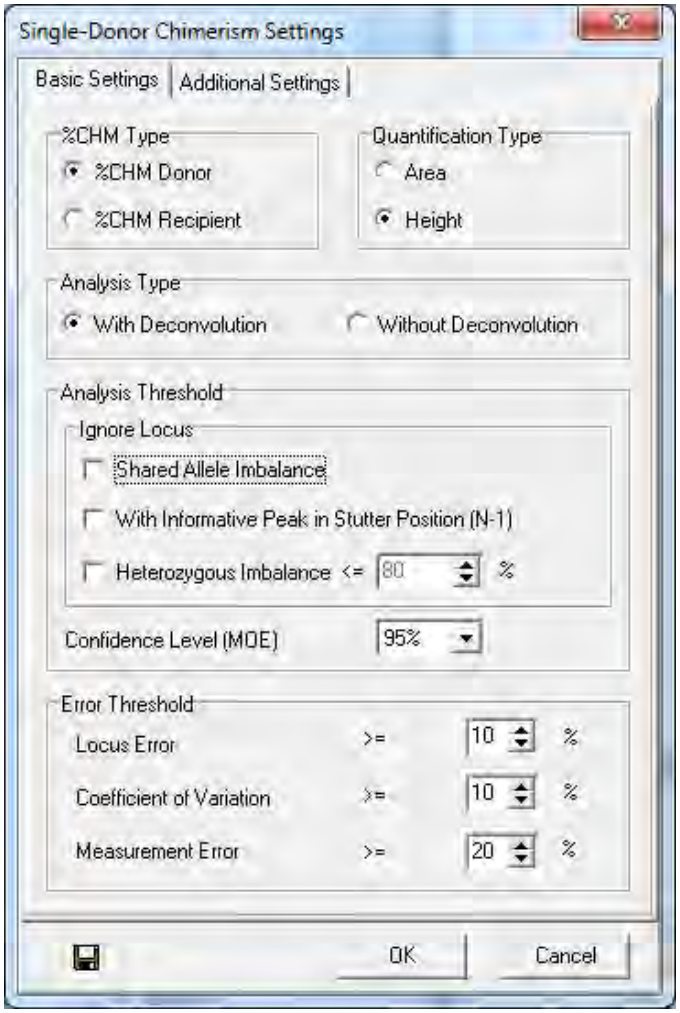
To maintain the integrity of the analysis going forward into the Chimerism Application and Calculations, make edits in the Main Analysis dialog and save them in the Chimerism project (.SGC) file.

Automated Chimerism Detection and Quantification

From the main tool bar, select **Applications**. Choose **Single Donor** or **Double Donor Chimerism Analysis** to launch the linked chimerism application directly from the main screen.

There is no need for copy, paste, or data transfer. The dialogs for **Single Donor Chimerism Settings** and **Double Donor Chimerism Settings** provide flexible analytic options. Details of the equations used for all calculations are provided in Chimerism equations.

Figure 36 Basic Single Donor Chimerism Settings

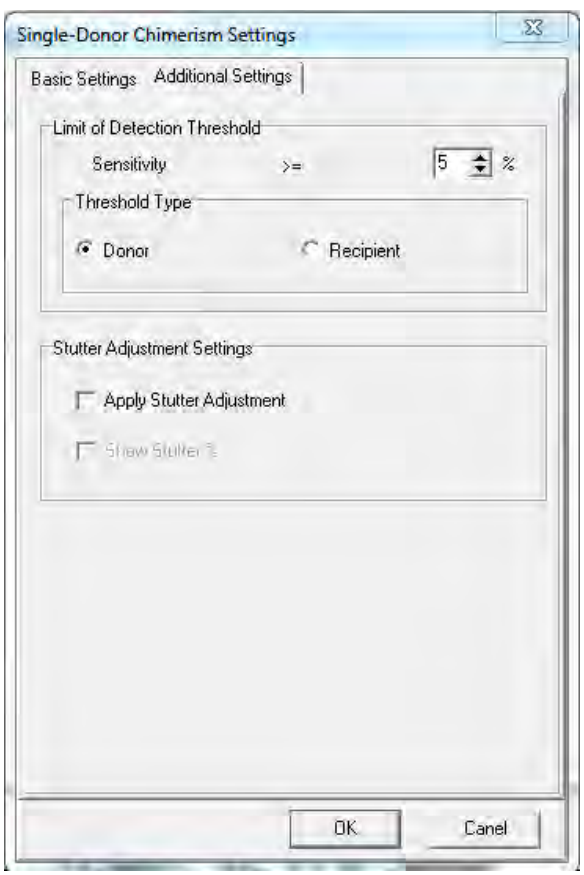


In the **Single-Donor Chimerism Settings** dialog, follow the next steps:

1. Select **%Chimerism Type**, and then choose **%CHM Donor** or **%CHM Recipient**.
2. Select **Quantification Type**, and then choose (Peak) **Area** or **Height**.
3. Select Analysis Type, and then choose either With Deconvolution or Without Deconvolution.
4. The **Ignore Locus** pane provides three (3) additional conditions under which markers should be ignored:
   - **Shared Allele Imbalance**: If this option is selected, the marker is ignored if a single source peak in the marker has a higher RFU than a shared peak.
   - **With Informative Peak in Stutter Position (N-1)**: The marker is ignored if a donor, recipient, or shared peak is in a stutter position. If selected, the stutter adjustment option is disabled.
   - **Heterozygous Imbalance**: Ignores a locus if the smaller of two (2) donor or recipient peaks is shorter than the specified percentage times the height of the larger of the two (2) peaks.
5. Set the desired **Error Threshold** for either **Locus Error**, **Coefficient of Variation**, and **Measurement Error**.

   The **Single-Donor Chimerism Settings** dialog displays.

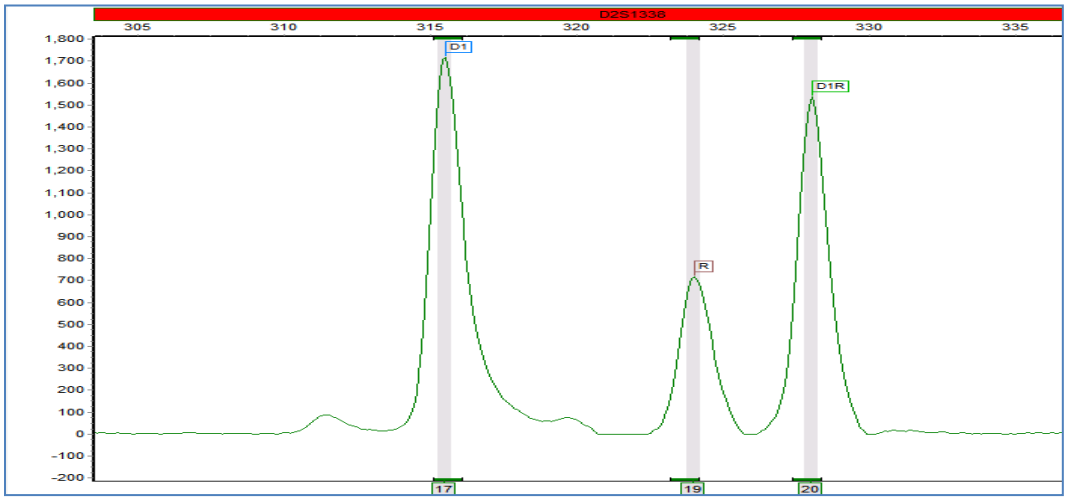
   Figure 37 Additional Chimerism Single Donor Chimerism Settings



6. Specify the percentage for **Limit of Detection Threshold**. Any sensitivity above the threshold % is flagged red. Also, specify the threshold type, donor or recipient. It is best to use the same type as the **%CHM Type** selected in the **Basic Settings**.
7. **Apply Stutter Adjustment** subtracts marker stutter-specific values-- specified in the panel-- in the stutter alleles. The stutter % value for each marker is displayed if **Show Stutter %** is selected along with **Apply Stutter Adjustment**.
8. At the bottom-left corner, click **Save** to save your selections as the default settings.
9. Select **OK** on Figure 37 to review the results of chimerism calculations.

   The results of the Chimerism Calculations graph display to show an example of SAI for locus D2S1338.

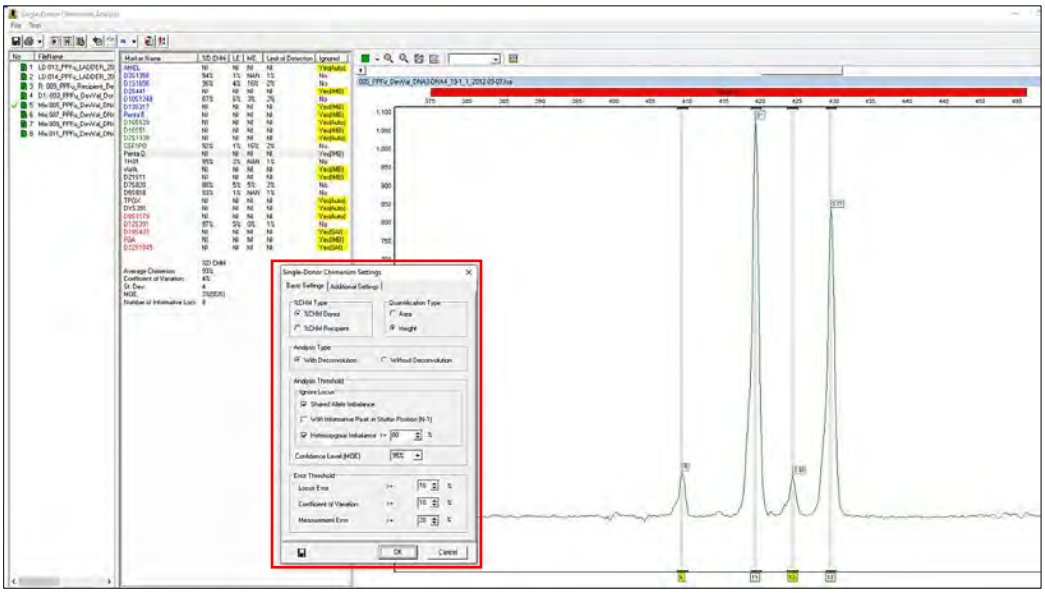
   Figure 38 Example of an Unsuitable Marker for Chimerism Quantification



   Quantification is suspect when the PCR bias results in a smaller fragment from one individual who is more efficiently amplified than the larger fragment that is shared between donor and recipient.

   An IMB reason for ignoring a locus in the Chimerism analysis is detailed next in the example_decreased_hetimb_allowableTo70_donorHetBalanceWithinSpecs.

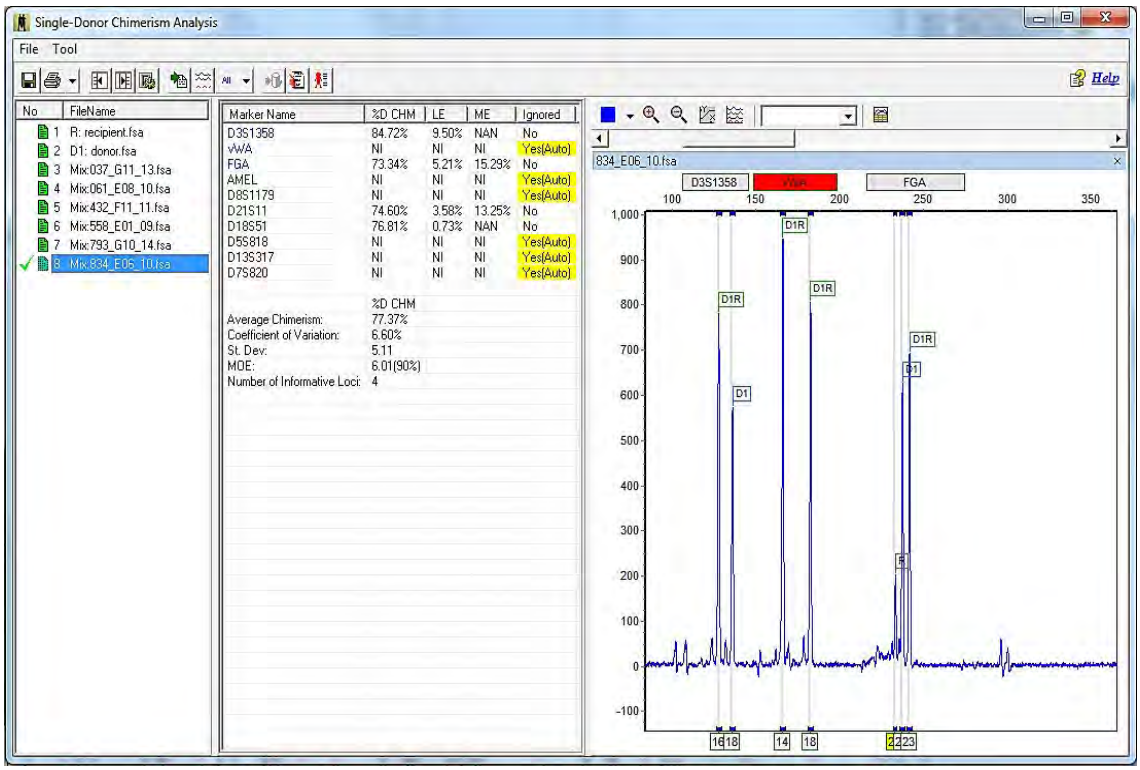
Figure 39 Impact of Heterozygous Imbalance Setting on Marker Selection



Review Results

In the **Single Donor Chimerism Analysis** dialog displayed next, the automated chimerism calculations are displayed in the center pane.

Figure 40 Single Donor Chimerism Analysis



Perform the following actions as detailed next.

- To view the results for a given file, select a file name in the left panel. Loci that do not meet the selected chimerism analysis settings are ignored and highlighted in yellow in the report table.
- A marker is ignored in all samples by right-clicking and selecting **Ignore marker in all Samples**.
- The locus labels of any uninformative loci are highlighted red in the electropherogram.

  Review the following designations in Figure 40:

  - **NI:** Non-informative. A marker is non-informative based on peak pattern and parameters chosen in analysis settings.
  - **NAN**: No available number. No results are calculated for the following peak pattern.
  - **ME**: Measurement error.
  - **LE**: Locus error.
  - **MOE**: Margin of error.

Alternative Method Procedure

The alternative workflow is designed for problematic data, such as files that have extensive pull up peaks or shifts in migration from capillary electrophoresis variation over time. Optimally, these issues are resolved by CE system maintenance; however, the alternative workflow in ChimerMarker provides the flexibility to allow accurate chimerism detection and monitoring in some sub-optimal data sets, as listed next:

Initial analysis begins with genotyping (determining size and allele calls) of the single source donor and recipient sample for each case. Perform the steps provided in the following places:

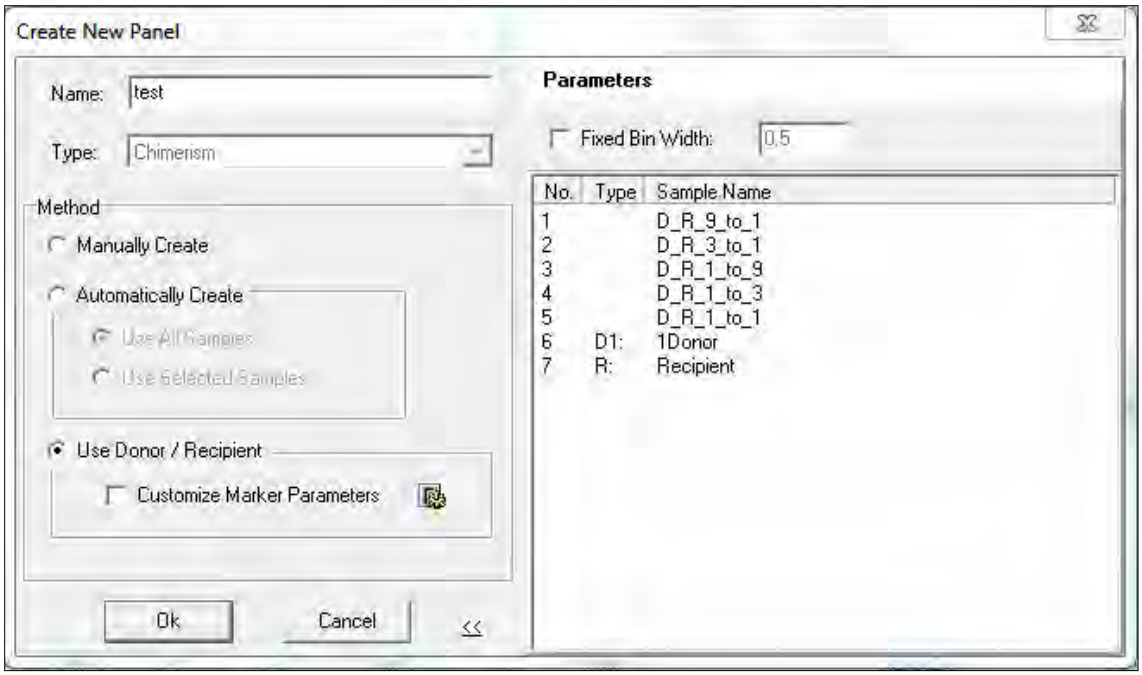
1. Chapter 3 Genotyping General Procedure.
2. After genotyping the original single source samples, the case-specific chimerism panel is built, as described in Chapter 6 Panel Editor.
3. Return to the main analysis screen and activate the **Run Wizard.**
4. Use the Chimerism panel for the case under analysis and re-analyze the data using the new panel. Information about the origin of each fragment (donor, recipient, or shared peak) from the Chimerism panel is displayed as flags on each peak. D indicates a donor peak (D1 or D2 if two (2) donors are involved), R indicates a recipient peak, and D1R indicates the peak is shared (occurs in both the donor and the recipient).

Select Pre-Transplant Sample Identifiers

ChimerMarker uses the allele calls of the donor(s) and recipient pre-transplant samples to detect and label peak origin automatically in all post-transplant samples (donor, recipient or shared).

This information is set automatically with a file-naming system (see Figure 41) or by right-clicking on the sample name and selecting a set sample type.

Figure 41 Create New Panel



Analysis Preferences and Multi-Lineage Analysis

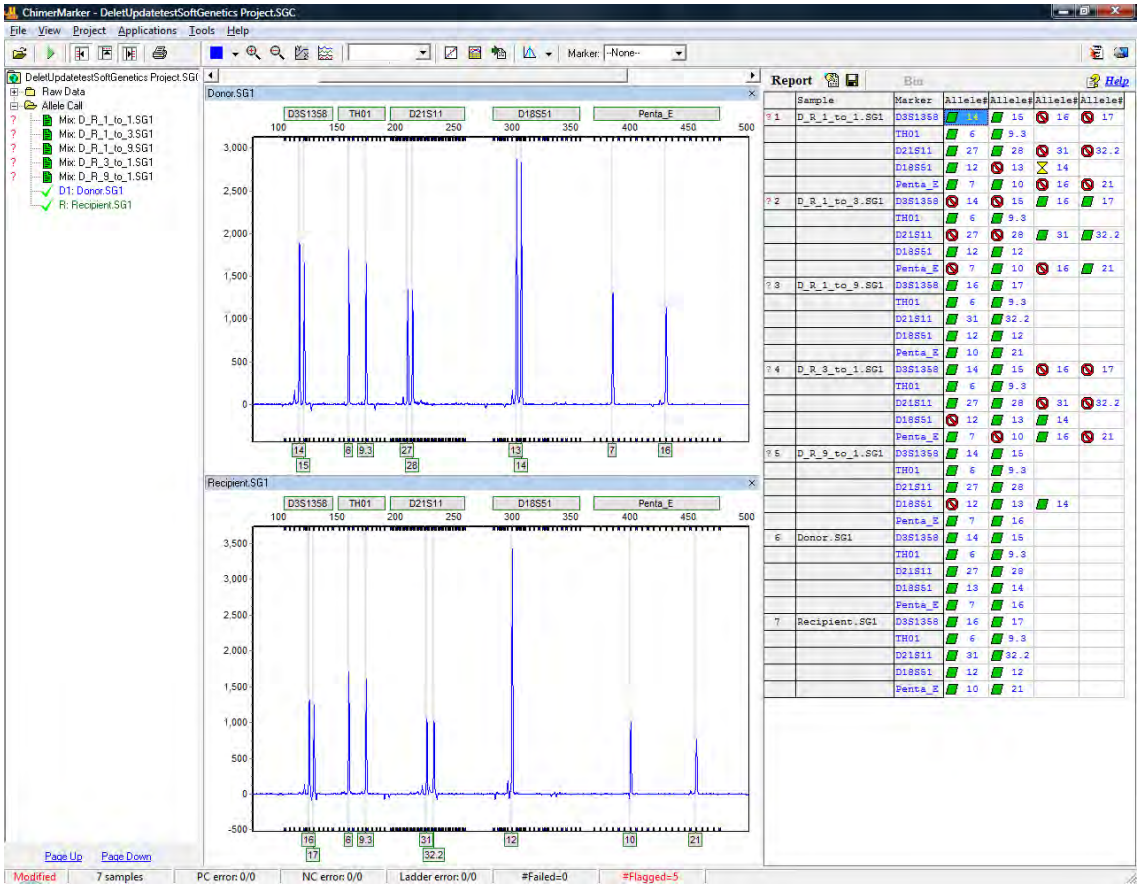
Program preferences include labeling peak ratios by height or area, with multi-lineage capabilities for chimerism analysis of Tcells, Bcells, and other cell type populations. By conducting multi-lineage assessment, valuable information about the current graft status of the host is seen. In addition, there are functions for the comparison of samples at different time points to conduct longitudinal studies and produce a comprehensive graph. Case-specific panels are automatically constructed using Donor and Recipient allele calls.

Genotype the Single Source Samples

Perform the steps for importing and processing data from Chapter 3 Genotyping General Procedure and Chapter 4 Genotyping Main Analysis Overview.

Review the results in **Genotyping Main Analysis Review** dialog.

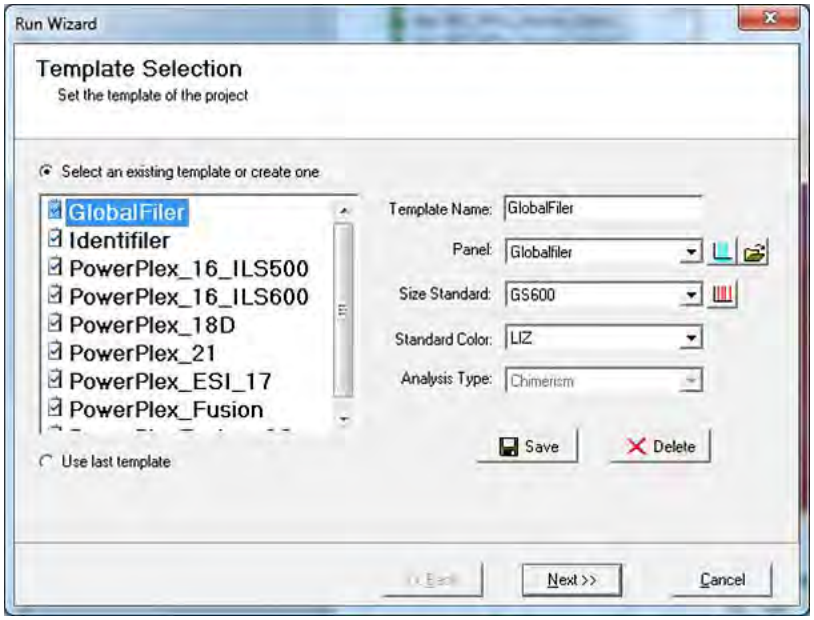
Figure 42 Genotyping Main Analysis Review



After reviewing the results and making any desired edits, as described in Chapter 4 Genotyping Main Analysis Overview, use **Tools** > **Panel Editor** to build the Chimertyping panel, as described in Chapter 6 Panel Editor. The identifiers are any combination of letters and numbers. For example, in place of RECIPIENT, labs may want to type the recipient's ID number or name.

1. From the **Project** drop-down or the green arrow icon, launch the **Run Wizard**.

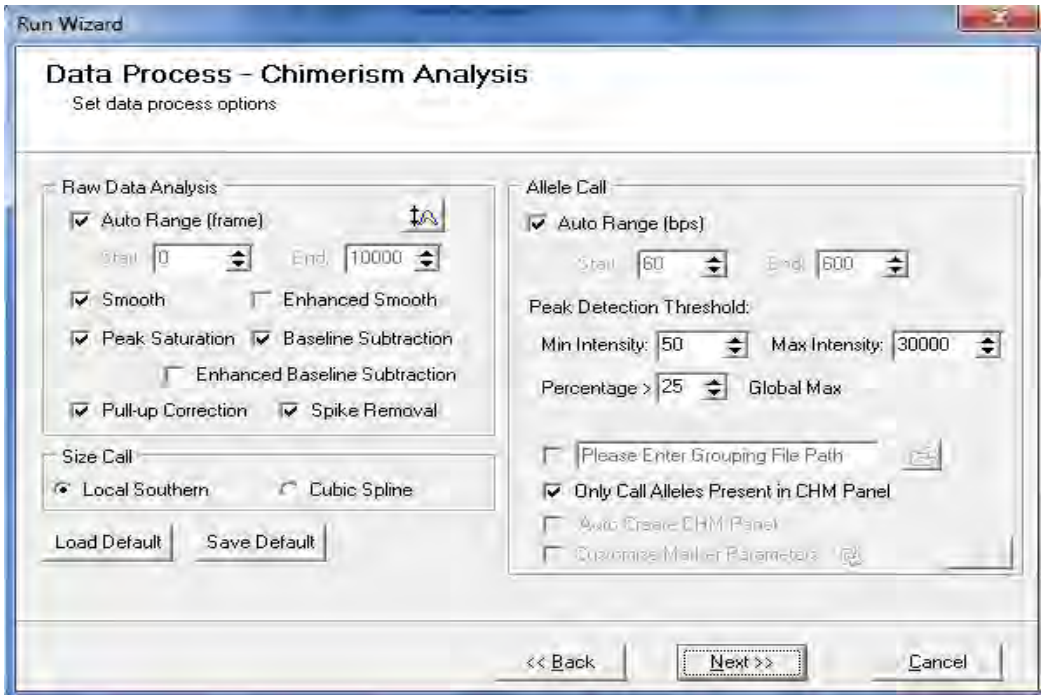
Figure 43 Run Wizard Template Selection



2. In the **Analysis Type** drop-down list, select the **Chimertyping** panel and click **Next**.

   The **Run Wizard Data Process – Chimerism Analysis** dialog displays.

Figure 44 Run Wizard Data Process



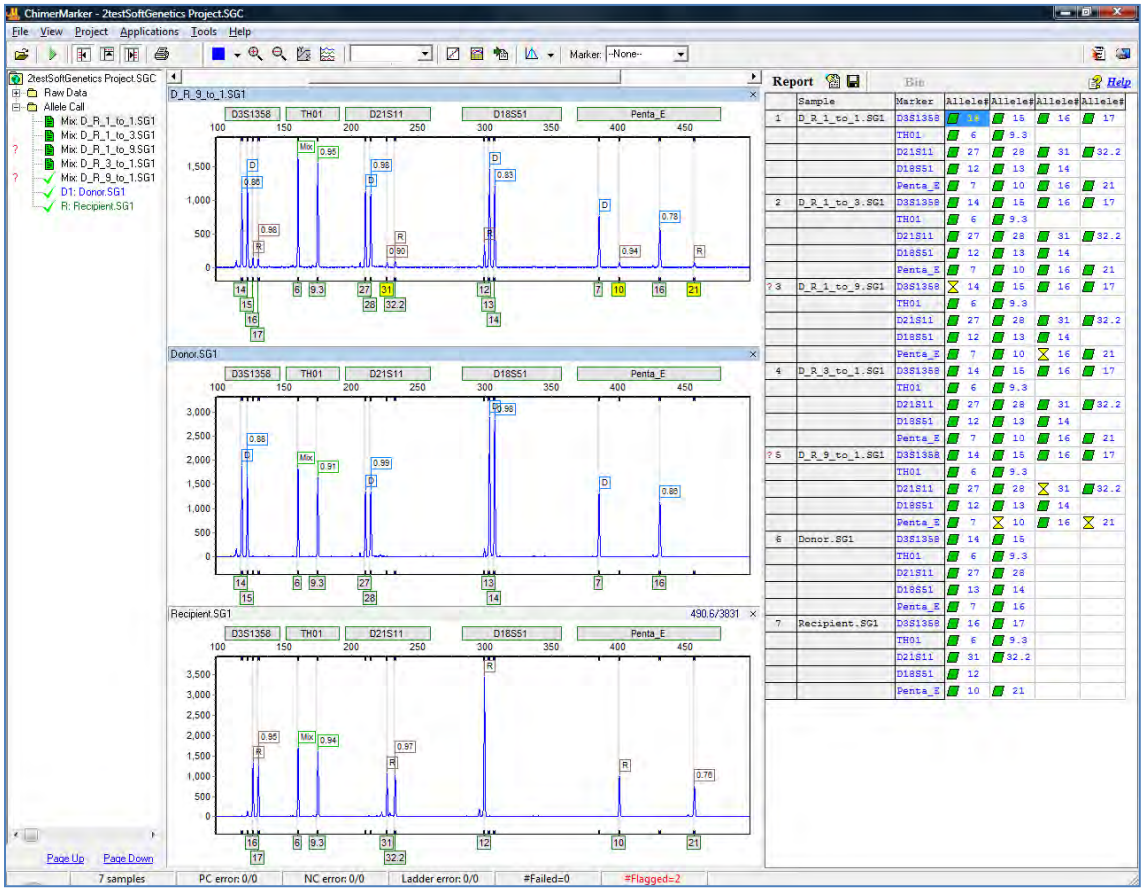
3. Select the **Only Call Alleles Present in CHM Panel** checkbox to filter out extraneous peaks. Adjust other parameters if needed to proceed with genotyping analysis.
4. Click **Next** and then click **OK**.

Review the results in the electropherograms and linked report table.

Review Results

The following figure shows sample review results. The **Donor** and **Recipient** indicated in the **File Name Tree** are based on user-specified names in **View** > **Preferences** > **Chimerism** tab.

Figure 45 ChimerMarker File Name Tree



Note: Post-Transplant Sample: ChimerMarker differentiates and labels peaks for Donor (D), Recipient (R), or Mixture (Mix) for shared alleles in each locus. Heterozygous Imbalances are also calculated for sister alleles of the same locus separately for donor and recipient.

If there is excessive heterozygous imbalance in one or more single source samples for a given allele, it is recommended by several publications on Chimerism analysis that these are unsuitable for Chimerism calculations and long-term monitoring. The heterozygous imbalance is displayed in the peak flags to assist researchers in rapid evaluation of peak height balance in heterozygous, single source samples. The heterozygous imbalance in the mixture sample is the peak ratio of sister peaks.

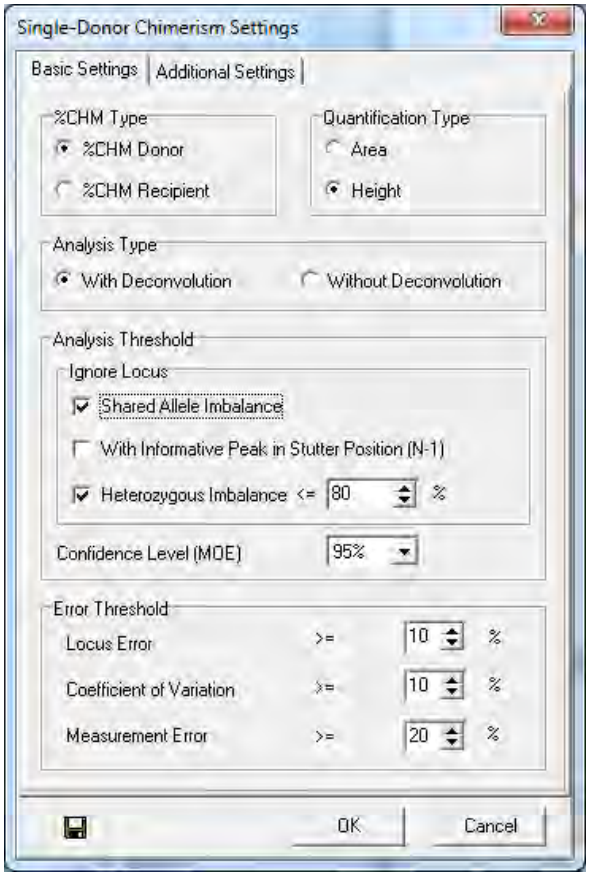
To maintain the integrity of the analysis going forward into the Chimerism Application and Calculations, make edits in the Main Analysis dialog and save them in the Chimerism project (.SGC) file.

Automated Chimerism Calculations

In Figure 45, select **Applications** from the main tool bar. Choose **Single Donor** or **Double Donor Chimerism Analysis** to launch the linked chimerism application directly from the main screen. There is no need for copy/paste or data transfer. The dialogs for **Single Donor Chimerism Settings** and **Double Donor Chimerism Settings** provide flexibility for analysis options. Details of the equations used for all calculations are provided at the end of this chapter in the section on Chimerism Calculations.

The **Single-Donor Chimerism Settings** dialog, **Basic Settings** tab displays.

Figure 46 Single-Donor Chimerism Settings Basic Settings Tab



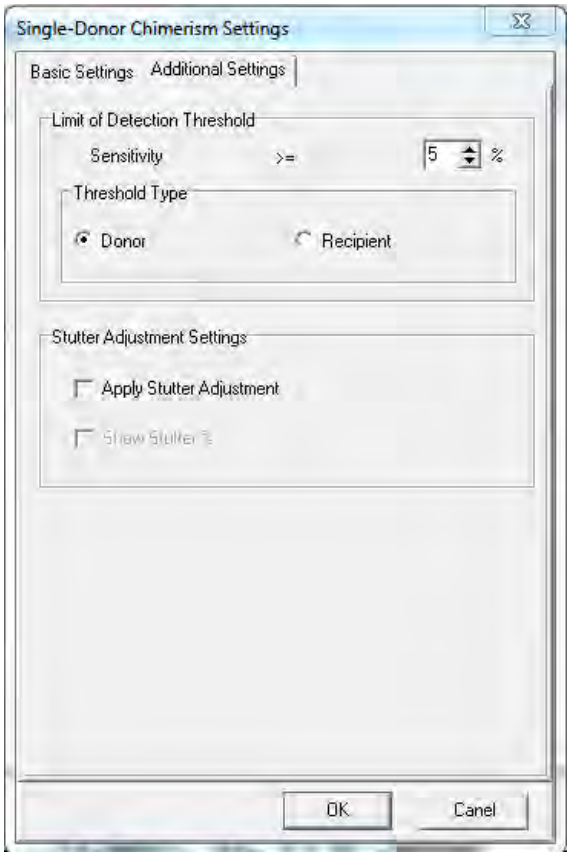
1. In the **% CHM Type** pane, select either **%CHM Donor** or **%CHM Recipient**.
2. In the **Quantification Type** pane, select either the (Peak) **Area** or **Height** radial button.
3. In the Analysis Type pane, select either With Deconvolution or Without Deconvolution.
4. In the **Analysis Threshold** pane, and the **Ignore Locus** sub-section, select from three (3) additional conditions under which markers should be ignored. These include the following listed next:
    - **Shared Allele Imbalance**: If this option is selected, the marker is ignored if a single source peak in the marker has higher RFU than a shared peak.

- With **Informative Peak in Stutter Position (N-1)**: The marker is ignored if a donor, recipient, or shared peak is in a stutter position. If selected, the stutter adjustment option is disabled.
- **Heterozygous Imbalance**: Ignores a locus if the smaller of two (2) donor or recipient peaks is shorter than the specified percentage times the height of the larger of the two (2)peaks.

5. In the Error Threshold pane for the values Locus Error, Coefficient of Variation, and Measurement Error.
6. Next to those values, specify the percent for the **Limit of Detection Threshold**. Any sensitivity above the threshold % is flagged red. Also specify the threshold type, donor or recipient. It is best to use the same type as the **%CHM Type** selected in the **Basic Settings** tab in Figure 46.

   Open the **Single-Donor Chimerism Settings** dialog, **Additional Settings** tab displays.

   Figure 47 Single-Donor Chimerism Settings with Additional Settings tab

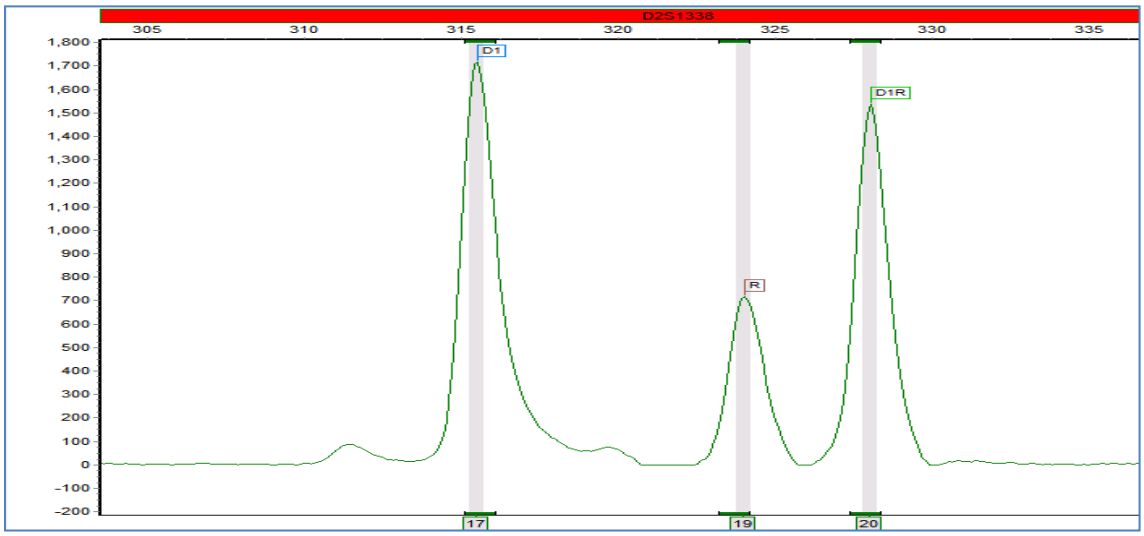


7. In the **Stutter Adjustment Settings** pane, if the **Apply Stutter Adjustment** checkbox is selected, marker stutter specific values that are shown in the panel are subtracted in the stutter alleles. The stutter % value for each marker is displayed if **Show Stutter %** is selected concurrently with **Apply Stutter Adjustment**.
8. At the bottom-left corner of Figure 46, click the **Save** icon to save your choices as the default settings.

9. Select **OK** in Figure 47 to review the results of Chimerism calculations.

The results of the Chimerism calculations graph next display an example of SAI for locus D2S1338.

Figure 48 SAI for locus D2S1338

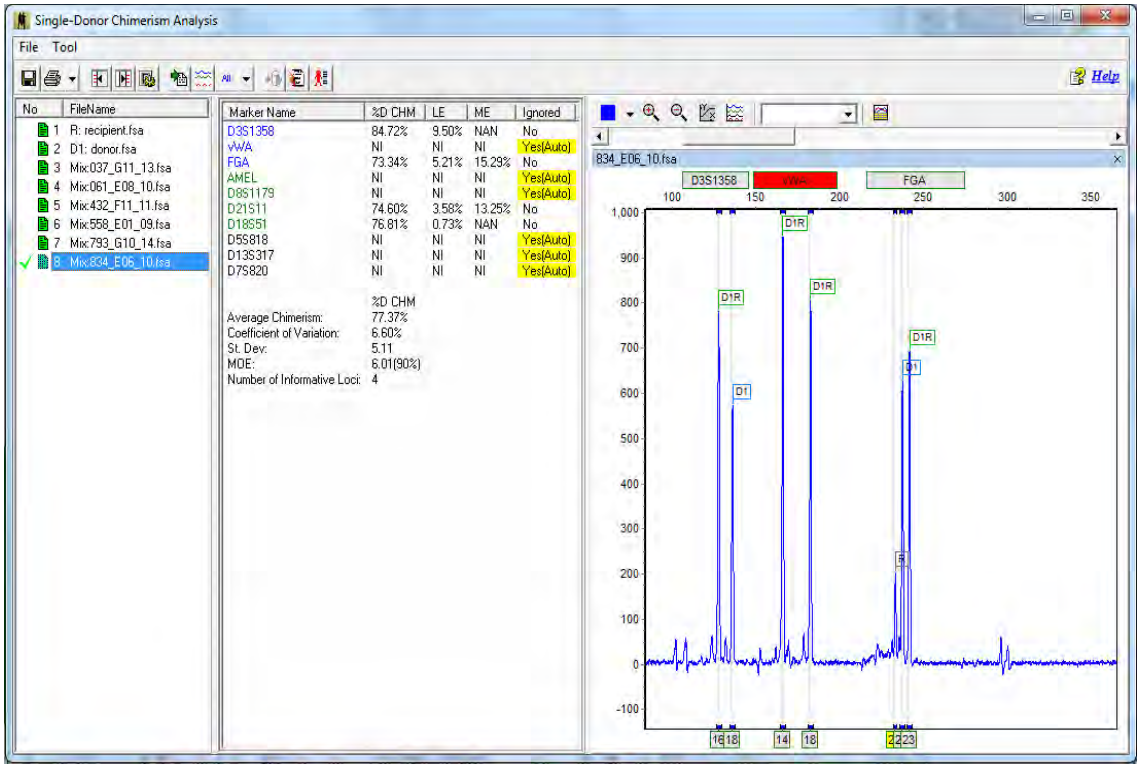


Quantification is suspect when a PCR bias results in a smaller fragment from one individual is more efficiently amplified than the larger fragment that is shared between donor and recipient.

Review Results

The **Single-Donor Chimerism Analysis** dialog displays.

Figure 49 Single-Donor Chimerism Analysis



The automated chimerism calculations are displayed in the center pane. To view the results for a given file, select the file name in the left pane.

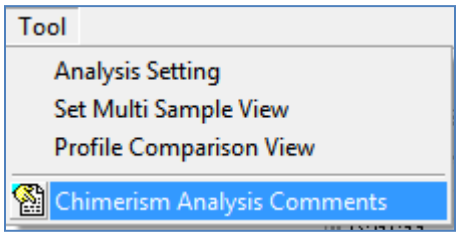
In the figure above:

- **NI:** Non-informative. A marker is non-informative based on peak pattern and parameters chosen in analysis settings.
- **NAN**: No available number. No results are calculated for the following peak pattern.
- **ME**: Measurement error.
- **LE**: Locus error.
- **MOE**: Margin of error.

Tools and Icons

The following tools and icons are displayed in the **Tool** dialog.

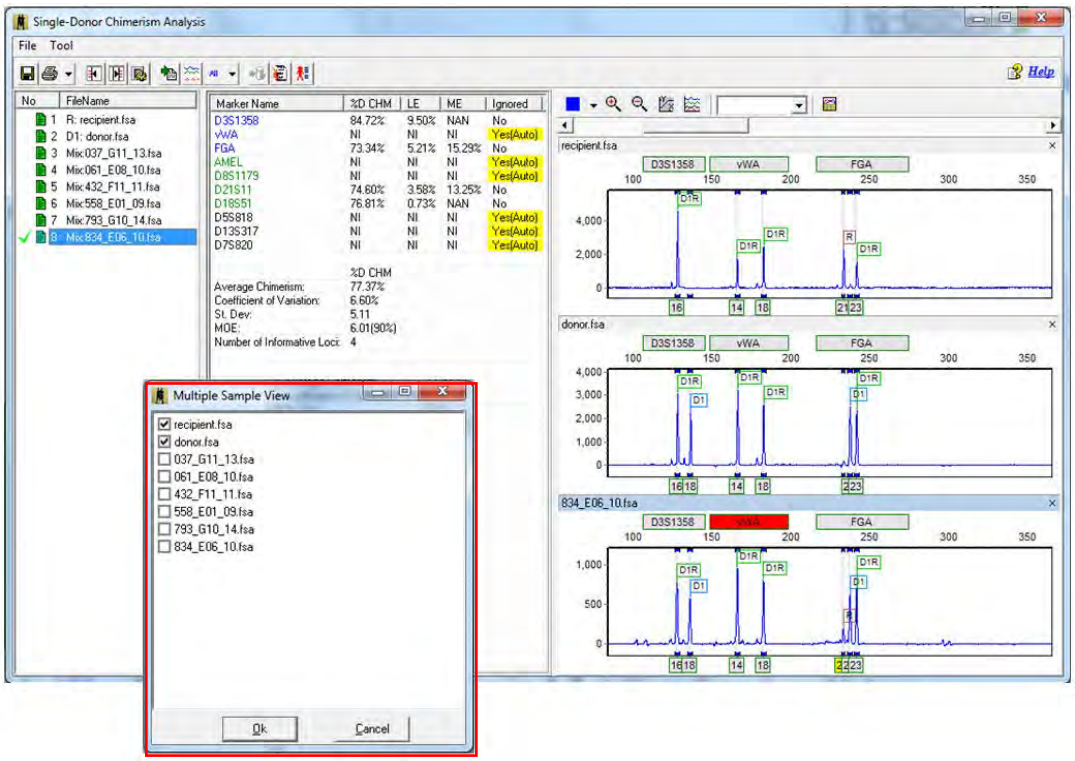
Figure 50 Tool Chimerism Analysis Comments



A description of each tool is provided next.

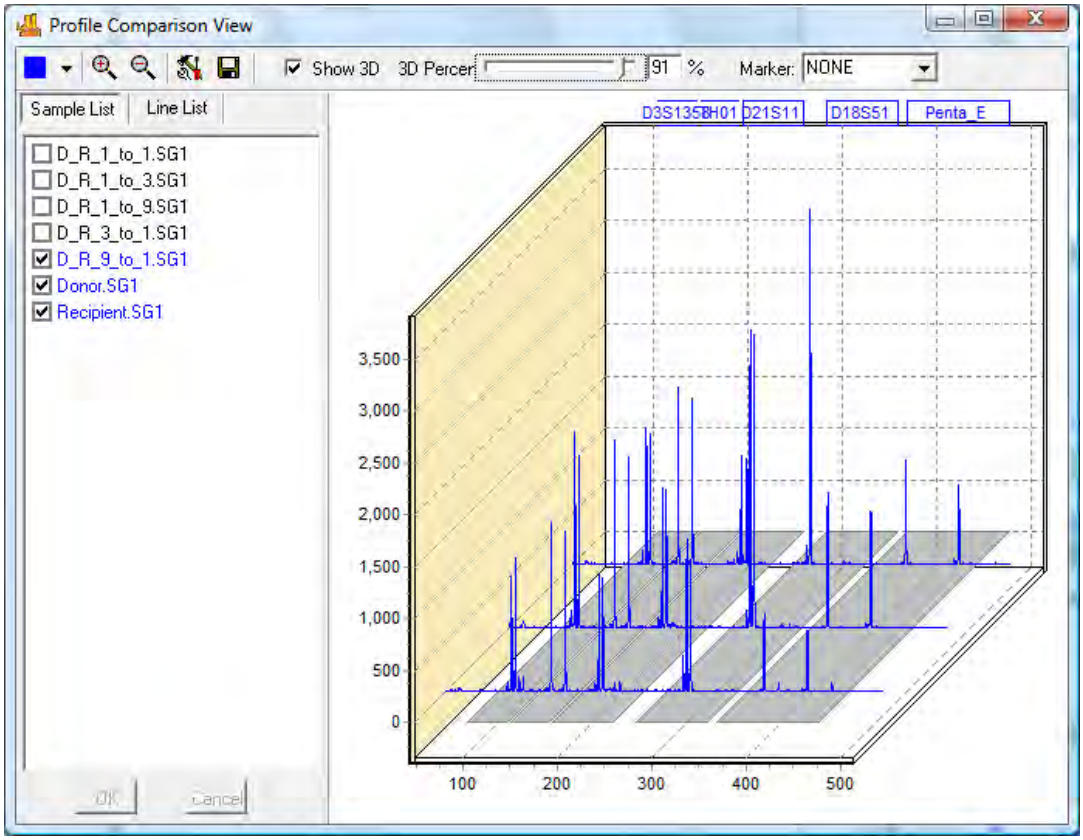
- **Analysis Settings:** Reopens the **Chimerism Settings** dialog, as illustrated by Figure 51.
- **Set Multiple Sample View**: View traces of selected files in the **Multiple Sample View** dialog. Selected files remain selected if the Chimerism application is closed and re-opened.

Figure 51 Single-Donor Chimerism Analysis Multiple Sample View



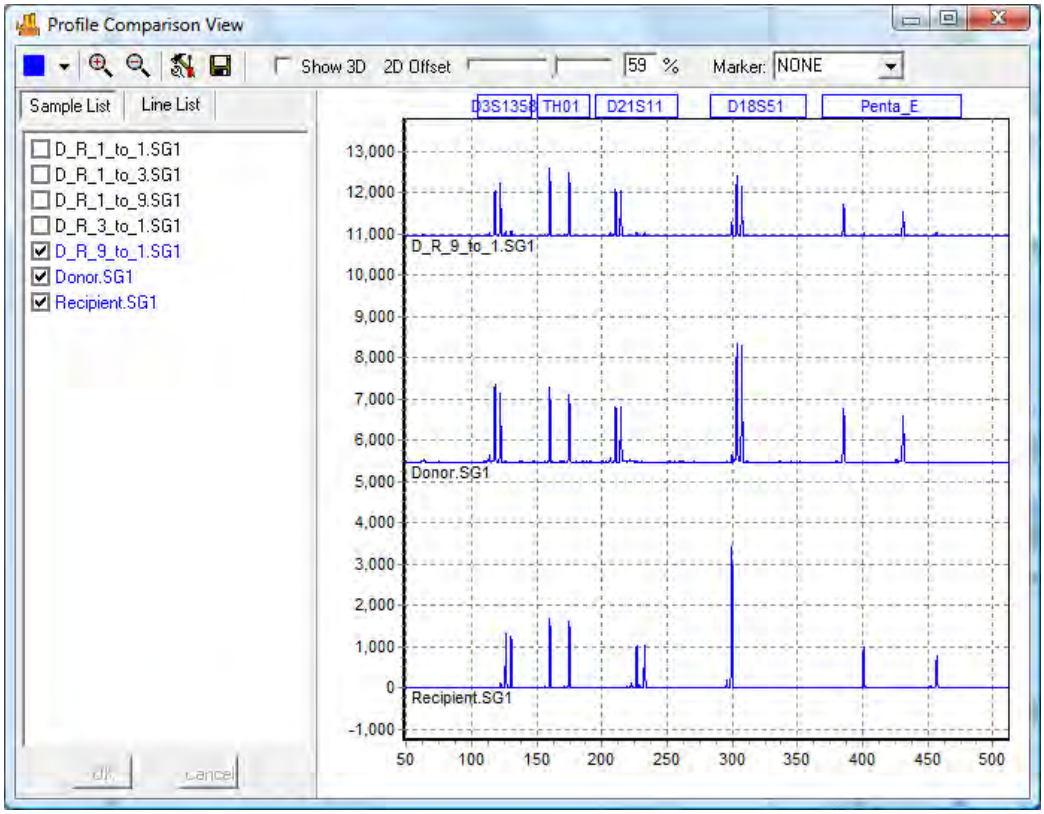
- **Profile Comparison View**: View selected traces and level of 2D or 3D offset, as illustrated in Figure 52.

Figure 52 Profile Comparison View Line List Tab



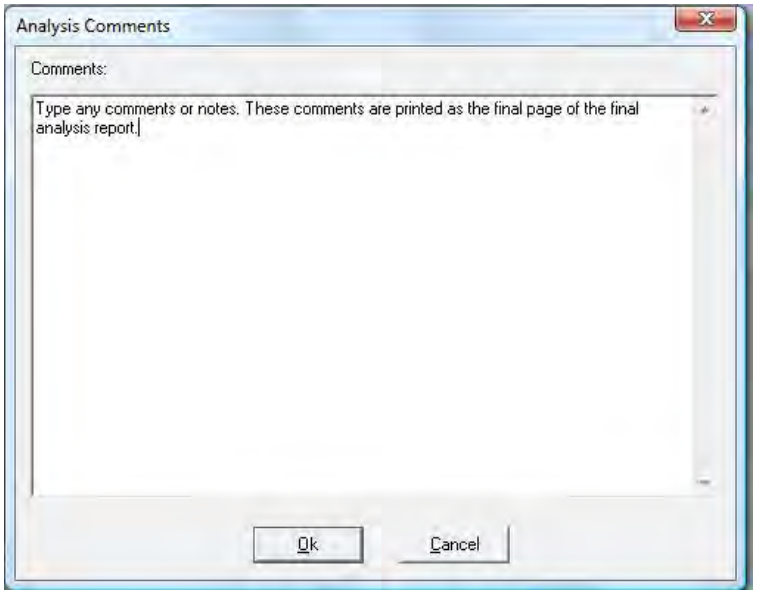
Select the **Show 3D** checkbox to initiate the 3D view. Unselect the **Show 3D** checkbox to restore the 2D view.

Figure 53 Profile Comparison View Line List



- **Chimerism Analysis Comments:** Record any case notes, save and print with the final report (saves up to 6400 characters).

Figure 54 Analysis Comments



- **ChimerMarker Print Settings:** Allow printer configuration.

Figure 55 ChimerMarker Print Settings

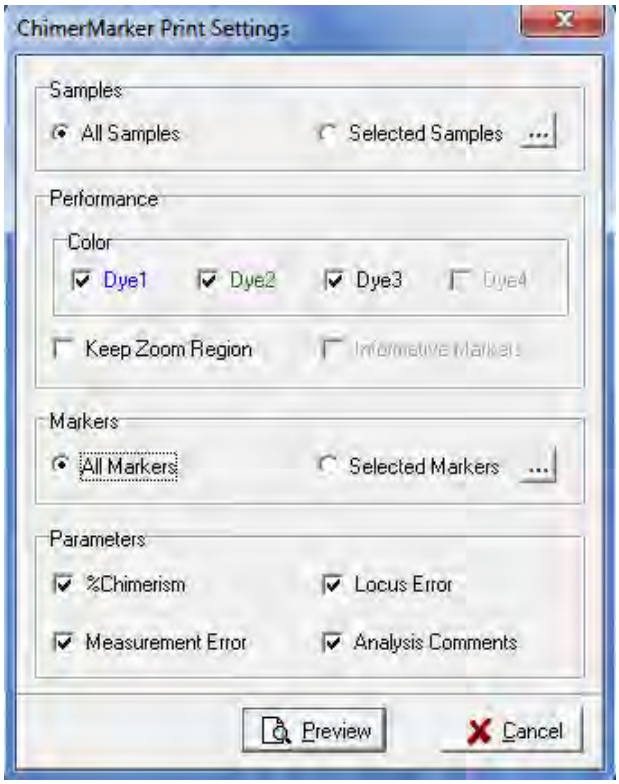


Table 2 Profile Comparison Icons

Icon	Name	Description
	Save	Save the report table in .xls or .txt file format.
	Show or Hide	Toggle this icon to show or hide the electropherograms.
	Analysis Parameter	Clicking this icon relaunches the Chimerism Analysis Settings Dialog.
	Multiple Sample View	Alternative path to display electropherograms of multiple samples.
	Profile Comparison	Alternative path to display profile comparison view.
All	Column Headings	Select any or all available column headings for the report table.

Icon	Name	Description
	Printing Options	Review comprehensive research reports, or print directly from Chimerism Analysis screen.
	Longitudinal Report	Activates the Longitudinal Report Options to monitor Chimerism trend.
	Help Icon	Quick reference with descriptions of abbreviations and statistical parameters used in chimerism results dialog.

Comprehensive Research Report

A comprehensive report for each sample at a given time point in the study; includes header, electropherograms (traces) and report table.

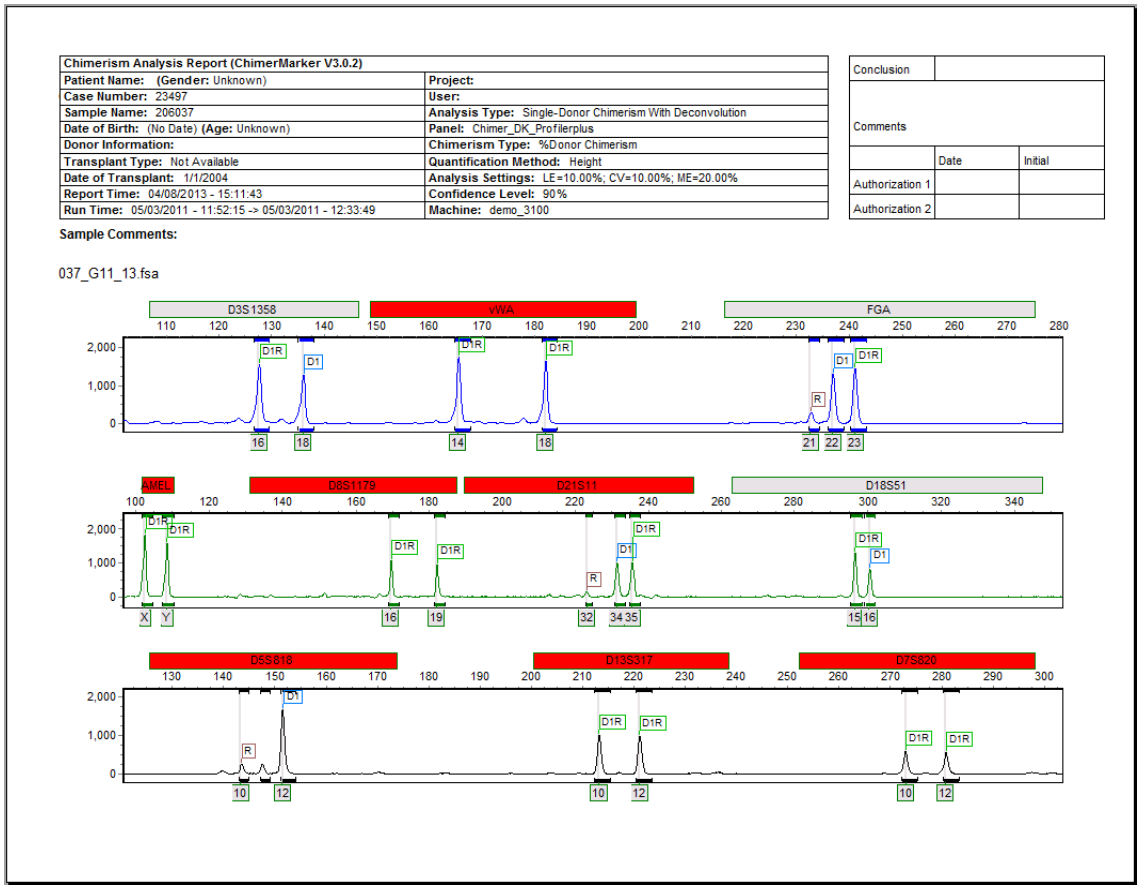
- The **report header** uses information from user management and the analysis settings to specify the technician, project, time, date, and parameters for electronic record keeping.
- The **electropherograms** (traces) show each dye-color separately with labels for D-(donor) and R (recipient).
- Results displayed include quality control metrics: coefficient of variation, standard deviation, % chimerism, measurement error, and locus error for each marker; and the total average % Chimerism.

  Nil represents uninformative loci (all peaks are shared) that are not included in the chimerism calculations or indicates that parameters could not be calculated based on data.
- ChimerMarker software also contains the functionality to allow the analyst to manually exclude loci from calculations, if needed.

Single Donor Report (page 1)

The single donor report page is illustrated next.

Figure 56 Single Donor Report Page 1



Chimerism Analysis Report (ChimerMarker V3.0.2)	
Patient Name: (**Gender:** Unknown)	**Project:**
Case Number: 23497	**User:**
Sample Name: 206037	**Analysis Type:** Single-Donor Chimerism With Deconvolution
Date of Birth: (No Date) (**Age:** Unknown)	**Panel:** Chimer_DK_Profilerplus
Donor Information:	**Chimerism Type:** %Donor Chimerism
Transplant Type: Not Available	**Quantification Method:** Height
Date of Transplant: 1/1/2004	**Analysis Settings:** LE=10.00%; CV=10.00%; ME=20.00%
Report Time: 04/08/2013 - 15:11:43	**Confidence Level:** 90%
Run Time: 05/03/2011 - 11:52:15 -> 05/03/2011 - 12:33:49	**Machine:** demo_3100

Conclusion		
Comments		
	Date	Initial
Authorization 1		
Authorization 2		

Sample Comments:

037_G11_13.fsa

Single Donor Report (page 2)

The single donor report page 2 is illustrated next.

Figure 57 Single Donor Report Page 2

Chimerism Analysis Report (ChimerMarker V3.0.2)	
Patient Name: **(Gender:** Unknown)	**Project:**
Case Number: 23497	**User:**
Sample Name: 206037	**Analysis Type:** Single-Donor Chimerism With Deconvolution
Date of Birth: (No Date) **(Age:** Unknown)	**Panel:** Chimer_DK_Profilerplus
Donor Information:	**Chimerism Type:** %Donor Chimerism
Transplant Type: Not Available	**Quantification Method:** Height
Date of Transplant: 1/1/2004	**Analysis Settings:** LE=10.00%; CV=10.00%; ME=20.00%
Report Time: 04/08/2013 - 15:11:43	**Confidence Level:** 90%
Run Time: 05/03/2011 - 11:52:15 -> 05/03/2011 - 12:33:49	**Machine:** demo_3100

Conclusion		
Comments		
	Date	Initial
Authorization 1		
Authorization 2		

Sample Comments:

037_G11_13.fsa

Marker	%D CHM	Ignored
D3S1358	90.43%	No
vWA	NI	Yes(Auto)
FGA	81.41%	No
AMEL	NI	Yes(Auto)
D8S1179	NI	Yes(Auto)
D21S11	NI	Yes(Auto)
D18S51	78.11%	No
D5S818	NI	Yes(Auto)
D13S317	NI	Yes(Auto)
D7S820	NI	Yes(Auto)

	%D CHM
Average Chimerism:	83.32%
St. Dev:	6.38
Coefficient of Variation:	7.66%
MOE:	10.76 (90%)
Number of Informative Loci:	3

The comprehensive report may be printed directly or saved in .png, .jpeg, or .pdf formats.

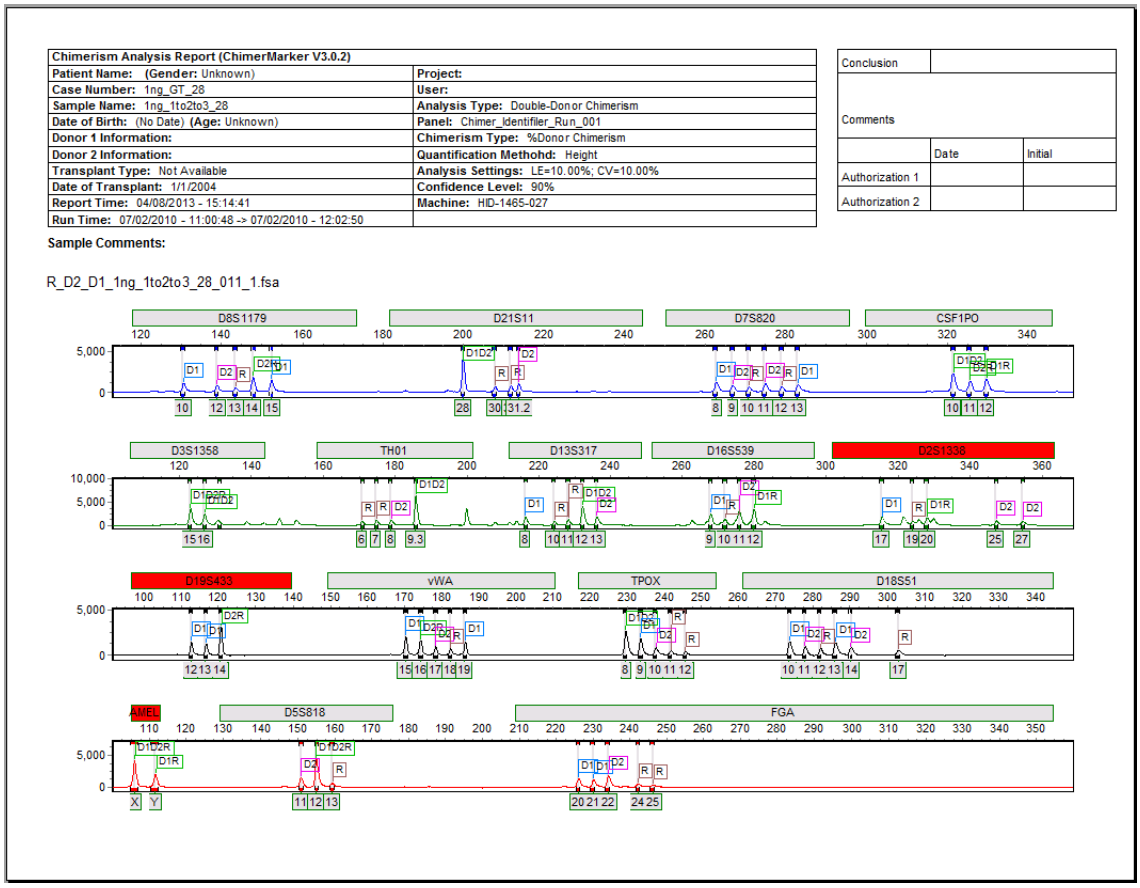
Double Donor Report: Report Results in 2-Page Format

A two-page report for cases with two (2) different donors provides the header and electropherograms on page 1 and the header and calculation results on page 2, including the option to report total average chimerism or to report chimerism from donor 1 and donor 2 separately.

Page 1: Electropherograms

The double donor electropherogram report is illustrated next.

Figure 58 Double Donor Report Electropherogram Page 1



Chimerism Analysis Report (ChimerMarker V3.0.2)	
Patient Name: (**Gender:** Unknown)	**Project:**
Case Number: 1ng_GT_28	**User:**
Sample Name: 1ng_1to2to3_28	**Analysis Type:** Double-Donor Chimerism
Date of Birth: (No Date) (**Age:** Unknown)	**Panel:** Chimer_Identifiler_Run_001
Donor 1 Information:	**Chimerism Type:** %Donor Chimerism
Donor 2 Information:	**Quantification Methohd:** Height
Transplant Type: Not Available	**Analysis Settings:** LE=10.00%; CV=10.00%
Date of Transplant: 1/1/2004	**Confidence Level:** 90%
Report Time: 04/08/2013 - 15:14:41	**Machine:** HID-1465-027
Run Time: 07/02/2010 - 11:00:48 -> 07/02/2010 - 12:02:50	

Sample Comments:

Conclusion		
Comments		
	Date	Initial
Authorization 1		
Authorization 2		

R_D2_D1_1ng_1to2to3_28_011_1.fsa

Page 2: Results Table

The Double Donor Electropherogram Report Page 2 is illustrated next.

Figure 59 Results Table Page 2

Chimerism Analysis Report (ChimerMarker V3.0.2)	
Patient Name: **(Gender:** Unknown)	**Project:**
Case Number: 1ng_GT_28	**User:**
Sample Name: 1ng_1to2to3_28	**Analysis Type:** Double-Donor Chimerism
Date of Birth: (No Date) **(Age:** Unknown)	**Panel:** Chimer_Identifiler_Run_001
Donor 1 Information:	**Chimerism Type:** %Donor Chimerism
Donor 2 Information:	**Quantification Methohd:** Height
Transplant Type: Not Available	**Analysis Settings:** LE=10.00%; CV=10.00%
Date of Transplant: 1/1/2004	**Confidence Level:** 90%
Report Time: 04/08/2013 - 15:14:41	**Machine:** HID-1465-027
Run Time: 07/02/2010 - 11:00:48 -> 07/02/2010 - 12:02:50	

Conclusion		
Comments		
	Date	Initial
Authorization 1		
Authorization 2		

Sample Comments:

R_D2_D1_1ng_1to2to3_28_011_1.fsa

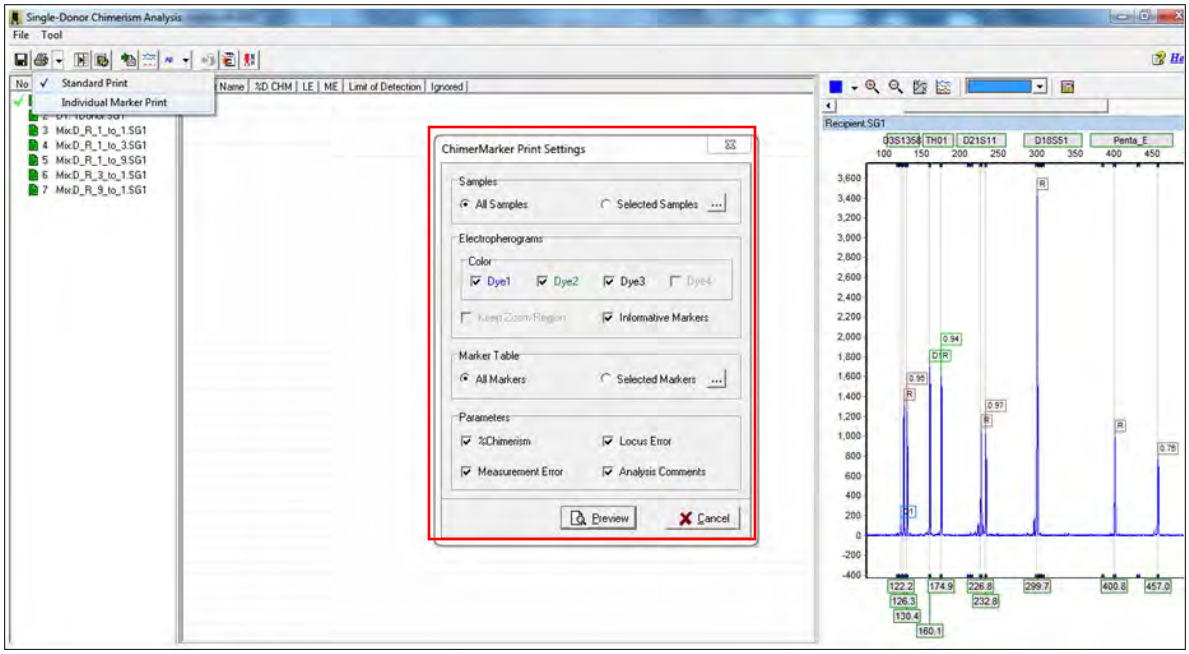
Marker Name	%Total D CHM	%D1 CHM	%D2 CHM	Total LE	D1 LE	D2 LE	Ignored
D8S1179	80.12%	44.20%	35.92%	2.05%	5.54%	9.44%	No
D21S11	77.84%	47.17%	30.66%	4.83%	0.82%	6.58%	No
D7S820	75.44%	39.77%	35.66%	7.77%	15.00%	8.66%	No
CSF1PO	87.53%	NAN	NAN	7.01%	NAN	NAN	No
D3S1358	100.00%	NAN	NAN	22.26%	NAN	NAN	No
TH01	80.29%	55.68%	24.61%	1.84%	19.00%	25.02%	No
D13S317	79.11%	36.18%	42.93%	3.28%	22.68%	30.80%	No
D16S539	73.93%	43.68%	30.25%	9.62%	6.65%	7.84%	No
D2S1338	NI	NI	NI	NI	NI	NI	Yes(Auto)
D19S433	NI	NI	NI	NI	NI	NI	Yes(Auto)
vWA	79.19%	51.75%	27.43%	3.18%	10.61%	16.41%	No
TPOX	85.97%	60.65%	25.32%	5.11%	29.62%	22.85%	No
D18S51	78.88%	48.42%	30.46%	3.56%	3.48%	7.20%	No
AMEL	NI	NI	NI	NI	NI	NI	Yes(Auto)
D5S818	81.17%	37.51%	43.66%	0.76%	19.84%	33.03%	No
FGA	83.79%	49.63%	34.16%	2.44%	6.07%	4.07%	No

	%Total D CHM	%D1 CHM	%D2 CHM
Average Chimerism:	81.79%	46.79%	32.82%
St. Dev:	6.66	7.56	6.39
Coefficient of Variation:	8.14%	16.16%	19.47%
MOE:	3.29 (90%)	4.13 (90%)	3.49 (90%)
Number of Informative Loci:	13	11	11

Print Individual Markers

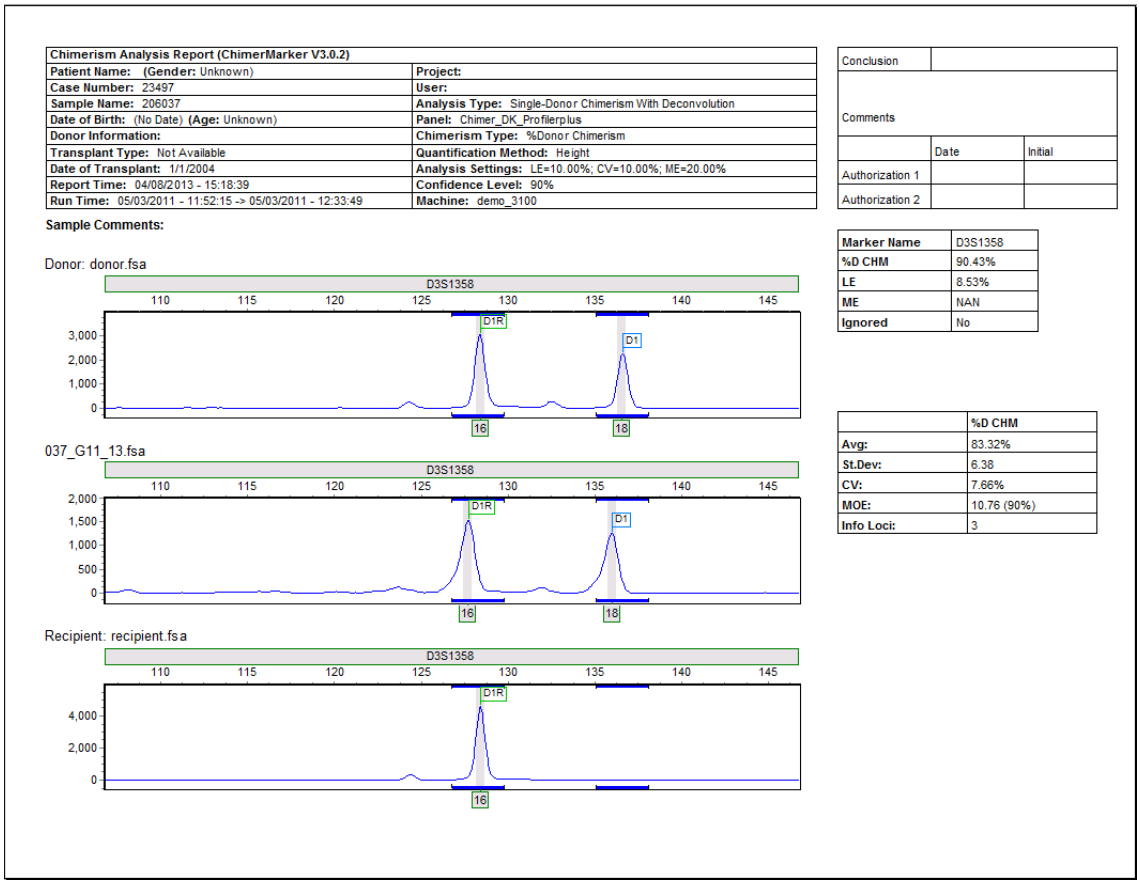
Individual markers for each sample are printed along with the corresponding Donor and Recipient markers. From the **Chimerism Analysis Results** page, select **Individual Marker Print** from the print drop-down arrow. Specify the Print settings and click **Preview** to see the report, as illustrated in the **ChimerMarker Print Settings** dialog popup illustrated next.

Figure 60 Selecting the Individual Marker Print Option



An example of the **Chimerism Analysis Report ChimerMarker** is illustrated next.

Figure 61 Example of One Page of the Individual Marker Report



Chimerism Analysis Report (ChimerMarker V3.0.2)	
Patient Name: (**Gender:** Unknown)	**Project:**
Case Number: 23497	**User:**
Sample Name: 206037	**Analysis Type:** Single-Donor Chimerism With Deconvolution
Date of Birth: (No Date) (**Age:** Unknown)	**Panel:** Chimer_DK_Profilerplus
Donor Information:	**Chimerism Type:** %Donor Chimerism
Transplant Type: Not Available	**Quantification Method:** Height
Date of Transplant: 1/1/2004	**Analysis Settings:** LE=10.00%; CV=10.00%; ME=20.00%
Report Time: 04/08/2013 - 15:18:39	**Confidence Level:** 90%
Run Time: 05/03/2011 - 11:52:15 -> 05/03/2011 - 12:33:49	**Machine:** demo_3100

Sample Comments:

Conclusion		
Comments		
	Date	Initial
Authorization 1		
Authorization 2		

Marker Name	D3S1358
%D CHM	90.43%
LE	8.53%
ME	NAN
Ignored	No

	%D CHM
Avg:	83.32%
St.Dev:	6.38
CV:	7.66%
MOE:	10.76 (90%)
Info Loci:	3

Each informative marker is grouped with the appropriate marker from Donor and Recipient sample for comparison. Both the marker result and the average result for the samples are displayed on the right-hand side. The header is the same in all print preview modes.

Longitudinal Report for Engraftment M/D of MR

The Longitudinal Report for Engraftment Monitoring / Detection of Malignant Relapse is detailed next. The Short Tandem Repeat (STRs) provide a highly sensitive measure of chimerism in post-BMT monitoring which is essential for long term tracking of engraftment and early detection of malignant cell replapse. Each patient project is easily appended (see Tools **Add Samples to Project**) with sequential samples from a monitoring study. The longitudinal graphs are easily appended with results from sequential samples. The report header provides verification of the user and the analysis parameters.

Add Samples to Project Procedure

Follow the next steps to add samples to a project.

1. Open a saved project or start a new project following the Chimerism Analysis procedures in Chapter 2 Chimerism Analysis.
2. Select **Project** > **Add Samples** to saved project to add serial sample(s) if appropriate.
3. Select **Applications > Select Single Donor** or **Double Donor Chimerism Analysis**.
4. Review Chimerism results.
5. Select the **Longitudinal Report** Icon. ( )
6. Select the desired samples to begin the longitudinal study or to add serial samples to an existing engraftment monitoring.

Icons and Functions

The next table shows the Chimerism Analysis Results page icons and their functions.

Table 3 Chimerism Analysis Results Page Icons

Icon	Name	Function
	Print report	Print the longitudinal report as zoomed view or global view.
	Save report	Save report in PNG, JPG, BMP, or PDF format. Save either the whole page or just the table.
	Display settings	Change the display settings on the Longitudinal Report for things like dates, statistics, and chart layers.
	Add patient information	Add patient name, sex, age, and date of transplantation.
	Chimerism display	Switch display of %D or Stutter Adjusted %D when in the Single Donor Application. Switch display of double donor chimerism from individual donors, total donor, or display all in the Double Donor Application.
	Graph display	Switch from bar graph to line graph and vice versa.
	Show/Hide table	Toggle between displaying graph and table or only the graph. Table contains all samples and stats of the samples.

Icon	Name	Function
	Refresh	Update the graphical display.

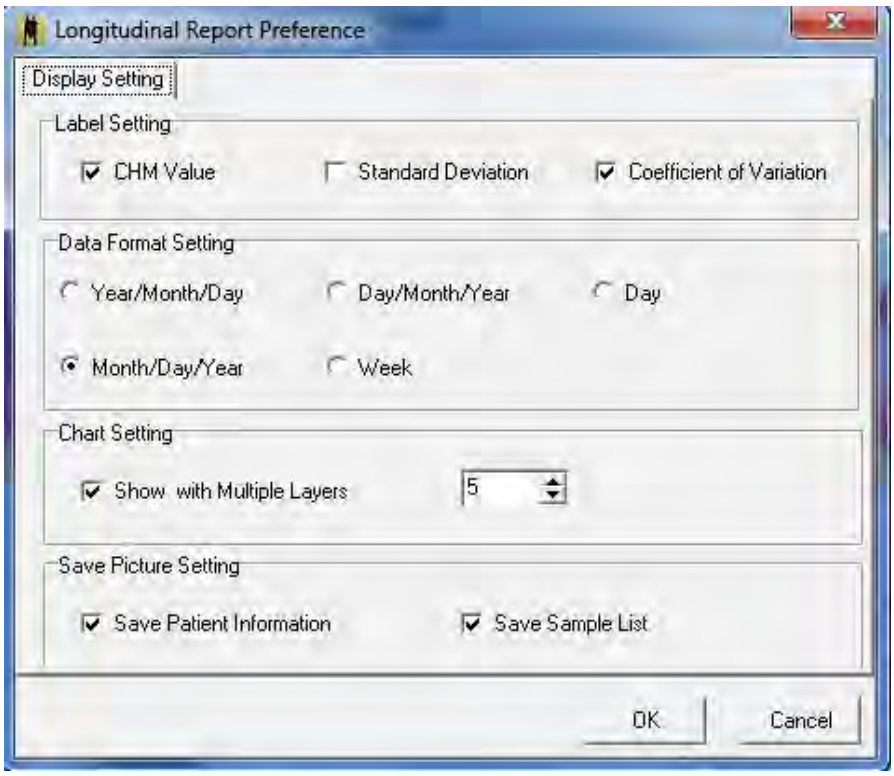
Add Functions are described next.

Show 3D: Display the graph in a 3D format.

Display Settings

The **Display Settings** dialog lists additional features and functions and is illustrated next.

Figure 62 Longitudinal Report Table Column Selections



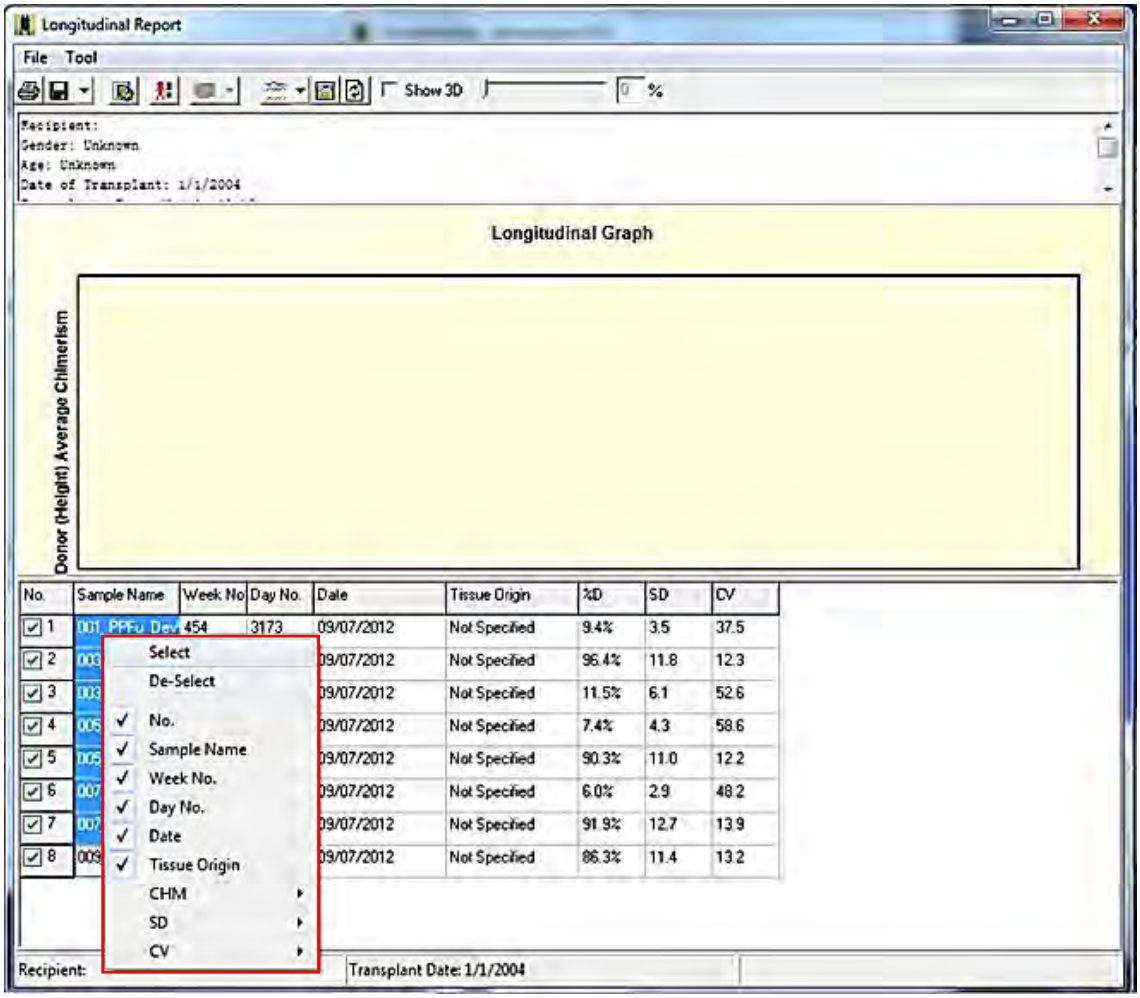
Display settings include the following:

- **Label Settings**: Select the statistics to display on top of the bar or line graph for each sample.
- **Date Format Setting**: Select the date display to show on the X-axis of the graph.
- **Chart Setting**: Specify the number of layers to display the statistics label with.
- **Save Picture Setting**: Specify if patient information and sample list should be saved with the image.

Add/Remove Samples

Multiple samples are selected by left clicking on a sample and highlighting other samples, as displayed by the **Longitudinal Report Graph** as illustrated next.

Figure 63 Longitudinal Report Table Column Selections



Once the desired samples are selected, right-click and choose **Select**. Use the **Refresh** icon to update the graphical display.

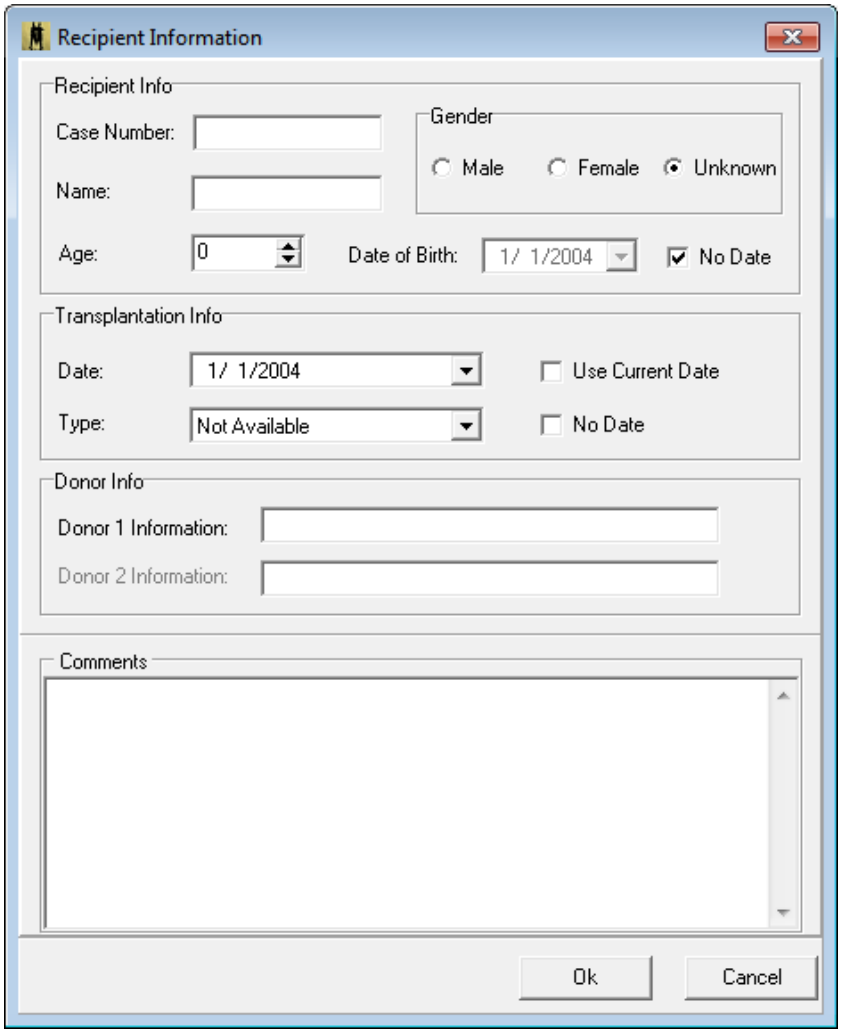
- **Date**: Specify dates for each sample.
- **Tissue Origin**: Choose the desired Tissue Origin from the drop-down options or manually enter them. The cell type information added in **View** > **Preferences** > **Chimerism** is displayed in the drop-down.

  Note: Up to 10 tissue types are monitored on a single graph.

Recipient Information

The **Recipient Information** dialog displays.

Figure 64 Recipient Information



The following entries are included in print report headers:

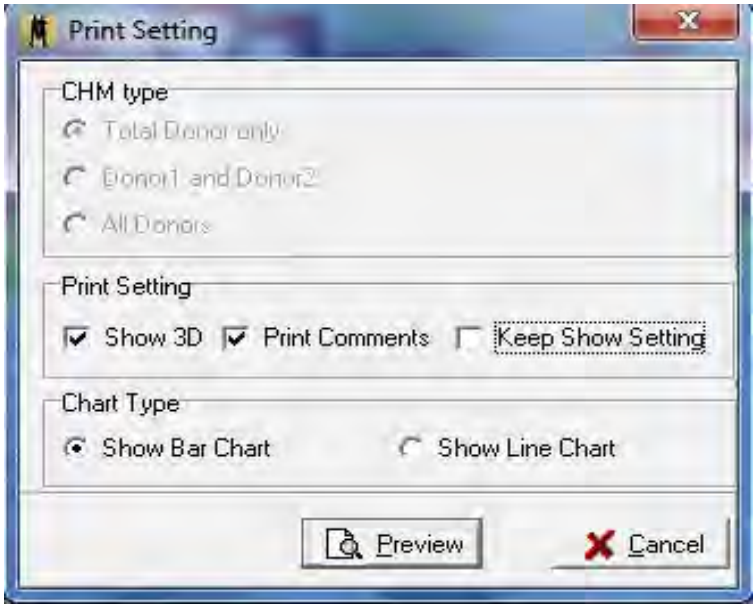
- In the **Recipient Info** pane, enter the following information.
  - Use the **Case Number** field, if applicable, to input the case number for this sample or project.
  - **Recipient Name**: Specify Patient Name/ID. This appears as the header on the graph.
  - **Gender**: Specify patient sex.
  - **Age**: Specify patient age.
  - **Note**: Age is automatically calculated from the date of birth.
  - **Date of Birth**: Specify the recipient's date of birth or check **No Date** if no date is available.

- In the **Transplantation Info** pane, enter the following information:
  - **Date of Transplantation**: Specify date of transplantation by using the drop-down menu, or check **Use Current Date** or **No Date**.
  - **Transplantation Type**: Specify the type of transplantation to be displayed on the report.
  - **Donor Information**: Use this section to briefly describe donors, (e.g. donor sex, relation to patient, etc.).
- In the **Comments** pane: Add remarks to the project, if needed. Any comments added are printed with the report.

Print Settings for Double Donor

The **Print Settings** dialog displays as illustrated next.

Figure 65 Print Settings



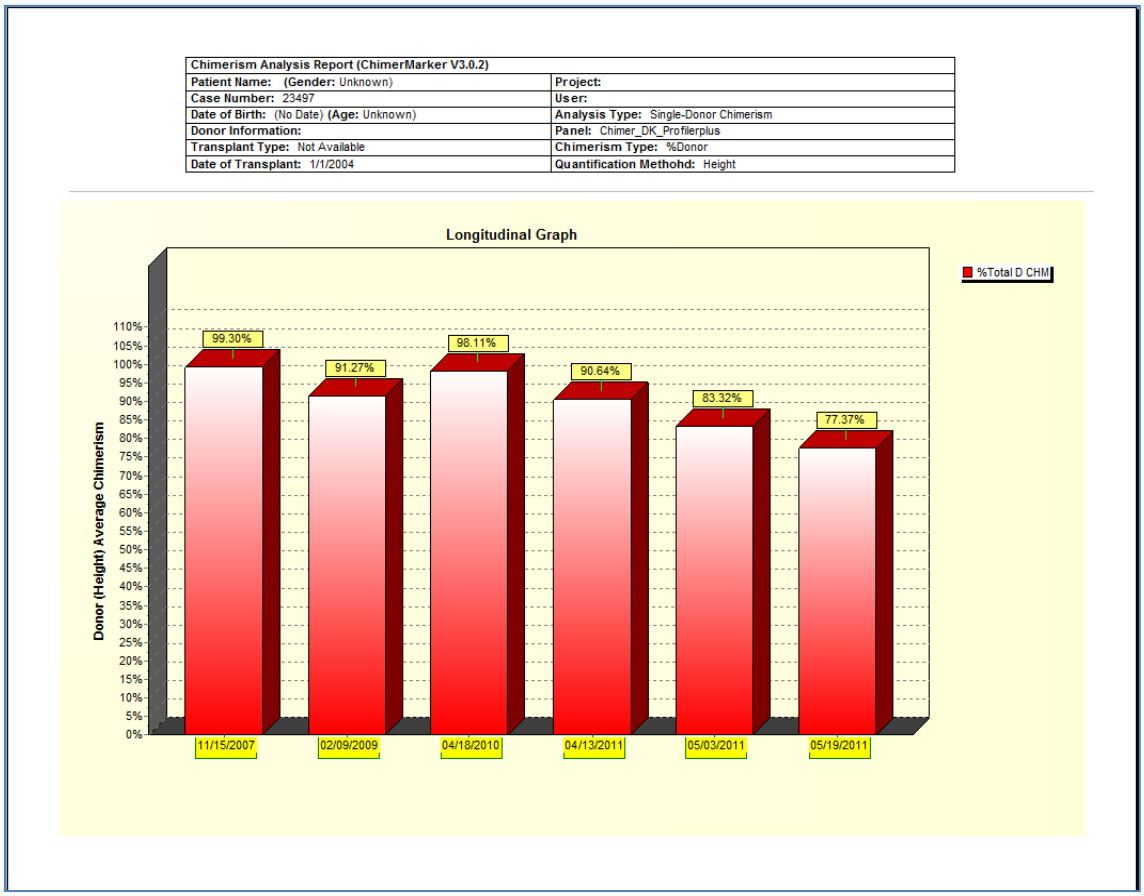
- In the **CHM Type** pane**:** select the type of donor chimerism to display.
- In the **Print Settings** pane: select the print settings format for the graph.

  Note: When selected, the **Keep Show Setting** checkbox prints the report with a zoom in on the graphical view.
- In the **Chart Type** pane, select the type of chart to print. Selecting the **Preview** provides a print preview of the selected settings.

What to Expect

The **Chimerism Analysis Longitudinal Report** dialog displays.

Figure 66 Chimerism Analysis Longitudinal Report Graphic



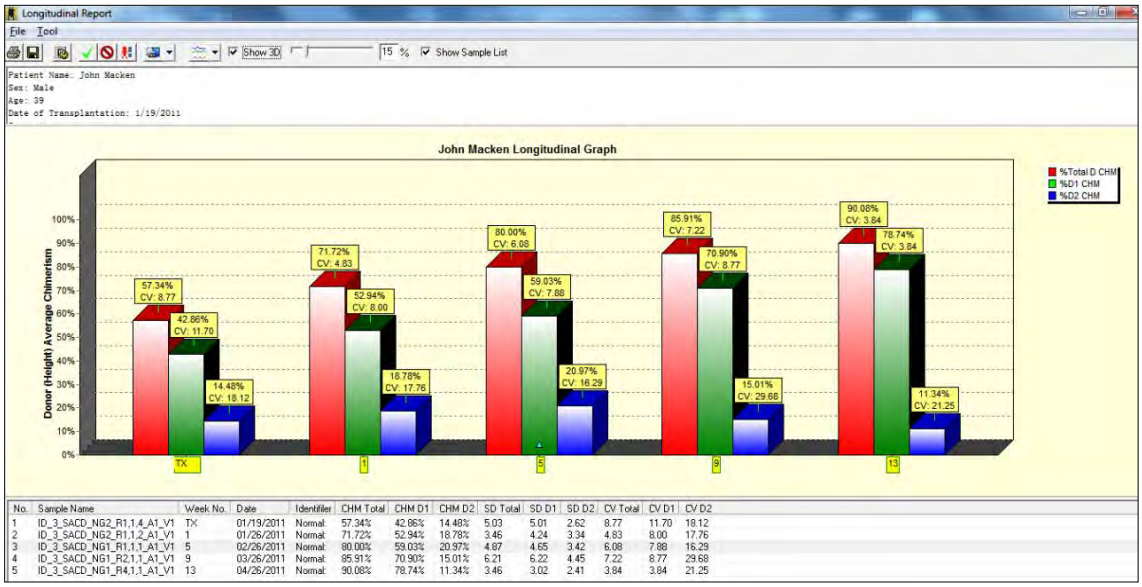
Chimerism Analysis Report (ChimerMarker V3.0.2)	
Patient Name: **(Gender:** Unknown)	**Project:**
Case Number: 23497	**User:**
Date of Birth: (No Date) **(Age:** Unknown)	**Analysis Type:** Single-Donor Chimerism
Donor Information:	**Panel:** Chimer_DK_Profilerplus
Transplant Type: Not Available	**Chimerism Type:** %Donor
Date of Transplant: 1/1/2004	**Quantification Methohd:** Height

The Longitudinal Report contains a header with User Identification from User Management (see Chapter 10 User Management), Analysis Parameters, and Chimerism Trend (donor or recipient) with the %chimerism on the Y axis and the sample date on the X-axis.

This graph is saved with the project and is easily appended with subsequent serial samples, printed from the print preview or saved in .png, .jpeg, .bmp, or .pdf format.

An example of a single donor bar graph, with quality control metrics and a sample date, is displayed as month/day/year.

Figure 67 Example of a Double Donor Longitudinal Graph with Table

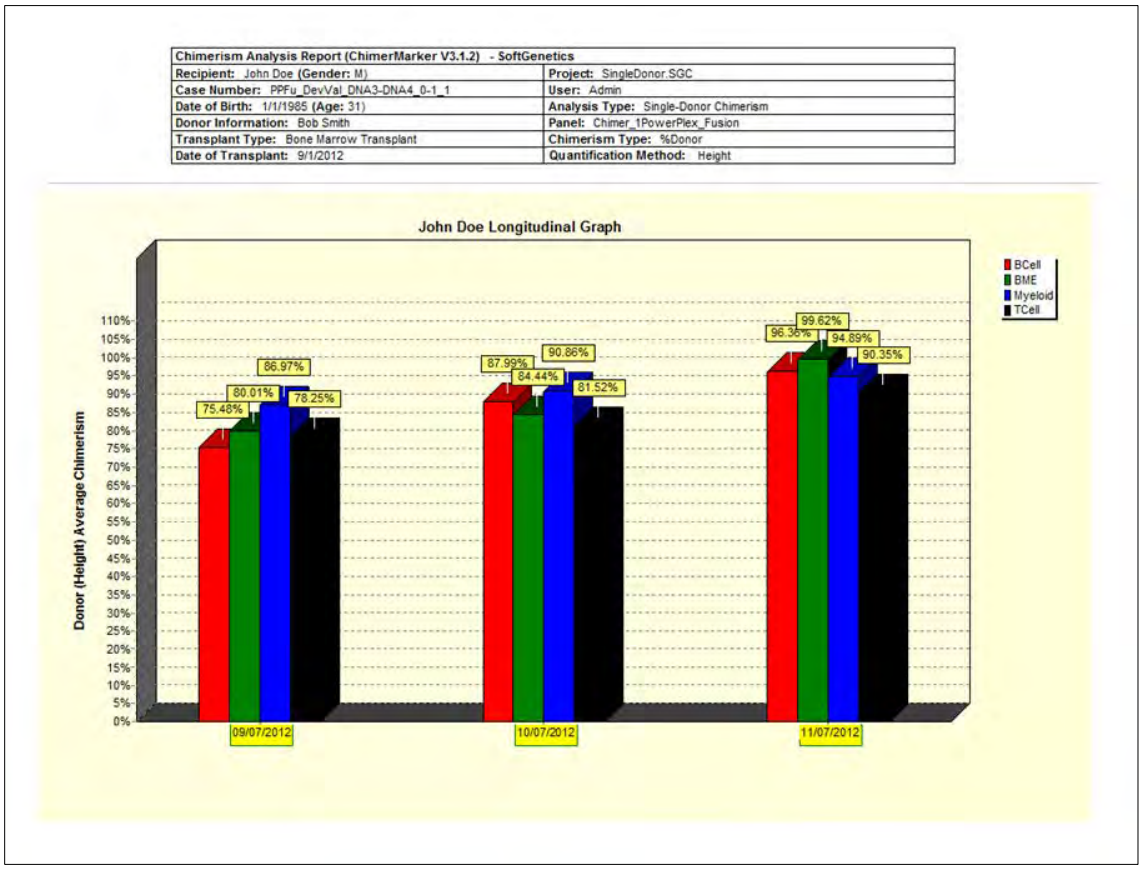


No.	Sample Name	Week No.	Date	Identifier	CHM Total	CHM D1	CHM D2	SD Total	SD D1	SD D2	CV Total	CV D1	CV D2
1	ID_3_SACD_NG2_R1,1,4_A1_V1	TX	01/19/2011	Normal	57.34%	42.86%	14.48%	5.03	5.01	2.62	8.77	11.70	18.12
2	ID_3_SACD_NG2_R1,1,2_A1_V1	1	01/26/2011	Normal	71.72%	52.94%	18.78%	3.46	4.24	3.34	4.83	8.00	17.76
3	ID_3_SACD_NG1_R1,1,1_A1_V1	5	02/26/2011	Normal	80.00%	59.03%	20.97%	4.87	4.65	3.42	6.08	7.88	16.29
4	ID_3_SACD_NG1_R2,1,1_A1_V1	9	03/26/2011	Normal	85.91%	70.90%	15.01%	6.21	6.22	4.45	7.22	8.77	29.68
5	ID_3_SACD_NG1_R4,1,1_A1_V1	13	04/26/2011	Normal	90.08%	78.74%	11.34%	3.46	3.02	2.41	3.84	3.84	21.25

This double donor monitoring report displays the trend of engraftment with an increase in overall chimerism and Donor 1 stability and a decrease in Donor 2 chimerism percentage. In this example, the analyst entered **Tx** to indicate the sample at transplant and then the weeks post-transplant for the sequential samples.

Another view of the Double Donor Monitoring Report is provided next.

Figure 68 Double Donor Monitoring Report



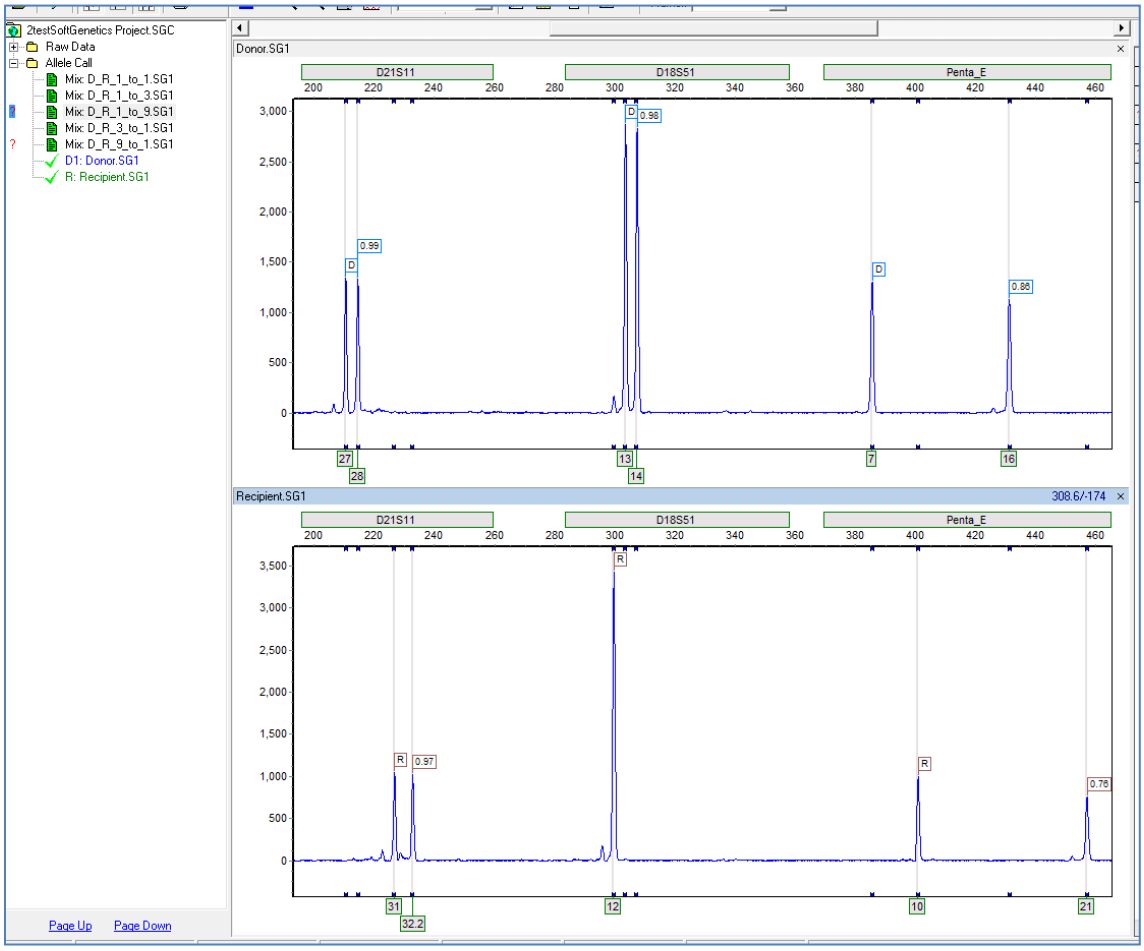
Chimerism Analysis Report (ChimerMarker V3.1.2) - SoftGenetics	
Recipient: John Doe **(Gender:** M)	**Project:** SingleDonor.SGC
Case Number: PPFu_DevVal_DNA3-DNA4_0-1_1	**User:** Admin
Date of Birth: 1/1/1985 **(Age:** 31)	**Analysis Type:** Single-Donor Chimerism
Donor Information: Bob Smith	**Panel:** Chimer_1PowerPlex_Fusion
Transplant Type: Bone Marrow Transplant	**Chimerism Type:** %Donor
Date of Transplant: 9/1/2012	**Quantification Method:** Height

This single donor example demonstrates an increase in stability across multiple tissue types. Each tissue type is graphed in a unique color. Up to 10 different tissue types are displayed on a single graph.

Chimerism Calculations

Metrics for variation in amplification provide a measure of variation from sources other than chimeras including, LCN, amplification variation --- PCR conditions, PCR bias, random events, and CE conditions.

Figure 69 PCR Bias Impact on Chimerism Quantification



Example of variation PCR bias include the following.

- Alleles in loci that have a smaller size range are able to be amplified more than alleles in loci of a larger size range in the same amount of time. One of the causes of heterozygous imbalance is PCR bias, which is the amplification of the smaller fragment more times than the larger one.
- In the example provided in Figure 69, there is almost no amplification difference between the two (2) closely spaced alleles at D21S11 for both Donor and Recipient genotypes. In contrast, the alleles at Penta E for both of these individuals have a larger molecular weight difference, resulting in the smaller of the two (2) fragments being amplified more than the larger fragment.

- This is a source of variation (heterozygous imbalance) due to the PCR process. In this example, both D21S11 and Penta_E are acceptable candidate loci used to track chimerism because there are no shared alleles between the donor and the recipient.

  D21S11 is preferable over Penta_E because there is less imbalance at D21S11(97 – 99% in D21S11 compared to 76 – 86% in Penta_E) in the original individual samples.

Equations

Quality Metrics: These equations pertain to the quality control metric calculations in the Chimerism Analysis. These metrics include measurement error, locus error, and coefficient of variation. All calculations are multiplied by 100 to convert results to percentages.

Measurement Error (ME): Estimate ratio of Allele 1 in donor and recipient to Allele 2 of donor and recipient at a locus. At optimal efficiency, the measurement should be 1, indicating that there is no imbalance between either allele. If ME is high, the DNA measurement between the sister alleles of donor and recipient is large. (Similar to heterozygous imbalance but consider the entire locus):

- ME=[(A-B)/(C)]*100.
- A= R1+ D1 (allele 1 of recipient + allele 1 of donor).
- B=R2 + D2 (allele 2 of recipient + allele 2 of donor).
- C= larger of A or B.

Locus Error (LE)

Estimate relative deviation of a locus %CHM from the Average %CHM:

- LE=[(Average %CHM - Locus %CHM)/ Average %CHM]* 100.
- Locus %CHM= the chimerism level calculated for that individual locus using the alleles only from that locus.
- High LE=high variation of locus from the rest of the sample.
- Low LE= low variation of locus from the rest of the sample.

Coefficient of Variance (CV)

Ratio of the standard deviation to the average percent chimerism of the sample. It gives a measure of variation within the sample relative to the sample's average:

- CV= [Standard déviation (σ)/Average %CHM (μ)]* 100.
- If the CV is small, all data points in the sample varies by only a small fraction.
- If CV is large, indicate that the data varies greatly or there is an outlier in the sample.

Margin of Error (MOE)

Calculated by obtaining value in T-table according to degree of freedom and confidence interval multiplied by the Standard Error of the Mean:

- MOE= SE* T-score.

- Standard Error of the Mean (SE)= St. Dev/ Square root of (N), where N= number of informative markers.
- Large MOE indicates sampling variability in the data and results are more open to interpretation. Small MOE indicates less sampling variability in the data and results may be less open to interpretation.

Limit of Detection

The limit of detection is calculated as listed next:

- Homozygous: [(T)/Total DNA in Marker]* 100.
- Heterozygous: [(T*2)/ Total DNA in Marker]*100.

  **T= Threshold set in panel under Minimum Heterozygote Intensity.

Stutter Adjustment

The stutter adjustment is details are listed next:

- X= Stutter % specified in panel.
- A=Stutter contribution.
- Y= DNA of minor allele not affected by stutter.

Equation:

- Major allele x (X)= A (Stutter contribution RFU).
- Minor allele - A= Y (DNA not affected by stutter).

  **(Y) is used for Chimerism calculations in equation below.

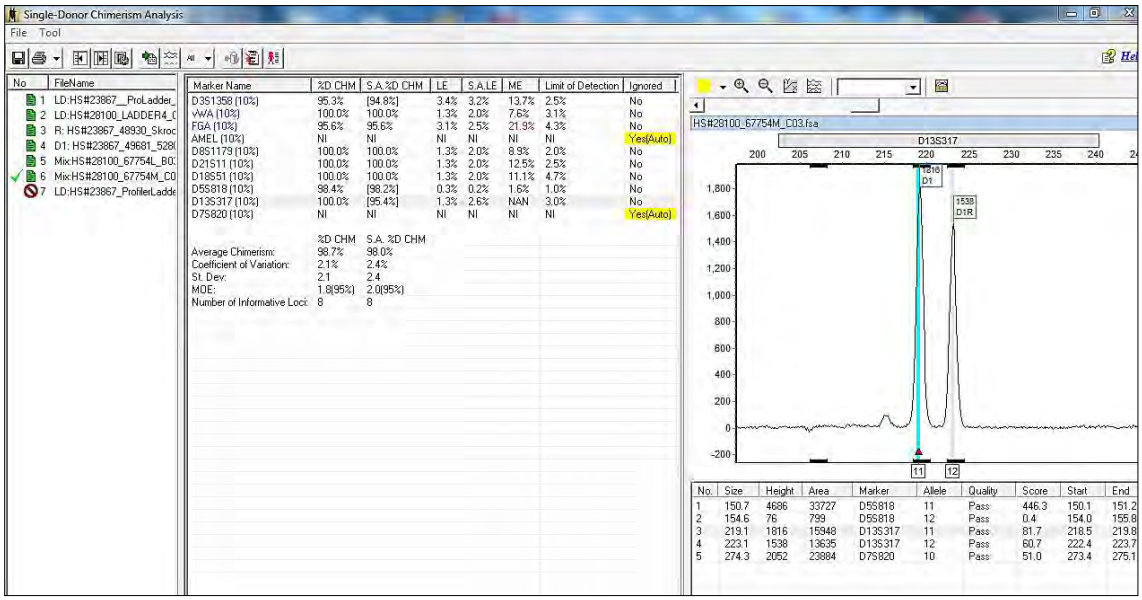
Calculation for Stutter Equation Adjustments

This section provides examples of stutter equation adjustments.

For S.A. when the main peak is shared, there are two (2) peaks in sample:

Total corrected height/total uncorrected height is illustrated next.

Figure 70 Stutter Correction Example 1



- Correcting 1816 height for the stutter effect:

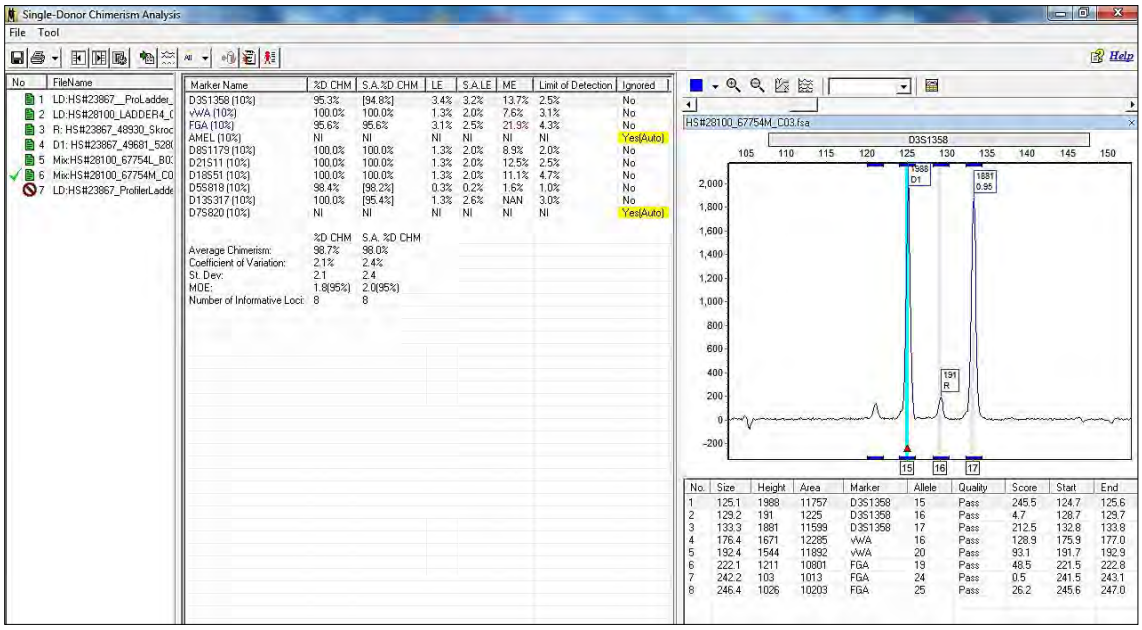
  1816 – (1538 x 0.1) = 1662.2
- 1662.2 + 1538 = 3200.2 = total corrected height:

  (3002.2/3354) x 100 = 95.4 %

When peaks are not shared the correction is:

Corrected Donor peak height/ total uncorrected heights is illustrated next.

Figure 71 Stutter Correction Example 2



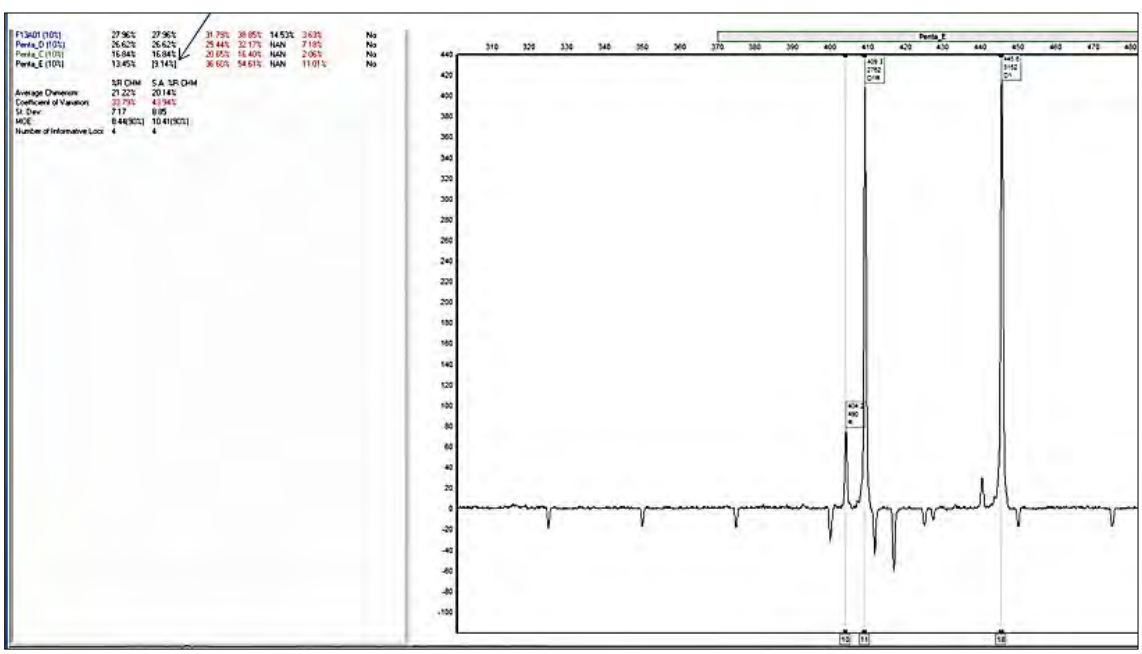
1988 – 19 = 1969 (corrected D1 peak in stutter position to R peak):

1969 + 1881 / 1988 + 191 + 1881

(3850/4060) x 100 = 94.8%

Main peak is shared peak, without deconvolution, three (3) peaks in sample: Calculate percent chimerism due to the stutter and subtract that from the uncorrected percent stutter.

Figure 72 Stutter Correction Example 3



Ht. of allele 10 allowable by the stutter setting / total height:

(2762 x 0.10)/490 + 2762 + 3152

(276.2/6404) x 100 = 4.31 %

%Chimerism from peak heights:

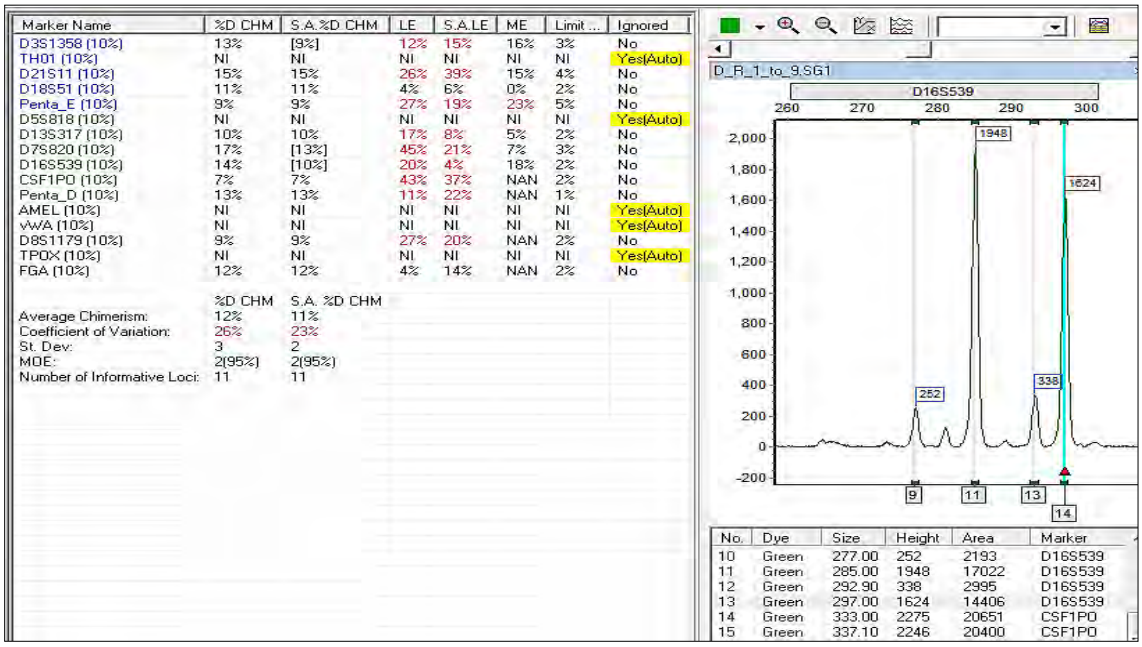
(490/ 3152 + 490) x 100 = 13.45%

13.45 – 4.31 = 9.14%

In this next case there are eight (8) markers that are not affected by stutter. Ignoring the three (3) loci that are affected by stutter is one option.

Alternatively, especially for cases where most of the loci are affected by stutter, the stutter adjusted percent chimerism is obtained in the S.A. %D CHM column.

Figure 73 Stutter Correction Example 4



Marker Name	%D CHM	S.A.%D CHM	LE	S.A.LE	ME	Limit ...	Ignored
D3S1358 (10%)	13%	[9%]	12%	15%	16%	3%	No
TH01 (10%)	NI	NI	NI	NI	NI	NI	Yes(Auto)
D21S11 (10%)	15%	15%	26%	39%	15%	4%	No
D18S51 (10%)	11%	11%	4%	6%	0%	2%	No
Penta_E (10%)	9%	9%	27%	19%	23%	5%	No
D5S818 (10%)	NI	NI	NI	NI	NI	NI	Yes(Auto)
D13S317 (10%)	10%	10%	17%	8%	5%	2%	No
D7S820 (10%)	17%	[13%]	45%	21%	7%	3%	No
D16S539 (10%)	14%	[10%]	20%	4%	18%	2%	No
CSF1PO (10%)	7%	7%	43%	37%	NAN	2%	No
Penta_D (10%)	13%	13%	11%	22%	NAN	1%	No
AMEL (10%)	NI	NI	NI	NI	NI	NI	Yes(Auto)
vWA (10%)	NI	NI	NI	NI	NI	NI	Yes(Auto)
D8S1179 (10%)	9%	9%	27%	20%	NAN	2%	No
TPOX (10%)	NI	NI	NI	NI	NI	NI	Yes(Auto)
FGA (10%)	12%	12%	4%	14%	NAN	2%	No

	%D CHM	S.A. %D CHM
Average Chimerism:	12%	11%
Coefficient of Variation:	26%	23%
St. Dev:	3	2
MDE:	2(95%)	2(95%)
Number of Informative Loci:	11	11

No.	Dye	Size	Height	Area	Marker
10	Green	277.00	252	2193	D16S539
11	Green	285.00	1948	17022	D16S539
12	Green	292.90	338	2995	D16S539
13	Green	297.00	1624	14406	D16S539
14	Green	333.00	2275	20651	CSF1PO
15	Green	337.10	2246	20400	CSF1PO

The next graphic illustrates the rubric used to determine the loci to ignore when **Ignore Locus with Informative Peak in Stutter Position** is used.

Figure 74 Rubric for Determining Loci to Ignore when Ignore Locus with Informative Peak in Stutter Position is Used

%CHM Reported	N-1	N	option	option	Ignore
Donor					
	D1	R	With/out Deconvolution	With Informative Peak in Stutter Position	Ignore(Stutter)
	D1	D1R	With/out Deconvolution	With Informative Peak in Stutter Position	Ignore(Stutter)
	D1R	D1	With Deconvolution	With Informative Peak in Stutter Position	Ignore(Stutter)
	D1R	R	With Deconvolution	With Informative Peak in Stutter Position	Ignore(Stutter)
%CHM Reported	N-1	N	option	option	Ignore
Recipient					
	R	D1	With/out Deconvolution	With Informative Peak in Stutter Position	Ignore(Stutter)
	R	D1R	With/out Deconvolution	With Informative Peak in Stutter Position	Ignore(Stutter)
	D1R	D1	With Deconvolution	With Informative Peak in Stutter Position	Ignore(Stutter)
	D1R	R	With Deconvolution	With Informative Peak in Stutter Position	Ignore(Stutter)

- To manually ignore (disable) a locus, right-click on the cell in the **Ignore** column and select **Ignore**.
- To ignore multiple loci with a single action, hold the shift or ctrl keys down, select the desired loci, then right-click and select **Ignore markers**.

Decision Pathway for Single Donor Chimerism Calculations

Figure 75 Decision Pathway Single Donor Cases with a Shared Allele Analyzed without Deconvolution

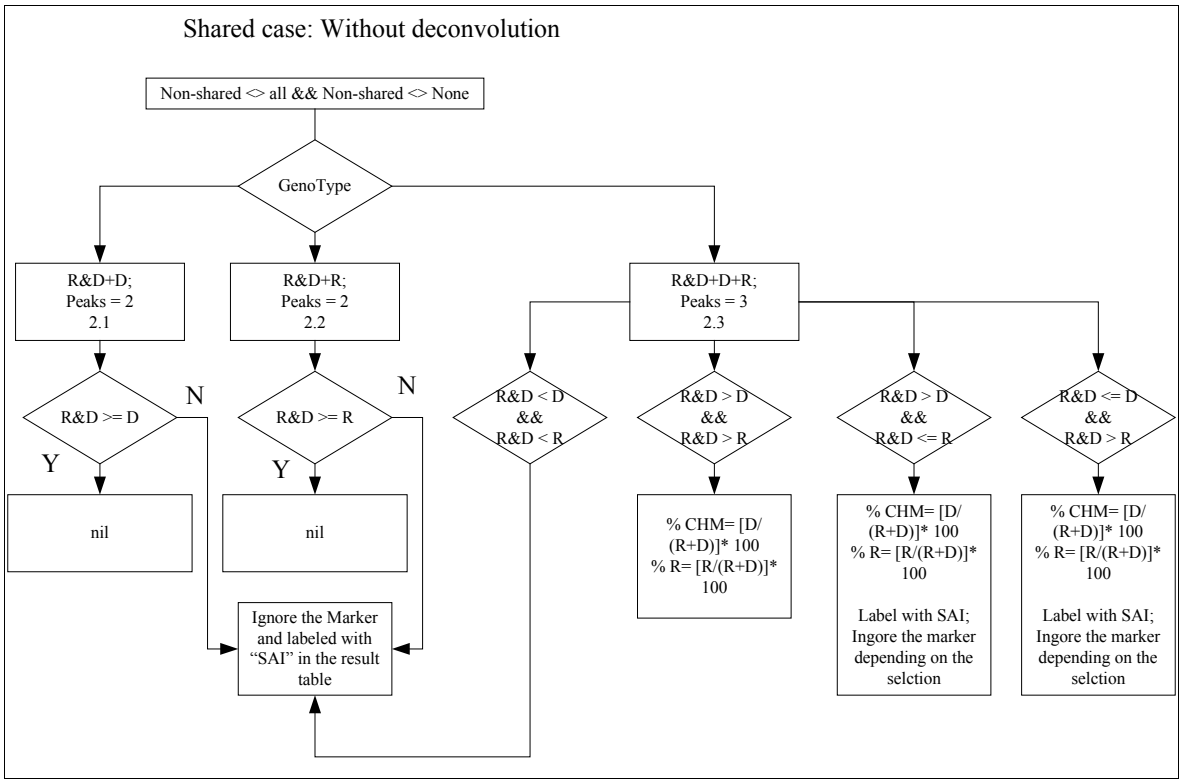
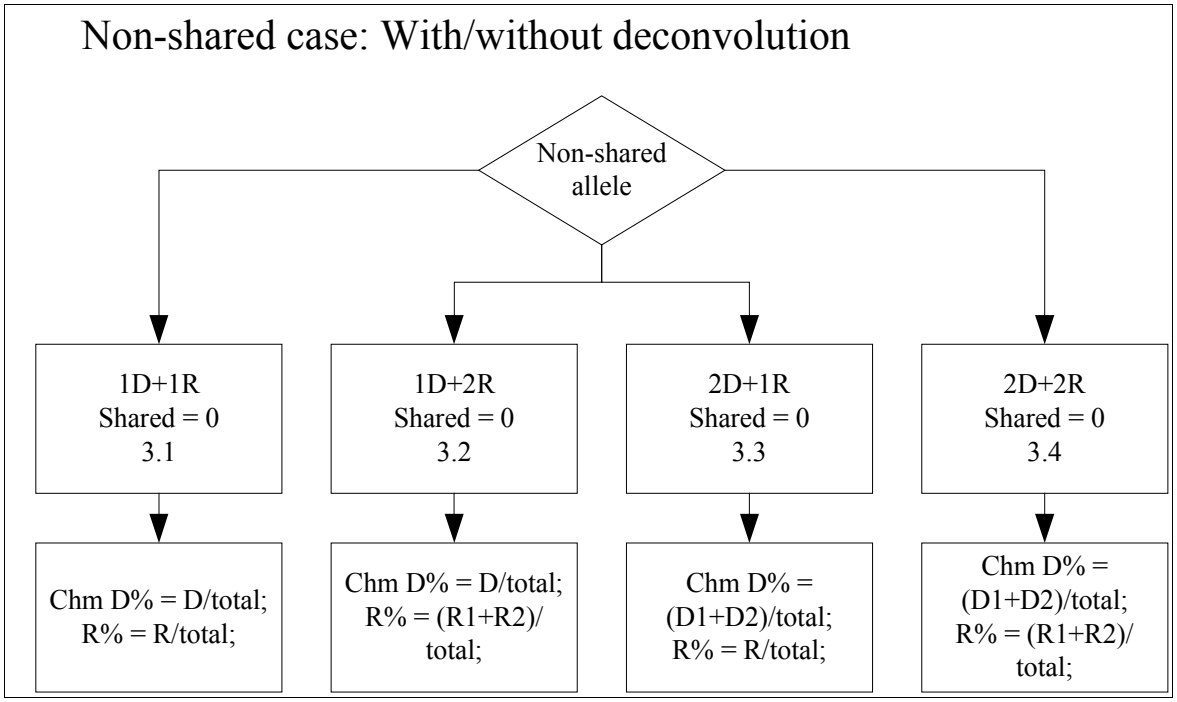


Figure 76 Single Donor Cases with No Shared Alleles Analyzed with or without Deconvolution



Decision Pathway for Double Donor Chimerism Calculations

The next graphics provide illustrations of deconvolution examples.

Figure 77 Case 1.0 IR + 1D1 + 1D2

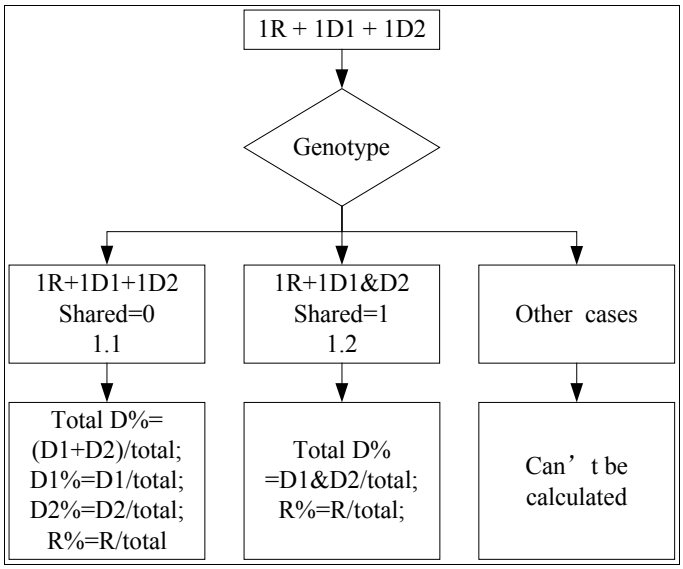


Figure 78 Case 2.0 1R +1D1+2D2 Homozygous R and D1, Heterozygous D2

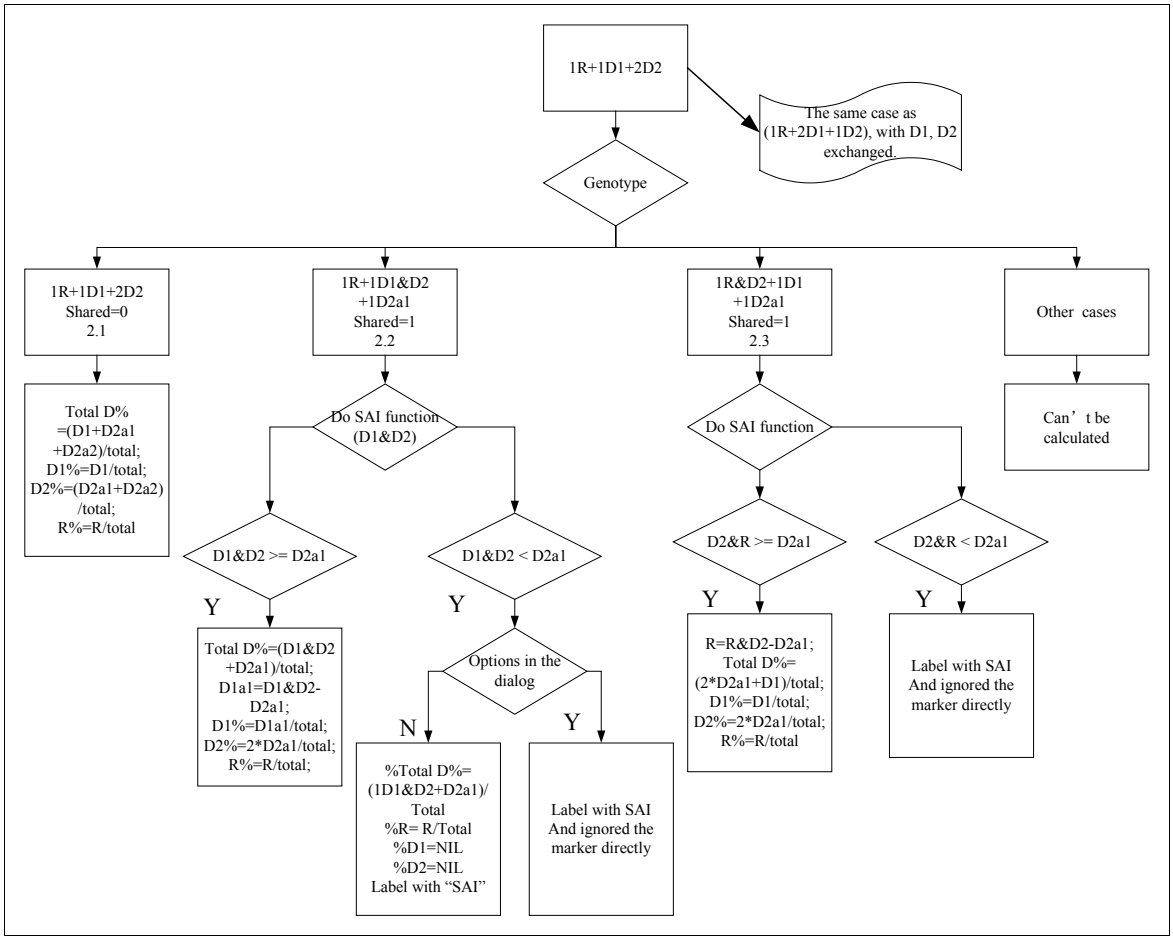


Figure 79 Case 3.0 1R+2D1+2D2 Homozygous R, Both Donors Heterozygous

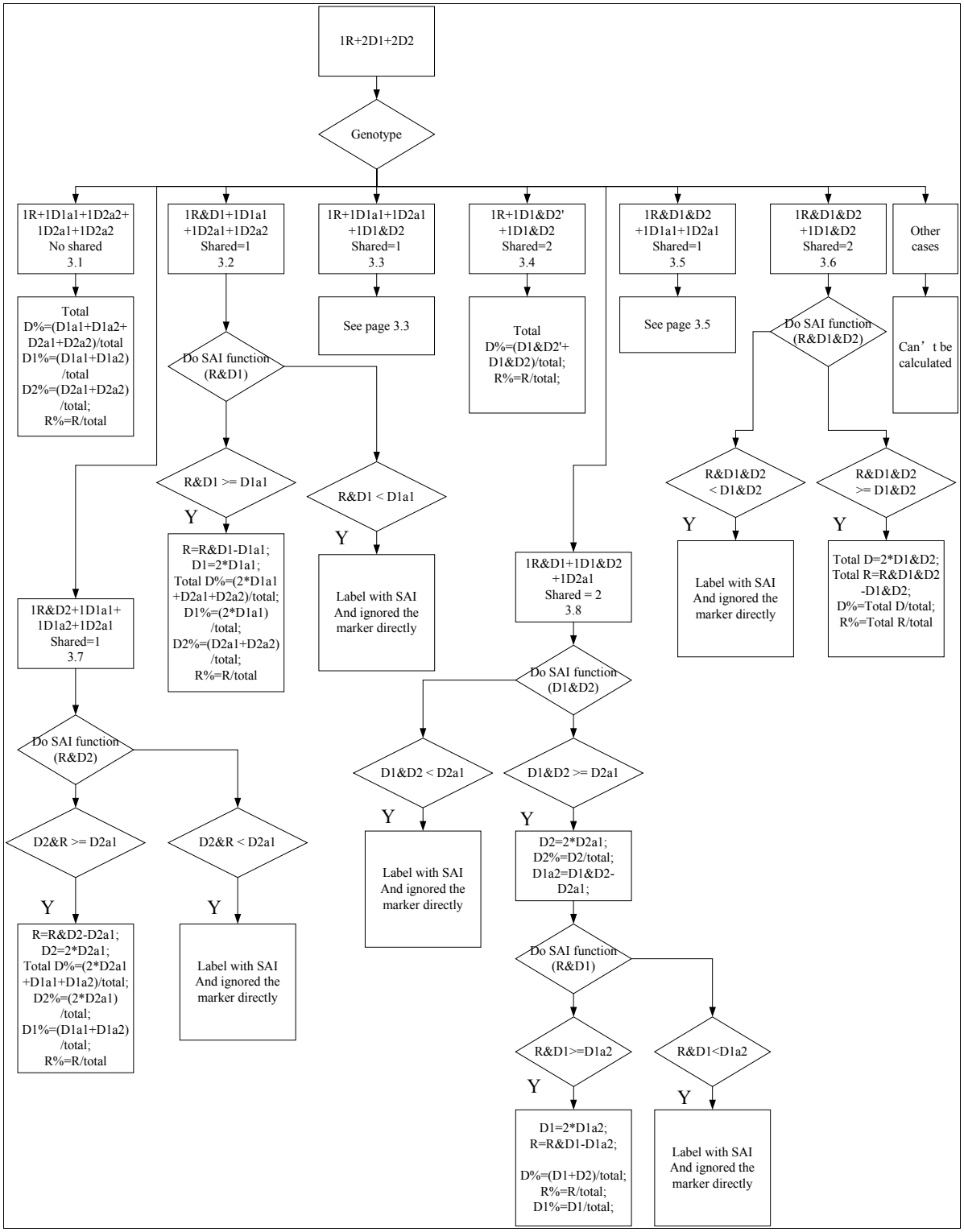


Figure 80 Case 3.3 1R+1D1a1+1D2a1+1D1&D2 Shared = 1

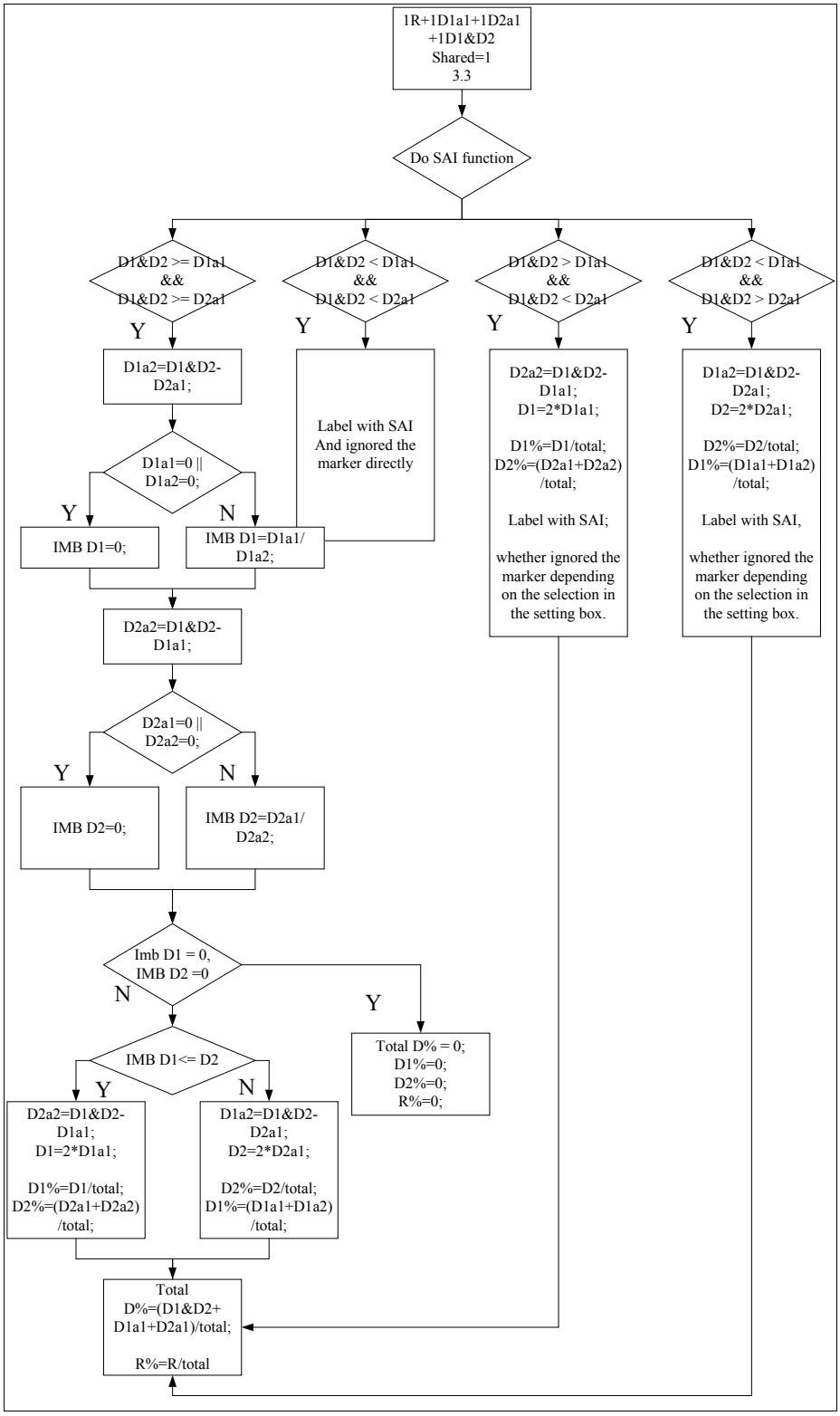


Figure 81 Case 3.5 1R&D1&D2+1D1a1+1D2a1 Shared = 1

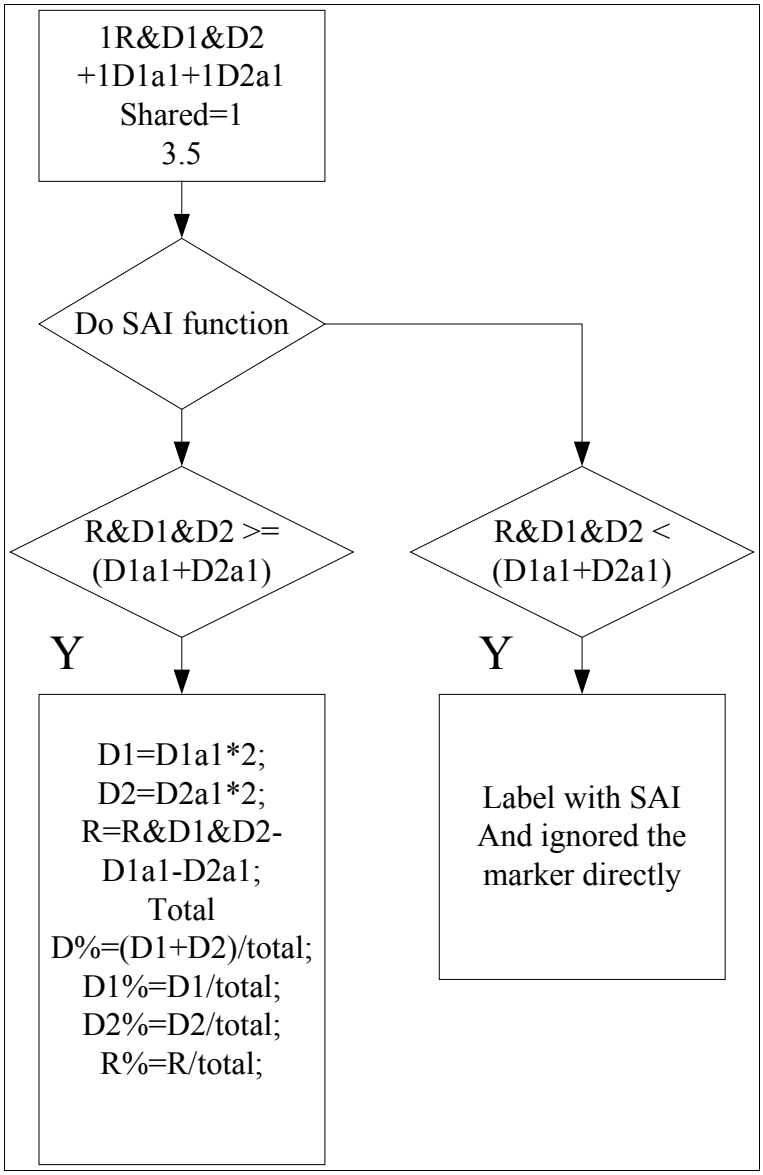


Figure 82 Case 4.0 2R+1D1+2D2 Heterozygous R and Donor2, Homozygous D1

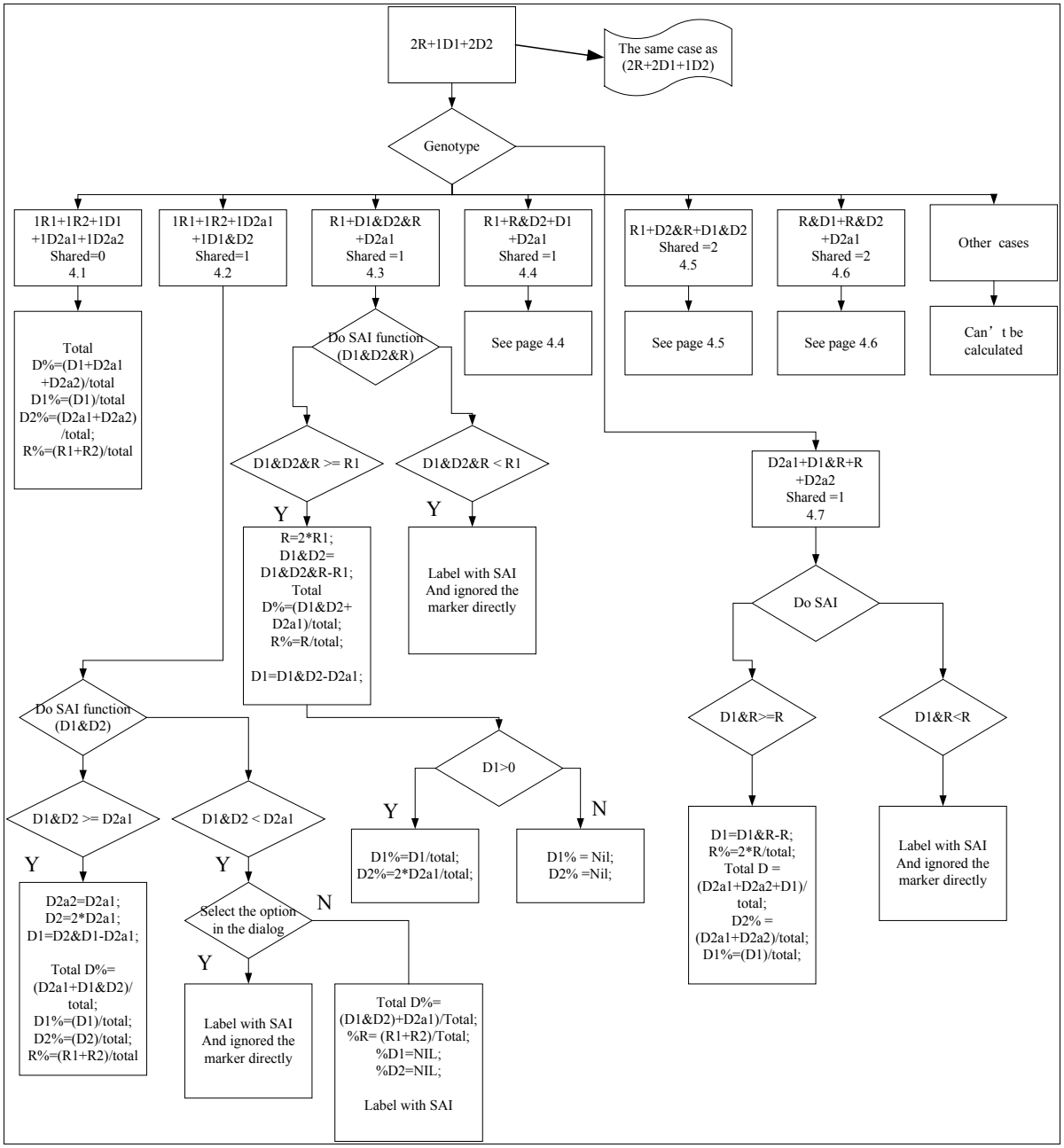


Figure 83 Case 4.4 R1+R&D2+D2a1 Shared = 1

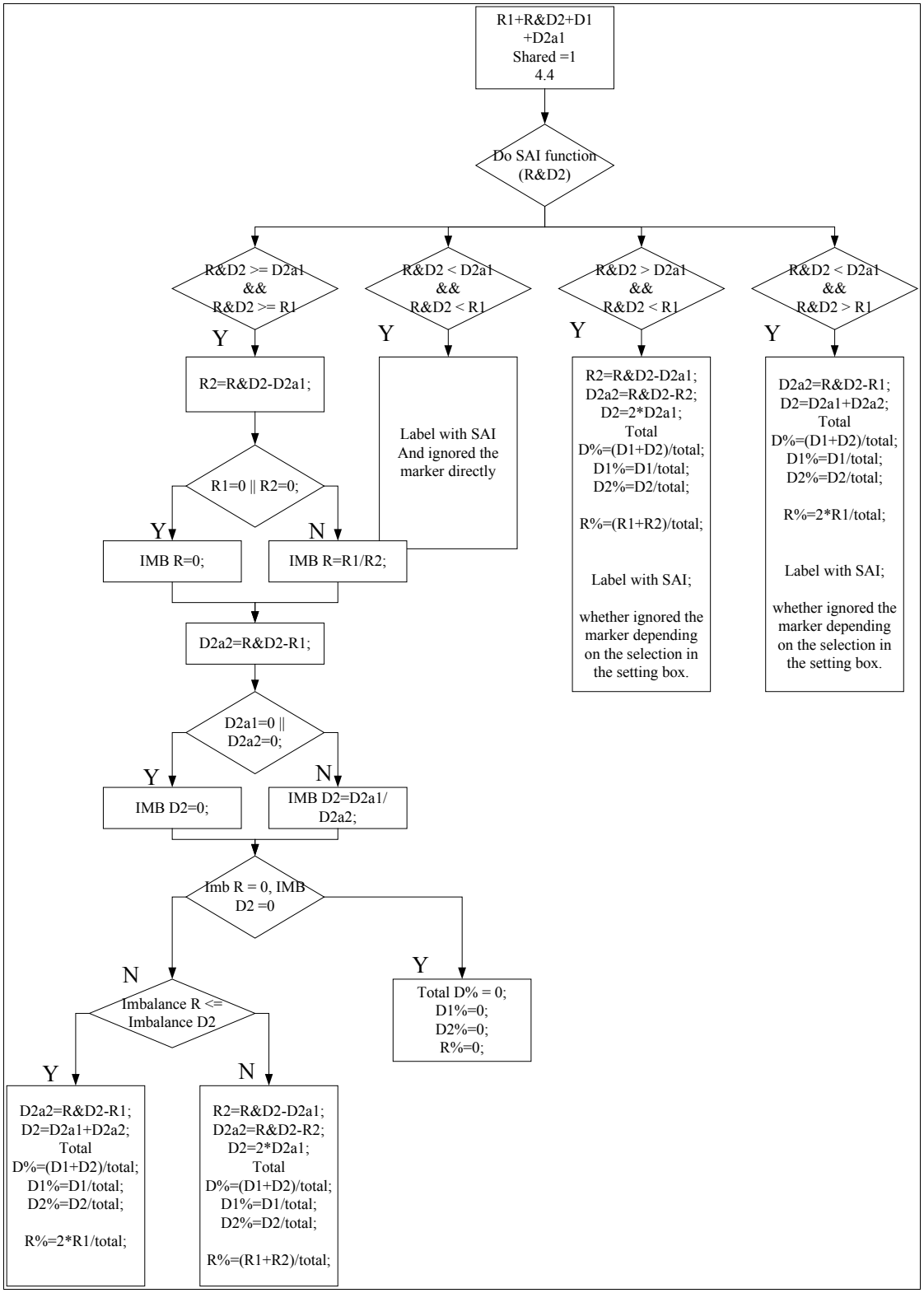


Figure 84 Case 4.5 and Case 4.6 Shared = 2

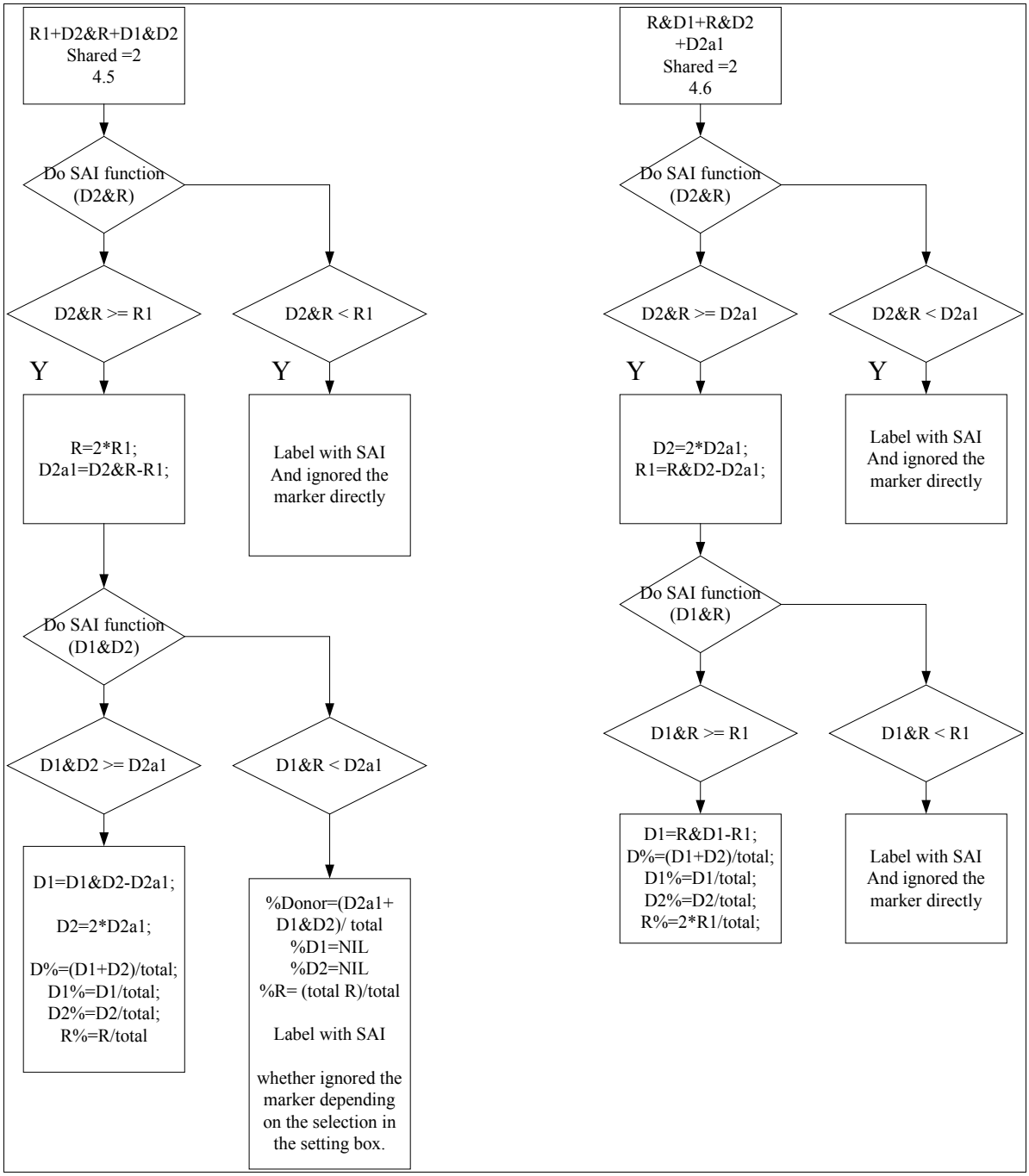


Figure 85 Case 5.0 2D1+2D2+2R All donors are heterozygous

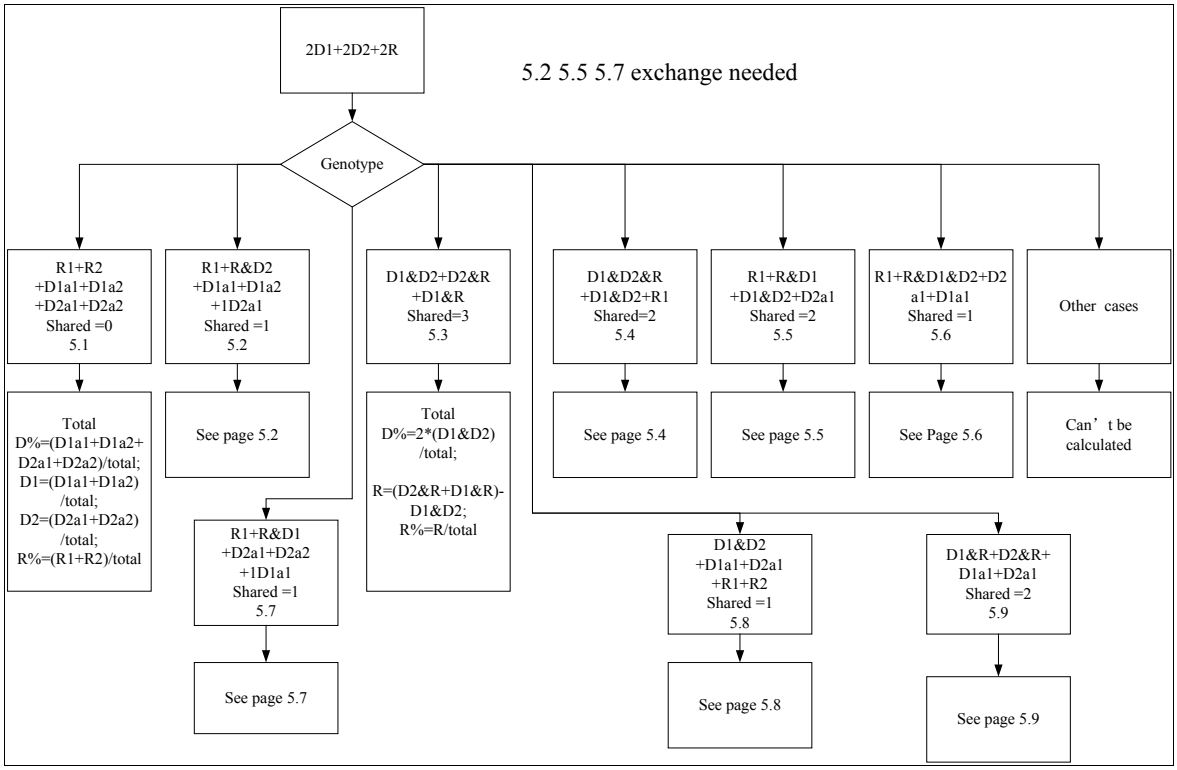


Figure 86 Case 5.2 R1+R&D2+D1a1+D1a2+D2a1 Shared =1

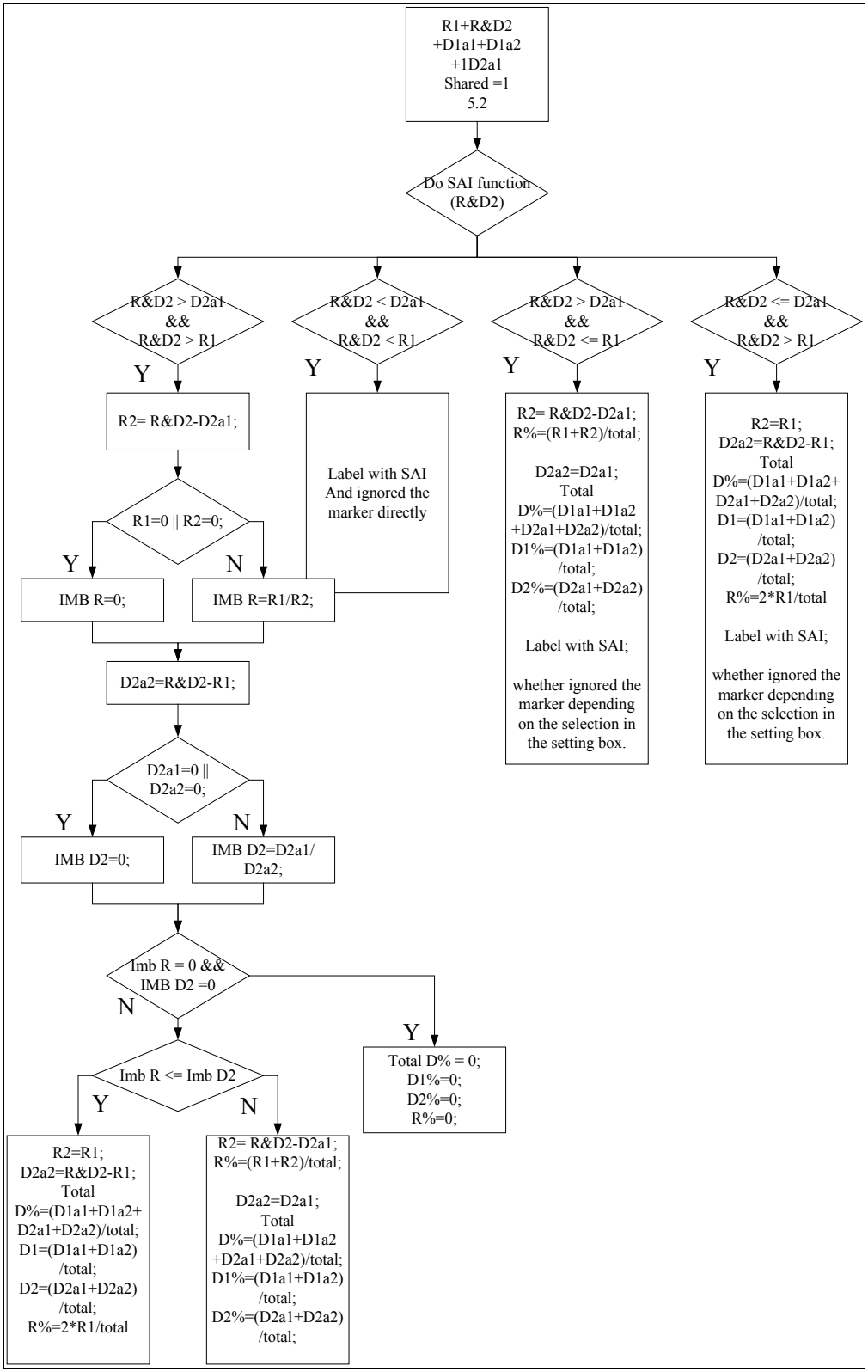


Figure 87 Case 5.4 D1&D2&R+D1&D2+R1 Shared = 2

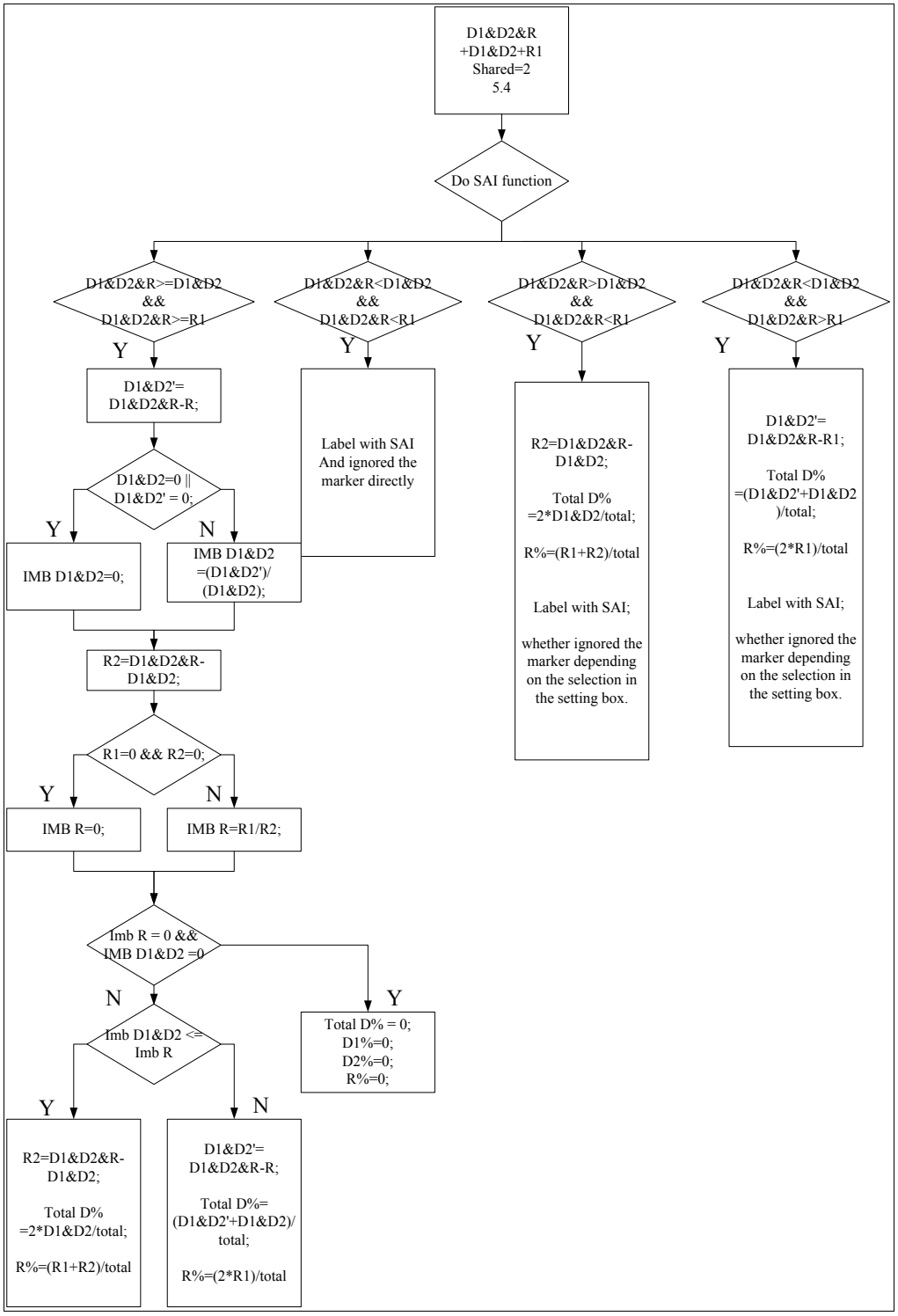


Figure 88 Case 5.5 R1+R&D1+D1&D2+D2a1 Shared = 2

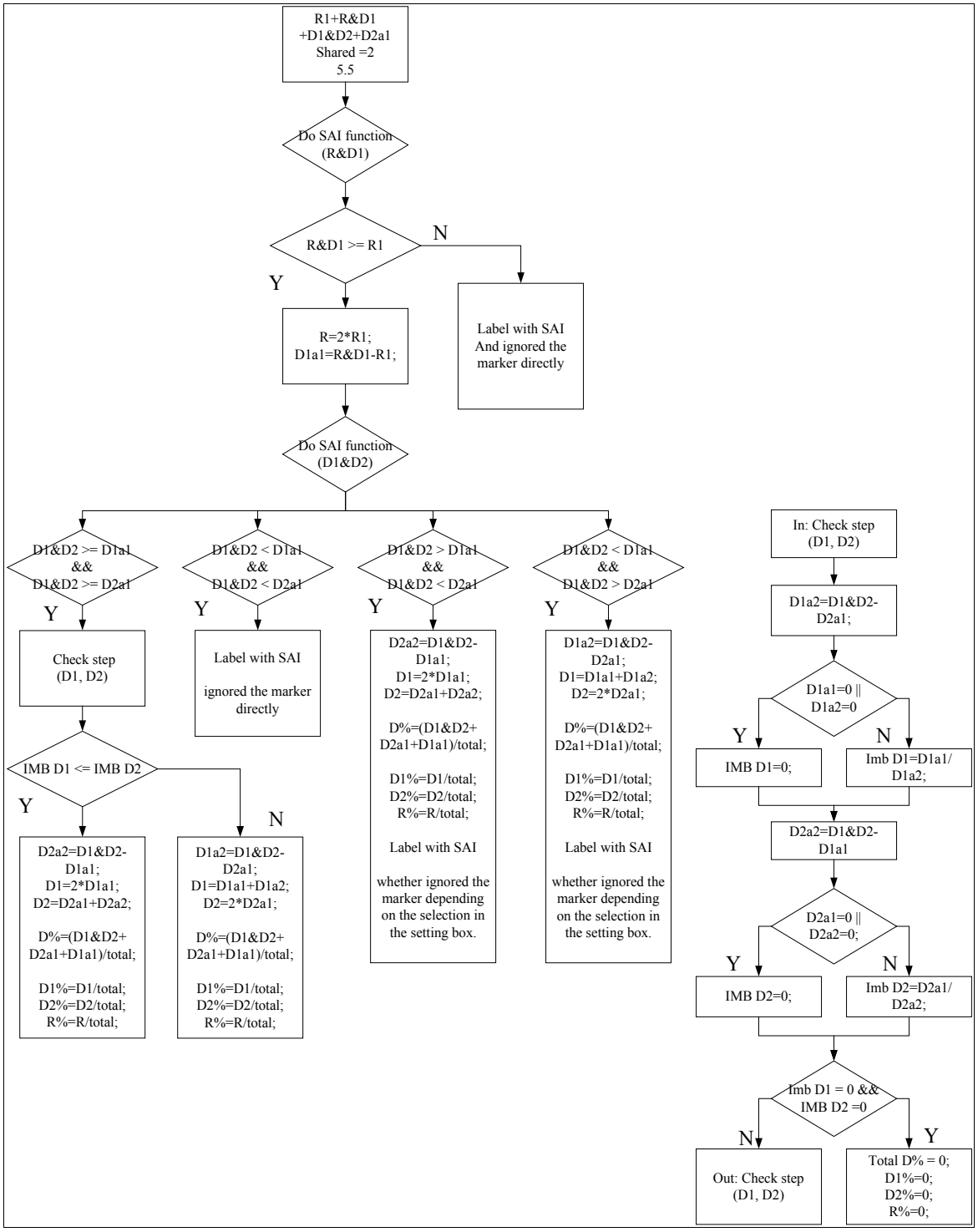


Figure 89 Case 5.6 R1+R&D1&D2+D2a1+D1a1 Shared = 1

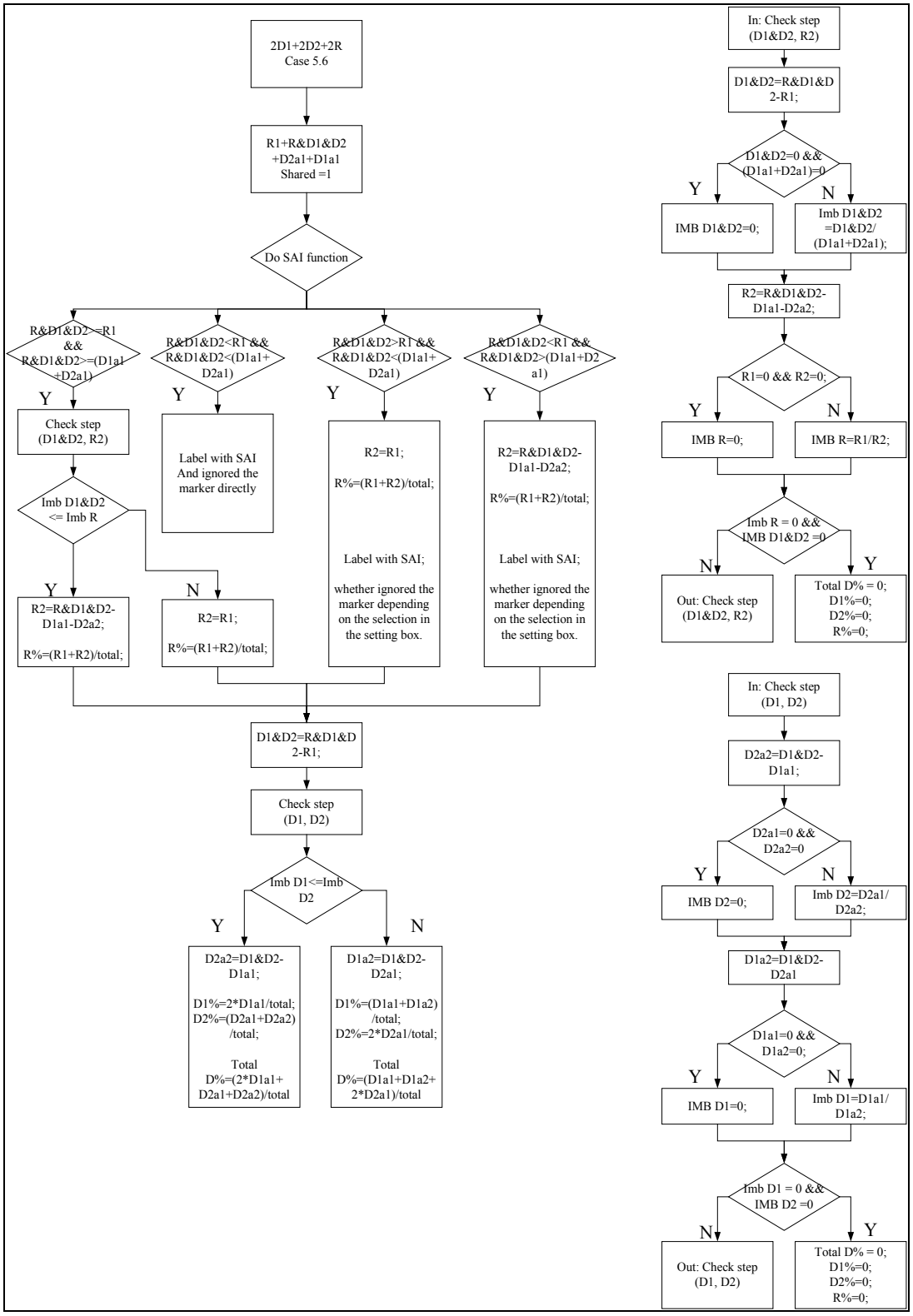


Figure 90 Case 5.7 R1+R&D1+D2a1+D2a2+D1a1 Shared = 1

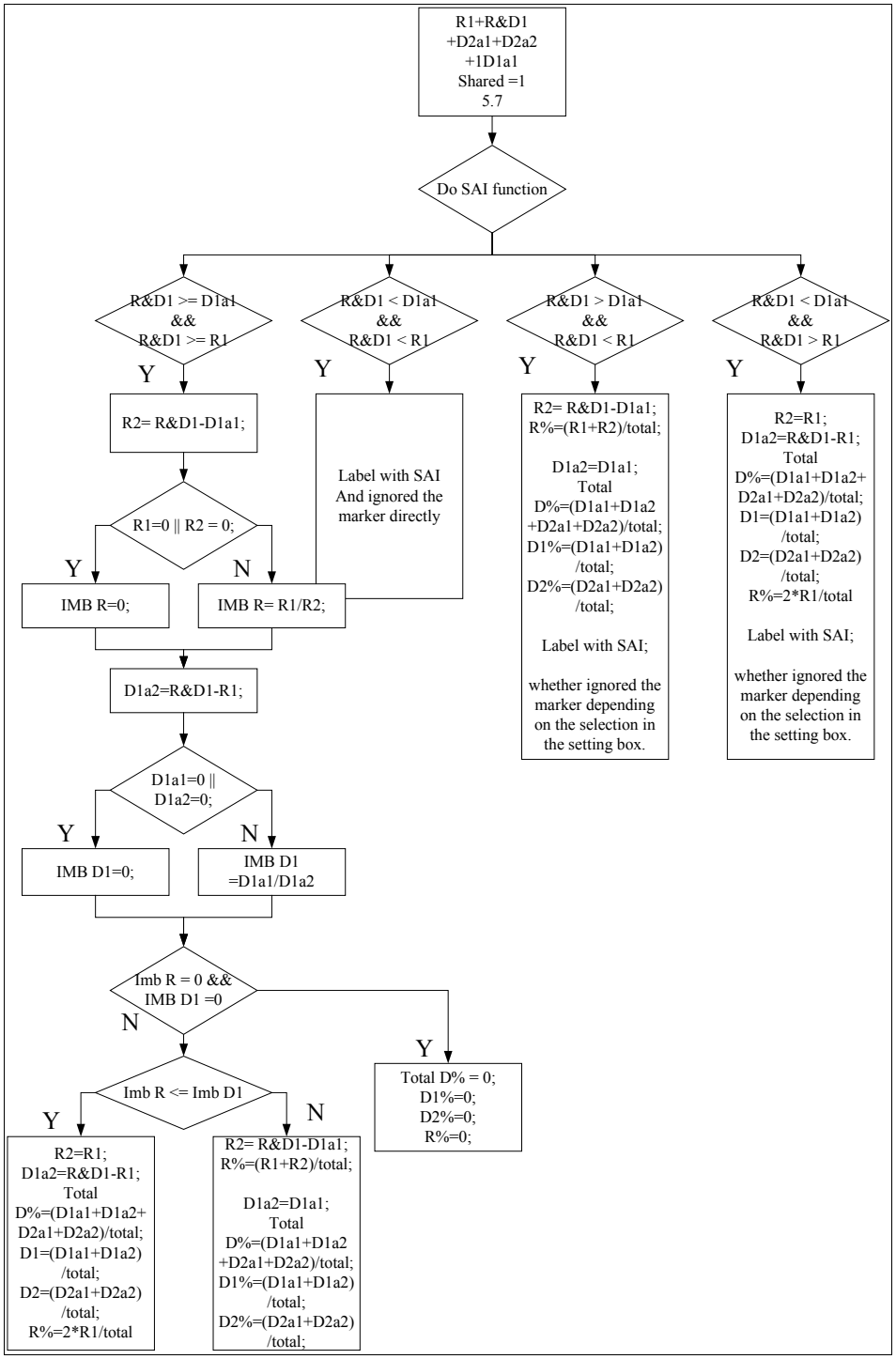


Figure 91 Case 5.8 D1&D2+D1a1+D2a1+R1+R2 Shared = 1

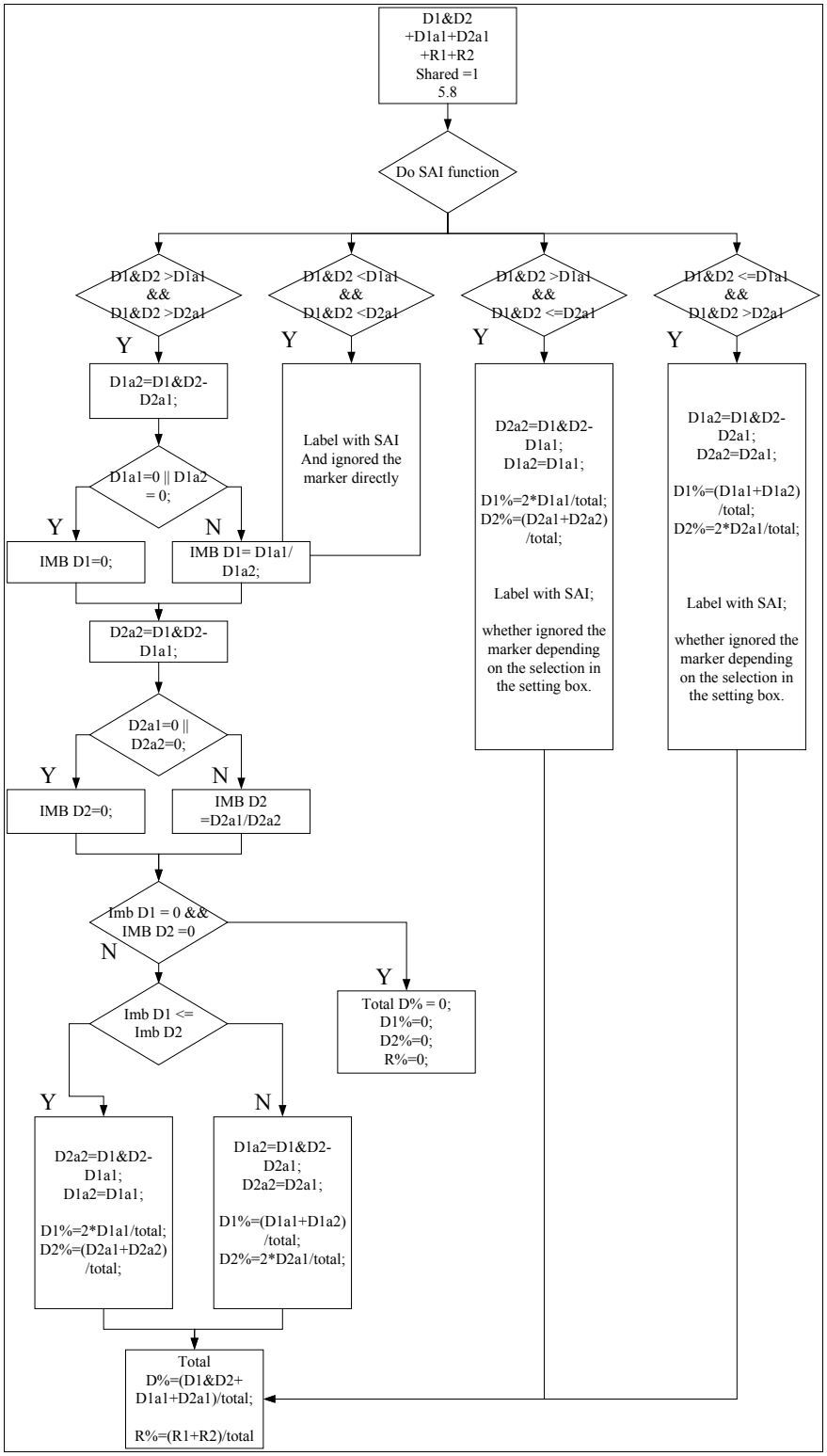


Figure 92 Case 5.9 D1&R+D2&R+D1a1+D2a1 Shared = 2

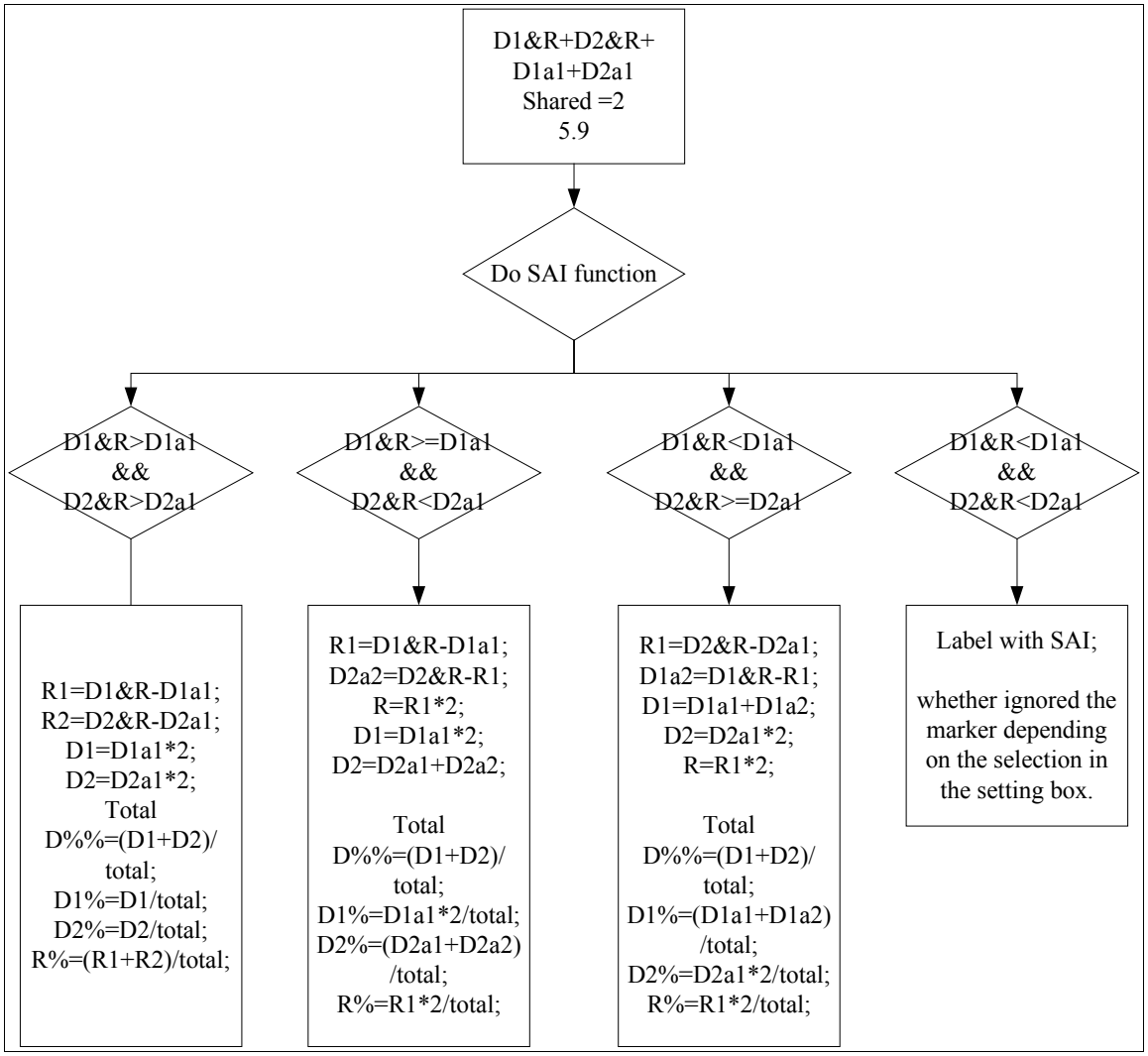
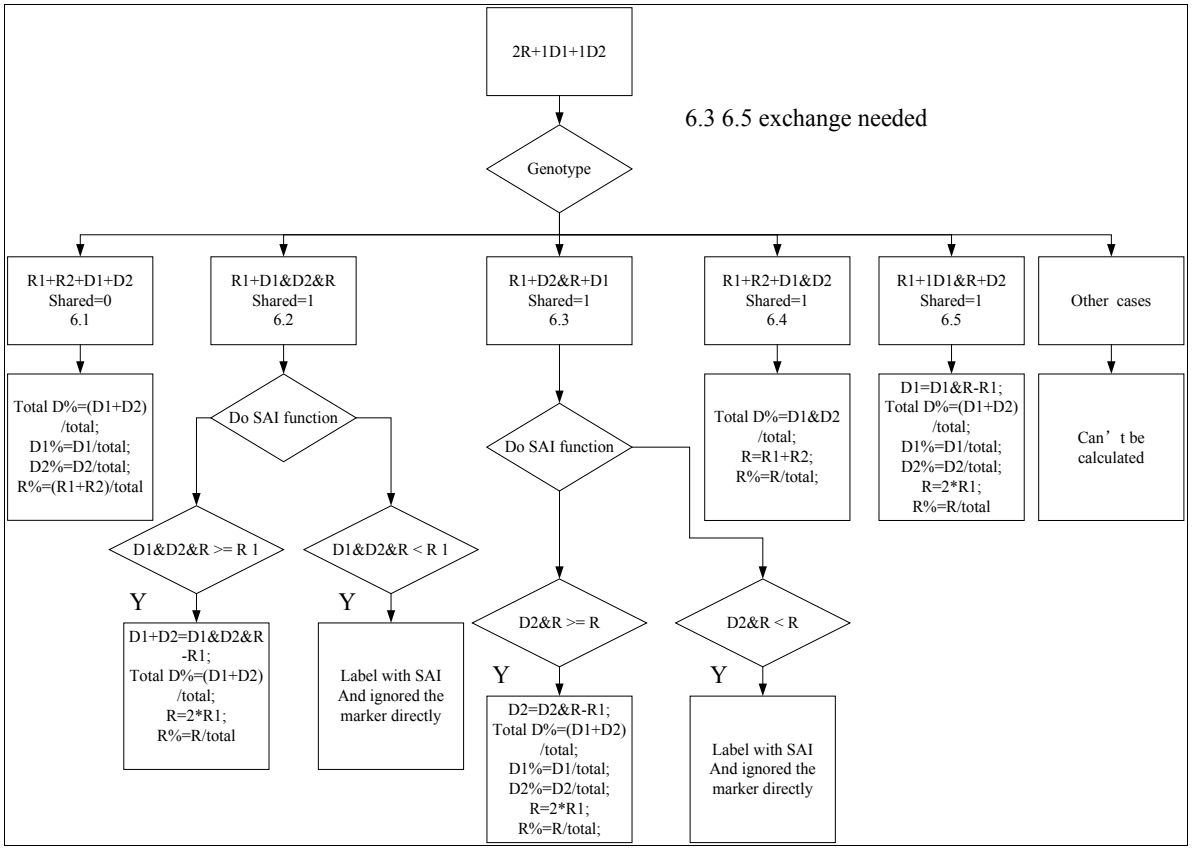


Figure 93 Case 6.0 2R+D1+D2 Heterozygous R, Both Donors Homozygous



Chapter 3 Genotyping General Procedure

Overview

A detailed description of importing data, all raw data and analysis icons, capillary electrophoresis review and details for genotyping is provided in this chapter and Chapter 4 Genotyping Main Analysis Overview. For streamlined workflow for chimerism detection, quantification, and monitoring, go to the section Automated Chimerism Detection and Quantification.

Import Data Files

After installing ChimerMarker software, ready to begin fragment analysis. First, raw data files must be uploaded to the program. Below is the list of file types supported by ChimerMarker.

ABI - .fsa, .hid

MegaBACE - .rsd

Beckman-Coulter - .esd

Generic - .scf, .sg1

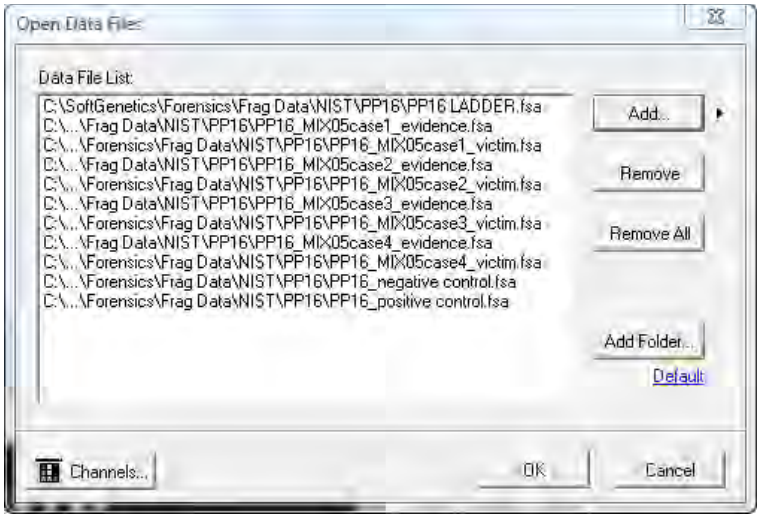
Fragment Analysis Procedure

1. Launch ChimerMarker
2. Click **Open Data**.

   The **Open Data File** dialog displays, as illustrated in Figure 94.

In the **Data File List** pane, the list of open data files is displayed.

Figure 94 Open Data File Dialog



3. Click **Add**.

   The **Open Data Files** dialog re-displays.

4. Select **Open** in the **Open** dialog.

   The files selected display in the **Data File List** field.

5. Navigate to the directory that contains the raw data files.
6. Select all files by **CTRL+A** or use **CTRL** and/or **SHIFT** keys to select individual samples.
7. Click **OK** button in the **Open Data Files** dialog and the samples are uploaded to ChimerMarker.

Features

There are several features available in the **Open Data Files** field to make any data upload easier. These include the following:

- **Add**: Is used to locate and select raw data files for upload. Click the arrow button next to the **Add** button to see the four (4) most recently accessed directories.
- **Remove**: Is used to remove samples from the **Data File List**. Highlight the sample using a single left-click in the **Data File List** then click **Remove**.
- **Remove All:** Removes all sample files from the **Data File List** field.
- **Add Folder:** Select **Add Folder** to upload raw data files from a specific folder in the file directory tree. Click the default hyperlink to navigate to set a default folder ChimerMarker opens when the **Add** or **Add Folder** buttons are clicked.
- **Channels:** Opens the **Set Channels** dialog with some 4, 5, 6-color tab options. It allows the user to choose from ABI, MegaBACE, and Beckman-Coulter standard dye color orders. The user can manually enter a dye color and name. The default

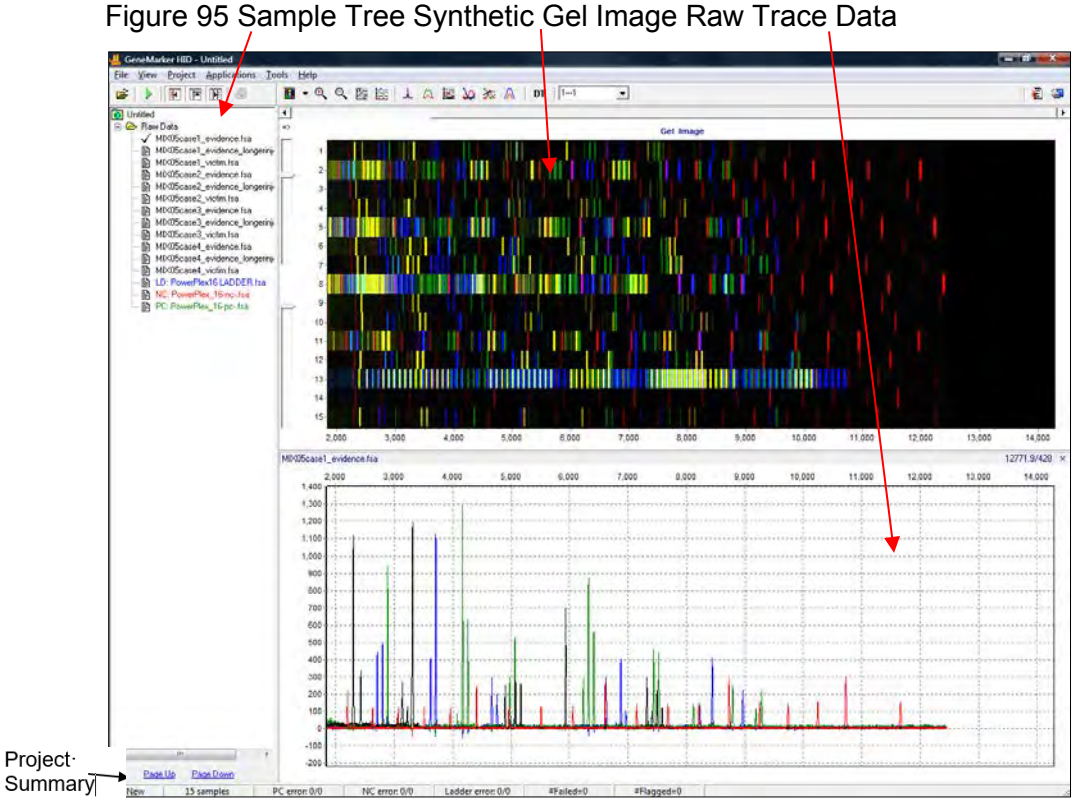
channel color setup is ABI. Set the dye color channels before clicking **OK** in the **Open Data Files** dialog.

Raw Data Analysis

Once the raw data files are uploaded, the Raw Data Main Analysis dialog appears. Double-click the samples in the Sample Tree to open the individual Raw Data Traces. The Synthetic Gel Image displays the unprocessed data in a traditional gel format with larger fragments located on the right. The Electropherograms display fluorescent signal intensities as a single line trace for each dye color. The signal intensities, recorded in Relative Fluorescent Units (RFUs), are plotted along a frame scale in the **Raw Data Analysis** dialog with fragment mobility from right to left. The largest size fragments are on the far right of the trace.

Raw Data Analysis

Figure 95 Sample Tree Synthetic Gel Image Raw Trace Data



Main Toolbar Icons

The main toolbar icons are described in the next table.

Table 4 Raw Data Analysis Main Toolbars and Icons

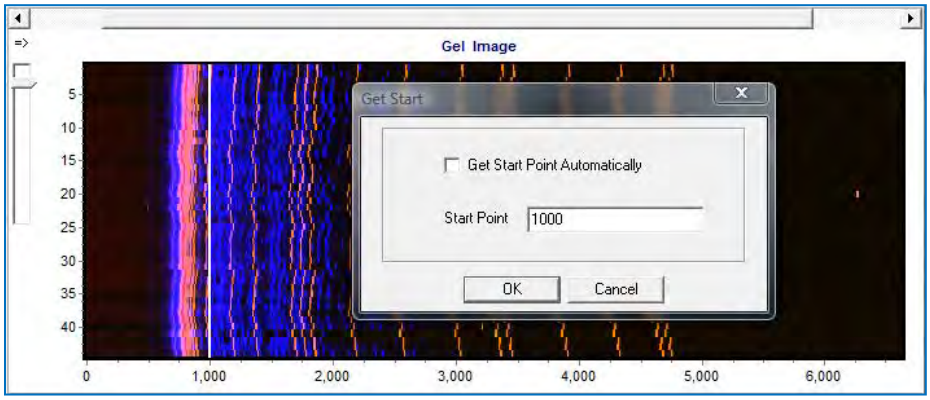
Icon	Name	Function
	Spike Removal	Removes peaks from voltage spikes caused by micro-air bubbles or debris in the laser path. This option is selected by default in the **Run Wizard**.
	Saturation Correction	A synthetic peak is created based on peak shape before and after saturation. The results of these are less accurate than that of non-saturated peaks. This option is selected by default in the **Run Wizard**.
	Smooth	This function smoothes the baseline by eliminating smaller noise peaks. This option is selected by default in the **Run Wizard**.
	Baseline Subtraction	Selecting this option will remove the baseline completely so that the Y-axis is raised above the noise level. This option is selected by default in the **Run Wizard**.
	Auto Pull-up Removal	Automatically removes peaks caused by wavelength bleed-through to other wavelengths. This option is selected by default in the **Run Wizard**.
	Manual Pull-up Correction	This allows the user to manually adjust larger pull-up peaks in case the **Auto Pull-up Removal** function has not corrected the problem. It is recommended to de-select **Pull-up Correction** in the **Run Wizard** when using this function.
DT	2nd Derivative Trace	This feature reduces high background noise and sharpens peaks. Baseline fluctuation caused from dye blobs or the DNA template in PCR are reduced with this function. It is recommended to de-select **Spike Removal** in the **Run Wizard** when this function is activated.

What to Expect

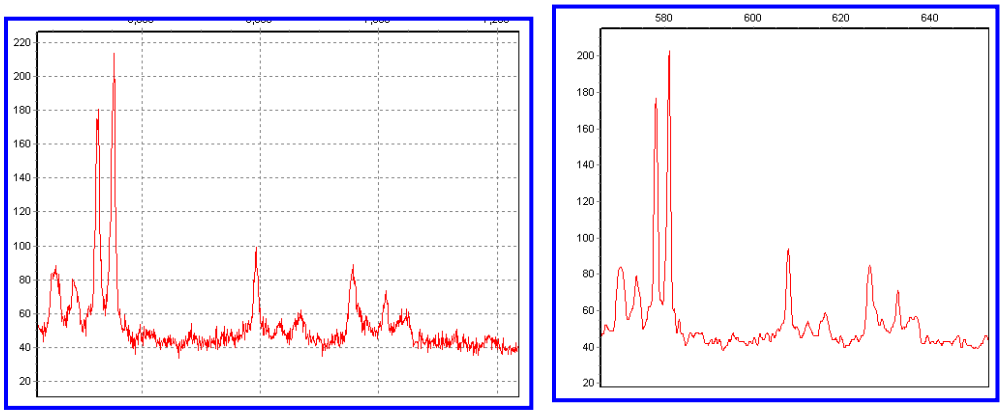
The raw data correction icons are selected individually in the **Raw Data Analysis** dialog. The images below demonstrate how the data appears before (left image) and after (right image) the parameter is applied.

- **Range**
  - **AutoRange**: Analyzes from 0 to end of trace for size call.
  - **Manual Range**: User-defined range.
  - Right-click in gel image and select **Get Start Point**.

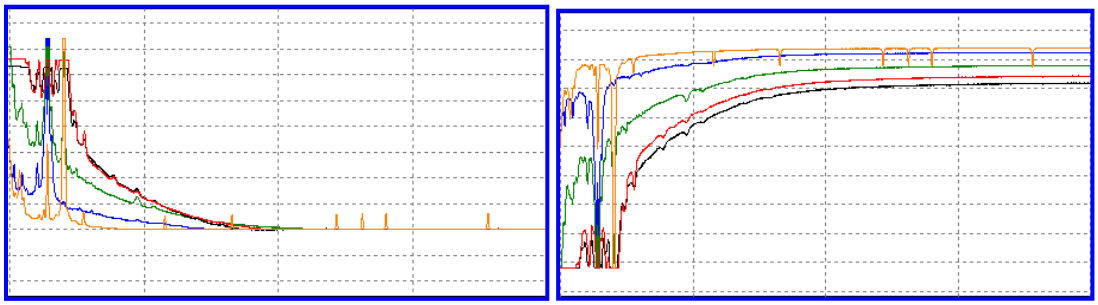
Figure 96 Raw Data Analysis Dialog



- **Smooth**
  - Fourier frequency transformation (FFT) to determine frequency domain.
  - Use only top 40% of lowest frequencies.
  - Smoothing broadens peaks and therefore you can lose resolution.
  - Enhanced Smooth - Same as smooth but use only top 20% of lowest frequencies.



- **Baseline Subtraction**
  - Use 20% of lowest intensities (to the right of the beginning of the range).
  - Looks at trace in 500-600 frame sections.



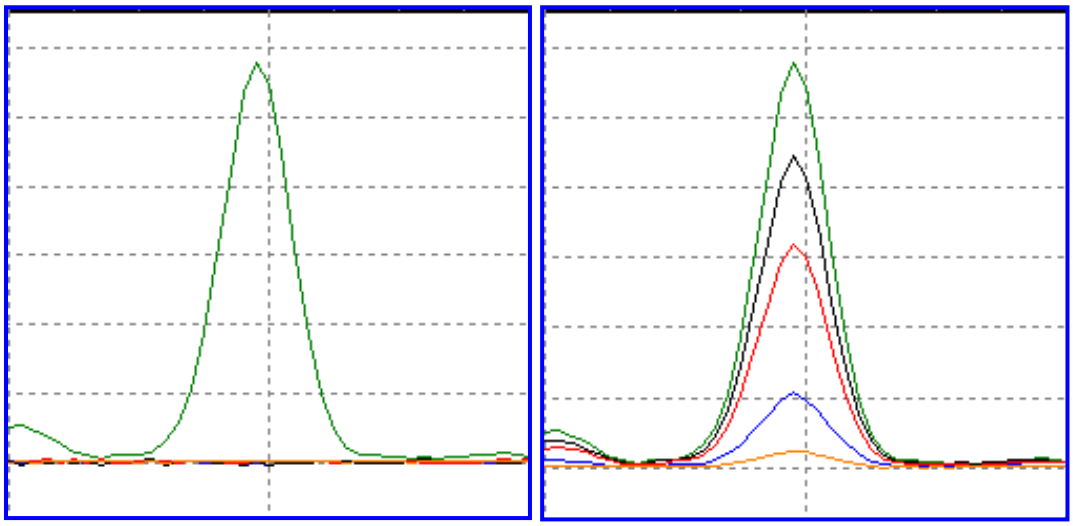
- **Pull-up Correction**

  Ax=B. A being the major coefficient. Use the Input matrix or use single dye adjustment up to 0.20 for small corrections.

  When a **Manual Pull-up** correction is chosen, a .txt or .mtx matrix file are uploaded and used to deconvolute dye colors.

  Note: De-select automatic **Pull-up Correction in the Run Wizard Data Process** field, if a manual matrix correction is applied.

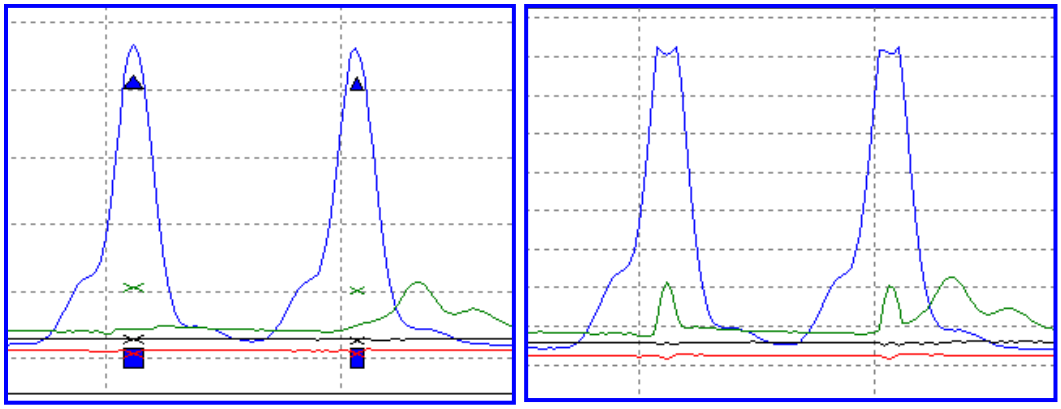
  Figure 97 Manual Pull-up Correction



- **Saturated Peak Correction**

  ABI instrument saturated peaks are typically >8000 RFU. The top of a saturated peak looks split. A small Pull-up peak may be present under the saturated peak.

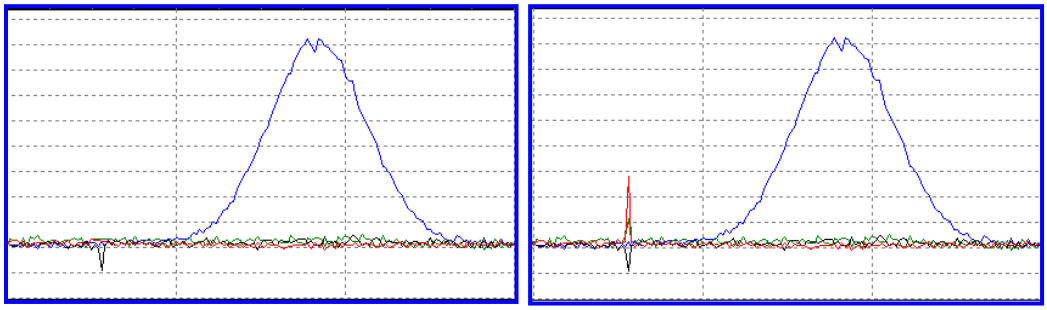
  ChimerMarker takes the small Pull-up peak and adds it to the split in the saturated peak.



- **Spike Removal**

  Caused by overheating of camera chip, voltage spike, etc. Spikes are usually only 1-2 frames wide; peaks are usually 5-10 frames wide. Create a first derivative trace of the raw data.

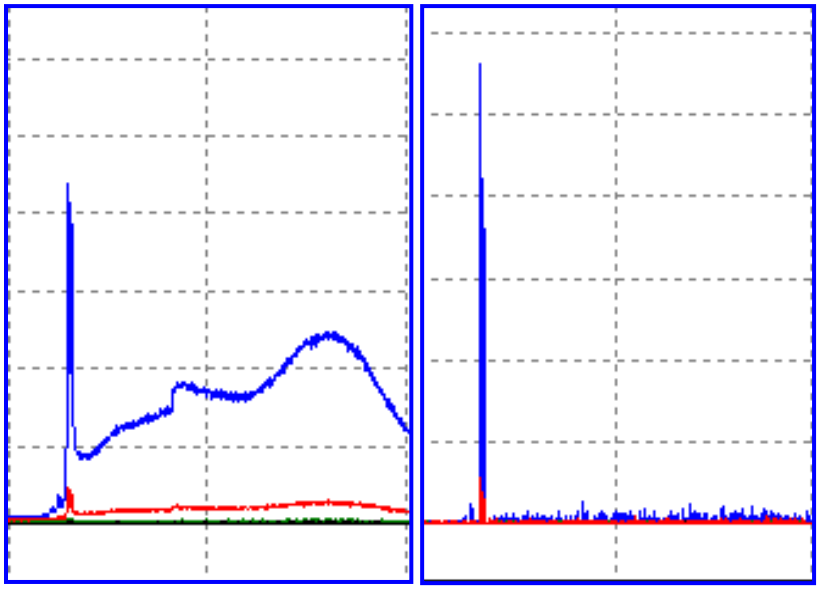
Spikes are the 1st DT outliers (3-5 sigma)



- **Second Derivative Trace**

  (A1-A2)-(A2-A3) = A1+A3-2(A2). Use when you have a fat base to your peaks (ex. Dye blob under peak, etc.).

  Note: Do not use 2nd DT with Spike Removal because real peaks look like spikes.



Process Data

After the raw data files have been uploaded to ChimerMarker, they are ready to be processed. The processing step includes application of a sizing standard, filtering of noisy peaks, and comparison to a known allelic Panel. ChimerMarker combines all these steps in one simple tool called the **Run Wizard**. To access the Run Wizard simply click the **Run Project** icon in the main toolbar.

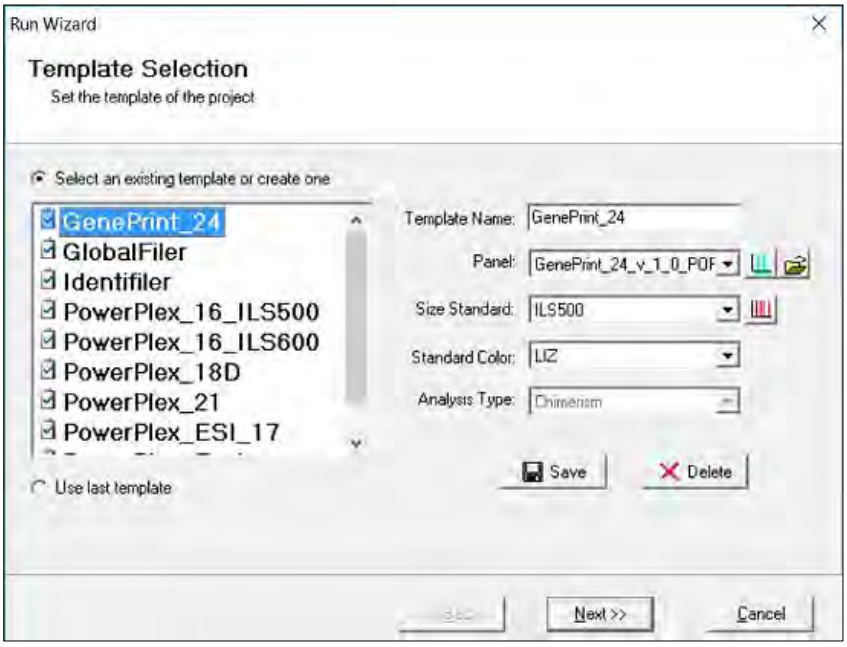
Run Wizard Template Selection

Follow the next steps to select templates using the Run Wizard.

1. Click the **Run Project** icon in the toolbar.

   The **Run Wizard Template Selection** dialog displays.

   Figure 98 Run Wizard Template Selection



2. Select a template (a previously saved set of size standard, standard color, and analysis type named for future use), or select a new combination of size standard, standard color, and analysis type.
3. Click **Next** when finished.

Icons and Functions

Template Name

Select from existing pre-made templates or create your own (see Figure 98) by entering a **Template Name** in the right-hand column and clicking **Save**. When a template is saved, only the settings in the **Template Selection** dialog of Run Wizard are saved.

To create a new template, click the **Select an existing template or create one** checkbox. Also, select a template from the list of available templates in the left column of the dialog, and then to save it for future use, click **Save**.

- To not use a template, select the appropriate size standard, standard color, and type of analysis.
- Select the **Use last template** checkbox to automatically select the last template used.

Panels

ChimerMarker comes preloaded with many common kits. Panels including Promina's PowerPlex kits and additional panels are imported by selecting **Open Files** next to the **Panel** field. A custom panel is created in the **Panel Editor** tool. See Chapter 6 Panel Editor.

Table 5 ChimerMarker Panels Icons

Icon	Name	Function
	Panel Editor	A Panel is selected from any available from the drop-down menu or is viewed and selected by clicking the **Panel Editor** icon.
	Import a Panel	If a Panel cannot be found in the Panel Editor tool, it is imported by clicking on the **Import a Panel** icon.

Size Standard

ChimerMarker comes preloaded with many common size standards including GeneScan 500 and LIZ600. A custom size standard is created by selecting the **Size Template Editor** icon next to the **Size Standard** field. See Chapter 5 Fragment Sizing Standard.

Table 6 ChimerMarker Size Template Icons

Icon	Name	Function
	Size Template Editor	This allows the user to check sample files against a selected size standard, modify and save the size standard for future use, or create a new size standard.

- **Standard Color**

  Select the dye color which contains the internal lane standard.

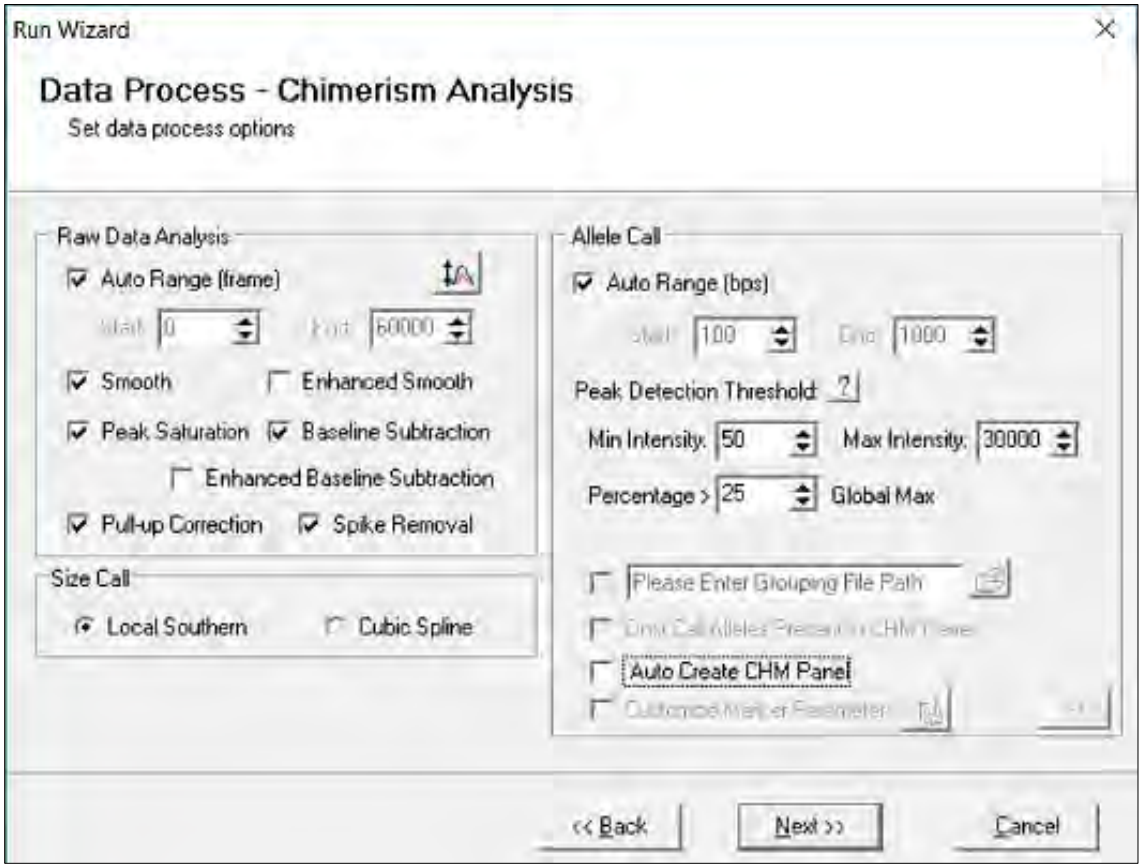
- **Analysis Type**

  The **Analysis Type** option is inactivated in ChimerMarker.

Run Wizard Data Process

The **Run Wizard Data Process** dialog displays.

Figure 99 Run Wizard Data Process Chimerism Analysis



Select the appropriate analysis settings in the **Data Process** dialog and click **Next** to continue.

Icons and Functions Raw Data Analysis

- **Auto Range (frame)**

  The range in camera frames automatically finds the process-able data range. If **Auto Range** is not selected, manually enter the start and end frame numbers of the data set for analysis.

  Note: If automatic size call fails due to high saturation, de-select **Auto Range** and manually input the required data range.

- **Smooth**

  Smooths the baseline by eliminating smaller noise peaks.

- **Enhanced Smooth**

  This feature is used only in cases where the data is extremely difficult to analyze and cannot be corrected with the **Smooth** function.

- **Peak Saturation**

  The software analyzes saturated data points by creating a synthetic estimate of the peak shape based on the curves prior to saturation. The results are less accurate than that of non-saturated peaks.

  Note: Saturated and Saturated Repaired peaks are not recommended for any quantitative calculations.

- **Baseline Subtraction (Classic)**

  This function removes the baseline completely so that the Y-axis is raised above the noise level. It uses 20% of lowest intensities (to the right of the beginning of the range) and looks at the trace in 500-600 frame sections.

- **Superior Baseline Subtraction**

  This is a similar algorithm to the classic baseline subtraction but it looks at the trace in 100 frame sections. This baseline subtraction method is excellent for samples that have a raised bubble that is occasionally present in the low molecular weight samples, and for samples with closely spaced peaks.

  Sample with a raised bubble baseline in the raw data, processed with classic baseline subtraction (left) and superior baseline subtraction (right).

Figure 100 Superior Baseline Subtraction

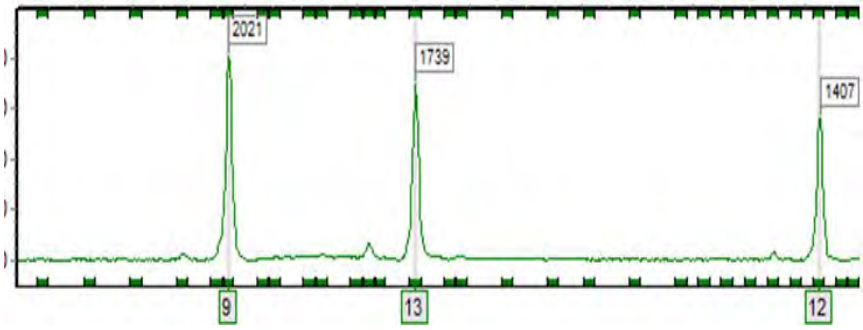
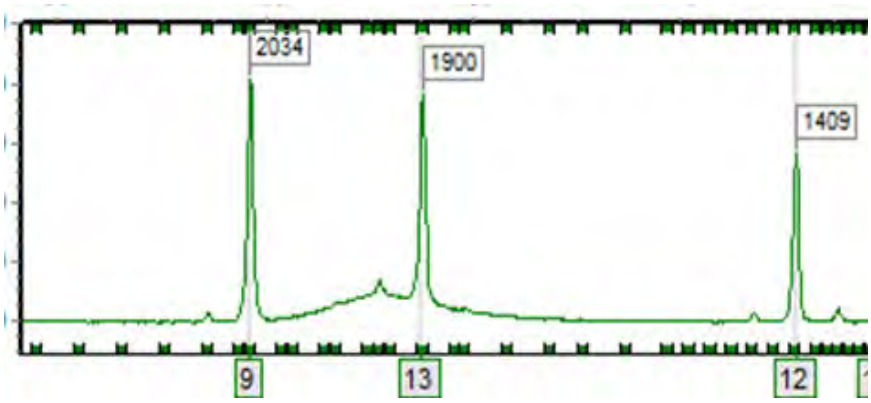


Figure 101 Sample with a Raised Bubble Baseline



- **Enhanced Baseline Subtraction**

  This feature is used only in cases where the data has excessive baseline in one or more of the dyes, or has an interfering slope from the ion front in the smaller marker ranges. The function uses the second derivative of the absolute value for every 30 data points and looks at the trace in 300 frame sections.

Figure 102 Enhanced Baseline Subtraction 1

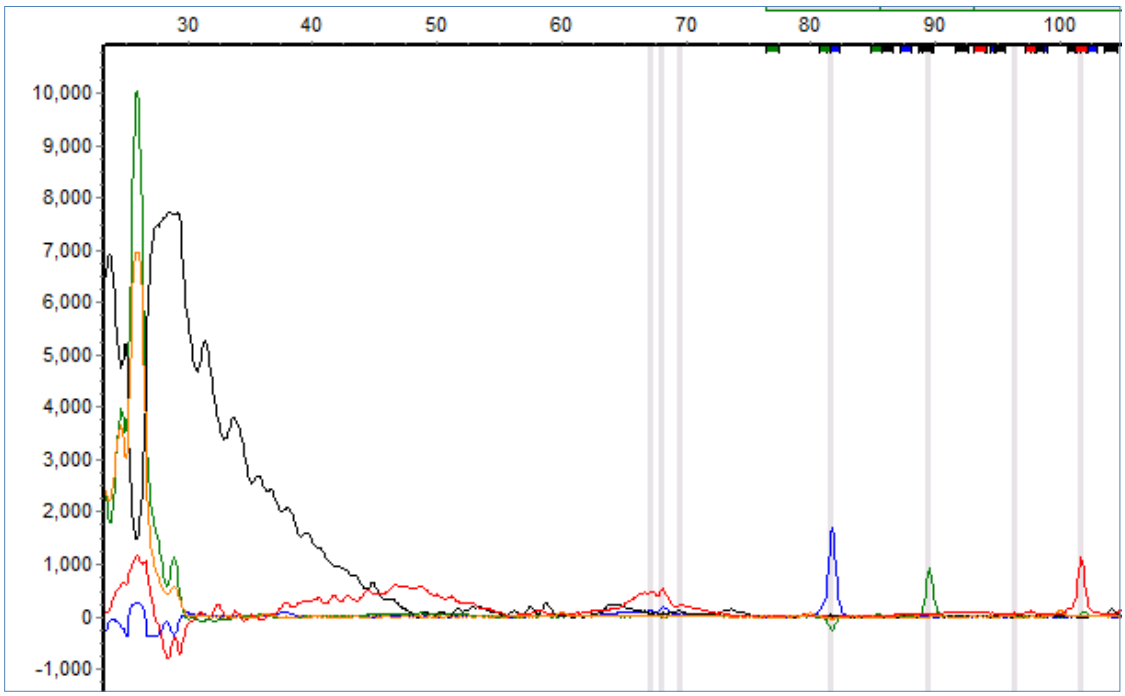
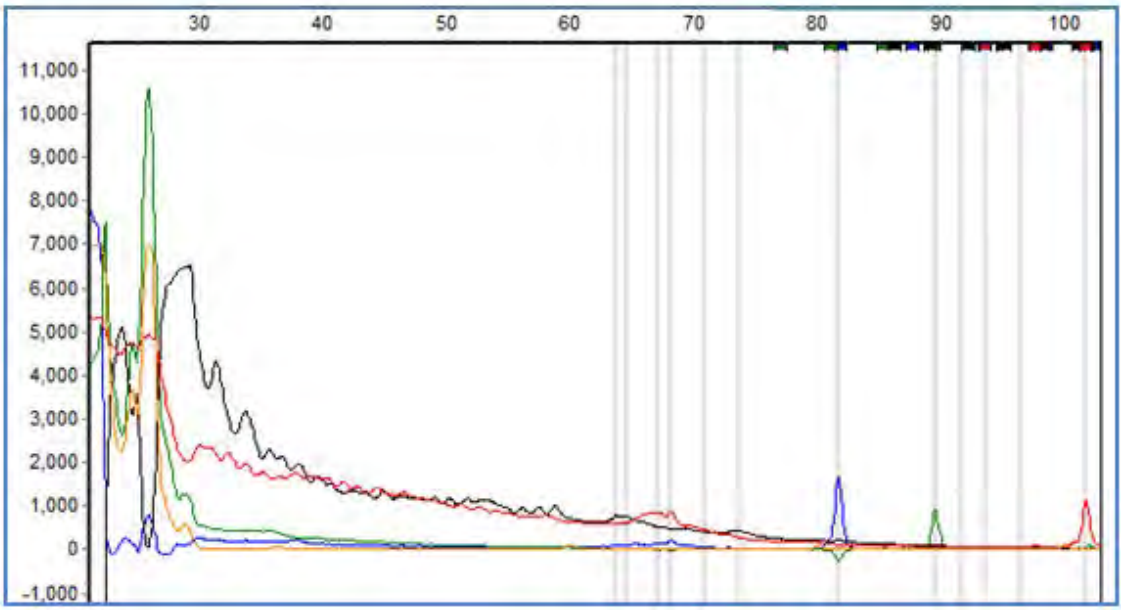


Figure 103 Enhanced Baseline Subtraction 2



In situations where there is an extended ion front in the mini-STR range, enhanced baseline subtraction is used.

- **Pull-up Correction**

  This function removes peaks caused by wavelength bleed-through to other wavelengths. The function should be disabled if a **Manual Pull-up Correction** was used in the **Raw Data Analysis** dialog.

- **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. Do not select **Spike Removal** when **2nd Derivative Trace** is applied.

- **Size Call**

ChimerMarker offers two sizing methods:

- **Local Southern**

  Used in most genotyping software applications and is recommended for most analyses. This method is based on the idea that smaller size fragments run faster. Plot a size v. time graph and overlay a size v. 1/time graph to determine linear trace. (Southern, E.M. “Measurement of DNA Length by Gel Electrophoresis.” 1979. *Analytical Biochemistry*. **100**, 319-323).

- **Cubic Spline Method**

  Cubic Spline is offered as an alternative method that may be more appropriate for some data. This method uses a cubic equation to connect known points on the size v. time graph. An example of a cubic equation: ax^3+bx^2+cx+d. (*The Astrophysical Journal*. December 1, 1994. **436**, pages 787-794.)

  Local Southern Method Cubic Spline Method

  Figure 104 Graphic Plot Local Southern

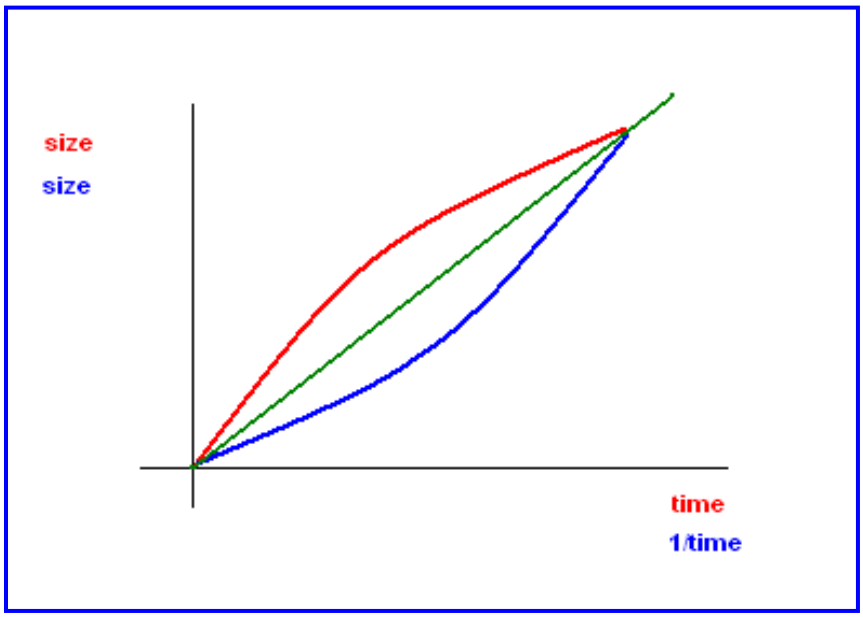
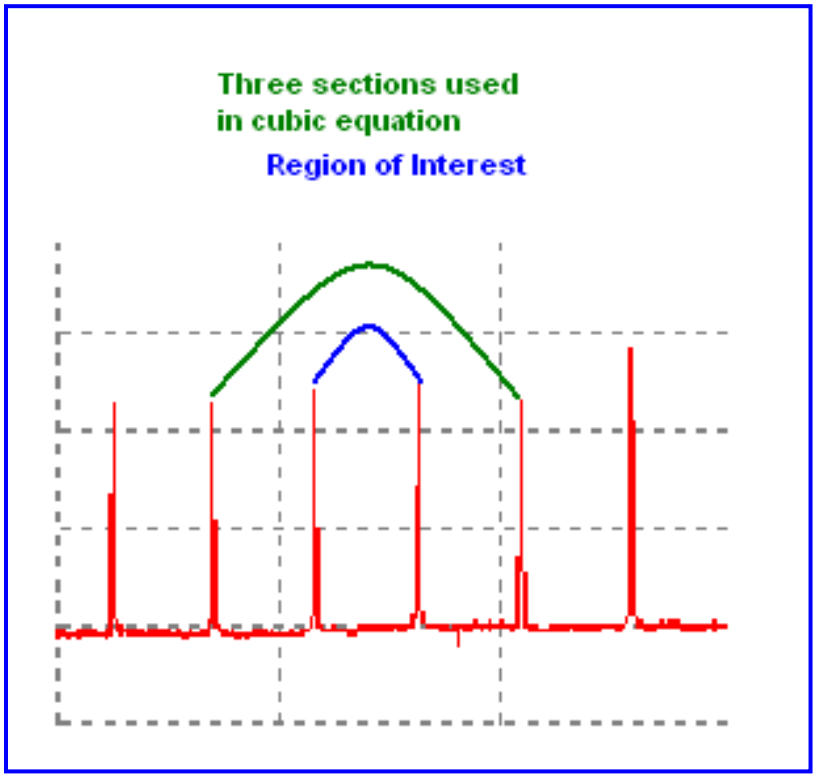


Figure 105 Graphic Plot Cubic Spline Methods



- **Allele Call**

  The Allele Call pane in Figure 99 allows the user to set allele calling range, detection thresholds, and filters.

- **Auto Range**

  The software identifies peaks in the process-able data range for each lane.

- **Manual Range**

  To select a specific analysis region, de-select **Auto Range** and input the desired base pair range. Peaks outside the Manual Range are not called.

- **Peak Detection Threshold**

  Note: The **Peak Detection Threshold** parameters are only applied to peaks *outside* of the Panel Markers. To adjust settings for peaks within Panel Marker ranges, see Chapter 6 Panel Editor.

  - **Min Intensity**

    Minimum RFU threshold of peak height used for peak detection. Peaks below this value are not called.

  - **Max Intensity**

    Maximum RFU threshold of peak height. Peaks above this value are flagged with a yellow Allele label, given a Quality Rank of Check, and marked with HI Quality Reasoning.

- **Percentage Global Max**

  Relative minimum intensity of allele peaks to the 5th highest peak in the dye color used for peak detection. Peaks below this value are not called.

- **Load Default**.

  Recalls any settings previously saved by the user. If there are no user-saved settings, the program loads the default settings for that analysis type.

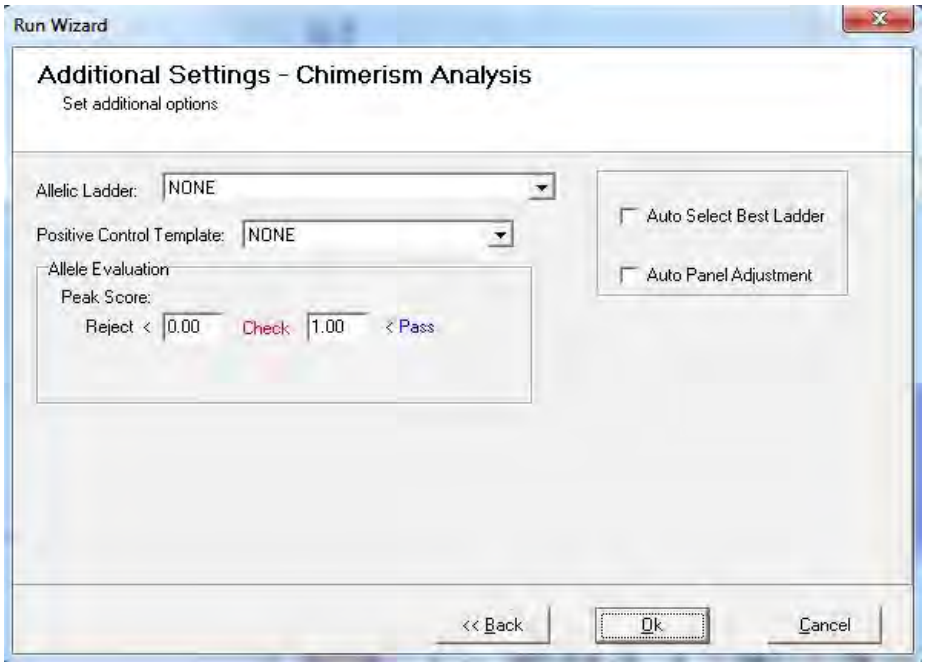
- **Save Default**.

  Saves any settings defined by the user that are different from the default. These settings are recalled for consistency of analysis on similar data sets.

Run Wizard Additional Settings

The **Run Wizard Additional Settings** dialog displays.

Figure 106 Run Wizard Additional Settings



Procedure

1. In the **Run Wizard Additional Settings** dialog, select the **Allelic Ladder** drop-down list, in the **Allele Evaluation** pane, adjust the **Peak Score** parameters.
2. Click **OK**.

   The **Data Processing** dialog displays.

   The data is sized, peaks are filtered, and the panel is applied.
3. When the **Data Processing** dialog completes, click **OK**.

Functions

- **Allelic Ladder**

  Permits the selection of a sample containing an allelic ladder. If the user selects one ladder, the ladder appears in **bold** font and is set to the top electropherogram in the **Main Analysis** dialog. All samples are analyzed using this selected ladder.

- **Allele Evaluation. Peak Score**

  User-definable confidence level of the allele call. Peak score is an algorithm that considers signal-to-noise ratio and peak morphology. Rejected samples appear in red, samples that need checking appear in yellow, and samples that have passed appear in green.

- **Auto Select Best Ladder**

  ChimerMarker identifies ladder samples in the dataset as defined in the **View** > **Preference** > **Chimerism** > **Ladder Identifier** field. Compare Ladder samples to the chosen Panel.

  Each ladder that is within the range of the selected panel will pass and appears in **bold** font in the **Sample File Tree**. **Auto Select Best Ladder** analyzes each sample file with the passing ladder that best matches that sample. The print report provides the file name of the reference ladder used for each sample.

- **Auto Panel Adjustment**

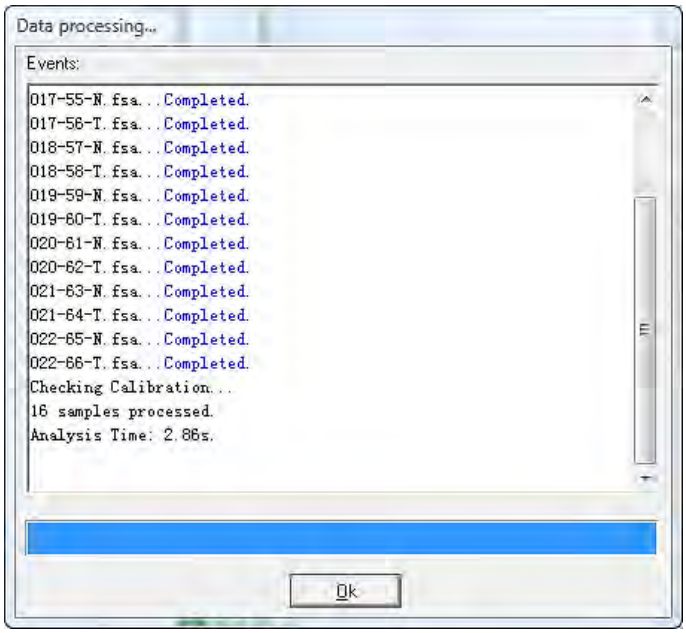
  When selected, the Markers and Bins of the chosen Panel are aligned with the peak positions of the Ladder samples in the dataset (within a five base pair shift). Ladder samples are identified by ChimerMarker as defined in the **View** > **Preference** > **Chimerism** > **Ladder Identifier** field. Major alleles and variant (or virtual) alleles are specified in the **Control Column** in the Panel editor. See Chapter 6 Panel Editor. This information is used for pattern recognition and automatic panel adjustment.

  Note: Panels that do not contain variant (virtual) alleles are manually adjusted in the Panel Editor by selecting the **Major Adjustment of Panel** icon then the **Minor Adjustment of Panel** icon.

Adjust Analysis Parameters

After clicking **OK** in the **Run Wizard Additional Settings** dialog, the **Data Processing** dialog displays.

Figure 107 Data Processing Dialog



The raw data is processed and sized, then the filtering parameters are applied, and finally a Panel is applied (if selected). Click **OK** when analysis is complete.

Review the results in the **Main Analysis** dialog. See Main Analysis Dialog. If there are many false positive peak calls, adjust the analysis parameters. There are three (3) options for adjusting the analysis parameters discussed below.

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

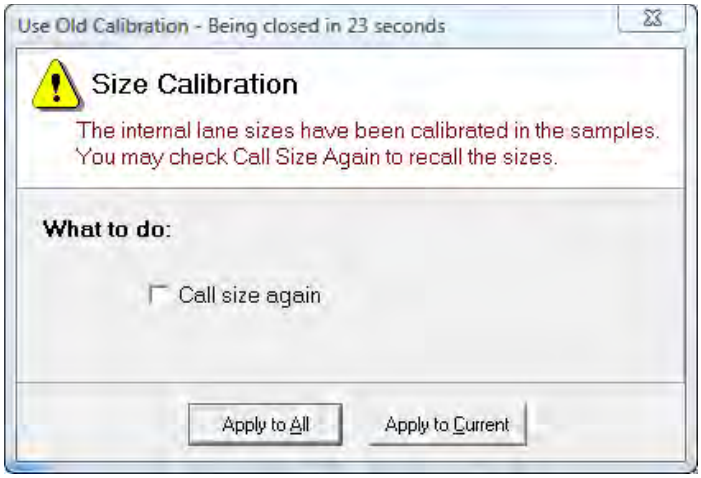
Re-Analyze with Run Wizard

To re-analyze with the Run Wizard tool, follow the next steps:

1. Select the **Run Project** icon in the main toolbar. The **Run Wizard** launches and the most recently selected parameters are displayed.
2. Adjust parameters as necessary and click **OK** in the **Run Wizard Additional Settings** dialog.

The **Use Old Calibration** dialog displays with the option to **Call Size Again**.

Figure 108 Size Calibration Dialog



Only select the **Call size again** checkbox if the Run Wizard Template Selection Size Standard selection was changed or any of the Run Wizard Data Process Raw Data Analysis parameters were changed.

3. Click **Apply to All**. The **Data Processing** dialog re-displays, and the data is re-analyzed with the new parameters.

Re-Analyze with Auto Run

To re-analyze with Auto Run, follow the next steps:

1. Select **Project** > **Options**. The **Project Options Settings** dialog displays. This dialog offers all the same parameters settings as are available in the Run Wizard.
2. Use the tabs to view the **Template Selection**, **Data Process**, and **Additional Settings** dialogs.
3. Click **OK** when finished.
4. Select **Project** > **Auto Run**. The data is re-analyzed using the new parameters.

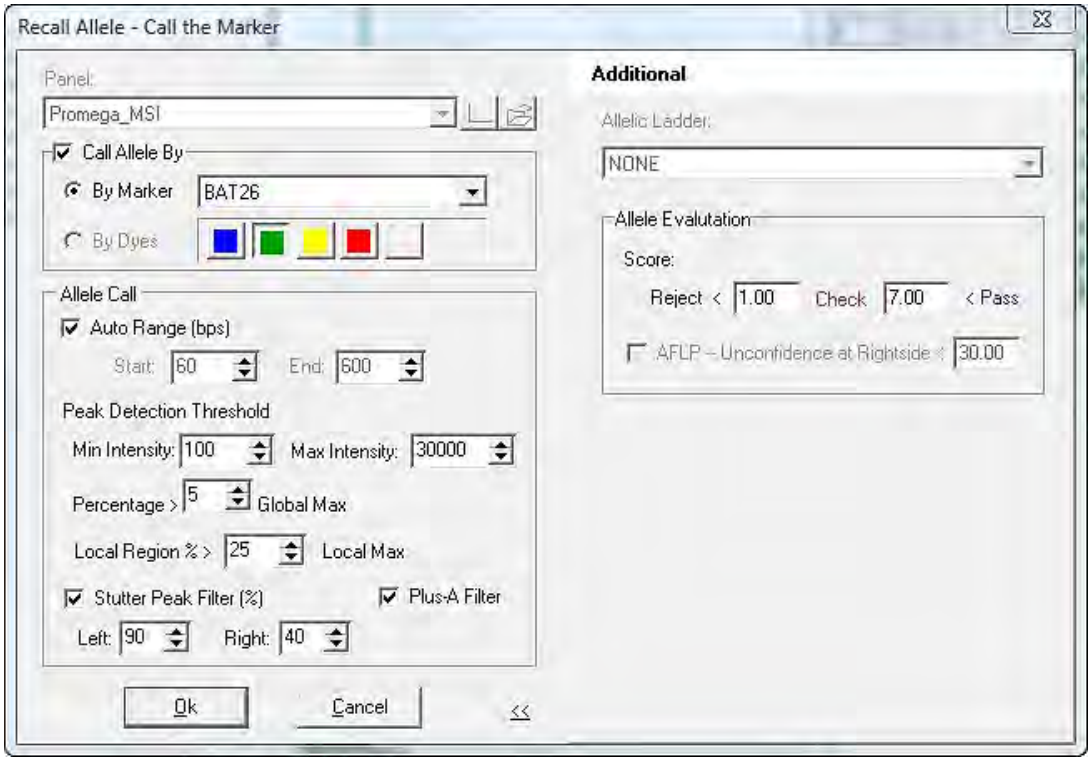
Note: The **Additional Settings Allele Evaluation Peak Score** parameters are changed in the **Project Options Settings** dialog and are applied to the data without having to re-analyze the data using the Run Wizard or Auto Run.

Re-Analyze Individual Samples

To re-analyze an individual sample, dye color, or marker, follow the next steps.

1. Select the **Call Allele** icon in the main toolbar. The arrow next to the icon opens the drop-down menu with additional options.
2. Select an option from the drop-down list and the **Recall Allele** dialog displays.

Figure 109 Recall Allele Call the Marker



3. Adjust parameters as necessary and click **OK**. The new parameters are applied.
   - **All Samples**

     This feature applies the new parameter settings to all samples in the dataset. This function is similar to Run Wizard and Auto Run.
   - **Open Samples**

     Applies the new parameter settings only to samples that are checked in the Sample File Tree.
   - **Current Sample**

     Applies the new parameter settings only to the sample highlighted in the Sample File Tree.
   - **Call the Dye**

     Applies the new parameter settings to the dye selected in the **Recall Allele** > **Call Allele by Dye** field.
   - **Call the Marker**

     Applies the new parameter settings to the marker selected in the **Recall Allele** > **Call Allele by Marker** field.

Chapter 4 Genotyping Main Analysis Overview

Main Analysis Dialog

The Main Analysis dialog of ChimerMarker has an easy to use layout, as illustrated in Figure 110. The sample files are displayed on the left, the **Synthetic Gel Image** is displayed at the top, **Electropherograms** are displayed below the gel image, and the **Report Table** is displayed on the right side column of the dialog.

To resize the frames in the dialog, follow the next steps:

1. Place the cursor over the partitions that separate the Synthetic Gel Image/Electropherogram/Sample File Tree/Report Table. The cursor changes to a two-headed arrow bisected by two vertical lines.
2. Hold down the left mouse button and drag the gray vertical line in the desired re-sizing direction.
3. To open and close the frames, use the **Show/Hide** icons in the main toolbar.

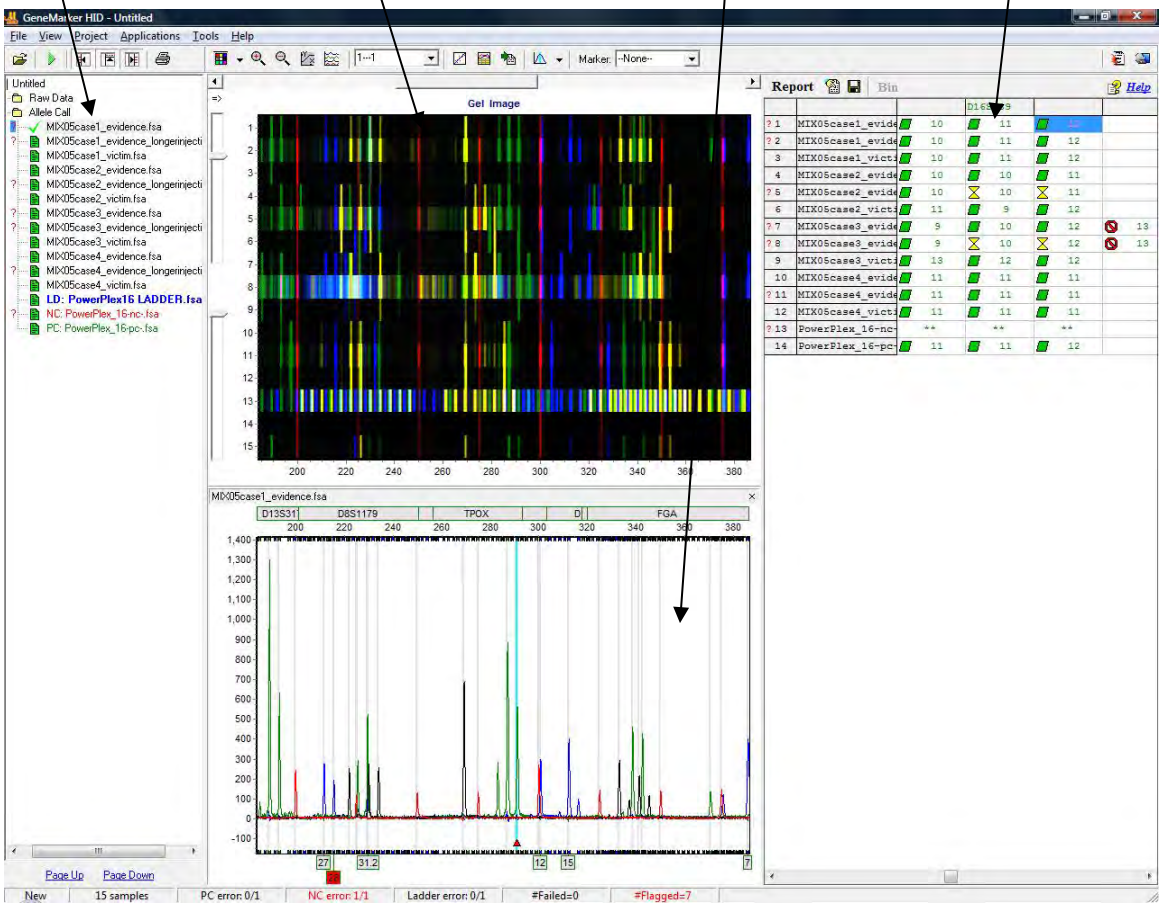
Sample File Tree

Report Table

Synthetic Gel Image

Electropherogram

Figure 110 ChimerMarker Main Analysis Dialog

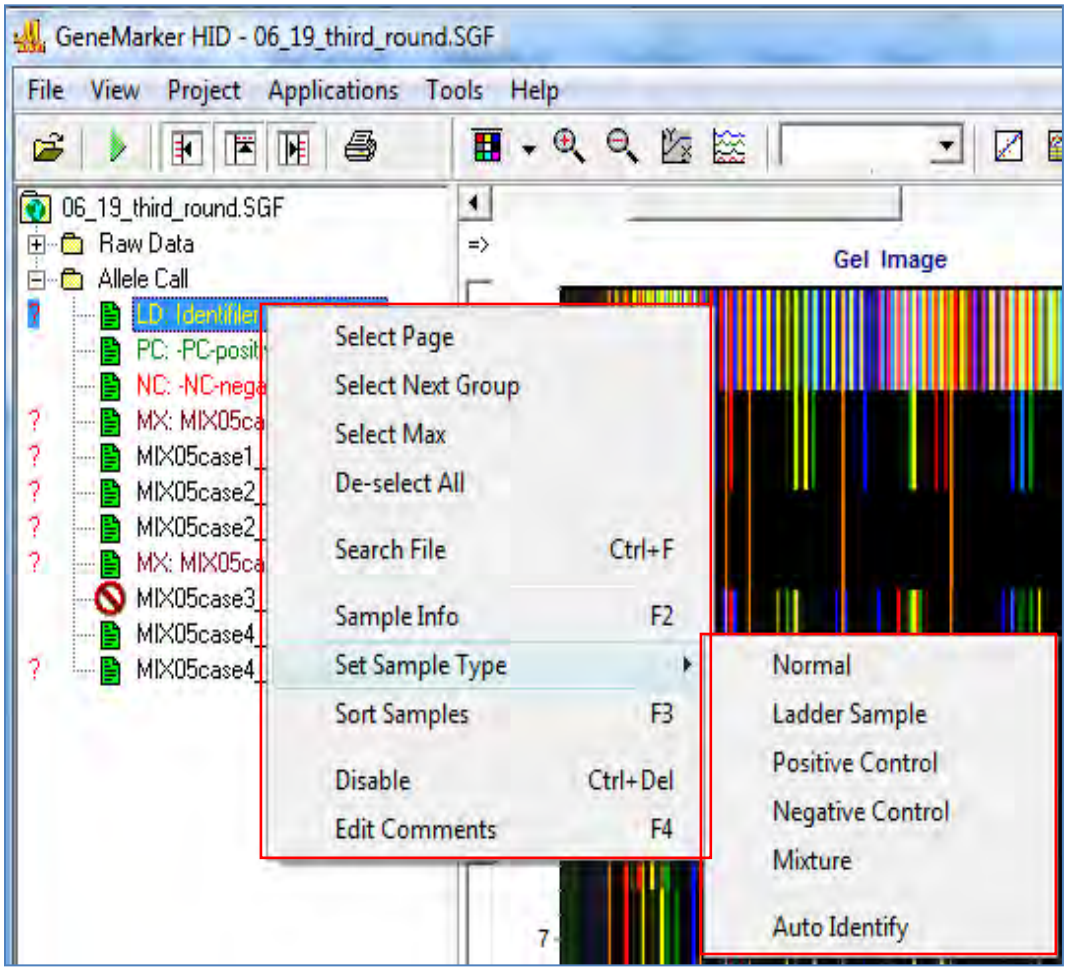


Sample File Tree

The **Sample File Tree** of the Main Analysis dialog contains two (2) folders.

- The first is the Raw Data folder, which when expanded, displays a list of all the dataset samples. When a sample is double-clicked, its preprocessed electropherogram trace displays in the Raw Data Analysis dialog. See Chapter 3 Genotyping General Procedure.
- The second folder, **Allele Call**, also contains a list of all the samples, but when the filename is double-clicked, the sample's electropherogram trace displays in the Main Analysis dialog. All sizing information and allele call filtering are applied. The Allele Call folder also flags each sample with a green sheet, yellow sheet or red strike-through indicating size calling success. See Chapter 5 Fragment Sizing Standard.

Figure 111 GeneMarker HID



Right-click the sample filename in the **Raw Data** or **Allele Call** folder to see additional options.

GeneMarker HID Options

On Figure 111, review the following options and menus on the popup menus:

- **Select Page**

  Opens electropherogram traces for the number of samples specified in the **View** > **Preference** > **Display Settings** > **Max Chart # In Page** field. **Hot Key** = **Page Up/Page Down**

- **Select Next Group**

  In descending order, selects the same number of samples previously selected by **Select Page**, grouping options (see Sample Grouping section below) or double-click the option.

- **Select Max**

  Opens electropherogram traces for the number of samples specified in the **View** > **Preference** > **Display Settings** > **Max # Open Charts**.

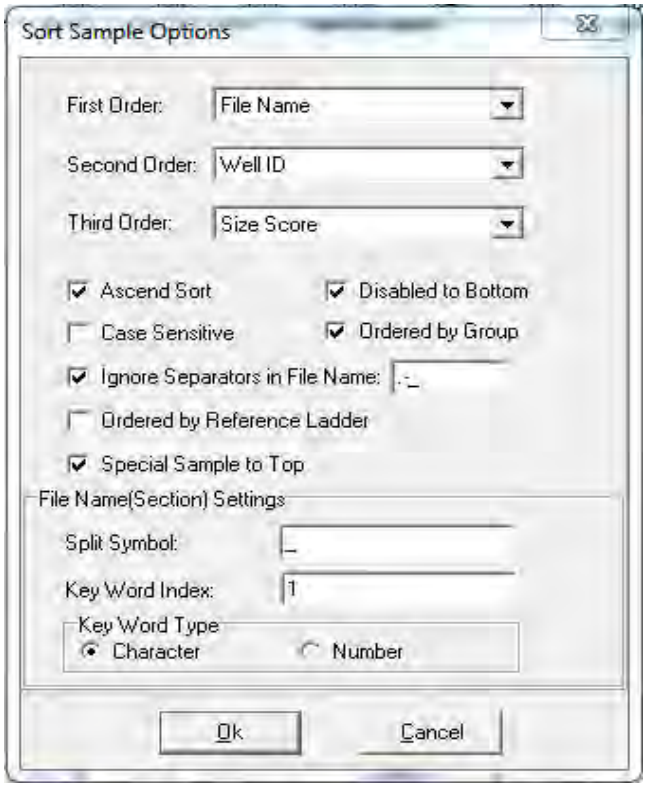
- **Deselect All**

  Unselects all selected samples in the **Sample File Tree** list and closes the electropherogram traces.

- **Sort Samples**

  Opens the **Sort Sample Options** dialog.

  Figure 112 Sort Sample Options Dialog



a. Select **First Order**, **Second Order**, and **Third Order** drop-down lists menu options including Sample Type, File Name, Lane Number, Well ID, Size Score, File Name (Section), and Run Date.

b. Specify the **Key Word Index** text field that is separated by the **Split Symbol** text field. For example, if 123_45_67 is the file name, and you want to group by "45", the key word index would be "2". Each key word index is separated by an (_) underscore. **Hot Key = F3.**

- **Search Options**

  Opens the **Search File** dialog using the popup menu from Figure 111.

  a. Enter any part of a filename to search for the sample in the list.

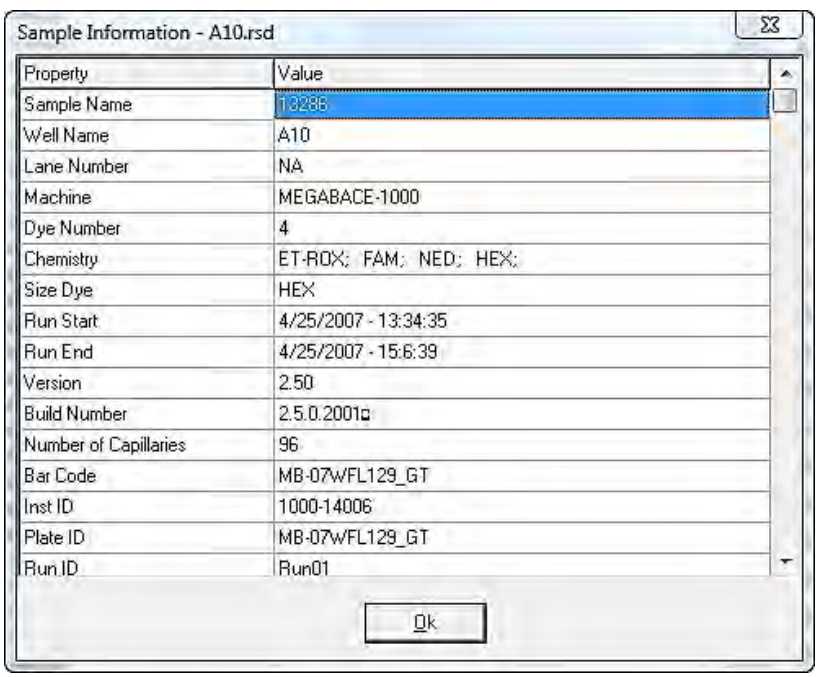
  b. Click the **Search** button.

  c. Left click and use **CTRL** or **SHIFT** key to highlight samples then click the **Open Selected** button. The electropherograms of the selected samples open in the **Main Analysis** dialog. **Hot Key = CTRL+F**

- **Sample Information**

  Opens the **Sample Information** dialog.

  Figure 113 Sample Information A10.rsd

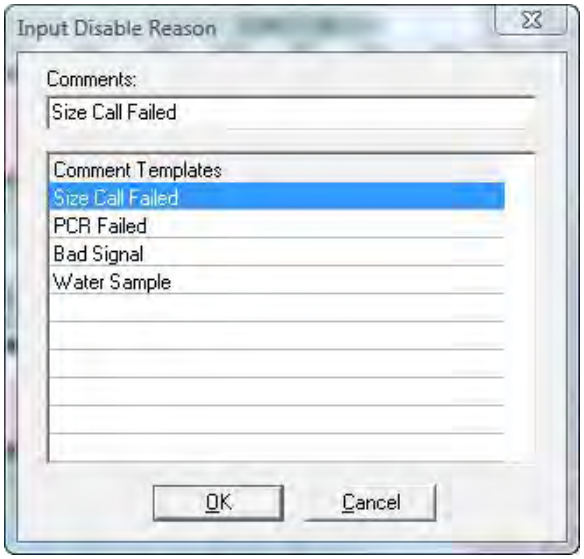


  A list of **Properties** displays in the left column. These properties include information like **Sample Name**, **Well Name**, **Lane Number**, **Machine**, and **Chemistry**. The list of properties varies depending on the file type. **Hot Key = F2**.

- **Edit Comments**

  To reach the comments field, open the **Input Disable Reason** dialog, as illustrated next and from the popup menu in Figure 111.

Figure 114 Input Disable Reason



Enter information in the **Comments** field. The last 10 comments are stored and selectable for future samples. The Sample Comments appear on the Print Report. See Chapter 7 Reports and Printing. **Hot Key = F4**

- **Disable**

  Opens the **Input Disable Reason** dialog in Figure 114 and marks the sample with a red strike-through. A disabled sample cannot be selected for display in the Main Analysis dialog and does not appear in the Report Table if the **View** > **Preference** > **Options** > **Show Disabled Samples in Report** is deselected.

  Select a **Comment Template** or enter a new comment in the **Comments** field and click **OK** to disable the sample. **Hot Key = CTRL+DEL.**

- **Add Samples**

  From the main toolbar, select **Project** > **Add Samples to Project**. The **Open Data Files** dialog displays.

  Select the **Add** button to select additional samples to add to the project and click **OK**. The added samples are sized and the allele calls are filtered according to the parameters set in the Run Wizard.

- **Sample Grouping**

  From the main toolbar, select **Project** > **Apply Sample Grouping**. The **File Name Group Editor** tool displays. See Chapter 9 Additional Tools. Follow the next steps:

  a. Select the **Group** and **Control** identifiers and click **Match**.

  b. Click **OK** to apply the matched groups. Group numbers display next to the filenames in the **Sample File Tree**.

  c. Use the **Select Next Group** right-click menu option *or* to open samples in a group, select **CTRL+PageUp/Down**. To disable the **Sample Grouping** feature, go to **View** > **Preference** > **Others** and uncheck **Enable Sample Grouping**.

Synthetic Gel Image and Electropherogram with Peak Table

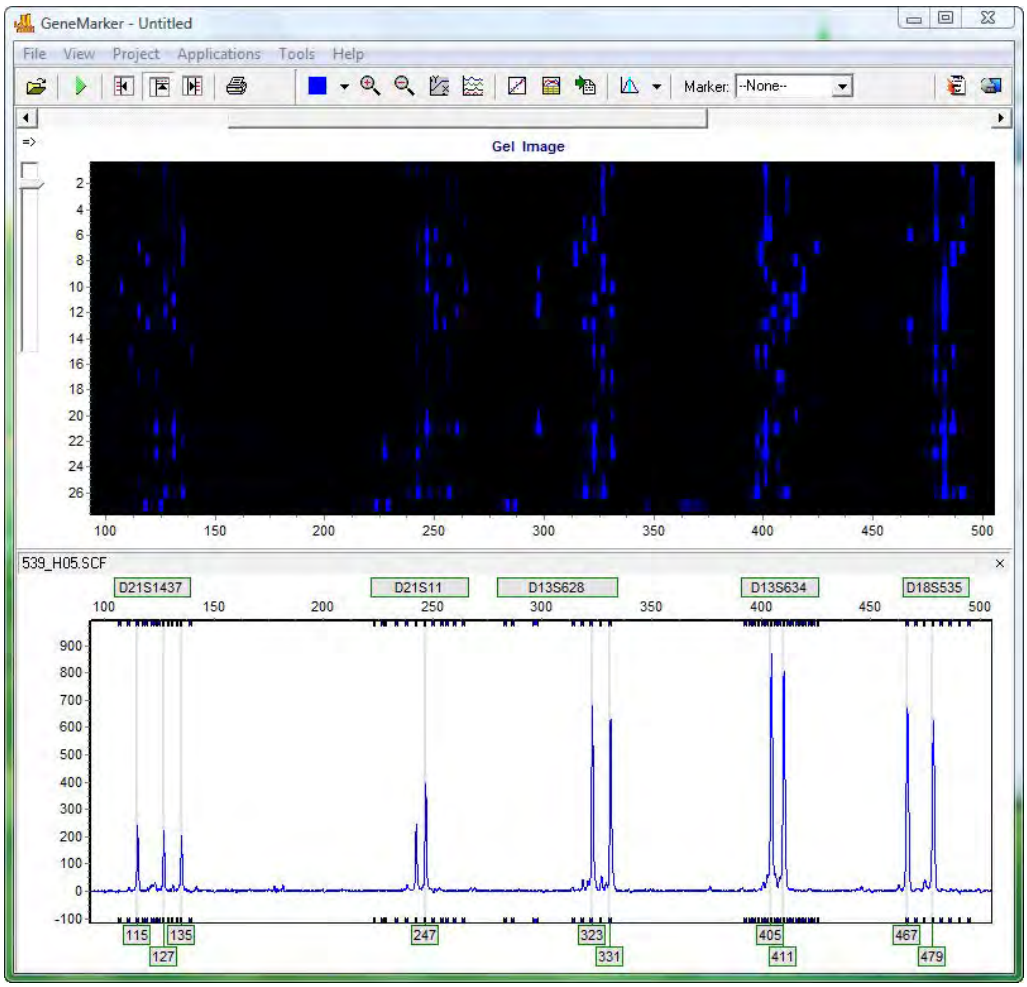
The Synthetic Gel Image and Electropherogram displays are associated in the Main Analysis dialog, as illustrated in Figure 110. Both display the fragment information in a visual form. When ChimerMarker is initially launched, all dye colors are displayed at once in the Synthetic Gel Image and Electropherogram. Single left-click the **Show Color** icon in the main toolbar to cycle through the dye colors or use the **Show Color** drop-down menu to disable individual colors or **Show/Hide All** colors.

Navigation

- **Zoom In/Out**

  In the Synthetic Gel Image or the Electropherogram, hold down the left mouse button and drag a dialog from upper left to lower right around the area to zoom in on. To zoom back out, hold down the left mouse button and drag a dialog in the opposite direction from lower right to upper left. The user may also use the **Zoom** icons in the main toolbar to zoom in and out. The Main Analysis dialog also allows the user to manually set the x and y axis with the **Set Axis** icon.

  Figure 115 GeneMarker Dialog



- **Horizontal Movement**

  The Synthetic Gel Image and the Electropherogram are synchronized to allow the user to view both images at once. To move the images in the horizontal direction, use the top slider bar (below the toolbar) to scroll the image in either direction, or hold down the right mouse button and drag the trace right or left.

- **Marker/Locus Specific Viewing**

  To scroll through individual markers/loci, select a marker from the **Marker** drop-down list in the main toolbar. To view subsequent markers, use the **Up/Down Arrow** keys.

- **Synthetic Gel Image Features**

  The Synthetic Gel Image displays all samples in the dataset vertically. The direction of fragment mobility is horizontal with the small size fragments on the left and the larger fragments on the right, so that the gel aligns with the electropherogram trace display. Move the mouse pointer over the Synthetic Gel Image to reveal the sample lane filename.

- **Image Utilities**

  Click the **Image Utilities** icon in the upper left corner of the Synthetic Gel Image. A fly-out menu appears with the following options:

  - Copy to clipboard.

    Copies the Synthetic Gel Image to the Windows clipboard for pasting into other applications such as Microsoft PowerPoint.

  - Save image.

    Allows the user to save the Synthetic Gel Image as a BMP image file.

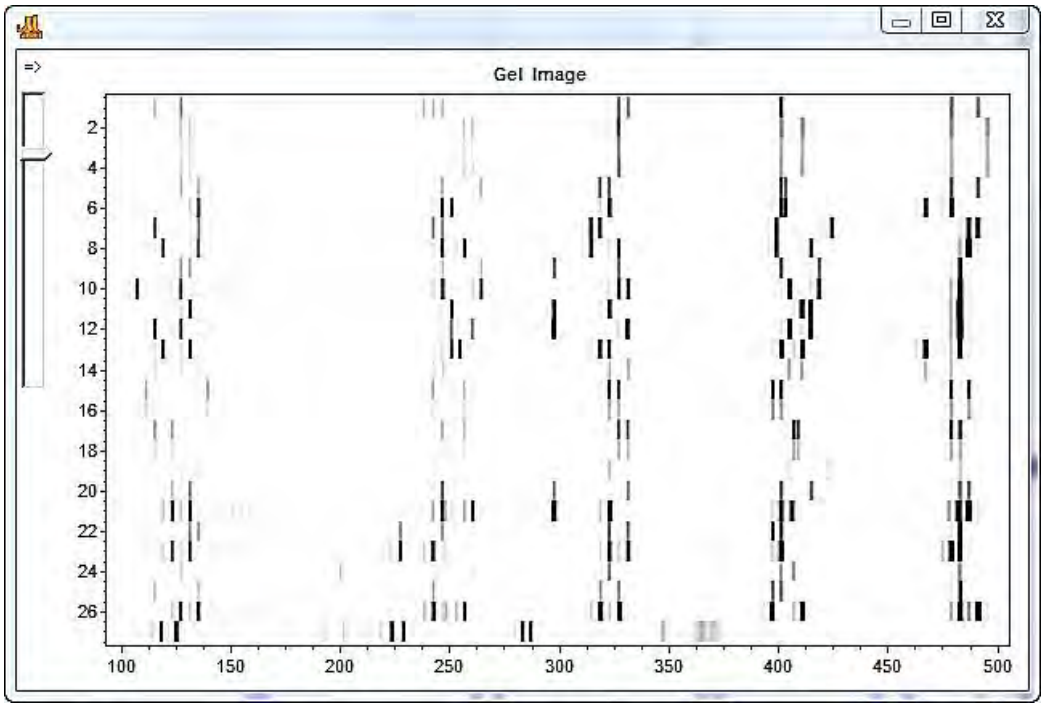
  - Show in dialog.

    Opens a separate window containing the Synthetic Gel Image. Maximize the separate window for closer gel image inspection.

- **Image Display Intensity**

  Move the intensity slide bar, located in the upper left corner of the Synthetic Gel Image, up and down to adjust the intensity of the fragments displayed.

  Figure 116 Gel Image



- **Greyscale**

  Go to **View** > **Preference** > **Display Settings** > **Gel Image**.

  - When only a single dye color is selected, select **Gray for Single Dye** to change the single dye Synthetic Gel Image to black and white.
  - When multiple dye colors are selected the fragments display in their respective colors. Click the **Background in White** option to reverse the black and white exposure for single dye color gel images.

Electropherogram and Peak Table Features

The Electropherogram displays fluorescent signal intensities from capillary electrophoresis instruments as a single line trace for each dye color. The signal intensities are recorded in Relative Fluorescent Units (RFUs) which are plotted along the y-axis.

Along the x-axis are the base pair sizes of the fragments. The frame units plotted along the x-axis in the original Raw Data Analysis dialog are converted to base pair size units as defined by the Size Standard selected and the Internal Lane Standard (ILS) of the individual samples. Fragment mobility is from right to left with the smallest size fragments on the far left of the trace.

The Peak Table contains information about the called peaks currently displayed in the Electropherogram.

Electropherogram Trace Display Range

The base pair size range (x-axis) is as set in the **Run Wizard Data Process Allele Call** options dialog. The RFU range (y-axis) is variable and re-adjusts according to the maximum peak height in the trace. To manually set x and y-axis ranges, use the **Set Axis** icon in the main toolbar.

- **Cursor Locator**

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

- **Allele Call**

  If a Panel is applied to the data, then grey horizontal bar Markers display above the electropherogram indicating locus ranges. Bin ranges appear as dye-colored brackets above and below the sample trace. Allele Labels display below the electropherogram and are associated with the center of each called peak, which is also marked by a light grey vertical line in the electropherogram. If a Panel is not applied, then Allele Labels for called peaks only indicate the base pair size of the peak.

- **Peak Table**

  The Peak Table is displayed below the Electropherogram.

Figure 117 GeneMarker HID

Display the Peak Table by selecting the **Show Chart/Table** icon in the main toolbar. Right-click in the **Peak Table** and select **Show Columns**. The **Show Columns** fly-out displays with column options.

- **Dye**

  Indicates the dye color of the peak.

- **Size**

  Indicates the base pair size of the peak (x-axis).

- **Height**

  Indicates the peak height in RFUs (y-axis).

- **Height Ratio**

  The value obtained when the peak's height is divided by the height of the highest peak in the dye color or Marker.

- **Area**

  Indicates the area under the curve of the peak. The area calculation begins and ends along the x-axis as indicated by the Start and End columns of the Peak Table respectively.

- **Area Ratio**

  The value obtained when the peak's area is divided by the area of the highest peak in the dye color or Marker.

- **Marker (Panel Only)**

  Indicates which Marker (Locus) the peak is contained in.

- **Allele (Panel Only)**

  Indicates which *Bin* the peak is contained in.

- **Difference (Panel Only)**

  Indicates the absolute value in base pairs of how far the peak center is from the *Bin* center.

- **Quality (Panel Only)**

  Assigns a Pass/Check/Undetermined quality ranking for each peak with regard to the peak score as set in the Run Wizard Additional Settings dialog and/or software editing of the original raw data, such as correction of saturated peaks (SAT Repaired). See Chapter 3 Genotyping General Procedure**.**

- **Score**

  The peak quality score is calculated based on signal-to-noise ratio and peak shape or morphology. Lower scores indicate poorer quality peaks. Additionally, the *Score* value is a based on an exponential curve.

- **Start/End**

  Indicate the beginning and end of the *Area* calculation for the peak.

- **Comments**

  Software and user edited comments appear in the Comments column.

- **Quality Reasons**

  Indicates the reason why a peak received a Quality rank of Check or Undetermined. For explanation of the two (2) and three (3) letter codes see below or click the **Help** icon above the Report Table.

  - **LS = Low Score**

    Quality Score is based on Signal-to-Noise Ratio and Peak Morphology and the Pass, Check, reject ranges are set in the **Run Wizard Additional Settings** dialog.

  - **OL = Off Ladder**

    Peak is outside of the marker range.

  - **OB = Out of Bin**

    Peak is within the marker range but outside of a bin.

  - **BC = Bin Conflict**

    More than one called peak present within a bin.

  - **SR = Saturated (Repaired)**

    Intense peaks with characteristic morphology are identified and “repaired” for allele calling.

  - **SP = Saturated (Pull- up)**

    Intense peaks may cause “pull-up” or additional peaks to appear in other dye colors.

  - **PL = Beyond Ploidy**

    When the number of peaks identified within a marker exceeds the maximum number of peaks expected as set in the **Panel Editor Edit Panel** dialog.

  - **LO = Low Intensity**

    Single peak called below the Minimum Homozygote Intensity threshold because a second peak was detected above N-x percentage value as set in the **Panel Editor Edit Marker** dialog.

  - **HI = High Intensity**

    Peak intensity approaches and/or exceeds the maximum peak intensity filter as set in the Run Wizard Data Process dialog.

  - **IMB = Heterozygote Imbalance**

    Peak intensity does not exceed the minimum percentage of the major peak within the marker as set in the Panel Editor Edit Marker dialog.

  - **IHE = Inconclusive Heterozygous**

    Peak intensity is within the heterozygous inconclusive range set for this locus in the **Panel Editor Edit Panel** dialog.

- **IHO = Inconclusive Homozygous**

  Peak intensity is within the homozygous inconclusive range set for this locus in the **Panel Editor Edit Panel** dialog. **Save Peak Table**.

  Click the **Save Peak Table** icon in the main toolbar to export the Peak Table information currently displayed in Excel (.xls) or a tab-delimited Text (.txt) format. All samples peak information for only the dye colors selected are exported in the table.

  Additionally, the user can right-click in the Peak Table and select **Copy Table** (**Hot Key = CTRL+C**) to place the current table information onto the Windows clipboard. The information can then be pasted into most common spreadsheet or word processing programs including Microsoft Excel.

- **Editing Peaks**

  Double-click the vertical grey peak center bar to select a peak. Right-click anywhere in the Electropherogram or Peak Table to see additional menu options.

- **Insert Allele**

  Right-click at the place in the electropherogram where you want to place an allele and select **Insert Allele**. The base pair size or bin name is applied in the Allele Label and the peak specifications are calculated and displayed in the Peak Table.

- **Delete/Undelete Allele**

  Right-click at the vertical grey bar indicating the center of the called peak or the peak cell in the Peak Table and select **Delete** (**Hot Key = DEL**). To call the allele again, right-click the peak and select **Undelete** (**Hot Key = SHIFT+DEL**).

- **Confirm/Un-confirm Allele**

  If a peak is given a low quality score, it receives a *Check* (yellow) or Undetermined (red) quality ranking. To give the peak a Pass (green) quality ranking, right-click the peak center bar and select **Confirm** (**Hot Key = CTRL+M**). The peak is marked Pass (green) and receives a **Confirmed** comment in the Peak Table. To un-confirm the allele, select **Unconfirm** from the right-click menu (**Hot Key = CTRL+ALT+M**).

- **Confirm/Un-confirm All**

  The Confirm All and Un-confirm All options perform the same actions as the Confirm/Un-confirm allele except that the Quality ranking for all peaks in that dye color for that sample is affected.

- **Edit Allele**

  Right-click an allele in the Electropherogram or Peak Table and select **Edit Allele**.

The **Edit Allele** dialog displays.

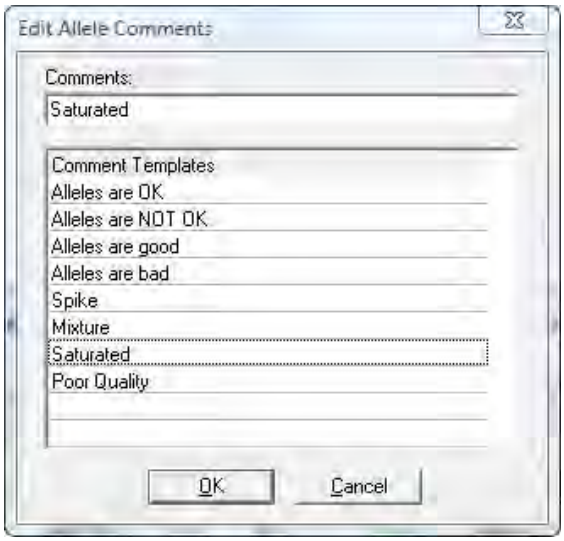
Figure 118 Edit Allele

Add or change the values in the Allele and/or Size fields. The Allele field is blank if no Panel is applied to the dataset. Check **Confirm the Allele** to automatically give the peak a Quality rank of **Pass** (green).

- **Allele Comments**

  Right-click an allele in the Electropherogram or Peak Table and select **Edit Comments**. The **Edit Allele Comments** dialog displays.

  Figure 119 Edit Allele Comments Dialog



Select a comment from the **Comments Template** list or enter a new comment in the **Comments** field. Click **OK** and the comment displays in the **Comments** column of the Peak Table. Only one (1) user-edited comment can be added to a peak. Comments automatically generated by the software cannot be removed. Additional user comments are added next to the software comment.

- **View History**

  Opens the **Show Edit History** dialog. Shows a record of all manual edits performed on the peak. The Show Edit History dialog is only active when the **Help** > **User Management** > **Settings** > **Record Data Edit History** option is selected. See Chapter 10 User Management**.**

Report Table

The Main Analysis dialog, Report Table contains additional information about sample peaks. See Chapter 7 Reports and Printing**.**

Navigation

The Report Table is linked to the other frames in the Main Analysis dialog.

1. Double-click on the desired allele or use the **Arrow keys** to move to the cell of interest and hit **Enter** key or use **Alt** + **Arrow keys** to move to different cells and zoom in on the peak in the Electropherogram.
2. Select multiple cells by holding down **SHIFT** key or hold down the left mouse button and drag over the desired cells.

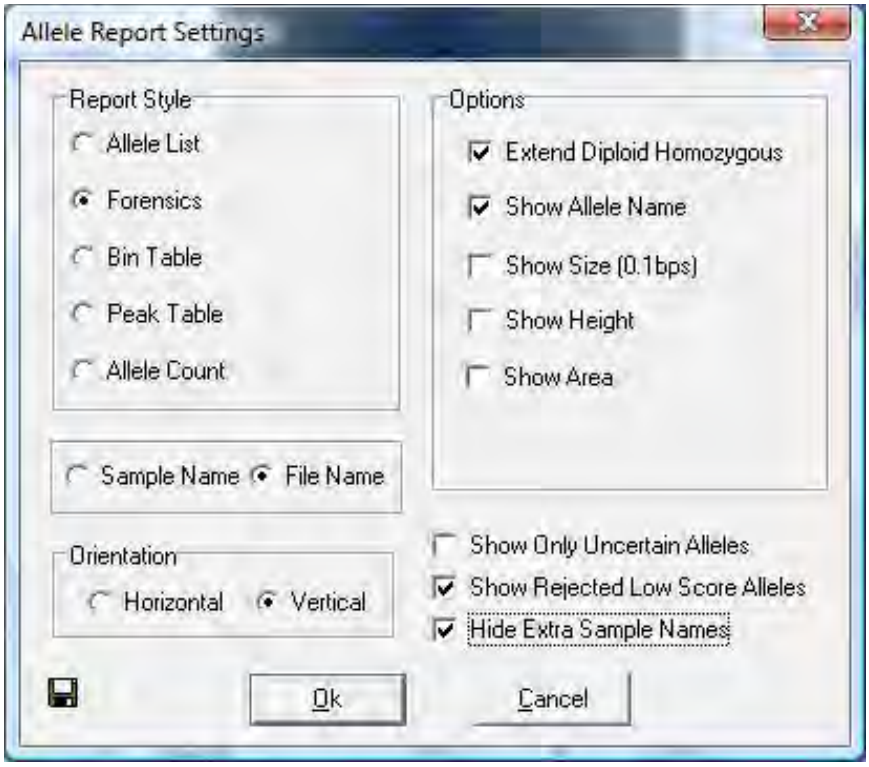
The rules by which the Report Table and other frames in the Main Analysis dialog are linked are controlled by options in the **View** > **Preferences** > **Others** tab.

Display Settings

Click the **Report Settings** icon in the Report Table toolbar.

The **Allele Report Settings** dialog displays.

Figure 120 Allele Report Settings Dialog



Select different Report Styles to see additional options. After selecting options from the **Report Style** pane, click the **Save as Default** icon in the bottom left corner.

Your options are saved and are recalled the next time that Report Style is selected. Additionally, select **View** > **Preferences** > **Others** > **Show Disabled Samples in Report** to include samples that are disabled in the Sample File Tree.

Sort Options

- **Sort by Marker**

  Select **Sort by Marker** from the right-click menu and choose from the fly-out menu to sort **Ascending** or **Descending**. If Ascending is chosen, then low-quality peaks are sorted to the top of the table. If Descending is chosen, then the lower quality peaks are placed at the bottom of the table. This option is only available with Marker Table and Allele Count Report Styles.

- **Sort by Column**

  Select **Sort by Column** from the right-click menu and choose from the fly-out menu to sort **Ascending** or **Descending**. If Ascending is chosen, then lesser values are sorted to the top of the table and greater values to the bottom the table and vice versa if Descending is chosen. This option is available with all Report Styles.

- **Editing Peaks**

  To edit peaks, first left single or double-click the cell in the Report Table then right-click to see menu options or use **Hot Keys**.

- **Delete Peaks**

  Right-click the peak cell in the Report Table and select **Delete Peaks** (**Hot Key = DEL**). The deleted peak is removed from the Report Table.

- **Confirm Peaks**

  If a peak is given a low quality score, it receives a Check (yellow) or Undetermined (red) quality ranking. To give the peak a Pass (green) quality ranking, right-click the peak cell and select **Confirm Peaks** (**Hot Key = CTRL+M**). The peak is marked Pass (green).

- **Save Report Table**

  To save all information currently displayed in the Report Table, click the **Save Report** icon in the Report Table toolbar. Choose a directory, enter a filename (ProjectName_AlleleReport is the default) and save as an Excel (.xls) or tab-delimited Text (.txt) file.

  To export only selected cells in the report table, follow the next steps:

  a. Select the cells by left mouse drag across the cell range or hold **SHIFT** key and select cells.

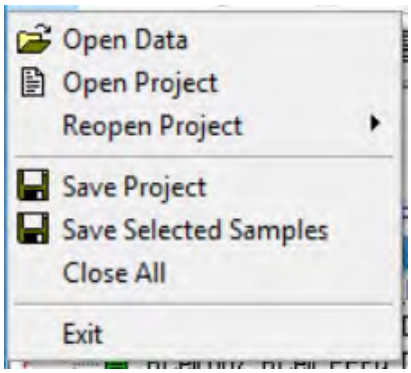
  b. Right-click on the highlighted cells and select **Copy** (**Hot Key = CTRL+C**).

     The information is saved to the Windows clipboard and can be pasted into any common word processor or spreadsheet program like Microsoft Excel. The row and column headers for those cells are copied with the highlighted cell information.

Menu Options

The following menu options are found in the menu bar of the Main Analysis dialog.

Figure 121 Menu Options



File Menu

The File menu contains functions for opening and saving raw and processed data.

- **Open Data**

  Launches the Open Data Files dialog where the user selects raw data files for upload into ChimerMarker. Accepted file formats include .fsa, .ab1, .abi, .scf, .rsd, .esd, .smd, .smr. See Chapter 3 Genotyping General Procedure**.**

- **Open Project**

  Opens a folder search dialog where the user can locate previously saved SoftGenetics ChimerMarker project files (.sgf, .sfp).

- **Re-Open Project**

  Saves the last four (4) projects that were opened by ChimerMarker and allows the user to launch any one of those four (4) projects directly.

- **Save Project**

  Saves a SoftGenetics ChimerMarker project (.sgf, .sfp) to a specified directory. Raw data files and analyzed data files with edits are saved within a project file.

- **Save Selected Samples**

  Saves a sub-project containing the selected samples in the project. This is helpful when starting next year's projects for long term monitoring cases.

- **Close All**

  Closes a project without exiting the program.

  Note: It is recommended to select **Close All** before exiting the program.

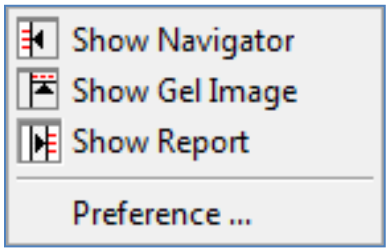
- **Exit**

  Closes the ChimerMarker program.

View Menu

The View menu contains options that determine how the data is displayed in the Main Analysis dialog.

Figure 122 View Menu



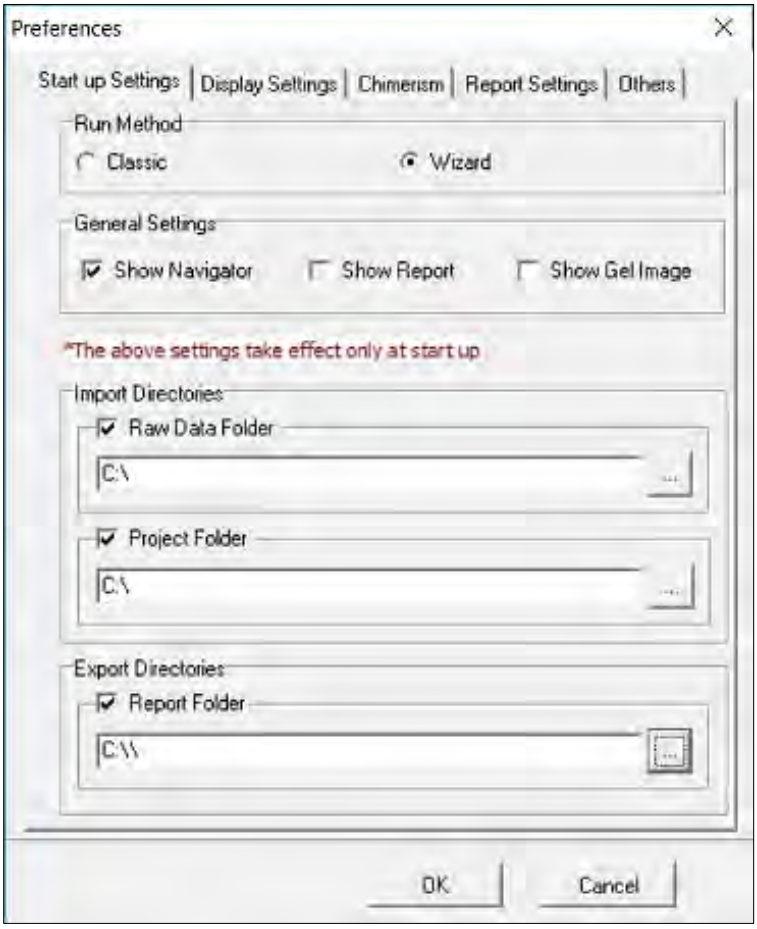
- **Show Navigator/ Gel Image/ Report**

  Toggles the Sample File Tree, Synthetic Gel Image, or Report Table frames open and closed in the Main Analysis dialog.

- **Preference**

  Activates the three-tab **Preferences** dialog, as illustrated next.

  Figure 123 Preferences Dialog



- **Start-up Settings**

  The **Start-up Settings** tab, illustrated on Figure 123, is effective only at start up, allows selection of either the **Run Method** pane or **General Settings** pane.

- **Run Method**
  - **Classic**: Appropriate for experienced users. The user moves through the program data input, settings, and display options without prompting, by simply following the program's sequential analysis flow.
  - **Wizard**: Activates the Run Wizard, which guides the user through the program's operation. This setting is best for the inexperienced user.

- **General Settings**

  Show Navigator*:* When selected, the Sample File Tree is automatically displayed in the Main Analysis dialog after data processing.

  - **Show Report**: When selected, the Report Table automatically is displayed in the Main Analysis dialog after data processing.
  - **Show Gel Image**: When selected, the Synthetic Gel Image is automatically displayed in the Main Analysis dialog after data processing.

- **Import Directories**
  - **Raw Data Folder**: Input the path to the directory the program opens to when importing files.
  - **Project Folder**: Input the path to the directory the program opens to when importing projects.

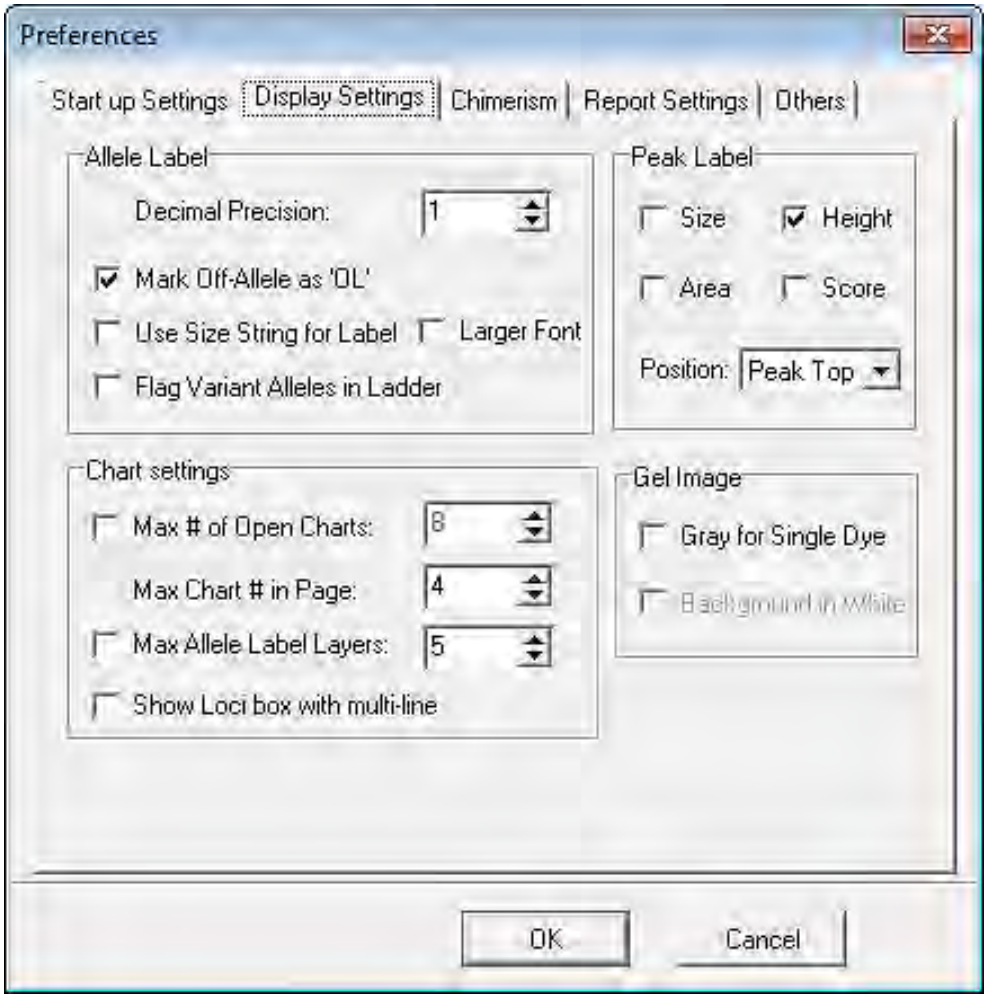
- **Export Directories**

  The Report Folder inputs the path to the directory the program saves reports to.

Display Settings Tab in Preferences

The **Display Settings** tab is used to set how the data is displayed in the electropherograms.

Figure 124 Preferences Display Settings Tab



- **Allele Label pane**
  - **Decimal Precision**: Select 0 to 2 decimal places for peak size labeling.
  - **Mark Off-Allele as 'OL'**: Select this option to label alleles that are outside of allele ranges as 'OL'.
  - **Use Size String for Label**: Select this option to label peaks in the electropherograms according to size instead of the allele label. To display a rounded size string, set the **Decimal Precision** to 0.
  - **Larger Font**: Doubles the font size of the allele label characters. Once selected, this increased font size is used in the Print Report.
  - **Flag Variant Alleles in Ladder**: Variant alleles called in the ladder sample are highlighted with a vertical yellow bar.

- **Chart Settings pane**
  - **Max # of Open Charts**: Select the maximum number of samples you would like to display as an electropherogram at one time (Max = 96). Use the Sample File Tree right-click option **Select Max** to open the number of samples specified.
  - **Max Chart # in Page**: Select the maximum number of sample electropherograms to display in the Main Analysis dialog at one time (Max = 8). Use the **Sample File Tree PageUp/Down** option to select subsequent groups of samples.
  - **Max Allele Label Layers**: Select the number of allele label layers to view at once (Max = 10). This determines how far to zoom in to clearly read neighboring allele labels and affects how the print report is displayed.
  - **Show Loci with multiline**: Select this option to display the names of all markers above the electropherogram when two (2) or more dyes are displayed.
- **Peak Label**

  Choose up to four labels (size, height, area, and score) to display as a flag next to individual peaks in the electropherogram.

  Choose to place the peak label at either the top of the peak, to the right side of the peak, or in the allele label in the electropherogram.
- **Gel Image**
  - **Gray for Single Dye**: When selected will display and print the gel image with a black background and white bands. When deselected the gel image will display a black background and colored bands (depending on dye color chosen to view).
  - **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**: Only available when Gray for Single Dye is selected. Will invert the gel image so that the background is white, and the band fragments is black.

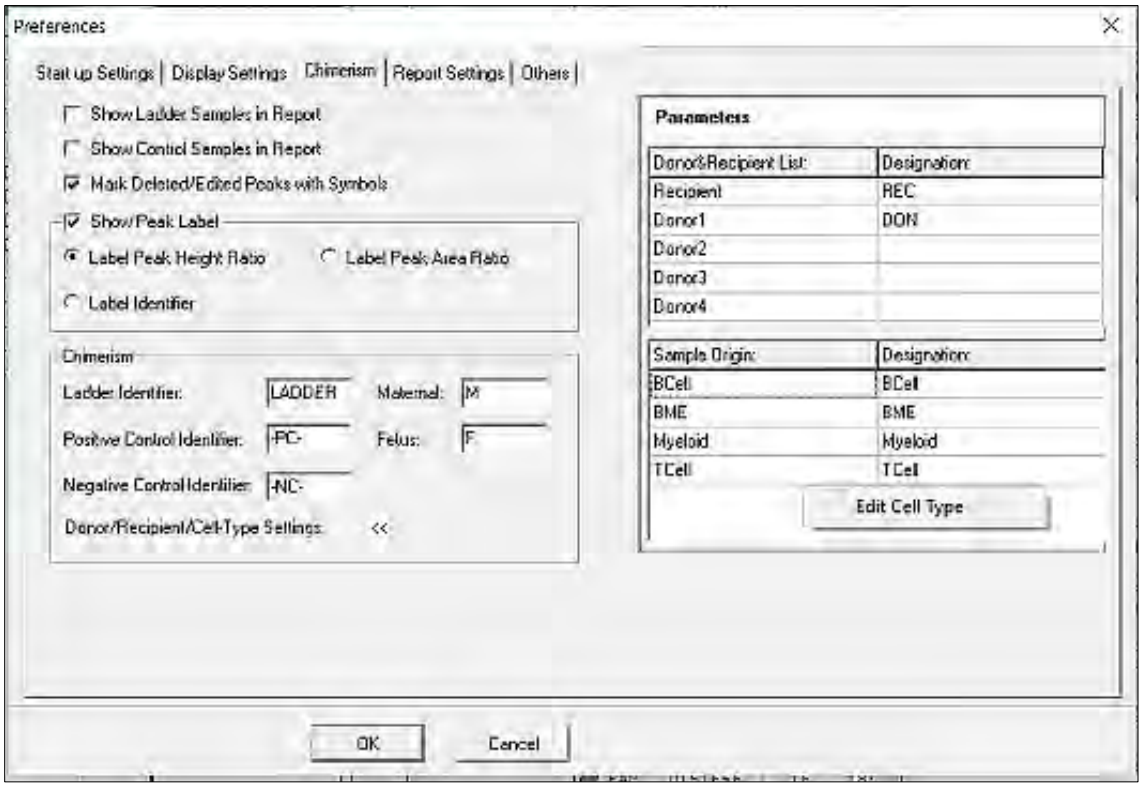
Chimerism Analysis Preferences, Multi-Lineage Analysis

The program preferences include labeling peak ratios by height or area; with multi-lineage capabilities for chimerism analysis of T-cells, B-cells, and other cell type populations. By doing multi-lineage assessment, valuable information about the current graft status of the host is seen.

In addition, there are functions for comparison of samples at different time points to conduct longitudinal studies and produce a comprehensive graph. Case specific panels are automatically constructed, using Donor and Recipient allele calls.

Select the desired Peak Ratio method – Peak Height or Area.

Figure 125 Chimerism Tab in Preferences



Enter the file identifiers used by your laboratory. The program uses these portions in the file name to differentiate between allelic ladder, **Positive Control Identifiers** and **Negative Control Identifier**, and **Donor/Recipient/Cell-Type Settings** (tissue type).

For example, if there is a sample from the recipient from BCell, a second sample from the recipient from BME and a third sample from the recipient from TCell the file names should include the use of these designators:

1. RECIPIENT_BCELL_.
2. RECIPIENT_BME_.
3. RECIPIENT_TCELL_.

The identifier may be any combination of letters and numbers (for example, in place of RECIPIENT labs may wish to type in the recipient's ID number or name. Edit the Sample Origin and Identifiers by right-clicking in the list and select **Edit Cell Type**. A text editor dialog displays where the Identifiers are entered.

- **Show Ladder Samples in Report**

  When selected, the samples designated as Ladders by the Ladder Identifier field will appear in the Report Table.

- **Show Control Samples in Report**

  When selected, the samples designated as Positive and Negative Controls by the Positive/Negative Control Identifier fields display in the Report Table.

- **Mark Deleted/Edited Peaks with Symbols**

  When selected samples that are deleted are marked with a **X** at the top of the peak. Samples that were edited are marked with an **E** at the top of the peak.

- **Label Peak Ratio**

  Select from displaying peak ratio from height or area in peak flags on the electropherogram.

- **Ladder Identifier**

  Enter common filename nomenclature for Ladder samples in the dataset (must be in all capital letters). Upon first analysis, ChimerMarker automatically scans the dataset filenames for the Ladder Identifier values and subsequently labels the Ladder samples with an **LD:** and displays the sample filename in blue font in the Sample File Tree. The default is **LADDER**.

  Note: The Ladder Identifier option affects the operation of the Auto Select Best Ladder and Automatic Panel Adjustment features in the Run Wizard Additional Settings dialog. After modifying the Ladder Identifier field, re-activate Run Wizard and proceed through Data Process. The Auto Select Best Ladder and Automatic Panel Adjustment features may now be selected.

- **Positive Control Identifier**

  Enter common filename nomenclature for Positive Control samples in the dataset (must be in all capital letters). Upon first analysis, ChimerMarker automatically scans the dataset filenames for the Positive Control Identifier values and subsequently labels the Positive Control samples with a **PC:** and displays the sample filename in green font in the Sample File Tree. The default is **-PC-**.

- **Negative Control**

  Enter common filename nomenclature for Negative Control samples in the dataset (must be in all capital letters). Upon first analysis, ChimerMarker automatically scans the dataset filenames for the Negative Control Identifier values and subsequently labels the Negative Control samples with an **NC:** and displays the sample filename in red font in the Sample File Tree. The default is **-NC-**.

  Note: To implement a change in the Identifier fields, right-click any sample in the Sample File Tree and select **Set Sample Type Auto Identify**.

- **Maternal**

  Enter common filename nomenclature for Maternal samples in the dataset (must be in all capital letters). ChimerMarker automatically scans the dataset filenames for Maternal identifier values and groups the samples accordingly in the MCC application.

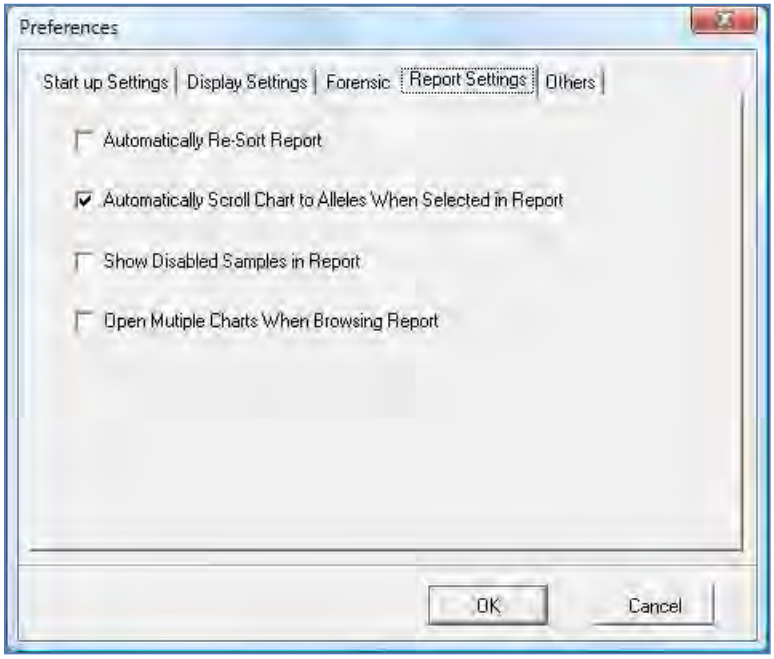
- **Fetus**

  Enter common filename nomenclature for Fetus samples in the dataset (must be in all capital letters). ChimerMarker automatically scans the dataset filenames for the Fetus identifier values and groups the samples accordingly in the MCC application.

Report Settings Tab in Preferences

The **Report Settings** tab allows users to select how data is displayed in the **Report Table**.

Figure 126 Preferences Report Settings Tab



The following settings are available In the **Report Settings** tab:

- **Automatically Re-Sort Report**

  Select this option to automatically re-sort the ChimerMarker report every time alleles are modified. Un-select this feature to leave the report sorted until you choose to re-sort.

- **Automatically Scroll Charts to Alleles**

  When Selected in Report*:* choose whether to scroll to alleles in the trace when selecting the allele in the report. Leave this feature selected to have the software automatically call up alleles in the trace when they are double-clicked in the report.

- **Show Disabled Samples in Report**

  ChimerMarker identifies samples that failed during electrophoresis or size calling. The default setting of unselected excludes the disabled samples from the report. Select the option to have failed or user-disabled samples identified in the report.

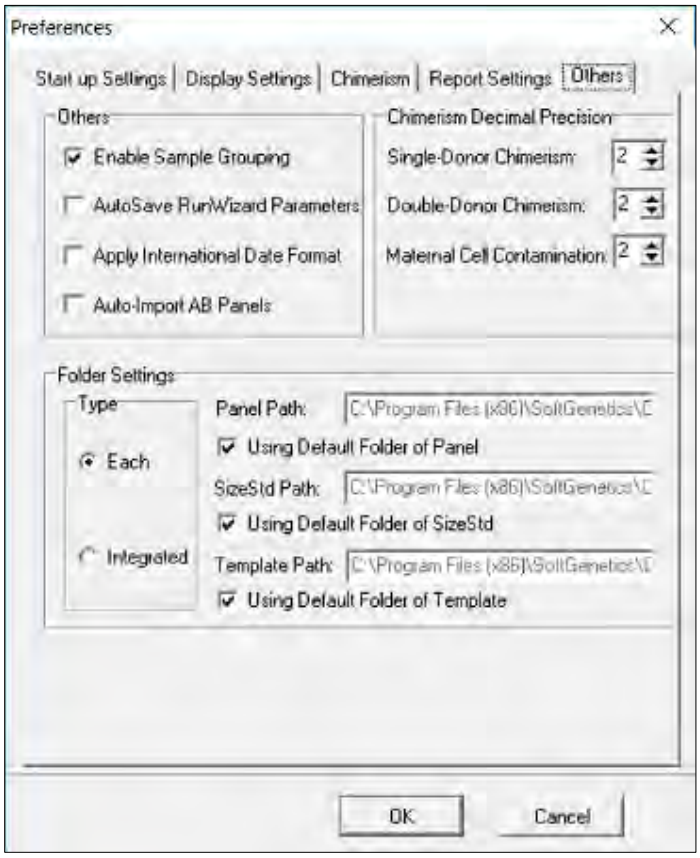
- **Open Multiple Charts When Browsing Report**

  Double clicking an entry in the allele report table opens a new electropherogram for each new sample selected.

Others Tab in Preferences

The **Others** tab has additional selections for the project.

Figure 127 Others Tab in Preferences



- **Enable Sample Grouping**

  Select this option to activate Apply Sample Grouping. De-select **Enable Sample Grouping** to inactivate the Apply Sample Grouping option. The Apply Sample Grouping information is saved. Recall by re-selecting **Enable Sample Grouping**. See Chapter 9 section Filename Group Editor.

- **Automatically Save Run Wizard Parameters**

  Automatically saves the Run Wizard parameters in an .ini file when the project is saved. The location is the same location as the saved project and the name of this file is the name given to the **SaveProjectName_RunWizardParameters.ini**.

- **Apply International Date Format**

  Check this option to configure the dates of the Reports for Applications in the International format day/month/year.

- **Decimal Precision**

  Input the desired decimal precision for each application (0 = integer, 1 = 1 decimal, 2 = 2 decimals, and so on.).

- **Folder Settings**

  By default, ChimerMarker imports panels, size standards, and templates from specific folders installed with the program. If the standard installation location is used, these folders are found in `C:\Program Files (x86)\SoftGenetics\ChimerMarker\V3.1.5\`. When at the Run Wizard, the selection of panels, size standards, and templates available from the dropdown menus is directly pulled from these folders by default.

  The folder settings tool gives the user the option to change these default locations. In other words, ChimerMarker can automatically import panels, size standards, and/or templates from an alternative location. This is particularly useful for users with a network setup and with files stored at a shared location. The folder settings tool gives the user two (2) options for changing the default folders.

- **Each**

  In the **Folder Settings** pane, when the **Each** radio button is selected (see Figure 127) the user may give new paths individually to the **Panel**, **Size Standard**, and **Template** folders. To use the default location installed with ChimerMarker for a given folder, simply check the **Using Default Folder** checkbox.

- **Integrated**

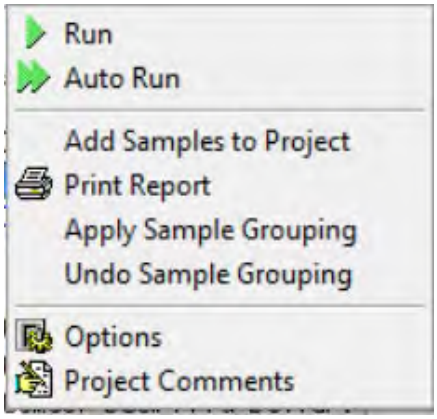
  With this option the user may give the location of a new directory, which contains a template folder, a size standard folder, and a panel folder. The program uses name recognition to identify each folder. The **Panel** folder must contain the word **panel**; the size standard folder must contain the word **size** and the template folder must contain the word **template**. Again, simply select the **Using Default Folder** checkbox to use the default (unmodified) setup.

  For both options, file paths must be entered exactly and should not include any spaces (unless a file name has a space in it). Network locations should be preceded by two back slashes (\\ ).

Project Menu

The Project menu contains options that control how the data is processed and printed.

Figure 128 Project Menu



- **Run**

  Activates the Run Wizard and begins the data processing setup. This allows the user to select or adjust program settings in a sequential manner. See Chapter 3 Genotyping General Procedure.

- **Auto Run**

  Select **AutoRun** to process data automatically with the process options currently selected. The same process action can also be accomplished by clicking the **Run** icon in the toolbar. If one or more of the parameters require changing to improve analysis, select **Project** > **Options**, to change the desired setting(s), and re-process the samples for analysis.

- **Add Samples to Project**

  The user can add samples to a project that has already been sized and analyzed. When selected, the **Open Data Files** dialog displays. Click **Add** to select individual files to the project and click **OK**. The raw data file is sized and processed with the same settings as the other files in the project and added to the bottom of the **Sample File Tree**.

- **Apply Sample Grouping**

  Selecting **Apply Sample Grouping** launches the **File Name Group Editor** dialog which allows the user to group their samples by Sections, Fixed Positions, Order, Run Date, or Cell Type.

  Note: Sample grouping is activated in **View**-> **Preferences**-> **Others**-> **Enable Sample Grouping**.

- **Undo Sample Grouping**

  Reverts the sample grouping applied to the samples in the project.

- **Report**

  Allows the user to Save Project, Print Report, or Close Project. See Chapter 7 Reports and Printing.

- **Print Report**

  Selecting **Print Report** launches the **Print Report Settings** dialog, which allows the user to define display settings in the Print Report. The software permits printing of the sample electropherograms. Choose to print all samples, selected samples, or print samples along with the allele table. See Chapter 7 Reports and Printing.

- **Options**

  Allows you to access and change parameters In the Project Option Settings dialog. This three-tab window contains settings identical to the Run Wizard. Adjust settings in the **Project Options Settings** dialog before selecting Auto Run. See Chapter 3 Genotyping General Procedure.

  Note: Auto Run does not need to be selected after adjusting the **Additional** > **Allele Evaluation** > **Peak Score** settings. The changes are automatically applied when the Project Option Settings dialog is closed.

- **Project Comments**

  Allows the user to write free-form comments regarding the analysis. These comments are saved with the project file and are displayed in the Print Report.

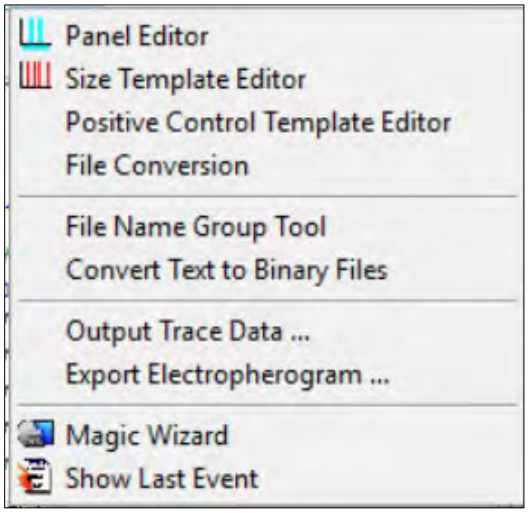
Applications Menu

The Applications menu contains individual modules for specific data and analysis types. These modules present advanced features and reporting options necessary for the application. Select the types of Chimerism Analysis (single donor or double donor) from this drop-down menu. The available modules are determined by the panel applied in the project.

Tools Menu

The Tools menu contains the Panel and Size Editors in addition to other miscellaneous modules.

Figure 129 Tools Menu



- **Panel Editor**

  Provides a variety of tools to adjust, edit, and create control Panels. See Chapter 6 Panel Editor.

- **Size Template Editor**

  Allows the comparison of sample files against a selected size standard, to modify and save the size standard for future use, or to create a customized size standard. See Chapter 5 Fragment Sizing Standard**.**

- **Positive Control Template Editor**

  This menu enables the user to enter positive control genotypes, making them available in the third screen of the Run Wizard for automated positive control concordance. See Chapter 3 Genotyping General Procedure and Chapter 9 Additional Tools.

- **File Conversion**

  This tool allows import of time and distance files from custom genetic analyzers for use with files formatted by the Convert Text to Binary File tool.

- **File Name Group Tool**

  Used specifically with the MSI and LOH applications, the Filename Group Tool allows users to define how reference samples and tumor samples should be grouped or paired. A Text (.txt) file is exported. See Chapter 9 Additional Tools.

- **Convert Text to Binary Files**

  For customers developing their own instrumentation, the Convert Text to Binary Files option allows users to upload four or five-color Text files (without headers) for conversion into SCF (four-color data) or SG1 (five-color data) trace files for analysis with ChimerMarker. See Chapter 9 Additional Tools.

- **Output Trace Data**

  Provides the option to output the raw or sized trace data as a TXT or SCF file. Select the samples to include, dye colors, data type, and the directory to output the trace files. See Chapter 9 Additional Tools.

- **Export Electropherogram**

  Allows the user to export the trace images to a specified folder.

- **Magic Wizard**

  Contains three option dialogues: Start Your Project, Run and Report.

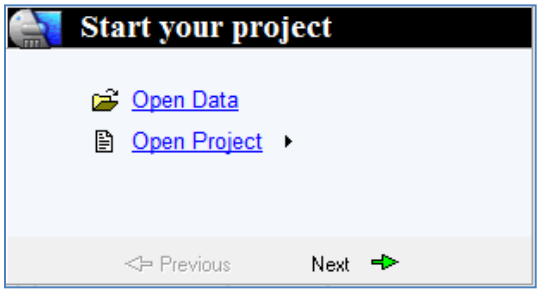
- **Show Last Event**

  Opens the last active Data Process action.

Start Your Project

The **Start Your Project** dialog allows easy access to the **Open Data** or **Open Project** upload dialogs.

Figure 130 Start Your Project



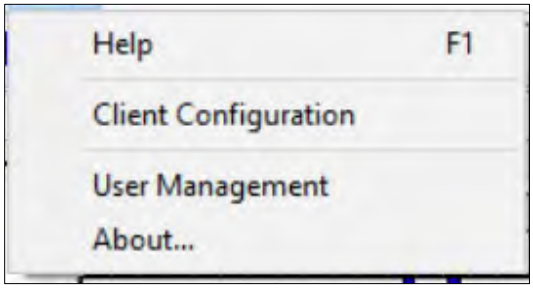
The user can re-open the four (4) previously opened projects by selecting the **black arrow** to the right of **Open Project**.

Help Menu

Help

Launches a searchable version of this manual.

Figure 131 Help Menu



- **Client Configuration**

  Active only for the network license type. Allows users to configure their client computer to the License Server Manager (LSM). See Chapter 1 Installing ChimerMarker.

- **User Management**

  Allows an administrator to assign access rights to different users. Also used to set up the password protection feature. See Chapter 10 User Management**.**

- **About**

  Displays information specific to the version of ChimerMarker running on the computer. Also contains links to email Technical Support and the SoftGenetics website.

Main Toolbar Icons

The main toolbar icons are detailed in the next table.

Table 7 Main Toolbar Icons

Icon	Name	Function
	Open Data	Opens data input dialog to begin analysis.
	Run Project	Opens Run Wizard for processing the data.
	Show/Hide Toggles	Displays or hides the Sample File Tree, Synthetic Gel Image, and Report Table frames, respectively.
	Print Report	Provides the user display options for the *Print Report.*

Icon	Name	Function
	Show Color	Allows the user to select all colors to view, hide all colors, or choose a single dye layer. Choose a single dye by single left mouse clicking on the icon.
	Show Color	Allows the user to select all colors to view, hide all colors, or choose a single dye layer. Choose a single dye by single left mouse clicking on the icon.
	Zoom In	Use the icon to zoom in on the image, or hold down the left mouse button and draw a dialog, from the top left corner to bottom right corner, around the area you wish to zoom in.
	Zoom Out	Use the icon to zoom out on the image or hold down the left mouse button and draw a dialog, from the bottom right corner to top left corner.
	Set Axis	The default setting automatically sets the Y-axis according to the maximum peak intensity of the samples. Two other options are available: auto fit the Y-axis using peak intensities of the alleles, or the user can select the ranges for the X- and Y-axis.
	Browse by All Colors	Displays a comparative view of sample electropherograms by dye color. Individual samples are selected from the drop-down menu.

Allele Call Icons

These icons are only available after the raw data has been processed and the **Sample File Tree Allele Call** folder is selected.

Table 8 Sample File Tree Allele Call

Icon	Name	Function
	Size Calibration	Displays calibration charts for linearity of lane analysis.
	Show Chart/Table	Toggles display to show only the Peak Table, the Peak Table and Electropherogram, or just the Electropherogram.
	Save Peak Table	Exports t4e Peak Table as an Excel (.xls) file or tab-delimited Text (.txt) file.

Icon	Name	Function
	Call Allele	Call alleles by sample(s), by marker, or by dyes. Permits slight modifications to the samples without having to activate Run Wizard again. Settings to change include Peak Detection Threshold, Stutter Peak Filter, and Peak Score Threshold.
Marker: Yellow_1	Marker Drop-down Menu	Allows the selection of a marker to view. This is available after the samples have been compared to a Panel.
	Event Log	Displays each lane's processing success or failure
	Magic Wizard	Activates the Start Your Project, Run and/or Report dialogs.

Report Table Icons

The icons are located directly above the Report Table.

Table 9 Report Table Icons

Icon	Name	Function
	Report Settings	Allows the user to customize Report Table display settings.
	Save Report	Exports the Report Table as an Excel (.xls) file or tab-delimited Text (.txt) file.
Bin	Customize Bin Column	Allows the user to select which bins to include/exclude in the Report Table.

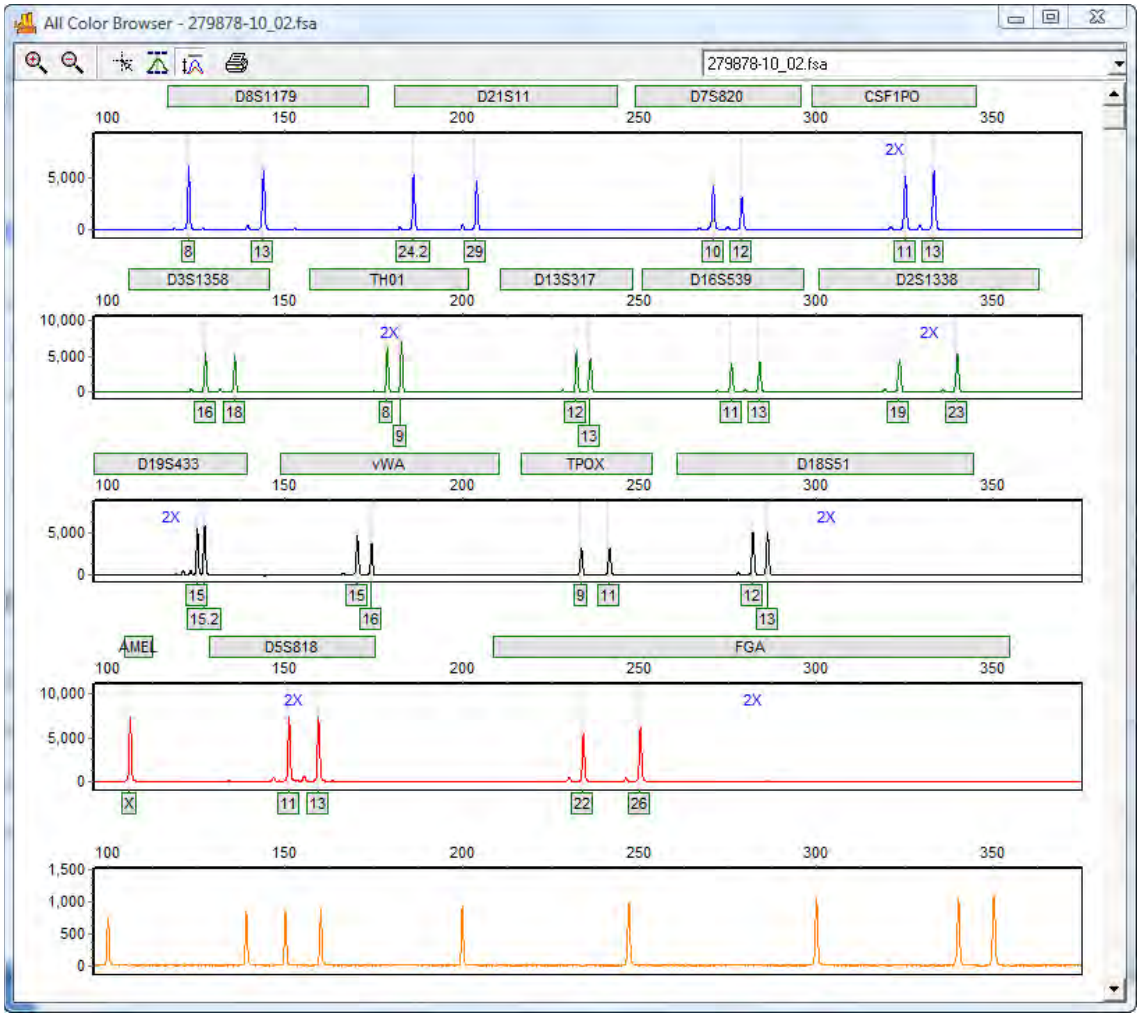
Additional Analysis Options

In addition to the Main Analysis dialog, there are two (2) other display options in which the sample data are viewed: Browse By All Colors and the Profile Comparison View.

Browse by All Colors

Click the **Browse by All Colors** icon in the Main Analysis dialog toolbar to display the **All Color Browser**.

Figure 132 All Color Browser



Navigation and peak editing options in the All Color Browser is similar to that in the Main Analysis dialog as illustrated in Figure 110.

To scroll through samples in the All Color Browser, select the drop-down menu from the menu bar in the upper right corner and select a sample from the list. Once a sample is selected in the drop-down menu, use the **Up/Down Arrow** keys to scroll through samples.

Icons and Functions Additional Analysis

The icons and functions are detailed in the next table.

Table 10 Icons and Functions Additional Analysis

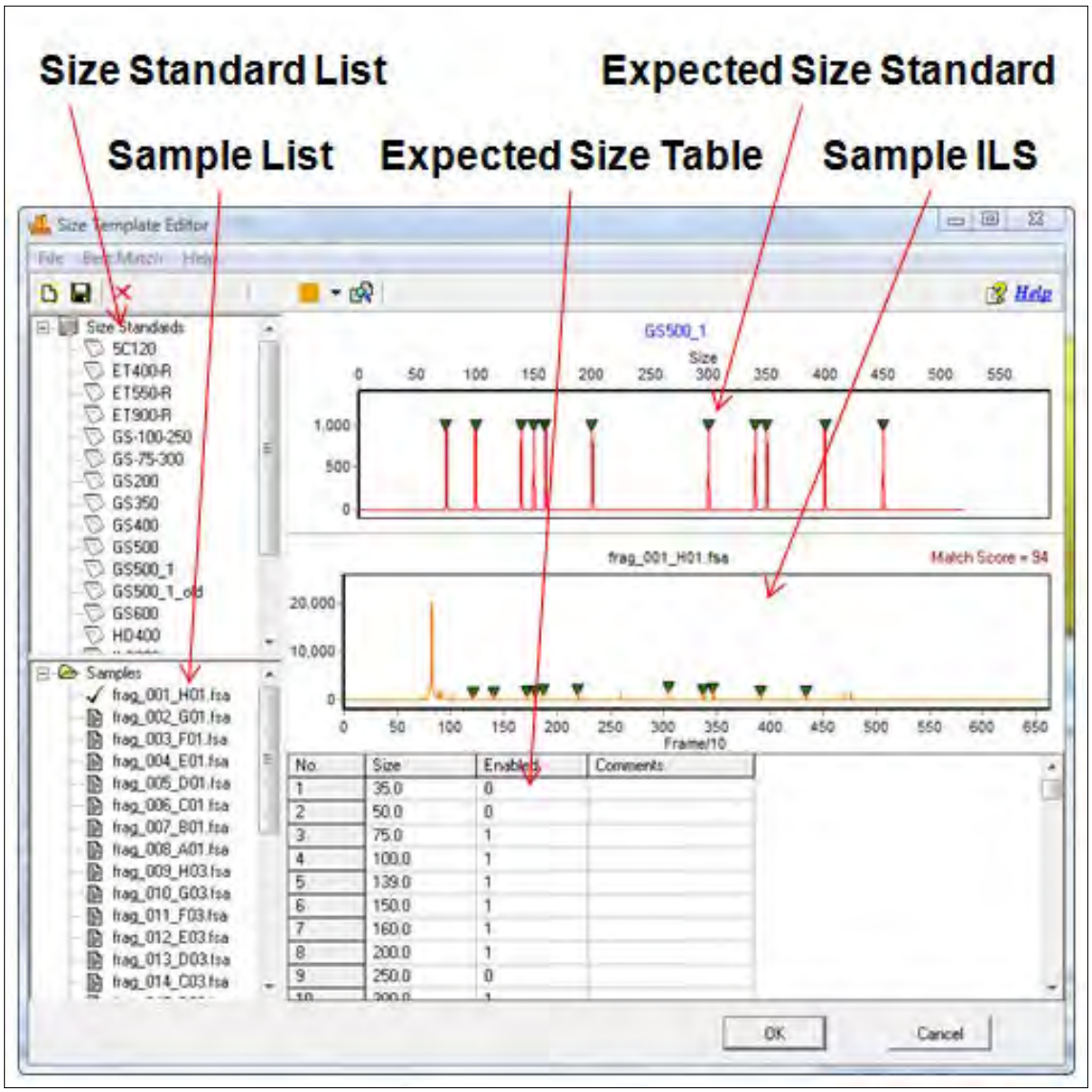
Icon	Name	Function
	Zoom In/Out	Use these icons to increase/decrease the zoom aspect of the electropherograms.
	Show/Hide Mouse Cross Lines	When selected, x and y-axis grid lines will appear at the tip of the mouse cursor along with the base pair size and RFU value of the mouse cursor position.
	Show/Hide Bin Ranges	When selected, the Bin brackets at the top and bottom of the electropherogram trace will appear.
	Auto Scale Markers	When selected, the RFU intensities of low peaks are adjusted 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 Print Report settings dialog. See Chapter 7 Reports and Printing.

Chapter 5 Fragment Sizing Standard

Size Template Editor

The Size Template Editor is a tool in ChimerMarker for creating and modifying Size Standards.

Figure 133 Size Template Editor



To open the Size Template Editor, select **Tools** > **Size Template Editor** from the menu bar or click the **Size Template Editor** icon in the **Run Wizard Template Selection** dialog.

Due to differential fragment mobility in capillary gel electrophoresis, a sizing standard must be applied. A Size Standard template is applied to each ILS and sizes between the known ILS peaks are interpolated.

Each sample run through a CE instrument contains an Internal Lane Standard (ILS). The ILS contains peaks of known size and is usually tagged with red or orange fluorescent dye. Since the ILS dye-labeled fragments migrate through the same capillary as the other dye-labeled sample fragments, they are subject to the same environmental conditions and can therefore be used as a guide to determine the size of the other fragments in the sample.

Note: ChimerMarker is optimized to size fragments with linear mobility. Larger fragments or those run through a high viscosity gel (for example, agarose) do not migrate linearly and therefore cannot be analyzed with ChimerMarker at this time.

The available options in the Size Template Editor include the following:

- **Size Standard List**

  The Size Standard List contains all pre-defined Size Standards and any custom-made Size Standards. Single-left click a Size Standard in the list to select it. The Expected Size Standard trace and Size Table display on the right.

- **Additional Options**

  To see additional options for each Size Standard, right-click the Size Standard name and the right-click menu will appear with the following options.

- **Delete Size Standard**

  Select **Delete** to delete the Size Standard from the Size Standard List and from the SoftGenetics ChimerMarker Size Standard directory.

  Note: This action is irreversible.

- **Export Size Standard**

  Opens the **Save As** dialog. Choose a directory folder and click **Save**. The Size Standard is copied to the selected directory and remains in the Size Standard List and SoftGenetics ChimerMarker Size Standard directory. The Size Standard is exported as an XML file, which is opened using Internet Explorer, Microsoft Excel, or Notepad.

- **Reload Size Standard**

  Click **Reload** to undo editing changes to the Size Standard. The most recently saved Size Standard is restored.

  Note: If the user selects **Save Size Standard** and then answers **NO** to the "*Size Standard has been changed, save changes?* question, the changes remain in the Expected Size Standard and Size Table until the user chooses **R7eload** or the ChimerMarker program is closed.

- **Sample List**

  The Sample List contains a list of all the samples in the dataset. Double-click the filename and the sample's ILS trace displays in the Sample ILS frame. Use the **Up/Down Arrow** keys to scroll through samples in the list.

- **Expected Size Standard and Size Table**

  The **Expected Size Standard** frame displays, as a trace, all the known fragment peaks that are expected to appear in the Sample ILS. Left click a green triangle atop a peak to select the peak. The green triangle turns yellow when the peak is selected.

- **Additional Options**

  Once a peak is selected, right-click anywhere in the **Expected Size Standard** frame. The right-click menu displays with the following options.

- **Insert Size**

  Right-click at the position in the **Expected Size Standard** frame or in the **Sample ILS** where the **Size** should be placed. The **Edit Size** dialog displays. ChimerMarker automatically interpolates the value in the **Size** field if there are two (2) or more sizes present in the trace. Adjust as necessary and click **OK**. A green triangle appears at the cursor position indicating where the new size was placed.

  Note: The height of the new Size in the Expected Size Standard trace is dependent on the height of the peak in the corresponding Sample ILS trace.

- **Edit Size**

  The **Edit Size** dialog displays.

  Figure 134 Edit Size Dialog

  Adjust parameters and click **OK**.

  - **Size**: Enter the expected base pair size of the ILS fragment.
  - **Comments**: Enter free-form text regarding the size.
  - **Enabled**: When selected, a "1" displays in the **Expected Size Table**. Deselect this option to disable the Size in the Size Standard. Disabled sizes are used for pattern recognition in the sample ILS but are not used to size fragments in the other dye colors. Disable a Size if its position is variable from sample to sample.

Note: If the **Enabled** value is changed in the **Size Table**, select another cell in the Size Table before saving the Size Standard or the change does not take.

- **Delete Size**

  Select **Delete Size** to remove the Size completely from the **Size Standard**. Alternatively, disable the Size by deselecting **Enabled** in the **Edit Size** dialog (See Figure 134) or by placing a zero (0) in the **Enabled** column of the Expected Size Table.

  Note: Sizing is often more successful when there are many sizes in the Size Standard.

- **Set Value to Column**

  Makes all values in the column equal to the value in the highlighted cell. Only available in the Expected Size Table.

- **Sample ILS**

  The Sample ILS frame displays the selected sample's ILS trace. Click the **Show Dye** icon in the toolbar to cycle through the other dye colors. Right-click at a peak without a green triangle indicator and choose **Insert Size**. The **Edit Size** dialog displays. Adjust as necessary and click **OK**. The green triangle now displays atop the peak and in the **Expected Size Standard** dialog.

- **Match Score**

  Appears in the upper right corner of the Sample ILS and corresponds to the degree of pattern match between the sample's ILS and the Size Standard selected. Perfect matches receive a score of 100, no correlation receives a score of zero (0).

  Navigation in the Sample ILS frame is like the navigation options in the Main Analysis dialog. See Chapter 4 Genotyping Main Analysis Overview.

Modify Size Standard Procedure

As mentioned previously, Size Standards are created to assign base pair size information to fragment peaks in a sample ILS. The other dye color fragment peak positions are then interpolated based on a linear size scale from the base pair sizes assigned to the peaks in the ILS. ChimerMarker's Size Template Editor tool allows users to apply pre-defined commercial Size Standards or create new custom Size Standards based on the dataset ILSs.

Pre-defined Size Standards include the following:

5C120	GS500
BTO_550	GS500_1
CEQ_400	GS600
CED_600	GS1200
ET400-R	HD400
ET550-R	ILS500
ET900-R	ILS600
GS-100-250	Liz_120
GS-75-300	Rox1000
GS200	SEQ_600
GS350	SNPlex_48plex_v1
GS400	

Pre-Defined Size Standards

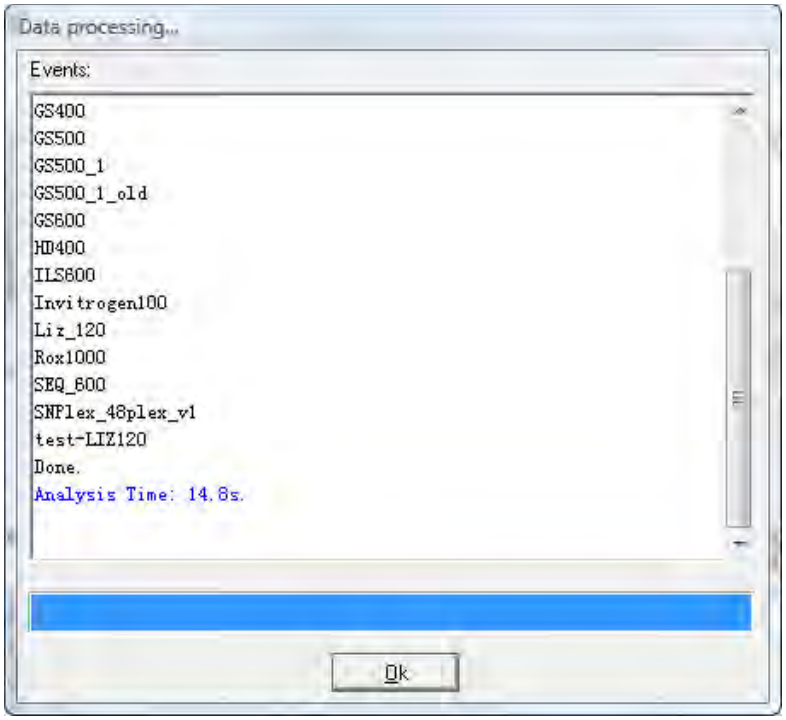
There are two (2) ways to choose a pre-defined Size Standard for the dataset. If the Size Standard name is known, simply single left-click the Size Standard name in the Size Standard List and click **OK** in Size Template Editor. The selected Size Standard displays in the Size Standard field of Run Wizard Template Selection dialog and is used to size the data.

Alternatively, if the Size Standard name is not known, follow the Best Match steps below.

1. In **Size Template Editor**, select **BestMatch > Match All**.

The **Data Processing** dialog displays.

Figure 135 Data Processing Dialog



ChimerMarker cycles through all Size Standards.

2. Click **OK** when the Data Process completes.

   The Size Standard with the best average Match Score across all samples in the dataset is highlighted in the Size Standard List and displays in the Expected Size Standard frame.

   Note: BestMatch does not always choose the correct Size Standard. User inspection is required.

3. Once the Size Standard is chosen, click **OK** in the Size Template Editor, Figure 137.

The selected Size Standard displays in the **Size Standard** field of **Run Wizard Template Selection** dialog and is used to size the data.

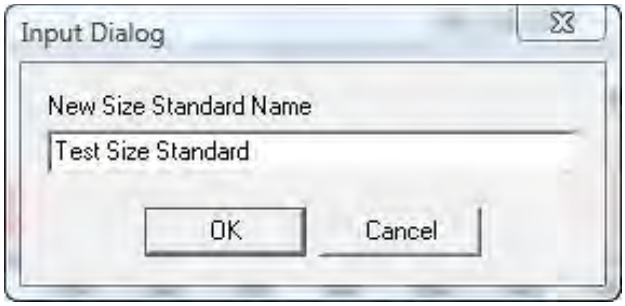
Custom Size Standard Creation

Follow the steps below to create a new Size Standard based on the dataset currently uploaded to ChimerMarker.

1. In Size Template Editor, select **File** > **New Size Standard or** click the **New Size Standard** icon.

The **Input Dialog** displays.

Figure 136 Input Dialog



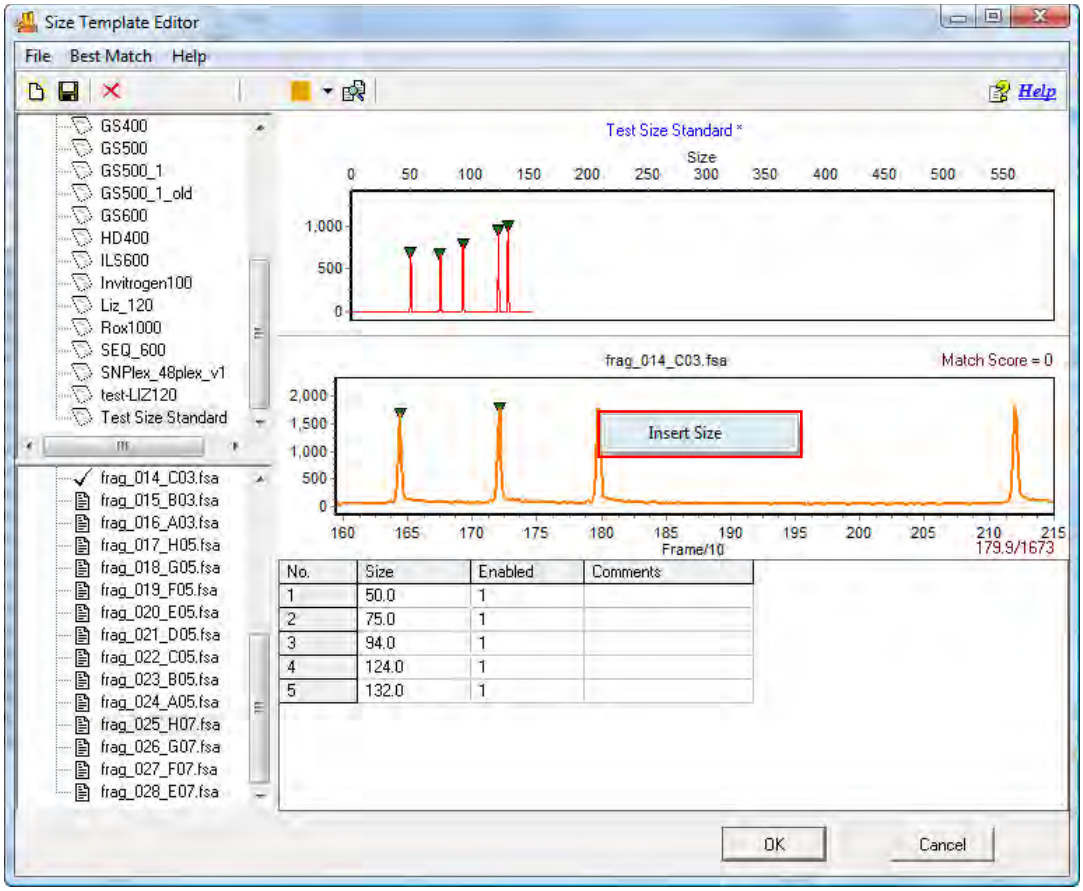
2. In the **New Size Standard Name** field type a Size Standard name and click **OK**.

   The Expected Size Standard frame is blank.

3. Right-click at a known peak in the Sample ILS frame.

4. On the **Size Template Editor** dialog, select the **Insert Size** dialog.

   Figure 137 Size Template Editor and Insert Size



5. Enter the base pair size of the peak in the **Size** field and click **OK.**

   A green triangle displays atop the peak in the Sample ILS and a new peak displays in the Expected Size Standard frame.

   Continue the **Insert Size** operation for the rest of the peaks in the Sample ILS.

   ChimerMarker interpolates Size values after two (2) peaks are added to the Size Standard.

   Note: It is recommended to use the interpolated Size values when creating a Size Standard due to the differential migration patterns of each sample.

6. When the Size Standard is complete, select **File** > **Save Changes** or click the **Save Changes** icon.

7. Click **OK** in Size Template Editor.

8. Proceed with Run Wizard data analysis.

Icons and Functions

The following are explanations of menu and icon options in Size Template Editor displayed in Figure 137.

- **Menu Options**

  The Size Template Editor contains three (3) menu options **File**, **BestMatch**, and **Help**.

  c. The **File** menu allows the user to create, save, and export Size Standards.

  d. The **BestMatch** menu contains options for selecting a Size Standard.

  e. The **Help** menu shows navigation hints for Size Template Editor.

- **File Menu and New Size Standard**

  Opens the Input Dialog (Figure 136) with a field to enter a new Size Standard name. Follow the steps above in **Custom Size Standard Creation**.

  a. **Delete Current Size Standard**

     Deletes the Size Standard that is currently highlighted in the Size Standard List.

     Note: This action is irreversible.

  b. **Save Changes**

     Saves edits and changes to the Size Standard in the SoftGenetics ChimerMarker Size Standard directory.

  c. **Save as New Size Standard**

     Opens the Input Dialog (Figure 136) with a field to enter a new Size Standard name. The Size Standard is added to the Size Standard List and saved in the SoftGenetics ChimerMarker Size Standard directory.

  d. **Import Size Standard**

     Opens a Windows Explorer dialog to the SoftGenetics ChimerMarker Size Standard directory. Use the **Import Size Standard** option to find previously exported Size Standard Files (.xml) on local or networked computers.

  e. **Export Size Standard**

     Exports the currently selected Size Standard in the Size Standard List as an XML file to a specified directory on a local or network computer.

  f. **Import ABI Size Standard**

     Opens a Windows Explorer dialog to the same folder the sample files were uploaded from.

  g. **Export ABI Size Standard**

     Exports the currently selected Size Standard in the Size Standard List as an XML file to a specified directory on a local or network computer.

h. **Exit**

Closes the Size Standard Editor tool. Save changes to the Size Standard before exiting.

- **BestMatch and Match Selected**.

  When selected, the Data Process dialog appears. Each sample in the dataset is compared to the currently highlighted Size Standard in the Size Standard List. The green triangle indicators are adjusted to give the best match possible.

  a. **Match All**

  When selected, the Data Process dialog appears. All samples in the dataset are compared to each Size Standard. The Match Scores for each sample are averaged together. The Size Standard with the highest average Match Score for the dataset is chosen as the Best Match.

  b. **Help**

  The Help menu contains a link to Hot Keys in Size Template Editor. Click **Hot Keys** and the **Size Editor Action Help** dialog appears.

Tool Bar Icons

The tool bar icons are detailed in the next table.

Table 11 Help Menu Icon Tool Bar Icons

Icon	Name	Function
	Size Template Editor	Found in the Run Wizard Template Selection dialog or in the Tools menu.
	Create New Size Standard	Opens the **Input Dialog** box with a field to enter a new Size Standard name. Allows for the creation of a new Size Standard.
	Save Changes	Saves modifications made to the Size Standard to the SoftGenetics ChimerMarker Size Standard directory.
	Delete	Deletes the Size Standard that is currently highlighted in the Size Standard List. **Note:** This action is irreversible.
	Show Dye	Allows the user to select a single dye color to view in the Sample ILS frame. Cycle through the colors by left-clicking the icon or use the drop-down menu.
	Size Match	Automatically places the green size marker triangles atop the peaks of the sample trace and matches it with the selected Size Standard.

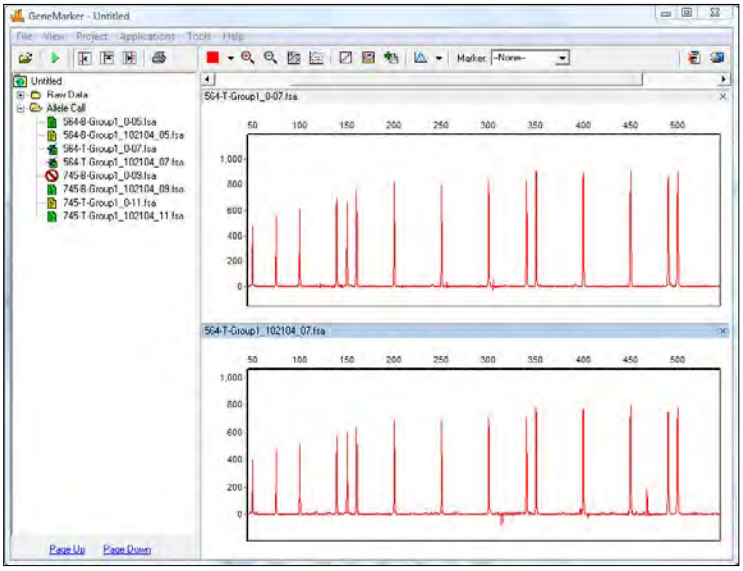
What to Expect

Once the Size Standard is created, it is applied to the dataset. **Save** the edited Size Standard in the Size Template Editor then exit Size Template Editor.

- If the Size Template Editor is accessed through the **Run Wizard Template Selection** icon, then the selected **Size Standard** displays in the **Size Standard** field.
- If the Size Template Editor was accessed through the **Tools** menu then click the **Run Process** icon in the Main Analysis toolbar. The Run Wizard displays.
    a. Select the **Size Standard** from the **Size Standard** drop-down menu in the **Run Wizard Template Selection** dialog.
    b. Proceed through the other Run Wizard dialogs and click **OK** when the **Data Process** is complete. The Size Standard is applied.

  The success of size calling for each sample is indicated by the green, yellow, and red sheet next to the sample filename in the Sample File Tree of the Main Analysis dialog. The lane sizing quality is determined by the Match Score, which in turn is a calculation of how closely the sample's ILS peaks match to the selected Size Standard.
- If a sample receives a low Match Score, the sample is marked with a yellow sheet.

  Figure 138 GeneMarker Low Match - Failed Samples



- If the size calling failed (the sample's ILS peaks could not be aligned with the Size Standard selected) then the sample is marked with a red strike-through.

When low score or failed samples occur, select the **Size Calibration Charts** icon in the main toolbar to correct the size calling.

Size Calibration Charts

The Size Calibration Charts tool is designed to aid the user in determining success or failure of size call after ChimerMarker's automatic sizing is performed. Select the **Size Calibration Charts** icon in the main toolbar of the Main Analysis dialog. As mentioned previously, once a Size Standard has been applied to the dataset, the Size Match *Score* indicators display next to the filename in the Main Analysis dialog Sample File Tree.

Samples with a high Match Score are indicated by a green sheet. Those with a low Match Score have a yellow sheet. Samples where size calling failed receive a red strike-through.

To analyze how each individual sample is matched to the Size Standard selected, access the Size Calibration Charts. Within Size Calibration Charts, the user can modify how each sample was sized and view the statistical information for disabled Size Standard peaks.

Sample List

The Sample List includes filename, Match Score, and disabled peak information for each sample in the dataset. Sort the list by single left-clicking the column header. The list re-sorts in ascending or descending order based on the values in the column selected.

- Single left-click a sample to view its **Sample ILS and Calibration Plots** on the right or use the **Up/Down Arrow** keys.
- Right-click the sample row and select **Mark as Failed** to disable the sample; select **Unmark Failed** to reverse the action. Disabled samples display as greyed-out in the Sample List.

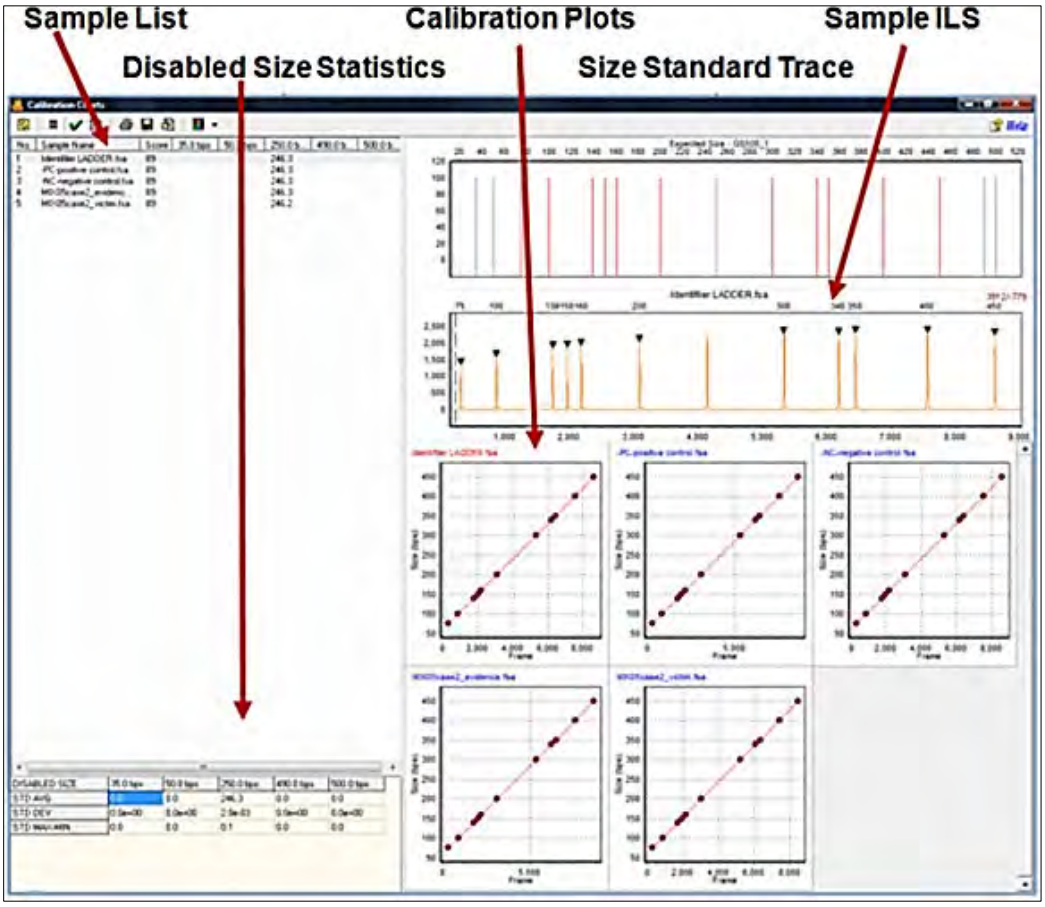
Score

The Score column displays the sample's Match Score, which corresponds to the degree of pattern match between the sample's ILS and the Size Standard selected. Perfect matches receive a score of 100; no correlation receives a score of zero (0) and the sample is considered to have failed size calling.

- **Disabled Size Columns**.

  The Sizes that were disabled in the Size Standard (see the section Size Template Editor) displays as column headers in the Sample List. If no Sizes are disabled, then only the Sample Name and Score columns display in the Sample List. The base pair size position of the disabled peak is reported for each sample. If the disabled peak is at the beginning or end of the Size Standard, no base pair size position is reported.

Figure 139 Size Calibration Charts



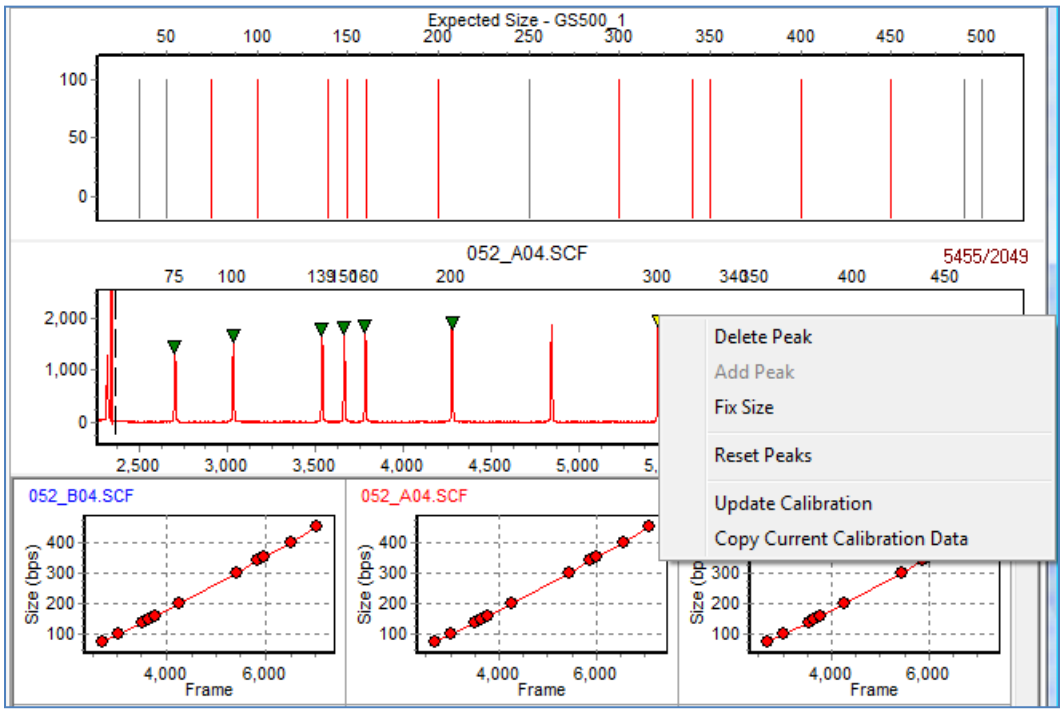
- **Disabled Size Statistics**

  If Sizes were disabled in the Size Standard (see the section Size Template Editor), then the Disabled Size Statistics table displays in the bottom left corner of the **Size Calibration Charts** dialog (Figure 139). The average base pair position, the standard deviation, and the difference between the maximum and minimum base pair positions across all samples are calculated for each ILS peak matched to the disabled peak's position. No statistics are calculated for disabled peaks at the beginning or end of the Size Standard.

- **Size Standard Trace**

  The Size Standard Trace displays a synthetic trace of the selected Size Standard.

  Figure 140 Size Standard - Expected Size



  Enabled Sizes are red; disabled Sizes are grey. Each peak in the Size Standard Trace represents the expected base pair size of peaks in the sample ILS.

- **Sample ILS**

  The Sample ILS displays the currently selected sample's ILS trace. Single left-click samples in the Sample List to see additional samples or use the **Up/Down Arrow** keys. The green triangle peak indicators appear atop peaks that correspond to the enabled Sizes in the Size Standard Trace. The base pair size associated with the green triangle peak indicator is located above the electropherogram. Edit the peaks selected for size calling in the Sample ILS frame as described below.

  Navigation in the Sample ILS frame is like navigation options in the Main Analysis dialog. See Chapter 4 Genotyping Main Analysis Overview.

- **Editing Size Call**

  Single left-click a green triangle peak indicator to select it. The triangle that is currently selected is yellow. To move the green triangle, hold down the **CTRL** key and left click and drag it to the desired position. Right-click the green triangle peak indicator or right-click the top of an unmarked peak to see additional options.

- **Delete Peak**

  Removes the green triangle peak indicator from the Sample ILS and the peak is not considered in the Match Score calculation. The Match Score calculation is updated when **Update Calibration** is selected.

- **Add Peak**

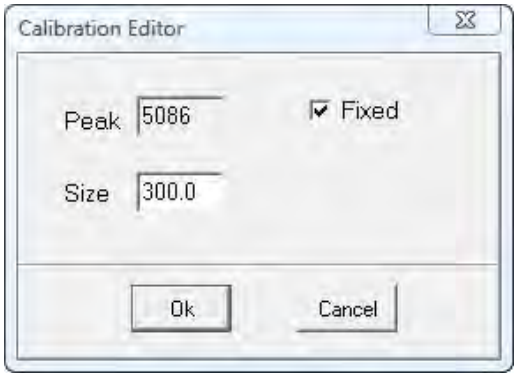
  Right-click at the peak position and select **Add Peak**. A green triangle peak indicator displays at the cursor position. To move the green triangle, hold down the **CTRL** key and left click and drag it to the desired position. The newly added peak is included in the Match Score calculation. The Match Score calculation is updated when **Update Calibration** is selected.

  Note: Add Peak is only available when no other green triangle peak indicator is selected.

- **Fix Size**

  When selected, the **Calibration Editor** dialog displays.

  Figure 141 Calibration Editor

  Enter the correct base pair size of the peak and click **OK**.

  Note: Only enter Sizes that occur in the selected Size Standard in the Calibration Editor *Size* field.

  - The peak is *fixed* at the specified base pair position and all green triangle peak indicators to the left and right of the fixed peak are adjusted to correctly align with the chosen Size Standard.
  - The **Fix Size** feature is useful when the selected Size Standard has uniformly spaced peaks and the sample ILS has additional peaks due to pull-up or other experimental abnormalities that influence the pattern recognition algorithm.

  Note: The **Fix Size** option is not active for manually added peaks or peaks outside the Size Standard range.

- **Reset Peaks**

  Select **Reset Peaks** (Figure 140) to eliminate manually added peaks and/or extra green triangle peak indicators after **Fix Size** in the list.

  Note: Deleted peaks are not recalled when **Reset Peaks** is selected.

- **Update Calibration**

  After editing peaks in the **Sample ILS**, select **Update Calibration**. The Match Score for the sample is recalculated based on the edited peak indicator positions. When

Size Calibration Charts is closed, the Size Match Score indicators next to the filenames in the Sample File Tree in the Main Analysis dialog are updated.

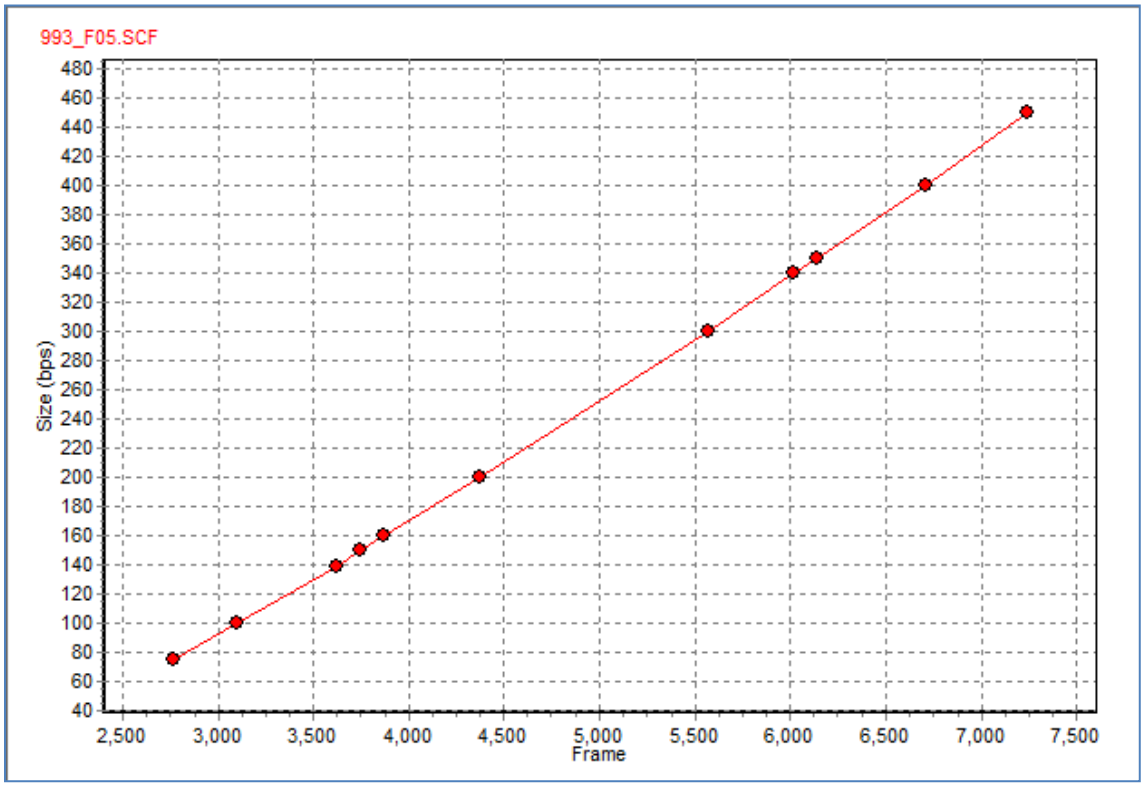
- **Copy Current Calibration Data**

  When selected, the frame position and base pair position of the green triangle peak indicators for the selected sample are copied to the Windows clipboard and are pasted into a spreadsheet or word processing program such as Microsoft Excel or Word.

Calibration Plots

- The Calibration Plots chart the migration linearity of the ILS fragment peaks for each sample.

  Figure 142 993_F05_S2F



- The charts plot the peak base pair positions on the y-axis as a function of time (raw data frame numbers) on the x-axis. As the linearity of the line decreases so does the Match Score for the sample. Incorrectly identified peaks result in a low Match Score.
- Double-click a **Calibration Plot** to select the sample in the Sample List and display the sample in the Sample ILS. The currently selected sample filename displays red in the upper left corner of the Calibration Plot.

Run Wizard Procedure

After a Size Standard is chosen and the data is processed by the Run Wizard, the Size Calibration Charts are used to correct improperly sized samples.

1. Click the **Size Calibration Charts** icon in the main toolbar.

   The Calibration Charts displays as in Figure 142.

2. Select a sample to edit in the Sample List.

   The sample's ILS appears in the Sample ILS frame.

3. Right-click in the **Sample ILS** frame and choose **Add**, **Delete**, or **Fix Size** to correct size call.

4. Right-click again and select **Update Calibration**.

   The changes are implemented for the sample and the Match Score is updated.

5. When editing is finished, close **Size Calibration Charts**.

   The Size Match Score indicators in the Sample File Tree of the Main Analysis dialog are updated.

Icons and Functions

The icons and functions are detailed in the next table.

Table 12 Icons and Functions

Icon	Name	Function
	Size Calibration	Found in the main toolbar of the Main Analysis dialog.
	View Mode	Change the layout of the Calibration Plots frame. Adjust the maximum number of rows and columns displayed. Maximum number of rows and columns is 5
	Chart Synchronize	When selected, both the Expected Size Standard and Sample ILS traces become synchronized. This option is not selected by default.
	Preprocess Raw Data	Select **Preprocess Raw Data** to smooth the samples raw data ILS.
	Auto Fit Y	Provides the option to automatically fit the Sample ILS's y-axis by the maximum peak height in the trace or by only the highest matched peaks.

Icon	Name	Function
	Print	Provides Print Preview and Print of size calibration page, providing a physical record of size calibration for the project.
	Save	Allows the user to save the size calibration pages as .png images.
	Manual Calibration	Provides the option of manually entering standard peak sizes if many peaks have been modified. This dialog contains three columns: Standard Size (fragment sizes of standards used for size calling), Peak Position (in frames), and Size (sizes are automatically entered, but easily edited).

What to Expect

It is important to verify sizing accuracy prior to analyzing a dataset. If a sample is not sized correctly, peaks may be called Off Ladder (OL) if a panel is applied. Incorrect sizing most dramatically affects larger size fragments.

Figure 143 Size Calibration Chart before Manual Correction

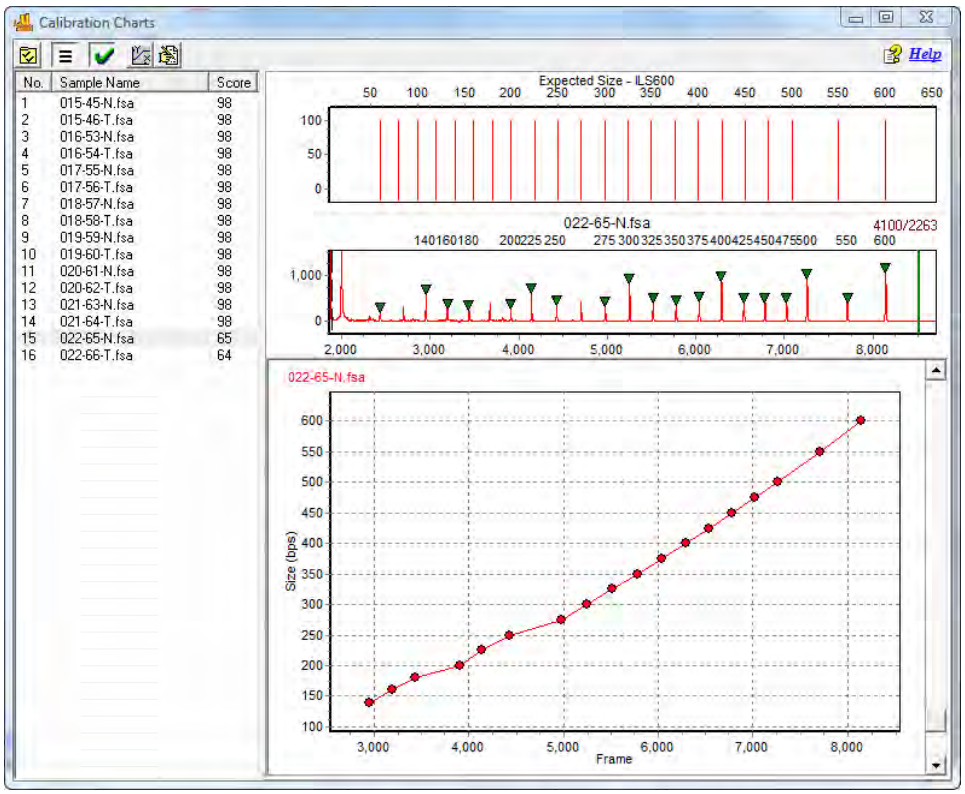
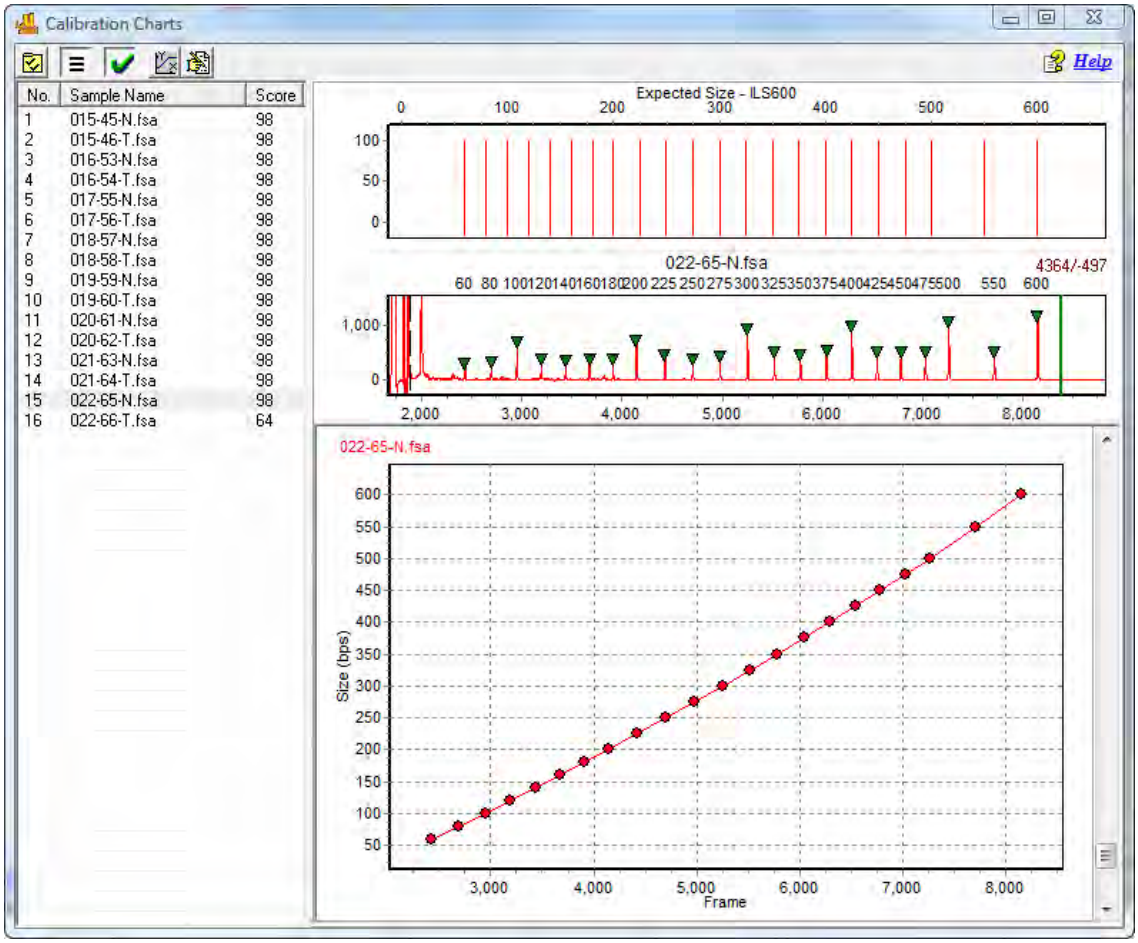


Figure 144 Size Calibration Chart after Manual Correction



Chapter 6 Panel Editor

Overview

The Panel Editor is accessed from the **Tools** menu in the Main Analysis dialog or using the **Panel Editor** icon in the **Run Wizard Template Selection** dialog.

Figure 145 Panel Editor

The purpose of a Panel is to outline the position of expected alleles. Loci or Markers give a range where a group of alleles is expected to display, and Bins indicate the specific base pair position of the expected allele. In ChimerMarker, Markers are indicated by a horizontal gray bar across the top of the electropherogram. Bins are indicated by the dye-colored brackets at the top and bottom of the electropherogram.

Note: Only in the Panel Editor do the vertical gray bars within the electropherogram indicate the center of the Bin. For all other views in ChimerMarker, the vertical gray bars in the electropherogram indicate the center of the detected peak.

Project Panel

The Project Panel list includes the template panel used for the project and all allelic ladder samples that fit the pattern recognition to the selected template panel. These ladder file names are in bold font in the Main Analysis dialog when Select Best Ladder is used for the Run Wizard. ChimerMarker selects the best fit ladder from this list as the reference ladder for each sample in the project. The reference ladder for a given sample is listed on the print report.

Panel Templates

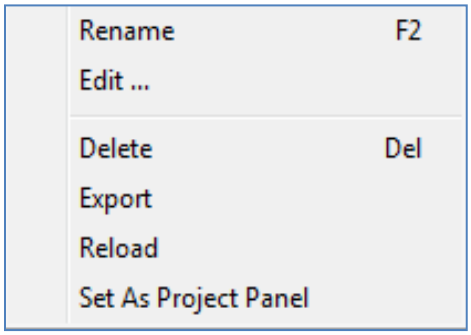
The Panel Template list includes a list of all pre-defined and custom Panels saved to the Panels folder in the SoftGenetics ChimerMarker directory. Single left-click on the panel name to display the trace. Single right-click on the Panel name to display the pop-up menu of options.

Double-click the **Panel** name to expand the folder and view the **Markers** associated with the Panel. Single left-click the Marker name to display that Marker in the **Overlay Trace** frame.

Additional Options

To see additional options for each Panel, right-click the **Panel** name and the right-click menu displays with the following options.

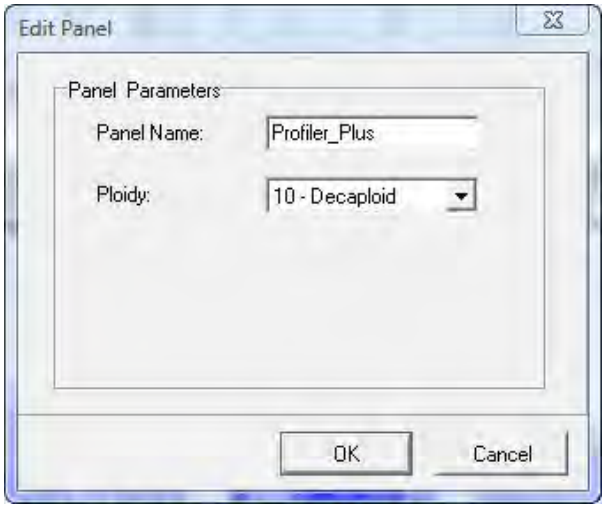
Figure 146 Panel Name Menu



- **Edit Panel**

  Opens the **Edit Panel** display.

Figure 147 Edit Panel Dialog



Editing the **Panel Name** field changes how the Panel is labeled in Panel Editor. Set the **Ploidy** field drop-down list from **Monoploid (1)** to **Decaploid** (10) to **Unlimited**. If the number of peaks within a Marker exceeds the Ploidy setting, the additional peaks are labeled **Off Ladder (OL)** and given the **Undetermined** (red) quality rank and **PL Quality Reasoning**. When **Unlimited** is selected the PL rule is never fired.

- **Delete Panel**

  Select **Delete** (**Hot Key = DEL**) to delete the Panel from the Panel List and from the SoftGenetics ChimerMarker directory.

  Note: This action is irreversible.

- **Export Panel**

  Opens the **Save As** dialog. Choose a directory folder and click **Save**. The Panel is copied to the selected directory and remains in the Panel List and SoftGenetics ChimerMarker Panel directory. The Panel is exported as an XML file, which are opened with Internet Explorer, Microsoft Excel, or Notepad.

- **Reload Panel**

  Click **Reload** to undo editing changes to the Panel. The most recently saved Panel is restored.

  Note: If the user makes changes to the panel and then answers **NO** to *The Panel has been changed, save changes to file?* prompt, the changes remain in the Overlay View until the user chooses **Reload Panel** or the ChimerMarker program is closed.

Sample List

The Sample List contains all the samples uploaded to ChimerMarker in the current project. Samples with a checkmark next to the filename are displayed in the Overlay View. Double-click the sample filename or right-click the sample and choose **Select/De-Select** to enable/disable it in the Overlay View. Right-click any sample in the list and choose **Select All/De-Select All** to display all or no sample traces in the Overlay View.

- **Sorting Options Sample Name**

  Sorts the samples in alphanumeric descending order. Sample Name sorting is the default option.

- **Size Score**

  Sorts the samples by the lane size score as it displays in the Size Calibration Charts See Chapter 4 Genotyping Main Analysis Overview. Samples with higher scores display at the top of the list.

Overlay Trace

The Overlay Trace displays all selected samples in the Sample List. The Marker bars display above the electropherogram and the Bins display within the electropherogram as brackets at the top and bottom. The center of the Bin is indicated by the vertical grey bar in the electropherogram (only in Panel Editor). Change the Overlay Trace view by clicking the **Trace Mode** icon in the toolbar. Other options include Max & Average and Gel Image.

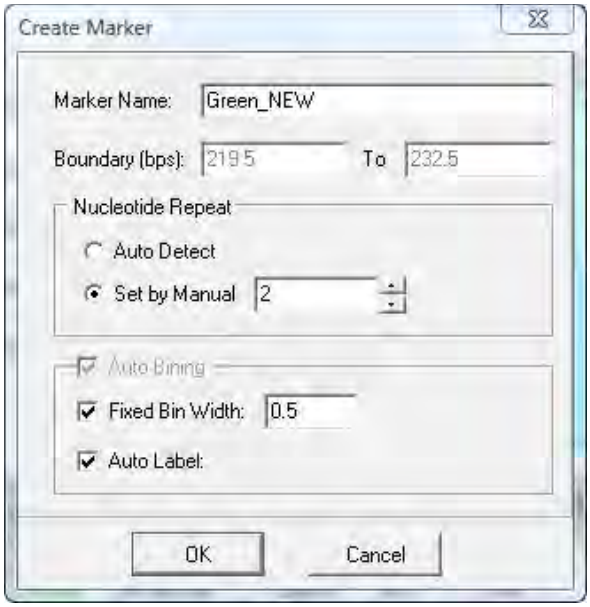
Navigation in the Overlay Trace frame is like the navigation options in the Main Analysis dialog. See Chapter 4 Genotyping Main Analysis Overview.

- **Marker Options Create Marker**

  Hold down **CTRL** key and left click and drag across peaks in the Overlay View. A light-blue hashed dialog is displayed. Right-click in the hashed dialog and select **Create Marker**.

The **Create Marker** dialog displays.

Figure 148 Create Marker



Adjust parameters and click **OK**.

- **Marker Name**

  Edit the **Marker Name** field to change how the Marker is labeled in the Panel.

- **Boundary**

  The base pair range of the Marker is defined by the range of the light-blue hashed dialog and is therefore inactive in the **Create Marker** dialog. To edit the Boundary, see section Edit Marker below.

- **Nucleotide Repeat**

  - **Auto Detect**: Based on the peaks present in the Overlay View, ChimerMarker will attempt to detect the number of nucleotides in each repeat unit of the alleles and place Bins at the appropriate interval.
  - **Set by Manual**: Select this option if the number of nucleotides in the allele repeat unit is known and ChimerMarker places Bins at the specified interval.

Auto Binning

- **Fixed Bin Width**

  Check this option to enter the number of base pairs on the right and left of the center of the Bins. If 0.5 is selected as the Bin Width, then the total Bin range is 1.0 base pairs.

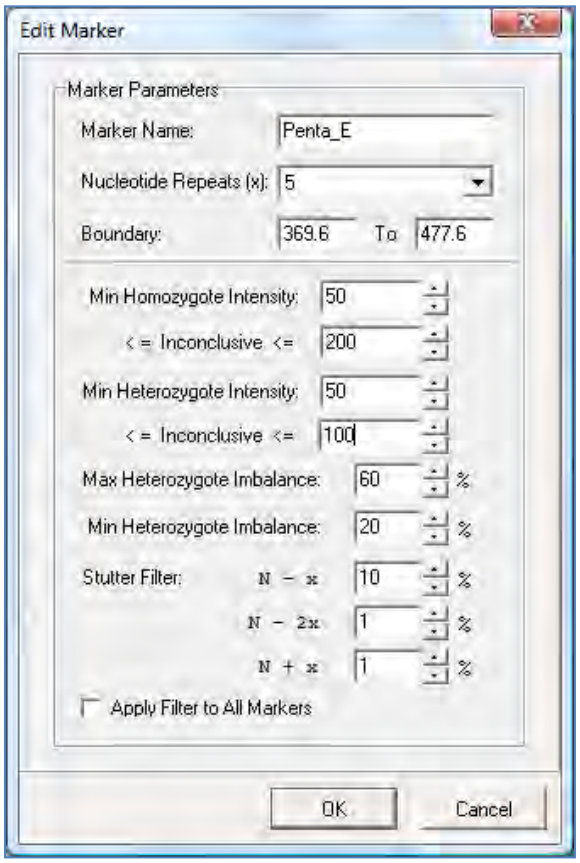
- **Auto Label**

  When deselected, the Bins are automatically labeled with the base pair size of the Bin position to the nearest tenth of a base pair. If selected, the base pair size is rounded up to a whole number value.

Edit Marker

Double-click the **Marker** bar or right-click the **Marker** bar and select **Edit Marker**. The **Edit Marker** dialog displays.

Figure 149 Edit Marker



Adjust parameters and click **OK**.

Note: Also access the **Edit Marker** dialog by right clicking the **Marker** name in the **Panel List** and selecting **Edit**. Marker parameter options include the following:

- **Marker Name**

  Edit the **Marker Name** field to change how the marker is labeled in the panel.

- **Nucleotide Repeats**

  Use the **Nucleotide Repeats** drop-down menu (1-6) or enter a value into the field to set the number of base pairs expected between each allele in the Marker.

- **Boundary**

  To move a Marker left or right, hold down **SHIFT** key and left click and drag the Marker bar. To adjust the base pair range over which a Marker is located, hold down **SHIFT** key and mouse-over the edge of the Marker bar until a double-headed arrow appears then left-click and drag the Marker edge to increase or decrease the range or right-click the **Marker** bar and select **Edit Marker**. The **Edit Marker** dialog displays.

  Adjust the **Boundary** field values as necessary. Additionally, if a Marker needs only slight adjustment to the right or left, right-click the **Marker** bar and select **Adjust Marker**. The Marker automatically moves to align with the closest peaks.

Filtering Parameters

- **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 determined by the number of peaks above the Min Heterozygote Intensity level.

- **Min Heterozygote Intensity**

  Sets the minimum RFU value at which the software calls peaks if there is more than one peak in a marker. The number of peaks in a marker is determined by the number of peaks above this minimum intensity level.

  Note: If the minimum homozygous and minimum heterozygous values are different from each other, a single peak above the(inconclusive heterozygous) Min Heterozygote Intensity but below Min Homozygote Intensity is called and labeled with a Check Quality and LO Quality Reason, when a second peak is detected above the N-x Stutter Filter value. The second peak is not called; however, because it is below the Min Heterozygote Intensity threshold.

- **Inconclusive Range**

  If desired, set an inconclusive range for homozygous and heterozygous peaks. Peaks within the inconclusive range is flagged with a Check Quality and IHO (inconclusive homozygous) or IHE (inconclusive heterozygous) Quality Reason. This setting is helpful for flagging peaks that are above the minimum detection level but are not high enough to include in statistics (within the stochastic range).

- **Max Heterozygote Imbalance**

  Uses the percentage of the highest peak in a marker to define the maximum peak threshold. For example, if the threshold is set to 60%, the height of all allele peaks must reach at least 60% of the height of the highest peak in that locus. If a peak does not reach that height, it is flagged with a Check Quality and IMB Quality Reason.

- **Min Heterozygote Imbalance**

  Uses a percentage of the highest peak in a marker to define the minimum peak threshold. If a peak does not reach the minimum imbalance threshold, **the peak is not called**. This function is the equivalent to a filter, allowing users to filter out peaks that are less than a given percent of the highest peak within the marker.

- **Stutter Filter**

  Forward and reverse stutter peaks commonly caused by PCR/chemical reactions are removed using the Stutter Filter. In human identification analysis, the stutter positions of interest are N-4, N-8, and N+4, relative to the highest peaks in the marker (given tetra-nucleotide repeat units). The “x” refers to the number of nucleotide repeats specified in the Marker Parameters – Nucleotide Repeats field. The settings are in percentage of the primary peak. Peaks in the N-x, N-2x, N+x positions that are below the stutter threshold is not called.

  Note: The stutter filter is meant to eliminate the extra steps of analyzing peaks caused by instrument anomalies. Set all Stutter Filter settings to “0%” if all peaks must be called.

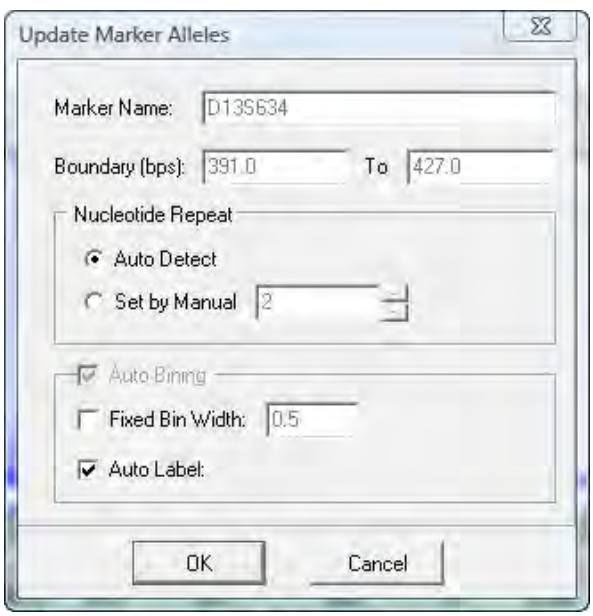
- **Apply Filter to All Markers**

  When selected, the values in the filtering parameters fields is applied to all Markers in the Panel. To optimize the stutter filter, set each marker with stutter values determined during lab validation procedures.

Edit Marker Bins

Right-click the **Marker** bar and select **Update Alleles**. The **Update Marker Alleles** dialog displays.

Figure 150 Update Marker Alleles



Adjust parameters and click **OK**.

The **Nucleotide Repeat** pane includes the following settings:

- **Auto Detect**

  Based on the peaks present in the Overlay View, ChimerMarker attempts to detect the number of nucleotides in each repeat unit of the alleles and place Bins at the appropriate interval.

- **Set by Manual**

  Select this option if the number of nucleotides in the allele repeat unit is known and ChimerMarker places Bins at the specified interval.

The Auto Binning pane includes the following settings:

- **Fixed Bin Width**

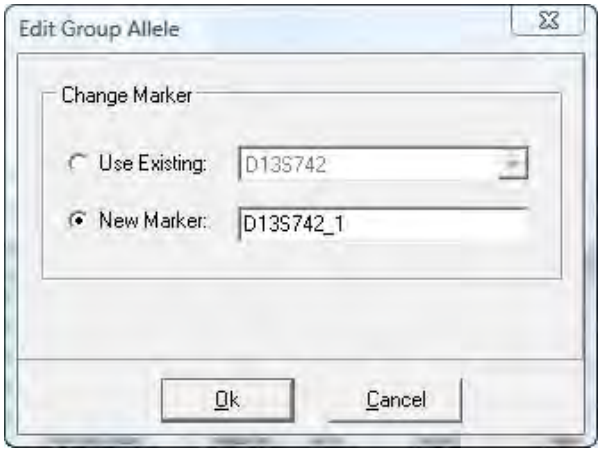
  Check this option to enter the number of base pairs on the right and left of the center of the Bins. If 0.5 is selected as the Bin Width, then the total Bin range is 1.0 base pairs.

- **Auto Label**

  When deselected, the Bins are automatically labeled with the base pair size of the Bin position to the nearest tenth of a base pair. If selected, the number is rounded up to a whole number value.

  To associate Bins with a different Marker, hold down **CTRL** key and left click and drag across peaks at the edge of a Marker. A light-blue hashed dialog is displayed. Right-click in the hashed dialog and select **Change Marker**. The **Edit Group Allele** displays.

  Figure 151 Edit Group Allele



  Select the **New Marker** radial button and a pre-defined name displays. Use this Marker label or create a new name and click **OK**. The highlighted Bins are now incorporated into the newly created Marker.

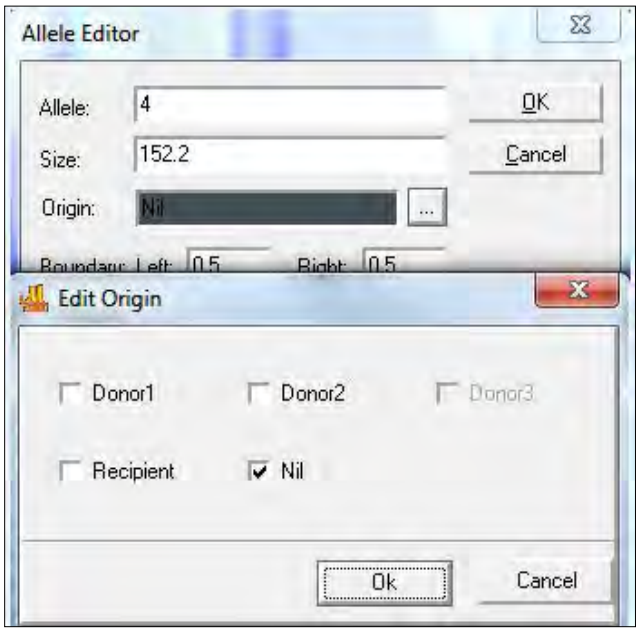
- **Delete Marker**

  Right-click the **Marker** bar and select **Delete Marker** or right-click the **Marker** name in the **Panel List** and select **Delete** (**Hot Key = DEL**).

Bin Options Create Bin.

To create a Bin position, right-click in the electropherogram at the exact position to place the new Bin. Select **Insert Allele**. The **Allele Editor** dialog displays.

Figure 152 Allele Editor and Edit Origin Dialogs



Adjust parameters and click **OK**.

- **Allele**

  Enter a name for the Bin. All peaks that appear within the Bin display this value in the Allele Label in the Main Analysis dialog.

- **Size**

  Indicates the base pair position of the center of the Bin.

- **Origin**

  This is filled in automatically based on donor and recipient peak information from the analysis. If the original electropherogram is not available the origin (Donor1, Donor2, Recipient may be selected for Chimertyping Panels). Nil is used for genotyping panels of single source data.

- **Boundary**

  Indicates the range of the Bin on either side (left and right) of the Bin center.

- **Marker**

  Select which Marker to associate the Bin with. The Markers to the right and left of the Bin position is displayed as well as the option to create a new Marker for the Bin. All Bins must be associated with a Marker.

- **Comments**

  Free-form text field to associate a comment with the Bin.

- **Control Gene**

  Select Control Gene if the Bin contains a major peak in the Ladder samples. Bins marked as Control Gene display the Allele Label below the Electropherogram trace of the Ladders in the Main Analysis dialog. Bins marked zero (0) do not display an Allele Label even if a peak is present.

- **Edit Bin**

  Right-click the vertical grey bar in the center of the Bin in the Overlay Trace. Select **Edit Allele** and the **Allele Editor** dialog displays. Adjust parameters and click **OK**. See the Bin Options Create Bin section above for explanation of Allele Editor options.

  a. To move a bin, hold down **SHIFT** key and left click and drag the vertical grey bar in the center of the Bin to the right or left. Let go of the **SHIFT** key and mouse button and the Bin remains in place.

  b. To edit the range of a Bin in the Overlay View, click the **Trace Mode** icon to view the Gel Image.

  c. Hold down **SHIFT** and mouse over the vertical white line of the Bin edge. When a double-headed arrow appears, hold down left-click and drag the Bin edge to adjust the range.

- **Delete Bin**

  a. Right-click the vertical grey bar in the center of the Bin in the Overlay Trace.

  b. Select **Delete Allele**. The Bin is deleted from the Panel.

  c. To delete multiple Bins, hold down **CTRL** key and left click and drag across peaks in the Overlay View. A light-blue hashed dialog is displayed.

  d. Right-click in the hashed dialog and select **Delete Alleles**. The Bins highlighted by the hashed dialog are removed from the Panel.

Panel Table

The Panel Table displays Marker and Bin information for the dye color displayed in the Overlay Trace frame.

Figure 153 Panel Table Overlay Trace

No.	Dye	Marker	Size	Left Range	Right Range	Allele Name	Control	Distance/kb	Comments
1	Blue	D3S1358	108.3	0.5	0.5	11	0	0.00	
2	Blue	D3S1358	112.3	0.5	0.5	12	1	0.00	
3	Blue	D3S1358	116.4	0.5	0.5	13	1	0.00	
4	Blue	D3S1358	120.4	0.5	0.5	14	1	0.00	
5	Blue	D3S1358	124.4	0.5	0.5	15	1	0.00	
6	Blue	D3S1358	126.6	0.5	0.5	15.2	0	0.00	
7	Blue	D3S1358	128.6	0.5	0.5	16	1	0.00	
8	Blue	D3S1358	130.6	0.5	0.5	16.2	0	0.00	
9	Blue	D3S1358	132.9	0.5	0.5	17	1	0.00	
10	Blue	D3S1358	134.6	0.5	0.5	17.2	0	0.00	
11	Blue	D3S1358	137.0	0.5	0.5	18	1	0.00	
12	Blue	D3S1358	139.1	0.5	0.5	18.2	0	0.00	
13	Blue	D3S1358	141.2	0.5	0.5	19	1	0.00	
14	Blue	D3S1358	144.5	0.5	0.5	20	0	0.00	

Edit all columns except Dye and Marker in the Panel Table. Right-click a highlighted cell and select **Set Value to Column** to make all values in the column equal to the value in the highlighted cell.

- **Dye**

  Indicates the dye color of the Bin.

- **Marker**

  Indicates which Marker the Bin is contained in.

- **Size**

  Indicates the position of the Bin center in base pairs.

- **Left/Right Range**

  Indicates the range of the Bin on either side of the Bin center.

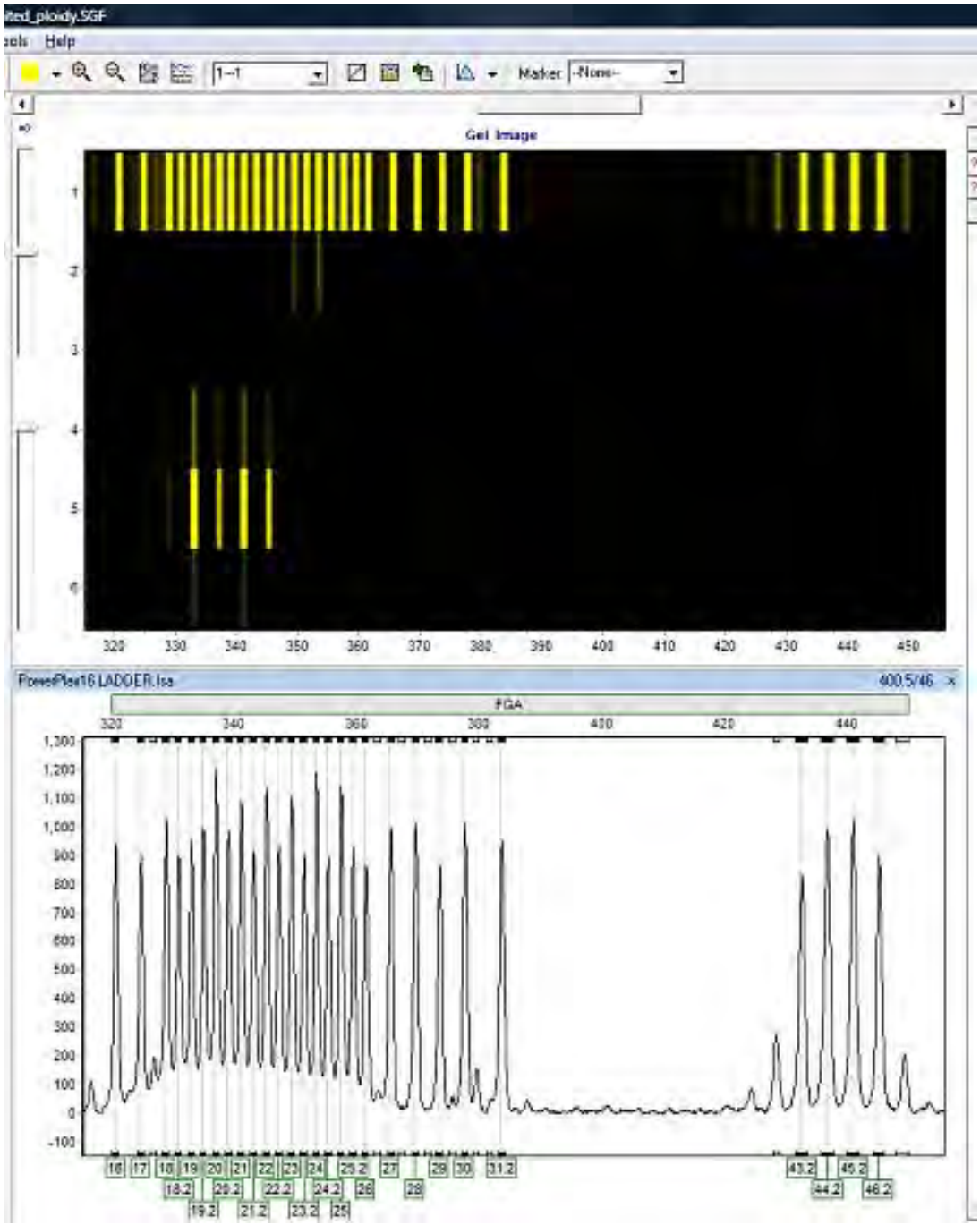
- **Allele Name**

  Peaks that appear within the Left/Right Range of the Bin are labeled with the Allele Name.

- **Control**

  Bins marked with a “1” are considered major alleles in the Ladder sample and are marked with an Allele Label and a darkened rectangle to mark the bin in the Main Analysis dialog.

Figure 154 dred_plcidy.SGF and PowerPlan



Bins marked with a zero (0) are minor peaks and do not receive an Allele Label. The bin is marked with a clear rectangle in the Main Analysis dialog.

Note: The designation of 0 and 1 in the Control column is used to assist with pattern recognition in the **AutoPanel Adjust** algorithm.

- **Distance/kb**

  Allows the user to input the distance (in kb) that each allele is from the beginning of the sequence. For example, “38.1” means that the allele is 38.1 kb from the beginning of the sequence.

  Note: This option is not applicable for forensic STR analysis.

- **Comments**

  Free-form text field to associate a comment with the Bin.

ChimerMarker Panels Procedure

As mentioned previously, Panels are created to outline the position, in base pairs, of expected peaks. In ChimerMarker, the Panels associated with several commercially distributed human identity kits are included. Examples of some of the pre-defined Panels include Promega’s Powerplex kits and Beckman's Vidiera. ChimerMarker also offers the opportunity to create a new custom Panel if the pre-defined Panels do not include a kit that the user is working with. A discussion next details how to use the pre-defined Panels or create a new Panel with ChimerMarker’s Panel Editor tool.

The Panels displayed by default include the following:

- GenePrint® 24
- PowerPlex® 16
- PowerPlex® Fusion
- PowerPlex® Fusion 6C
- PowerPlex®ESX16 and 17
- PowerPlex®EXI16 and 17
- PowerPlex® 16
  - PowerPlex®18D
  - PowerPlex®21
  - Globalfiler
  - Identifiler
  - Profiler
  - MentypeChimeraPanel

Pre-Defined Panels

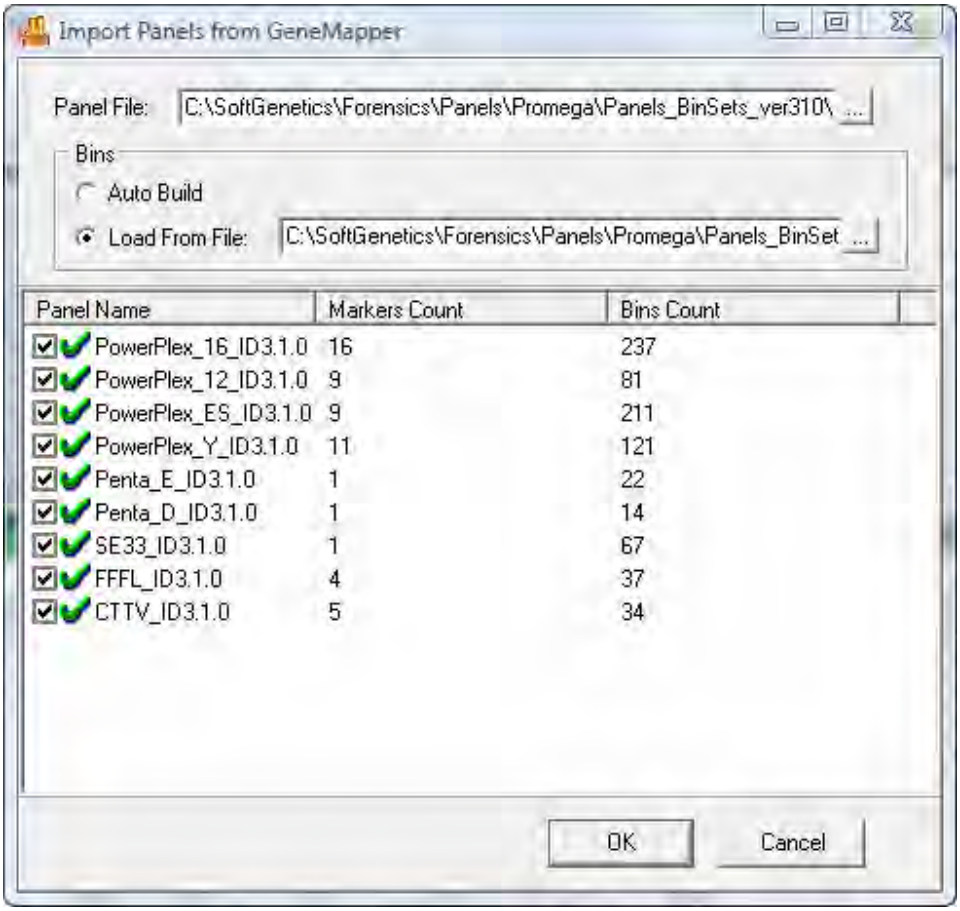
The pre-defined Panels display in the Panel List. Single-click any Panel in the **Panel List** to select it. In addition to the Panels displayed by default, the user has the option to import standard Panels and Bins text files. Follow the steps below to import Panels and Bins Text files.

Panels and Bins Files

1. In **Panel Editor**, select **File** > **Import ABI Panels** from the menu bar.

   The **Import Panels from GeneMapper** dialog displays.

   Figure 155 Import Panels for GeneMapper



2. Select the access button (**…**) next to the **Panel File** field. A Windows Explorer dialog displays.
3. Navigate to the location of the **Panels.txt** file and select **Open**.
4. Select the access button (**…**) next to the **Bins Load from File** field and locate the Bins.txt file.
5. Click **Open**.

   Note: Select **Bins Auto Build** if a Bins.txt file does not exist.

6. Click **OK**. All Panels in the Panels.txt file are uploaded into ChimerMarker.
7. Select a newly uploaded Panel from the Panel List.
8. Edit the Markers and Bins so that they align with the peaks in the dataset.
9. Save the edited Panel and close Panel Editor.
10. Click the **Run Project** icon in the Main Analysis dialog.
11. Select the Panel from the **Panel** field in the **Run Wizard Template Selection** dialog.
12. Proceed through Run Wizard and data analysis. See Chapter 3 Genotyping General Procedure and Chapter 4 Genotyping Main Analysis Overview.

Custom Panel Creation

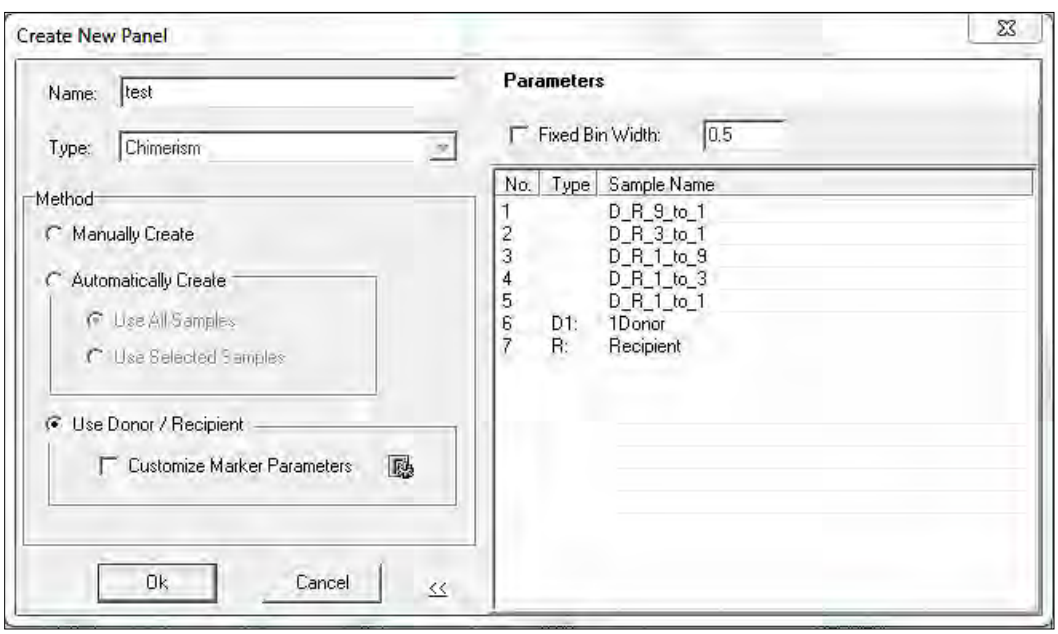
Follow the steps below to create a new Panel based on the dataset currently uploaded to ChimerMarker.

Automatic Panel Creation

1. In **Panel Editor**, select **File** > **Create New Panel** from the menu bar or click the **Create New Panel** icon.

   The **Create New Panel** dialog displays.

   Figure 156 Create New Panel



2. Enter a name for the Panel in the **Name** field. This is the Panel name that is displayed in the **Panel List**.

   The **Type** drop-down list by default displays the Analysis Type chosen initially in Run Wizard Template Selection. The only type option in ChimerMarker is **Chimerism**.

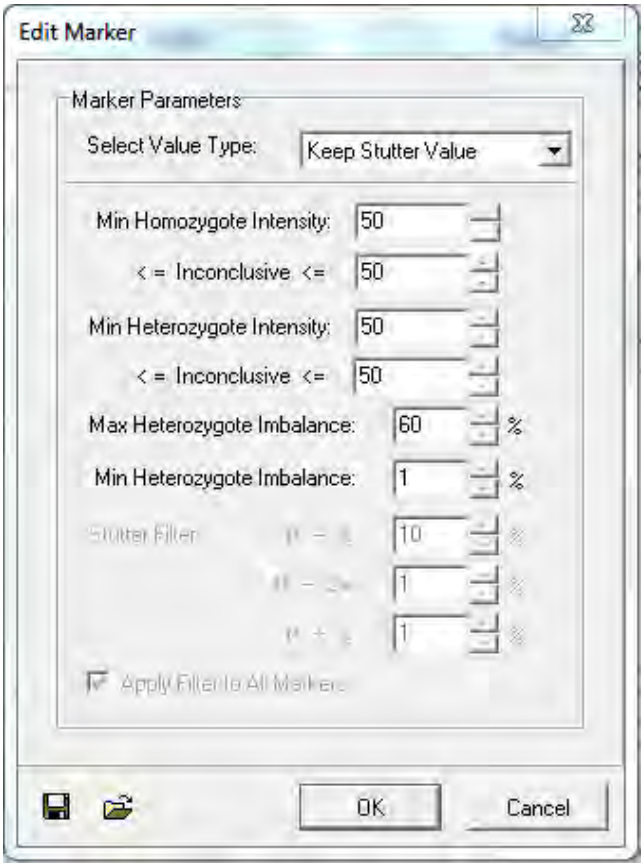
3. Select **Automatically Create**.

a. The **Use All Samples** radial button creates a Panel based on an overlay of all the sample peaks in the dataset.

b. The **Use Selected Samples** radial button creates a Panel based only on the samples selected in the Panel Editor Sample List.

4. Select **User Donor/Recipient** to use the donor and recipient allele calls to make a Chimertyping Panel.
5. If required, check the **Fixed Bin Width** checkbox and enter a value for the left and right Bin ranges.
6. Select the **Customize Marker Parameters** checkbox to input specific parameters for the new panel. Select this option to keep stutter-specific values for each marker.

Figure 157 Edit Marker



7. When finished, click **OK**.
8. The new Panel is created and added to the **Panel List.**

   Note: New Panels are created based on the Max & Average View Mode. More intense peaks are given higher priority for Bin placement when peaks do not overlap perfectly.
9. Edit the Markers and Bins as described in the previous section Chapter 6 Panel Editor overview.
10. Follow steps **10-12** above.

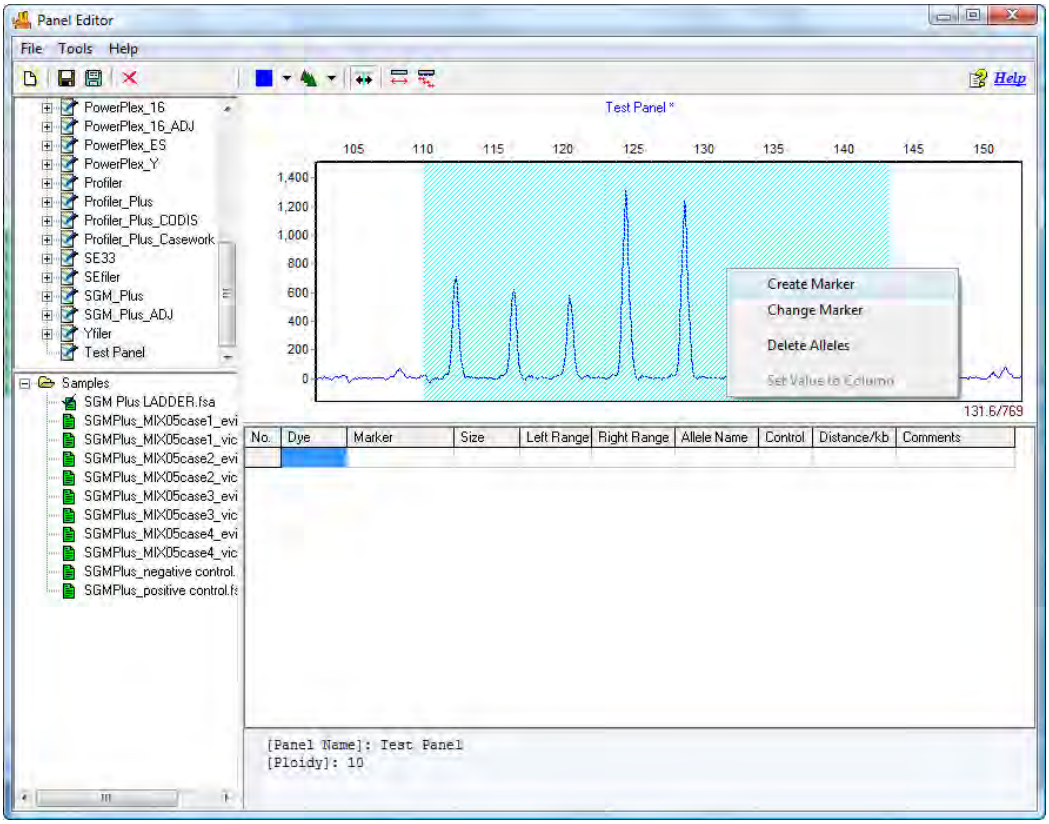
Manual Panel Creation

1. In **Panel Editor**, select **File** > **Create New Panel** from the menu bar or click the **Create New Panel** icon.
2. Enter a Panel name in the **Name** field.
3. Choose the appropriate **Analysis Type** from the **Type** drop-down menu.
4. Select **Manually Create**.
5. When finished, click **OK.**

   The Panel name displays in the Panel List; however, no Markers or Bins are associated with the Panel.

   Follow the steps in the previous Section Chapter 6 Panel Editor to create Markers and Bins.

   Figure 158 Panel Editor Create a Marker



Adjusting Panels

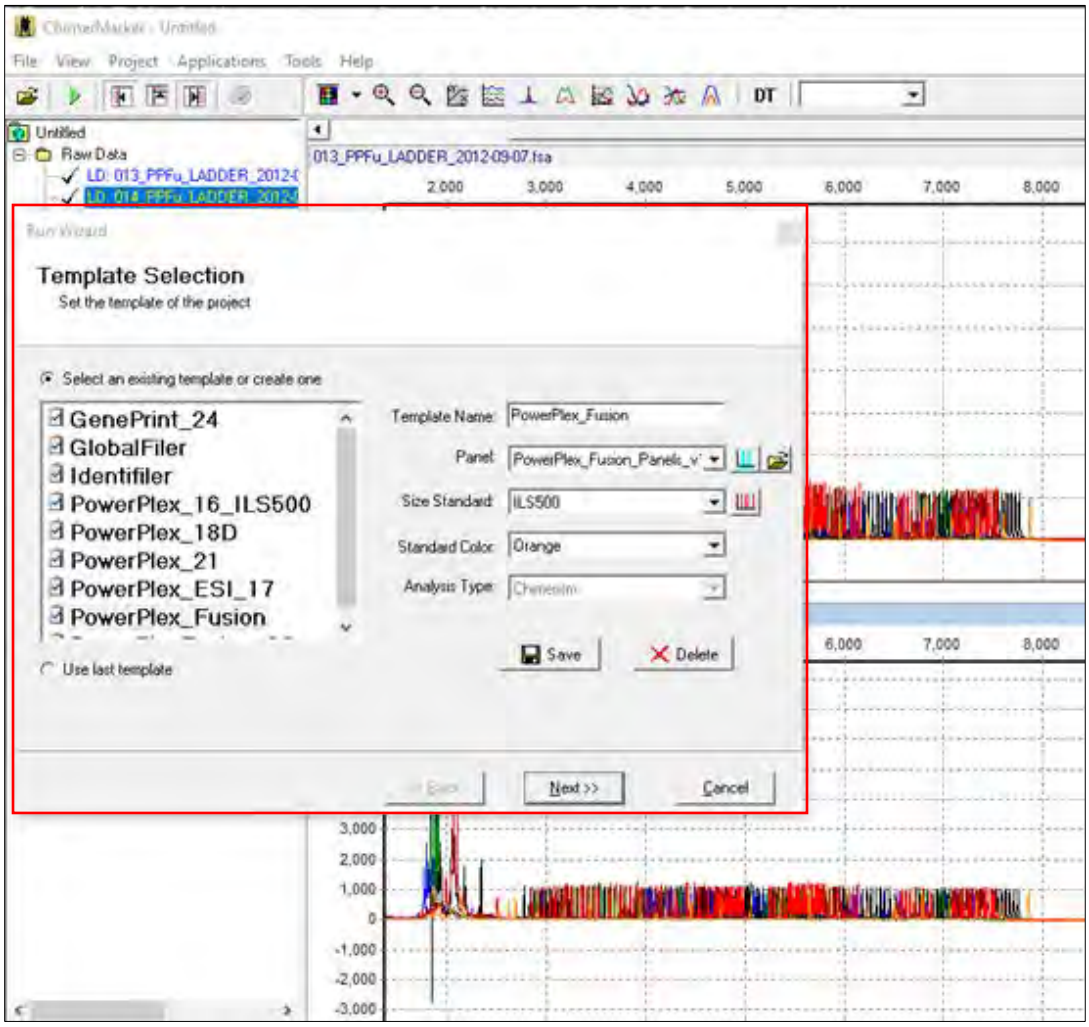
It is common for panel alignment to be shifted due to variations in genetic analyzers or run conditions (such as temperature, injection time).

Automated Genotyping Panel Calibration

Automated Genotyping Panel Calibration is the preferred method to calibrate genotyping panels. Genotyping panels may need to be recalibrated after CE instrument maintenance or if any changes have been made to the injection parameters.

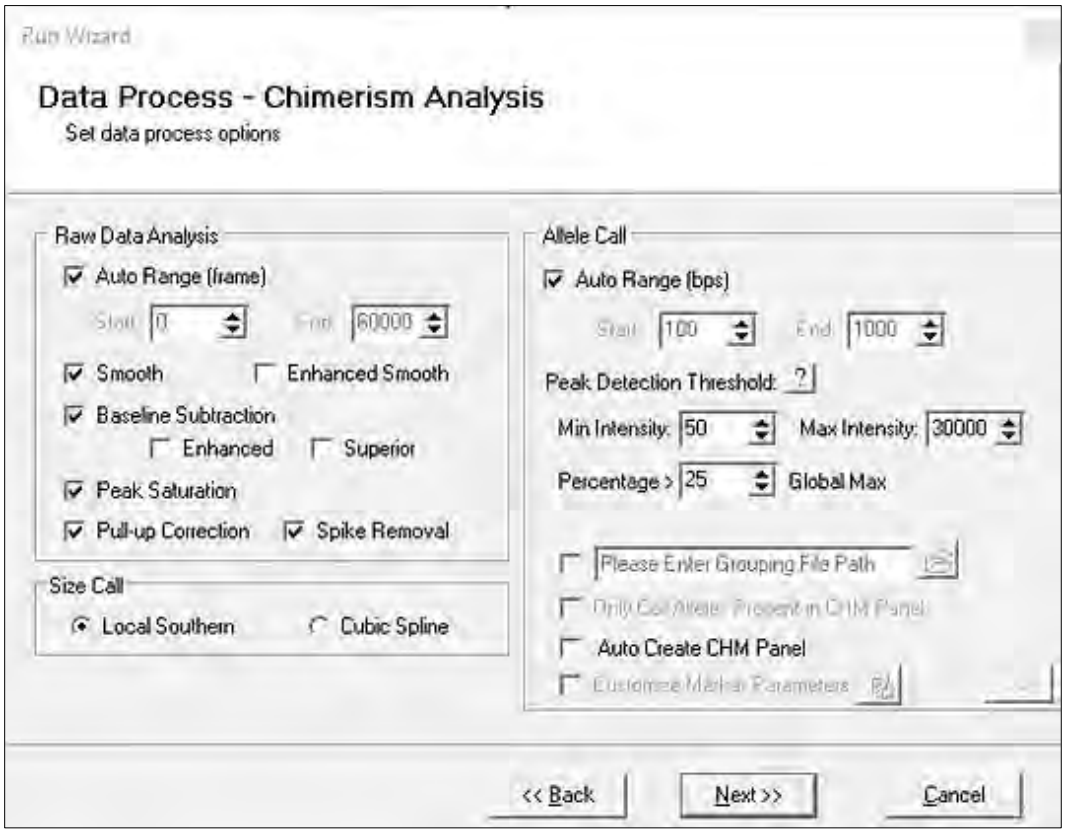
1. Import allelic ladder raw data and activate the **Run Wizard Analysis Template** dialog per the steps provided in Chapter 2 Chimerism Analysis.
2. Select the template appropriate for the chemistry being used and click **Next**.

Figure 159 ChimerMarker Template Selection



The **Data Process Chimerism Analysis** dialog displays.

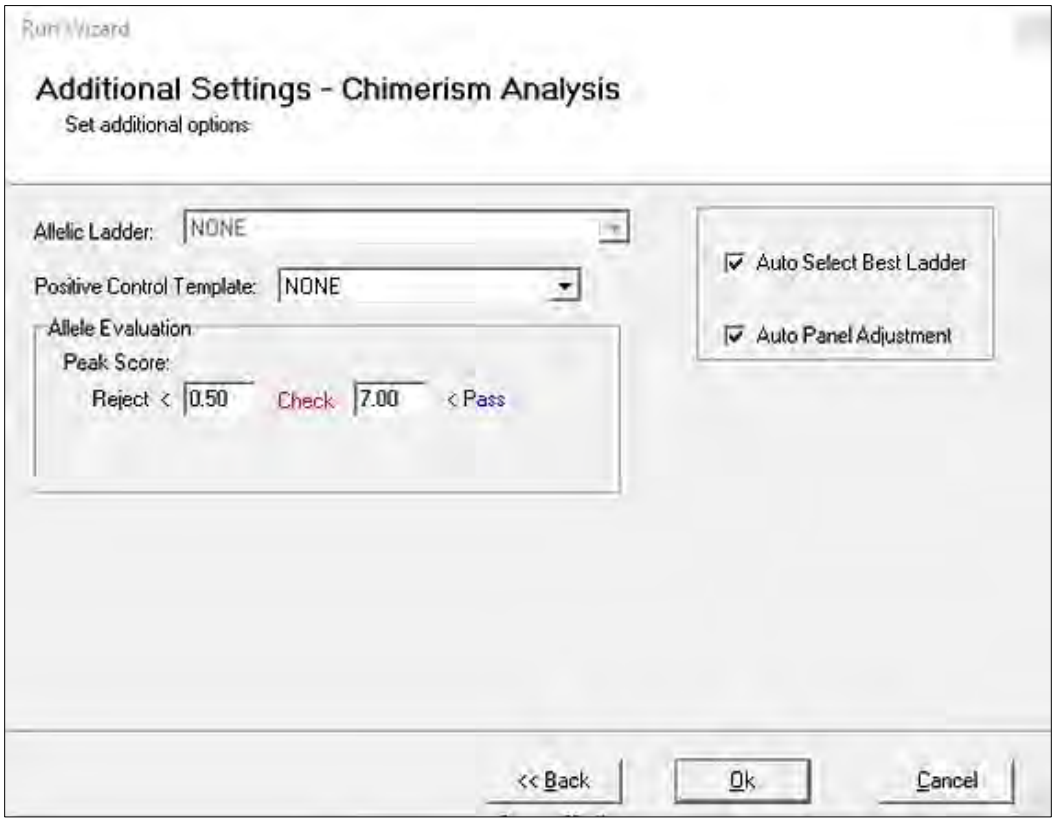
Figure 160 Data Process Chimerism Analysis



3. Uncheck **Auto Create CHM Panel**. This action also deselects and grays out other options, Click **Next**.

The **Additional Settings Chimerism Analysis** dialog displays, as illustrated next

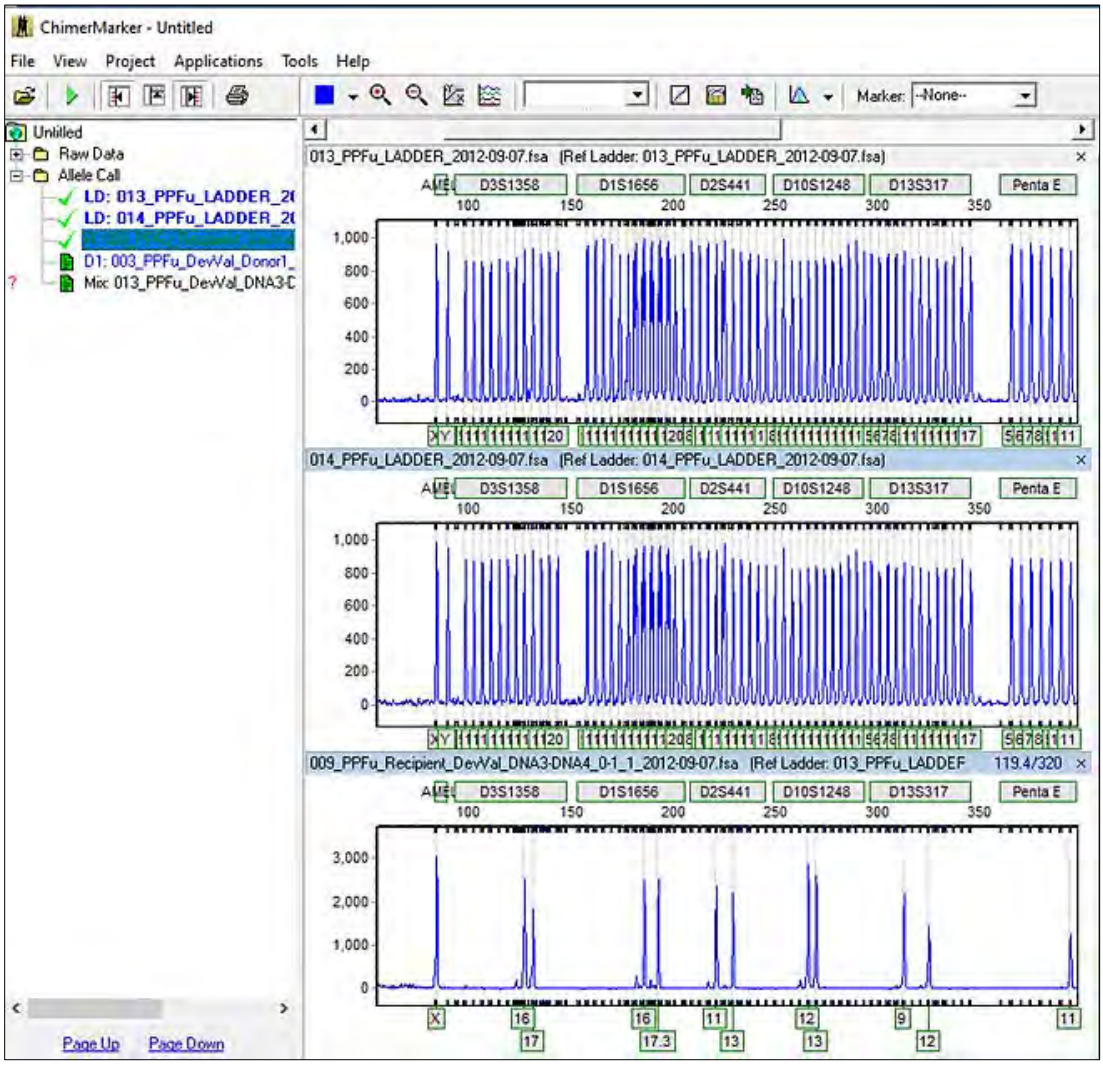
Figure 161 Additional Settings



4. Select both the **Auto Select Best Ladder** and the **Auto Panel Adjustment** checkboxes and click **Ok**.

   The **ChimerMarker** dialog displays.

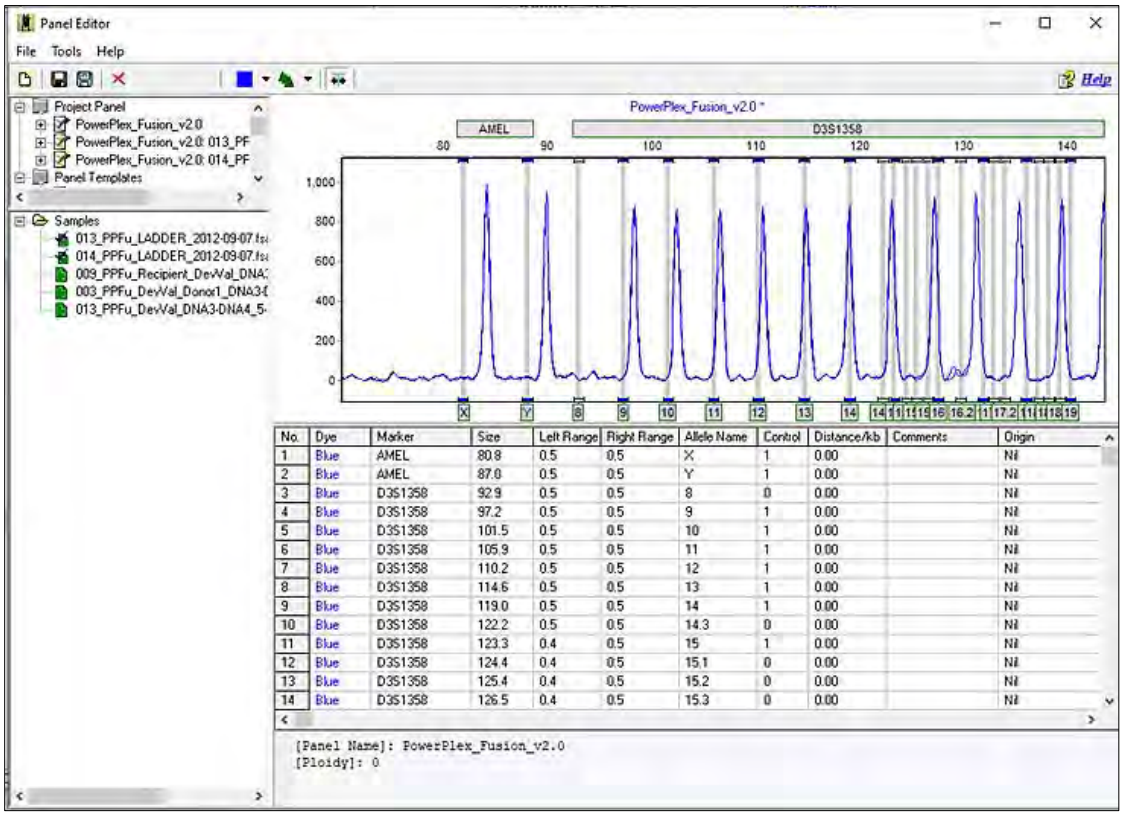
   Figure 162 ChimerMarker Dialog



5. Review the allelic ladders in the main analysis screen. That high-quality ladders are in displayed in **bold** font.
6. In the **ChimerMarker** dialog menu, select **Tools** > **Panel Editor**.

The **Panel Editor** dialog displays.

Figure 163 Panel Editor Dialog 1

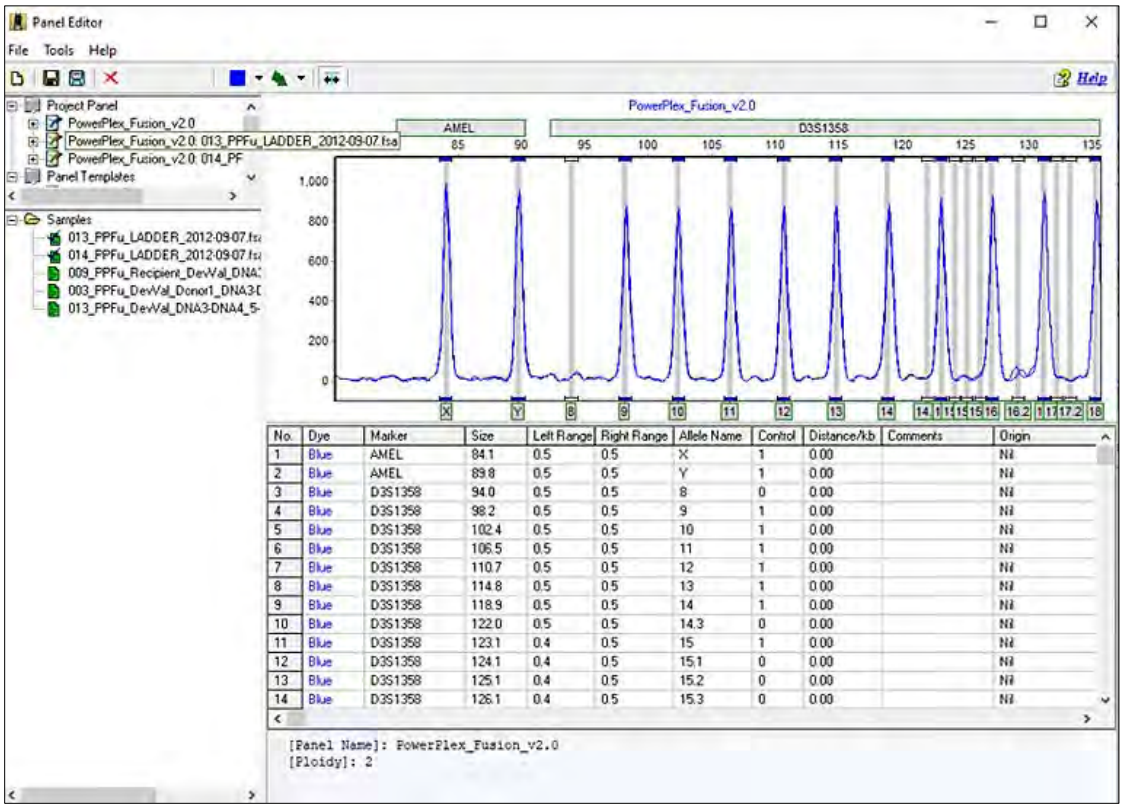


The first file selected at left is not in alignment with the migration of the project panels.

Note: The bins are not aligned in the electropherogram. This is because the migration shifts are expected from one (1) instrument and polymer to another.

7. Select one of the passing ladder files from the **Project Panel** list, not the panel file has been adjusted (calibrated) to the migration pattern of the allelic ladder from the lab.

Figure 164 Panel Editor Dialog 2



8. Select the **File > Save as > New Panel**.

   The **Input Dialog** displays.

   Figure 165 Input Dialog

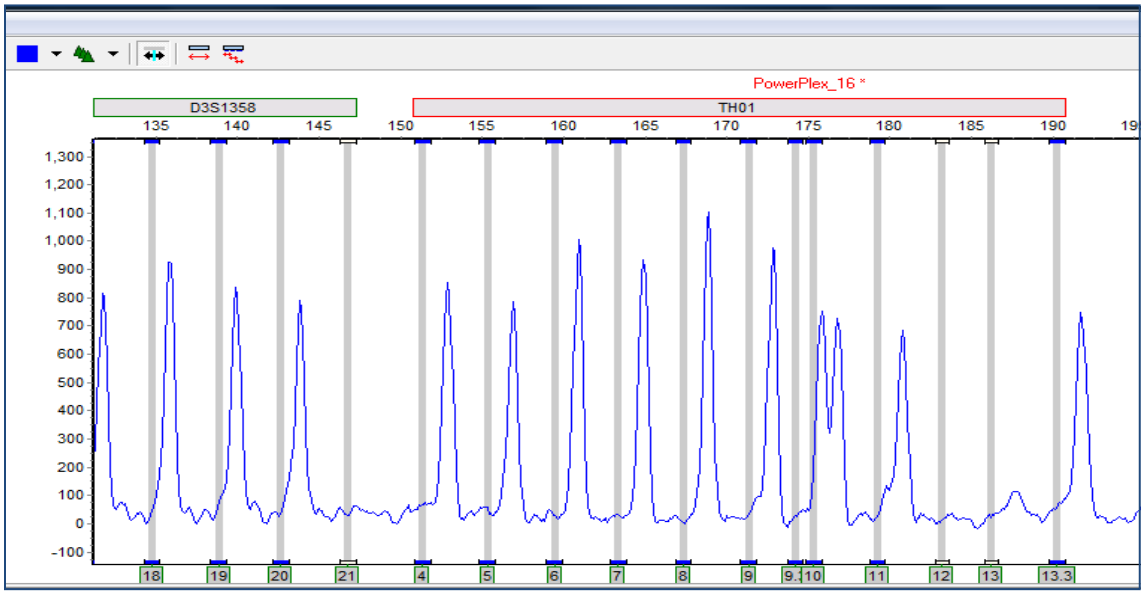
9. In the **New panel name** field, enter the name. Typically, the date and instrument type are included in the calibrated panel name.

   Use this calibrated panel in the analysis procedure detailed in Chapter 2 Chimerism Analysis.

Manual Genotyping Panel Calibration

Markers or bins are manually aligned to the allele ladder using the shift and mouse key.

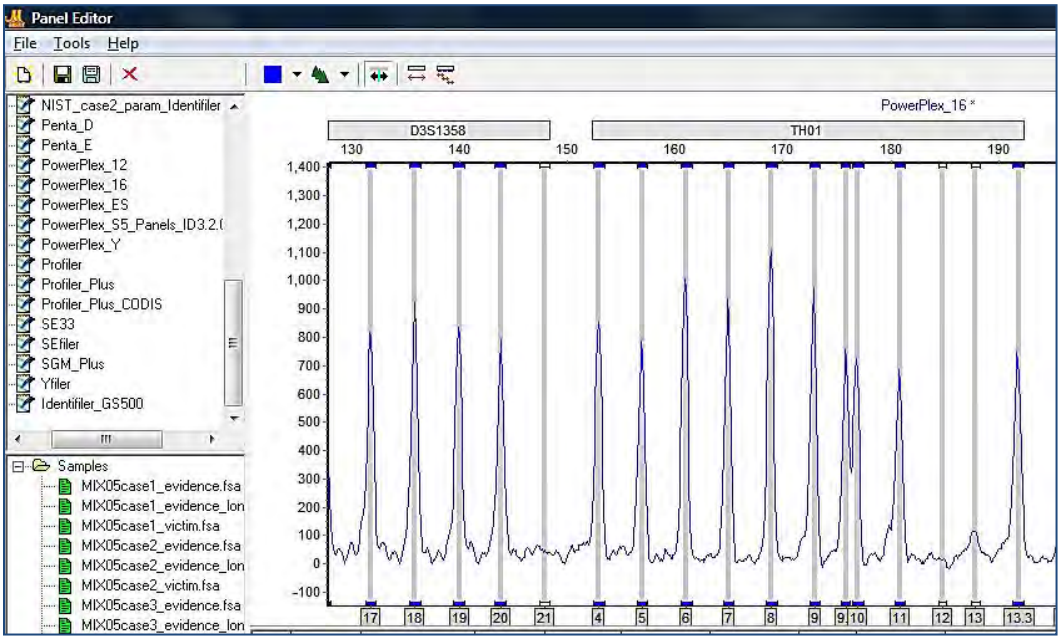
Figure 166 Adjusting Panels Manually - Before



Align all the bins within a marker, as detailed next:

1. Hold down the **SHIFT** key.
2. At the same time place the mouse over the gray marker name bar at the top of the electropherogram.
3. The marker rectangle is outlined in red and the panel name is in red font when the adjust feature is active.
4. Drag the marker to align the bins with the peaks of the allelic ladder.

Figure 167 Adjusting Panels Manually - After

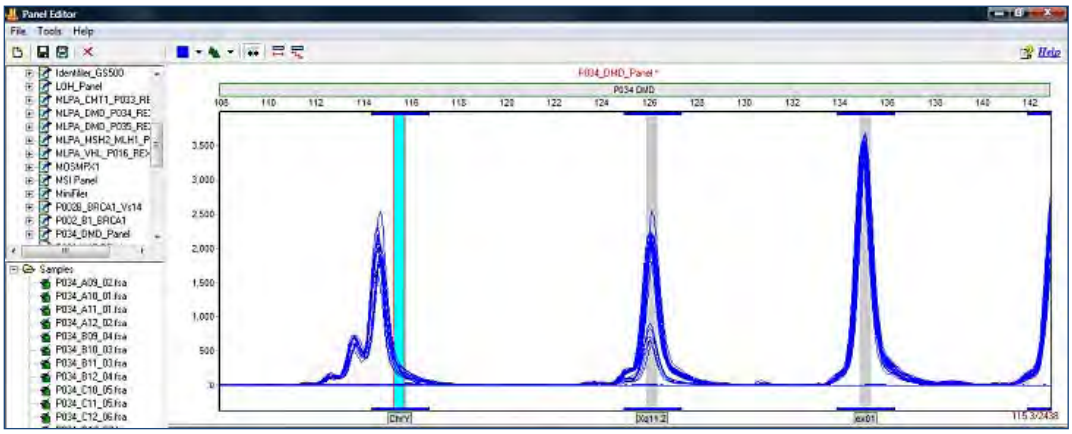


5. Save the panel with signal information to enable the major panel adjust feature to work in future projects. Use the turquoise **Save** icon.

Align an Individual Bin

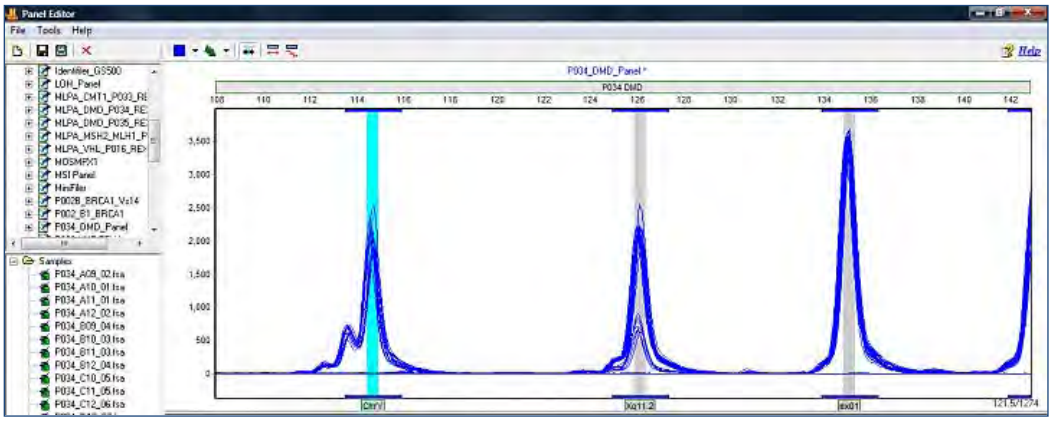
1. Select the gray vertical bar of the bin with the mouse. The bar turns blue.

   Figure 168 Adjusting Alignment of Individual Bins - Before



2. Hold down the **SHIFT** key and click on the gray vertical bar for the bin.

Figure 169 Adjusting Alignment of Individual Bins - After



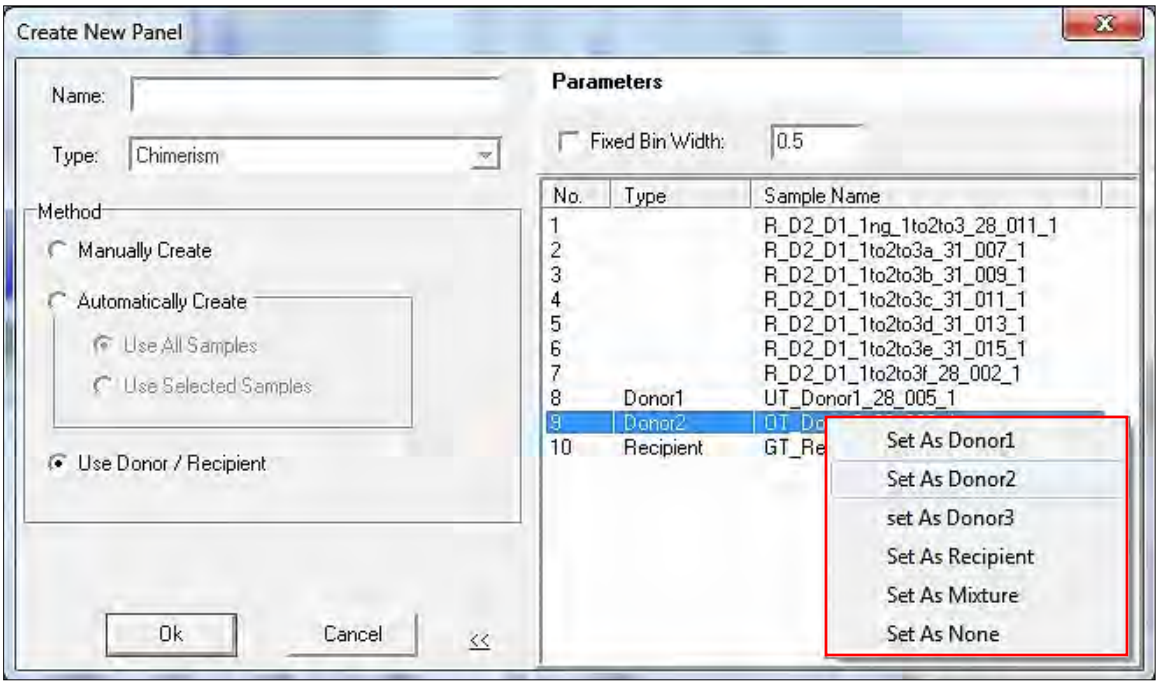
3. The vertical bar is outlined in red and the panel name is in red font.
4. Use the mouse to drag the gray vertical bar to the center of the peak.
5. Save the aligned panel with signal information.

Genotyping and Chimertyping Panels

ChimerMarker includes two (2) types of panel files: Genotyping and Chimertyping. As described above, genotyping panels contain all loci and alleles, which may be amplified by a given human identification chemistry (such asPowerPlex16 or PowerPlex ESI/ESX).

Chimertyping panels contain only the alleles of the donor(s) and recipient. If the selection is made to automatically construct a Chimertyping panel in the second screen of the run wizard, the program uses the allele calls of the pre-transplant samples to construct the Chimertyping panel for the patient. If preferred, these panels are easily constructed in the panel editor after the genotyping panel is applied to the project.

Figure 170 Alternative Method to Create Chimertyping Panel



File > Create Panel

In the **User Donor/Recipient** checkbox, select **OK** to automatically create a case specific Chimertyping panel. If a naming convention is not used to designate donor and recipient files, the designation may be made here by the user. Right click on the file name and set as the appropriate file type.

Applying a file type automatically creates a Chimertyping panel with the alleles found in all of the participants of the study (donors and recipients). When used in the chimerism analysis (see Chapter 2 Chimerism Analysis), the panel calls only those peaks that are consistent with the donor(s) and recipient alleles.

In addition to the columns discussed above for the genotyping panel table, Chimertyping panels have an Origin column. This documents the individual(s) that were the sources of these allele calls and is used to label the peaks in the electropherograms of mixture (post-bmt) samples in chimerism projects. See Chapter 2 Chimerism Analysis.

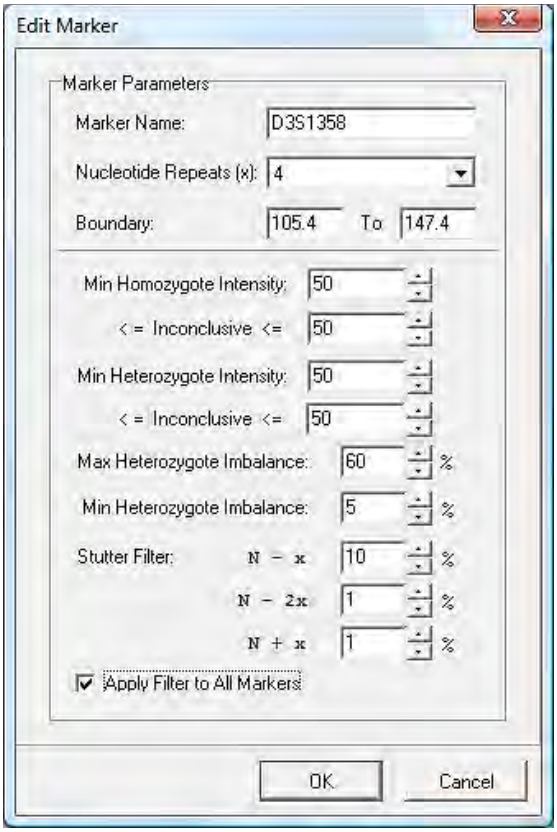
Editing a Chimertyping Panel

It is very likely that some of the peaks are minor peaks (only as small percentage of the RFU (height) of the major peaks. To ensure these minor peaks are not missed it is essential to set the minimum percentage to be accepted as a peak during the analysis. After the Chimertyping panel is created follow the next steps:

1. Select the right mouse-click on one of the Marker (Loci) labels above the electropherogram and select **Edit Marker**.

The **Edit Marker** dialog displays.

Figure 171 Edit Marker



2. Enter the appropriate value into the **Min. Heterozygous Imbalance** dialog.
3. Select the **Apply Filter to all Markers** checkbox to set this heterozygous imbalance to the same level in each marker or if your lab has determined that certain loci have different ranges, type in the appropriate value for each locus.
4. Save the panel with a name that indicates the study/case number and Chimertyping.

   Typical Human Identification Genotyping panels use a 20% minimum heterozygous imbalance filter. If minimum heterozygous imbalance is set to 20%, minor peaks must be at least 20% of the height of the major peak at that locus. For chimerism detection and monitoring it is necessary to detect minor peaks early on in studies.

   Set the Min. Heterozygous filter to the appropriate level for your study. In this example, the filter is set to 5% -- any peak that is 5% or more of the height of the tallest peak at the locus is called. Peaks that are **less than 5%** of the taller peak height **are not** called. To ensure that all peaks outside the stutter position are called, set the minimum heterozygous imbalance to one (1).

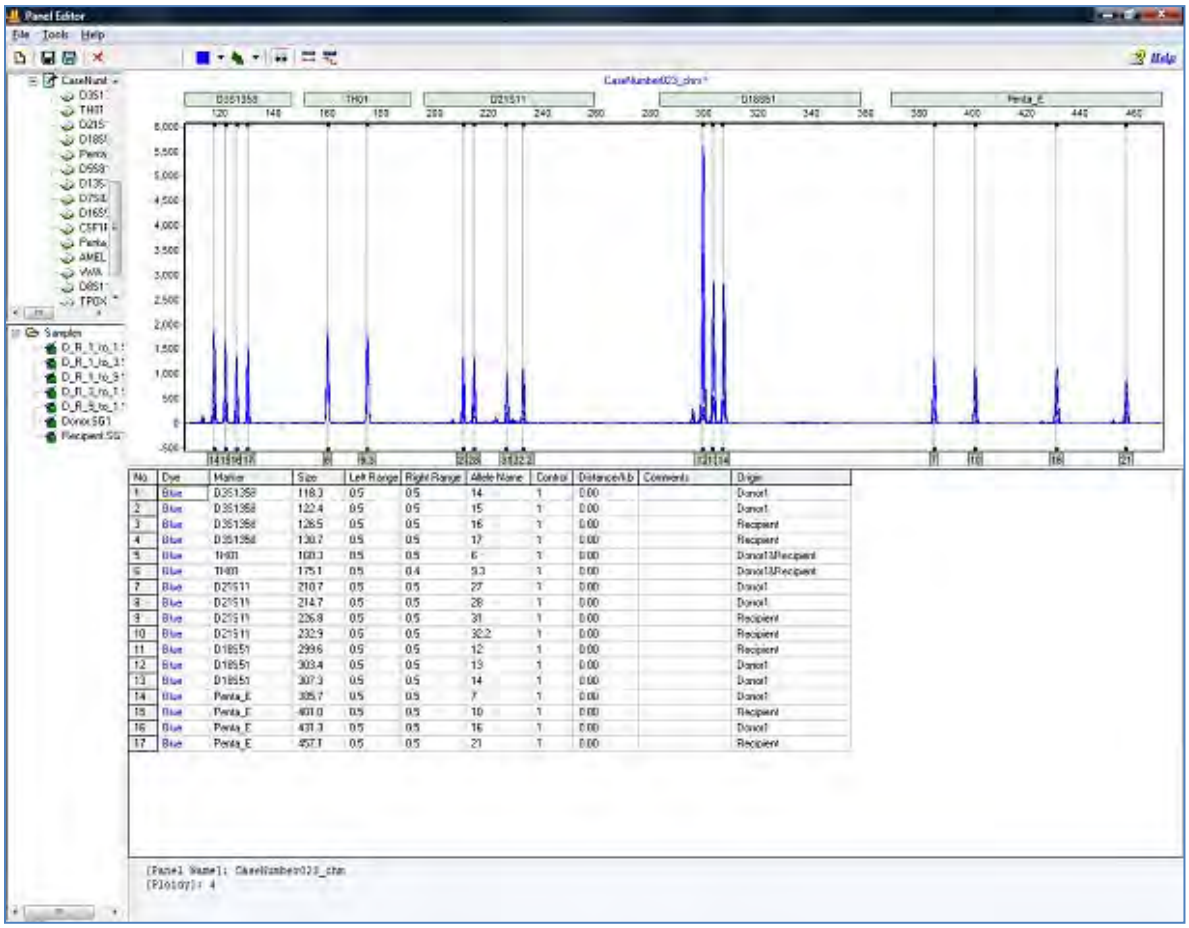
Options, Functions and Icons

The following are explanations of menu and icon options in Panel Editor.

Menu Options

The Panel Editor contains three (3) menu options including **File**, **Tools**, and **Help**.

Figure 172 Panel Editor



The **File** menu allows the user to create, save, and export panels. The **Tools** menu contains options for datasets with allelic ladder samples and exporting a panel. The **Help** menu contains navigation hints for **Panel Editor**.

File Menu Create New Panel

Launches the **Create New Panel** dialog 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 list.

- **Save Changes**

  Saves edits and changes to the Panel in the SoftGenetics ChimerMarker Panel directory. (**Hot Key = CTRL+S**)

- **Save as New Panel**

  Opens the Input Dialog with a field to enter a new Panel name. The Panel is added to the Panel List and saved in the SoftGenetics ChimerMarker Panel directory.

- **Import Panels**

  Opens a Windows Explorer dialog to the same folder the sample files were uploaded from. Use the **Import Panels** option to find previously exported Panel Files (.xml) on local or networked computers.

- **Import Pre-Defined Panels**

  Opens the SoftGenetics ChimerMarker Pre-Defined Panels folder.

- **Import ABI Panels**

  Launches the Import Panels from GeneMapper dialog. Opens Panels and Bins Text files and converts them to single Panel files in XML format for use in ChimerMarker.

- **Export Panel**

  Exports the currently selected Panel in the Panel List as an XML file to a specified directory on a local or network computer.

- **Exit**

  Closes the Panel Editor tool. Save changes to the Panel before exiting.

Tools Menu Match Ladder

Opens the **Select Ladder** dialog. Choose an allelic ladder sample from the drop-down menu. Click **OK** and the Panel adjusts slightly to align with the peaks in the selected ladder sample.

Note: Large differences between peak and Bin position cannot be resolved with the Match Ladder function.

- **Virtual Panel**.

  d. Select a Panel from the **Panel List** and click **Virtual Panel** in the **Tools** menu. The **Create Virtual Panel** process dialog displays.

  e. Click **OK** and a new Panel is added to the Panel List labeled VPanel_PanelName. This newly created Panel is an adjusted version of the original panel selected in the Panel List. ChimerMarker attempts to align the original Panel to the Ladder sample peaks based on an average calculation.

  Note: It is recommended to use Virtual Panel only when small adjustments to the Marker and Bin placement are required. Select the **Major Panel Adjustment** icon for larger adjustments.

- **Export the Project Panel**

  Exports the currently selected Panel in the Panel List as an XML file to a specified directory on a local or network computer.

- **Help Menu**

  The **Help** menu contains a link to Hot Keys in Panel Editor. Click **Hot Keys** and the **Panel Editor Action Help** dialog display.

  Figure 173 Panel Editor Action Help

Toolbar Icons Panel Editor

The toolbar icons are detailed in the next table.

Table 13 Help Menu Toolbar Icons

Icon	Name	Function
	Panel Editor	Found in the **Run Wizard Template Selection** dialog or the **Tools** menu.
	Create New Panel	Opens the **Create New Panel** dialog. Follow the steps in the **Create a Custom Panel** section above.
	Save Changes	Permanently saves Panel edits to the currently opened Panel file which is located in the SoftGenetics ChimerMarker Panel directory.
	Save Changes with Signal Info	Permanently saves all Panel edits, including height information which is used with the Major Panel Adjustment feature. **Note**: A Panel must be correctly aligned with peaks in the dataset before selecting **Save Changes with Signal Info** in order for the **AutoPanel Adjust** in the Run Wizard and the Major Panel Adjustment features to work correctly.
	Delete Current Panel/Marker	Deletes whichever Panel or Marker is currently highlighted in the Panel List. This action is irreversible.
	Show Dye	Allows the user to select a single dye color to view in the Overlay View. Cycle through the colors by left clicking on the icon.
	Trace Mode	Single left-click to cycle through the options or use the drop-down menu.
	Trace Overlay	Displays all traces of the selected samples in the Samples List one dye color at a time. Single click any trace in the **Trace Overlay** frame and the trace is come bold and the associated sample is highlighted in the Sample List.
	Max & Average	Displays two (2) traces in the electropherogram. The darker color line corresponds to the maximum peak height at that position and the lighter color line corresponds to the average of all selected sample traces at that position.
	Gel Image	Displays selected samples as a synthetic gel image. Bin ranges in the Gel Image mode appear as white vertical lines and are manipulated by holding down **SHIFT** and dragging the white lines left or right.

Icon	Name	Function
	Check Range in Edit	When activated, the software warns the user if they set the left or right range of an allele to overlap with another allele. This feature will prevent the user from setting allele boundaries too close to neighboring alleles. This option is selected by default.
	Major Adjustment of Panel	Uses previously defined size and height information located in the Panel file when the panel was previously saved with signal information, to identify Marker and Bin positions. To be used when a Panel must be adjusted by 1 - 5 base pairs in order to align with the dataset peaks. Best used for panels with evenly spaced alleles.
	Minor Adjustment of Panel	Aligns the center of the Bin to the center of the nearest peak (within one base pair of the Bin). **Note:** use adjust icons only if there are no variant allele bins in the panel.

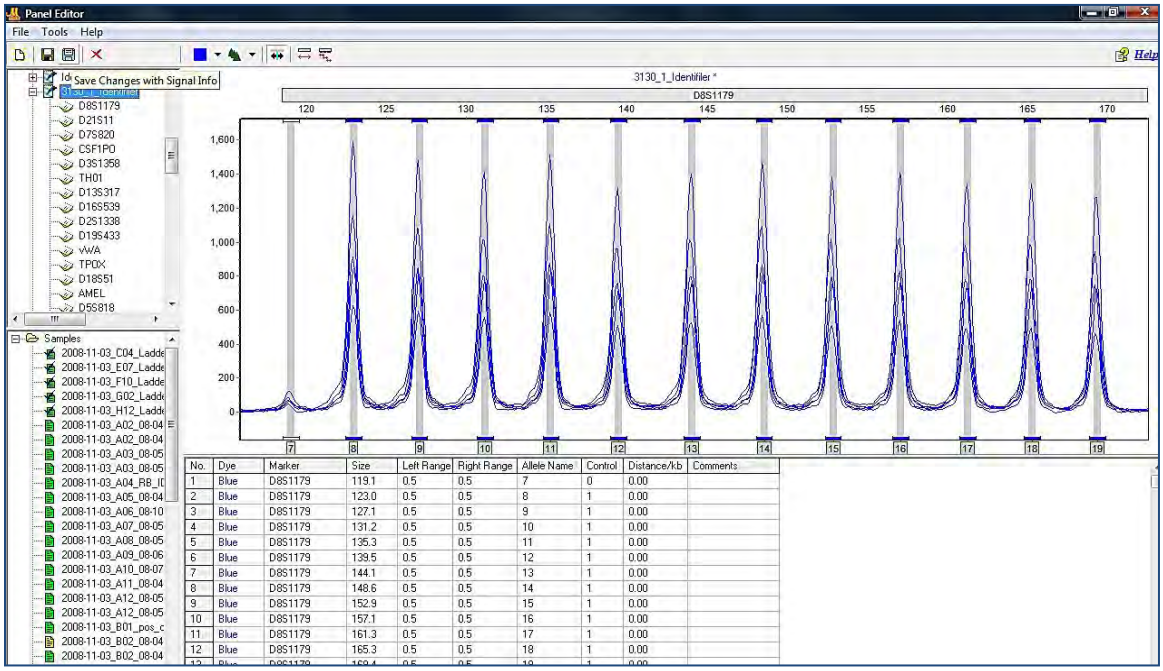
Saving a Panel with Signal Information

For optimal automatic panel adjustment, a panel should be adjusted manually the first time and then saved with signal information from several ladder samples from the same genetic analyzer and used for analysis of any samples from that analyzer.

To manually adjust alignment, hold down the **SHIFT** key and use the mouse to shift all of the bins for a marker with the entire gray horizontal bar containing the marker name; or, to shift individual bins hold down the shift and use the mouse to move the vertical bar into place. This signal information is used to adjust to any normal variation of different runs from the analyzer.

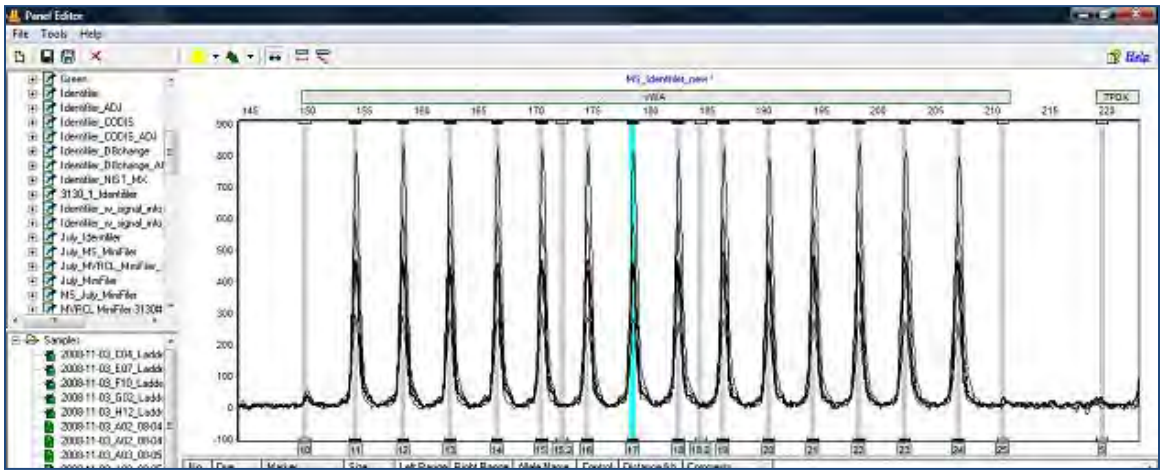
The Panel Editor is displayed next.

Figure 174 Panel Editor 1



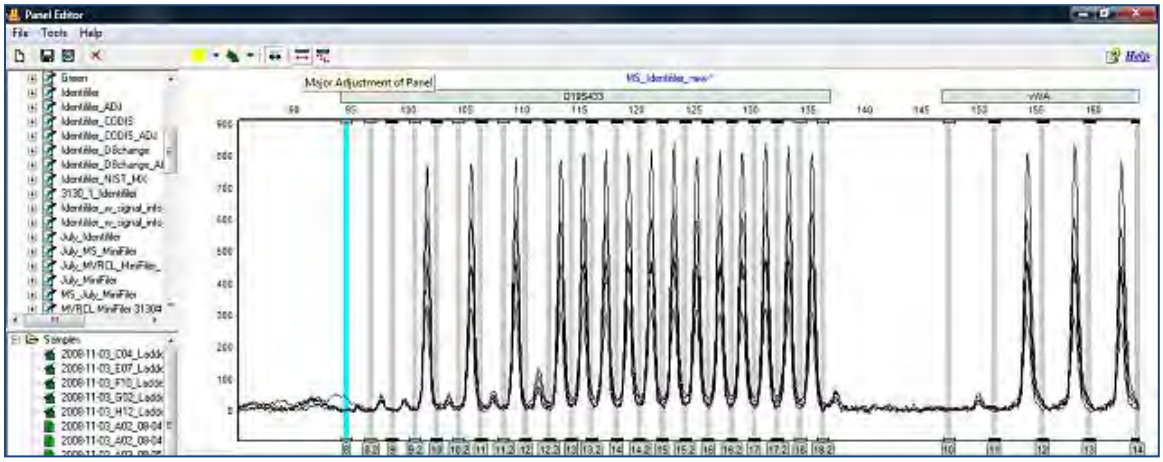
For example, if the laboratory routinely uses two 3130 genetic analyzers (3130_1 and 3130_2) there would be a panel saved with ladder signal from each genetic analyzer.

Figure 175 Panel Editor 2



Saving the panel with signal information from the ladders provides the program with the information needed to automatically adjust the panel with subsequent data.

Figure 176 Panel Editor 3



A common occurrence is a difference due to run time variation, causing a shift in the peaks of 1-5 base pairs. The major panel adjustment icon is used to automatically align the panel to the current ladders.

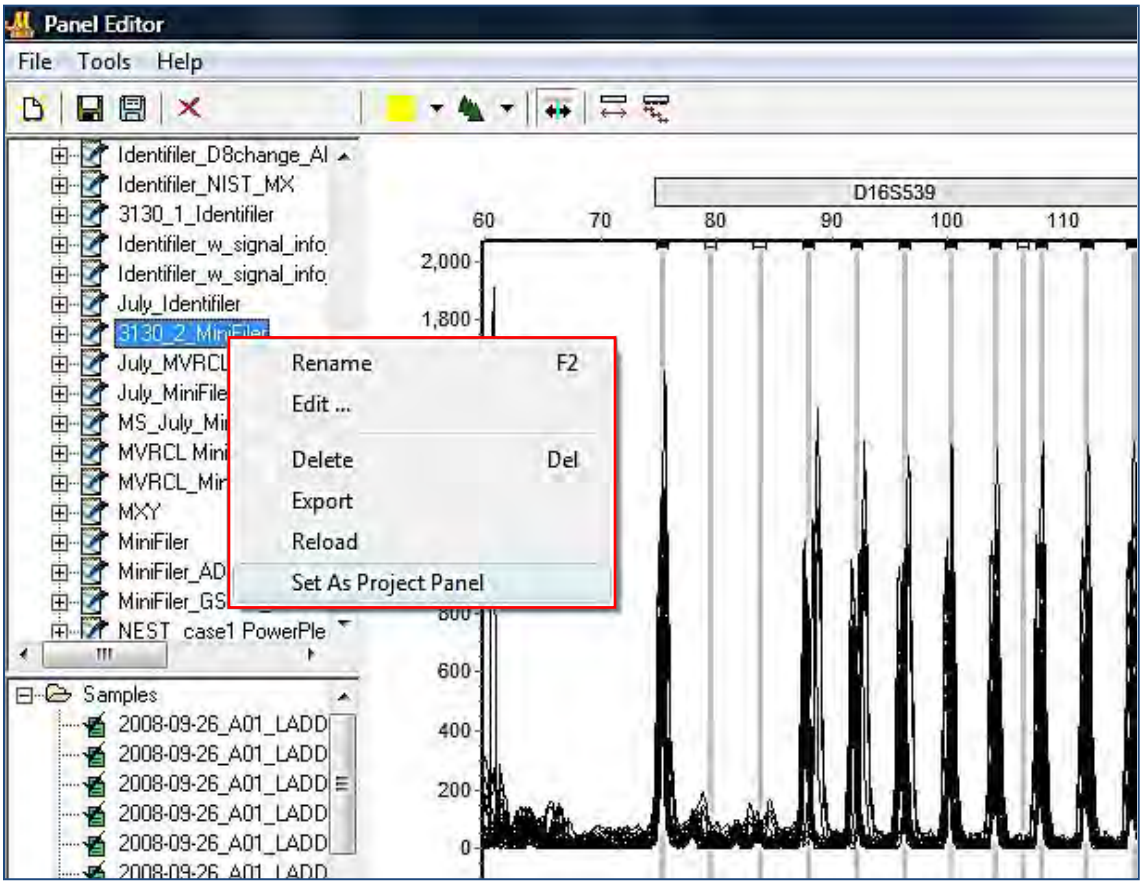
Project Panel

Once a panel is aligned and saved with ladder signal information, it is set as the project panel. This panel has signal information and is used to automatically adjust to multiple ladder files in a project.

1. Right click on the panel name in the list of panels and select **Set As Project Panel**, as illustrated in Figure 177.

The panel name is displayed at the top of the panel list under the heading **Project Panel**.

Figure 177 Panel Editor Popup Menu



2. Exit from the panel editor and the project panel is applied to the project.

What to Expect

Once a Panel is created, aligned and saved with signal information, it is applied to the dataset. Save the edited Template Panel with signal information in Panel Editor, then exit the Panel Editor.

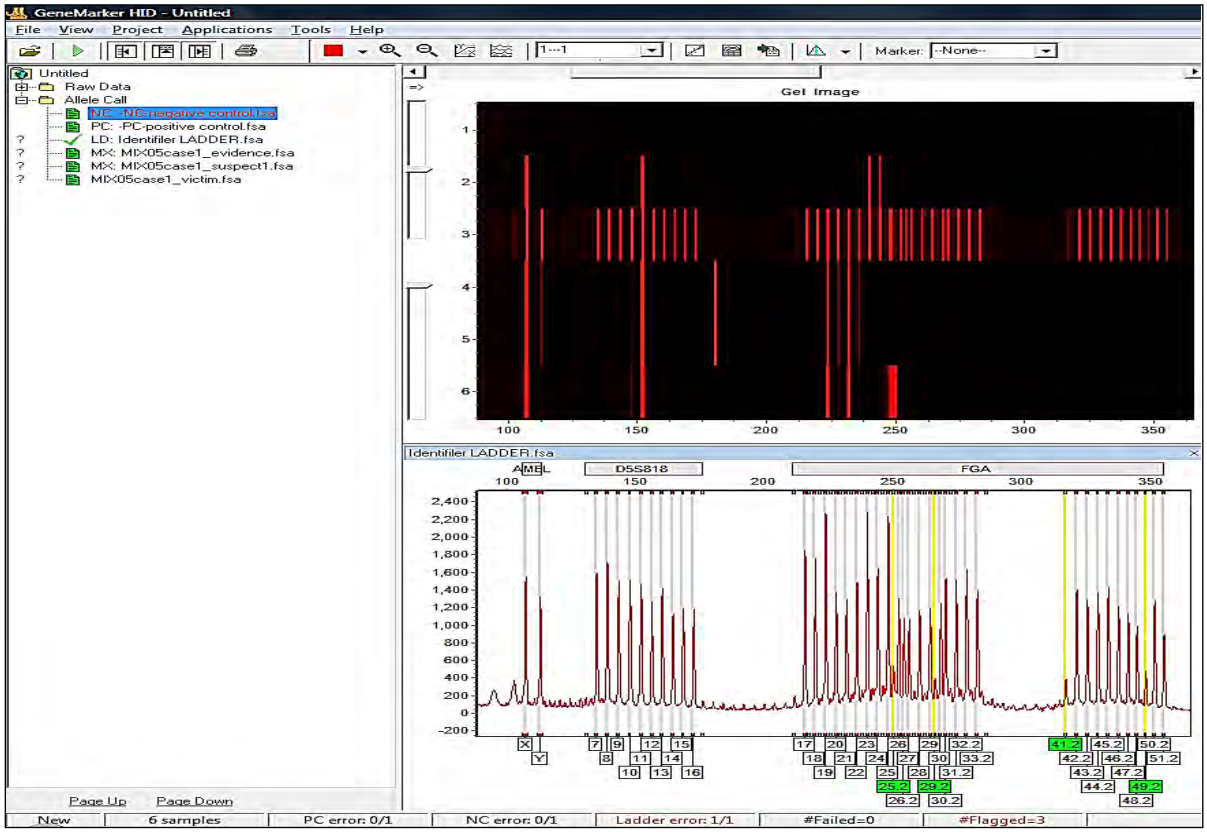
- If the Panel Editor is accessed using the **Run Wizard Template Selection** icon, then the selected panel displays in the **Panel** field.
- If the Panel Editor was accessed through the **Tools** menu, then click the **Run Process** icon in the **Main Analysis** toolbar and the Run Wizard displays.

Follow the next steps to apply the panel.

1. Select the Panel from the **Panel** drop-down menu in the **Run Wizard Template Selection** dialog.
2. Proceed through the other Run Wizard dialogues and click **OK** when the **Data Process** dialog is complete. The Panel is applied.

The summary bar at the bottom of the main analysis screen displays information about the results. Ladder errors may be flagged with a yellow vertical bar and green allele label to alert the analyst if a non-control, minor peak is higher than expected (Figure 178). If any peaks are not in their expected bin, they are flagged with red allele labels and red vertical bars (Figure 179). The analyst can return to the panel editor to determine if there were problems with the capillary for any ladder.

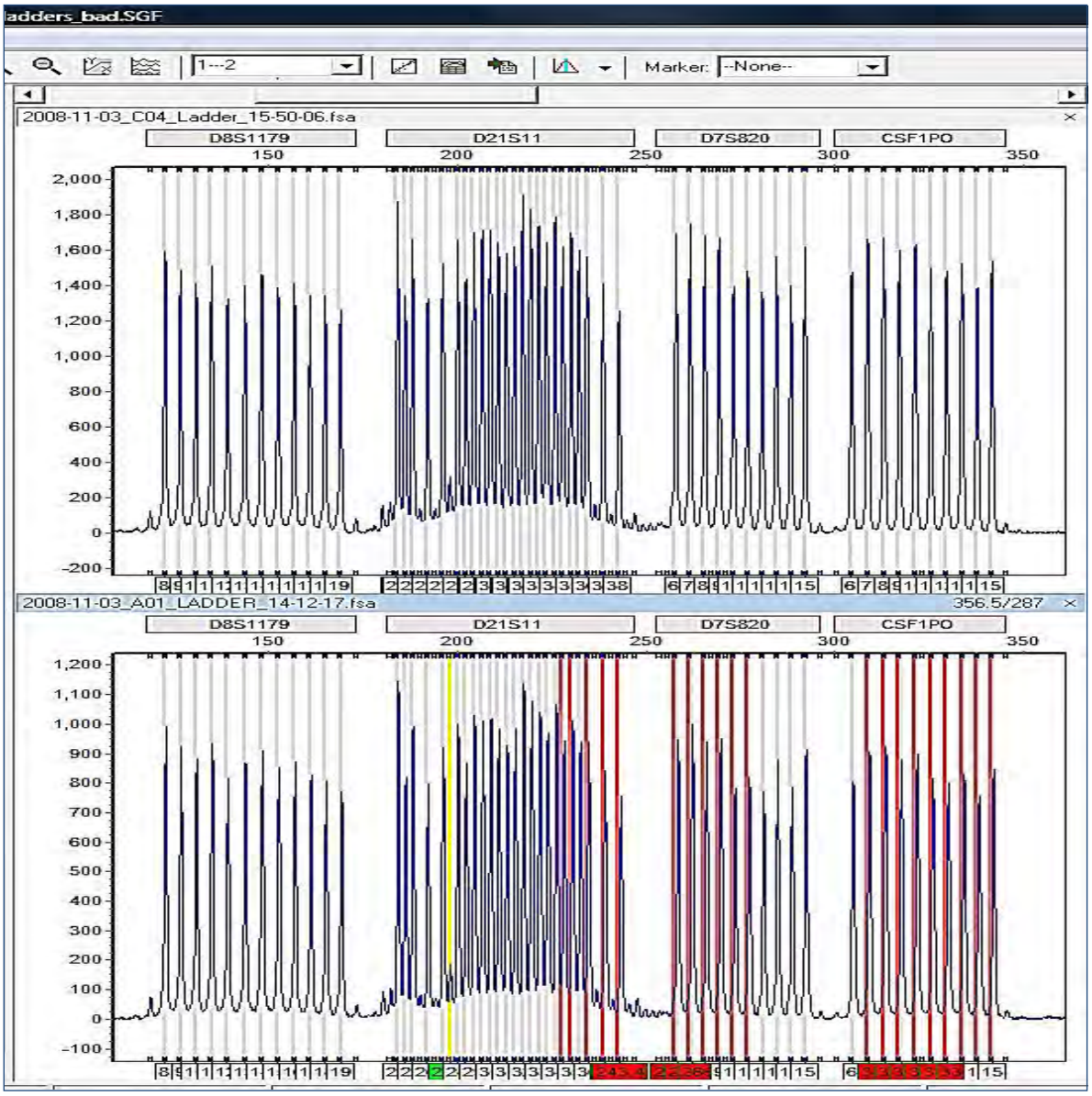
Figure 178 ChimerMarker Main Dialog



The analyst is alerted through yellow and green flagging that although the panel is aligned with the ladder bins, some of the minor peaks in the ladder have a higher RFU than expected.

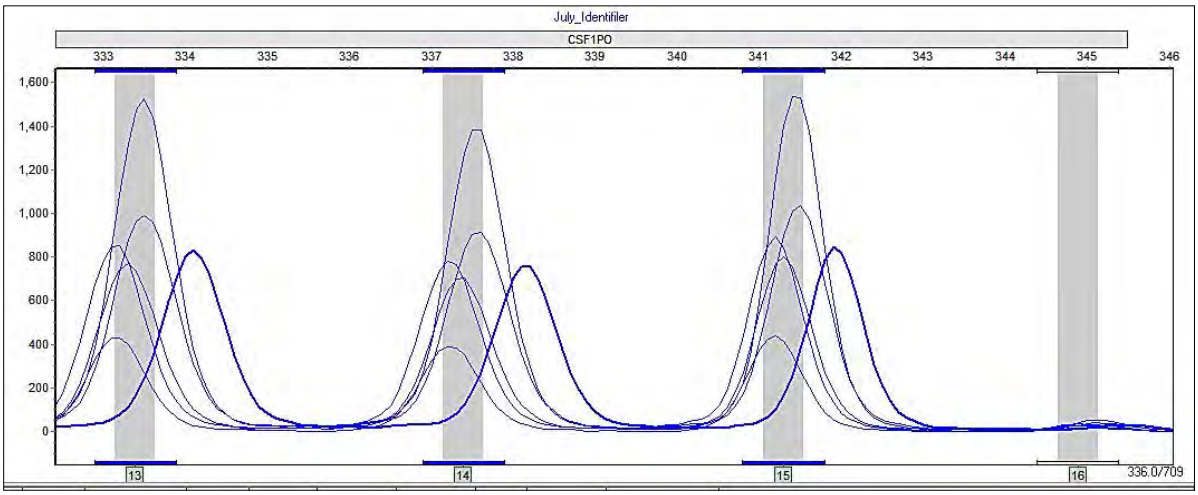
See Figure 179 for an illustration of the display.

Figure 179 Ladders



One of the ladder samples in this run has peaks that are out of the bins. These out of bin peaks are flagged with red allele label and red vertical bars.

Figure 180 Trace Overview



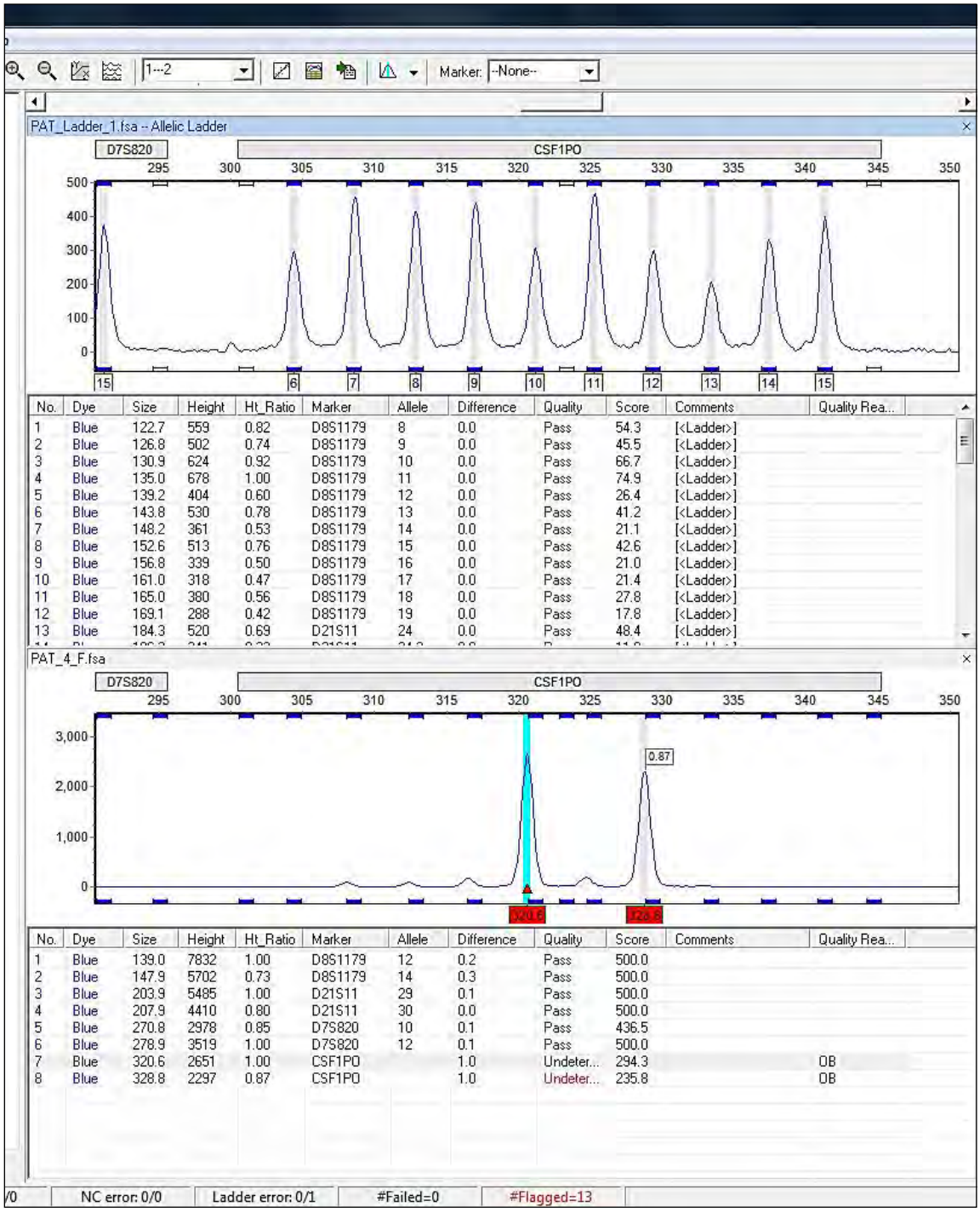
The trace overlay view in the panel editor shows the automatic adjustment of the panels to the ladder samples, illustrating that the ladder file in error is an outlier. The analyst may elect to disable this ladder sample in the **Main Analysis** dialog.

After the Panel is applied to the dataset, the **Markers** and **Bins** appear in the Electropherogram and Report Table.

- In the Electropherogram, the Markers are horizontal grey bars, the Bins appear as dye-colored brackets above and below the trace, and the center of the peaks are marked with a vertical grey bar.
- Peaks that fall outside of the Markers are marked **Off Ladder** (OL).
- Peaks that fall within the marker but outside of a bin are marked OB.

The allele ladder that best matches a sample file is used by the **Auto Select Best Ladder** in the Run Wizard.

Figure 181 Auto Select Best Ladder



The file name of the ladder used for a sample is displayed in the allele report. See Chapter 7 Reports and Printing.

Chapter 7 Reports and Printing

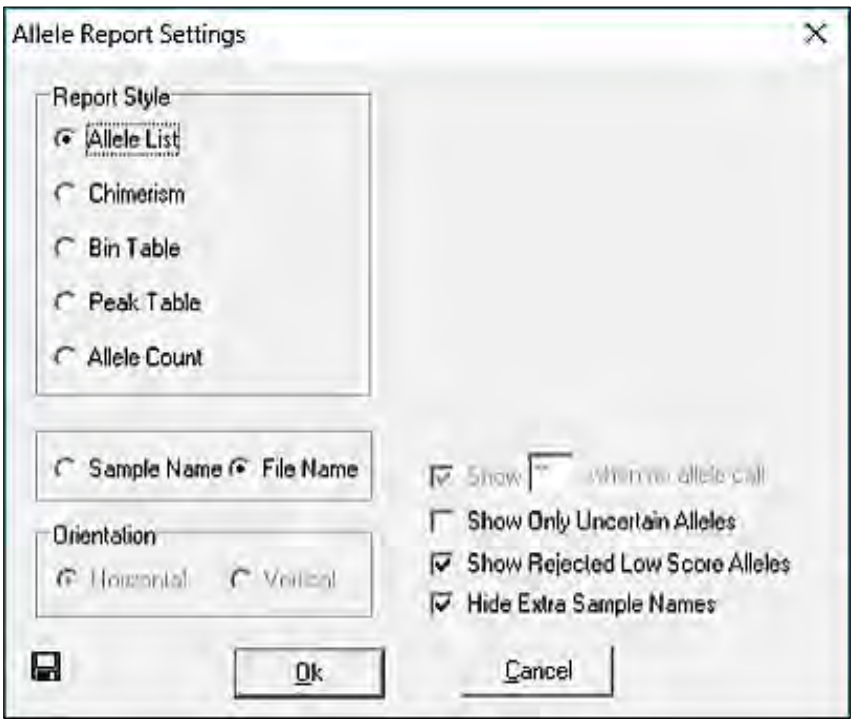
Report Table

The general features of the Report Table were outlined in Chapter 4 Genotyping Main Analysis Overview**.** In this section each Report Style available in the Report Table is discussed.

Allele List Reports

The Allele List Report Style displays the base pair size (or Allele Label, if a Panel is applied) of the called peaks.

Figure 182 Allele Report Settings



The sample names are listed in rows in the far-left column and peaks are numbered in columns at the top of the table.

Features

Report sample by Sample Name or File Name.

- **Show Only Uncertain Alleles** checkbox

  When selected, displays only the peaks with Quality ranks of **Check** (yellow) and **Undetermined** (red).

  Figure 183 Report Sample - Allele List

		AL #7	AL #8	AL #9	AL #10	AL #11	AL #12
1	051_G03.SCF	331	401	479	491	OL	128
2	052_A04.SCF	411	479	495	OL	120	141
3	052_B04.SCF	327	401	411	479	495	OL
4	052_H03.SCF	411	479	495	OL	OL	178
5	061_B05.SCF	401	403	479	491	OL	124
6	062_C05.SCF	467	479	124	178	182	281
7	063_D05.SCF	399	424	487	491	OL	124
8	064_E05.SCF	399	415	487	OL	124	136
9	065_F05.SCF	401	418	483	OL	128	132
10	066_G05.SCF	405	418	483	OL	132	136
11	067_H05.SCF	483	OL	128	136	182	186
12	068_A06.SCF	405	415	483	OL	132	136
13	069_B06.SCF	401	411	467	483	OL	124
14	539_H05.SCF	405	411	467	479	OL	120
15	819_A06.SCF	397	401	479	487	OL	128
16	819_B06.SCF	257	323	327	397	401	479
17	820_C06.SCF	406	409	479	483	OL	128
18	820_D06.SCF	406	409	479	483	OL	OL
19	821_E06.SCF	254	323	405	422	483	OL

- **Show Rejected Low Score Alleles**

  When selected, the peaks with peak scores below the **Run Wizard Additional Settings** the **Allele Evaluation Peak Score Reject** setting is displayed in the table.

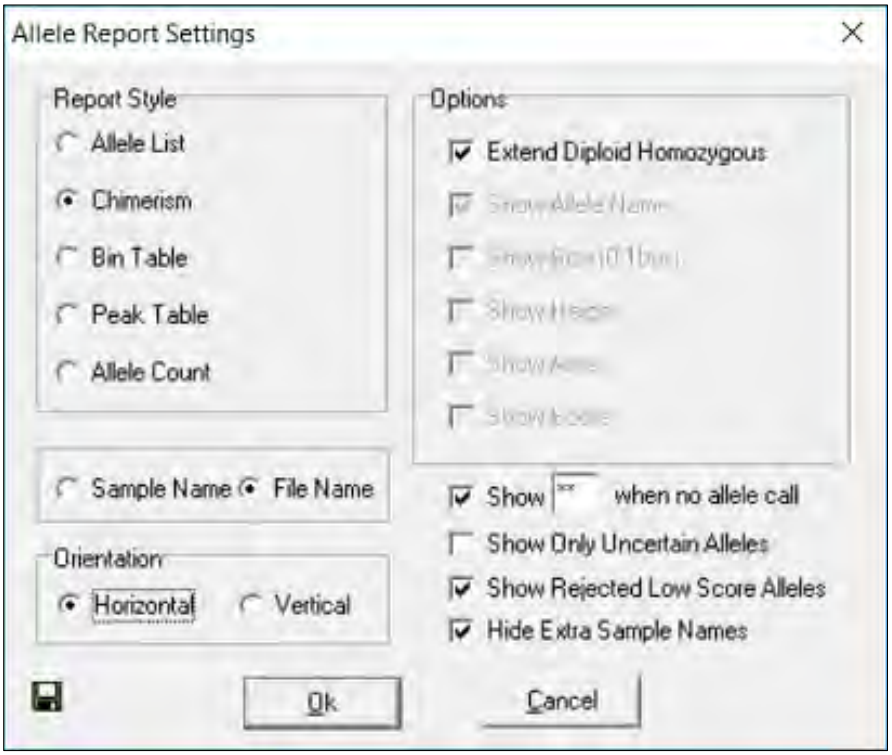
- **Hide Extra Sample Names**

  When data is displayed in Vertical Orientation (see **Orientation** pane Figure 184), the sample names are repeated for each row of data that the sample is associated with. If **Hide Extra Sample Names** is selected, then the sample name appears only once in the first of the rows it is associated with.

Chimerism Reports

The Chimerism Report Style displays the Quality rank and Allele Label of each called peak. Samples are listed in rows in the far-left column and Panel Marker names indicate the columns at the top of the table. Chimerism is the default Report Style in ChimerMarker.

Figure 184 Allele Report Settings



Chimerism Features Options

- **Extend Diploid Homozygous**

  Repeats the same Allele Label in the second allele position of the marker when only one peak is detected in the marker. Only active when the Edit Panel Ploidy option is set to 2-Diploid.

- **Show Allele Name/Size (0.1bp)/Height/Area**

  Allele Name is displayed in the Report Table by default regardless of table Orientation. Select to display **Size**, **Height**, and/or **Area** of the peak all within the same cell. Parentheses separate the peak statistics from the Allele Name. Only enabled when **Vertical Orientation** is selected.

The **Report Sample Chimerism** displays.

Figure 185 Report Sample Chimerism

		D21S11				D13S628	
1	051_G03.SCF	238	242	OL		327	331
2	052_A04.SCF	257	260			327	327
3	052_B04.SCF	257	260			327	327
4	052_H03.SCF	257	260			327	327
5	061_B05.SCF	247	264			319	323
6	062_C05.SCF	247	251			323	323
7	063_D05.SCF	242	247			314	319
8	064_E05.SCF	247	257			314	327
9	065_F05.SCF	247	264			297	327
10	066_G05.SCF	247	264			327	331
11	067_H05.SCF	251	251			297	323
12	068_A06.SCF	251	260			297	331
13	069_B06.SCF	251	254			319	323
14	539_H05.SCF	247	247			323	331
15	819_A06.SCF	242	257			323	327
16	819_B06.SCF	242	257			323	327
17	820_C06.SCF	247	257			327	331
18	820_D06.SCF	247	257			327	331
19	821_E06.SCF	242	254			323	323
20	845_A05.SCF	247	247			297	331
21	846_B05.SCF	247	260			297	323
22	847_C05.SCF	227	247			323	331
23	848_D05.SCF	227	242			323	331
24	849_E05.SCF	242	OL		260	323	323
25	852_F05.SCF	242	242			319	327
26	853_G05.SCF	242	257			319	327
27	993_F05.SCF	224	228			283	287

Chimerism Table Orientation

- **Horizontal**

  Sample names appear on the left in rows and Markers appear at the top in columns.

- **Vertical**

  Sample names appear in the far-left column in rows with Markers listed in the second column. Alleles in order of base pair size appear at the top in columns.

- **Show when no allele call**

  Allows user to specify a symbol or short word to enter in the cell of the allele report when there is no peak (no amplicon) at that marker. If not selected these cells is empty in the allele report.

- **Show Only Uncertain Alleles**

  When selected, displays only the peaks with Quality ranks of **Check** (yellow) and **Undetermined** (red).

- **Show Rejected Low Score Alleles**

  When selected, the peaks with peak scores below the Run Wizard Additional Settings the Allele Evaluation Peak Score **Reject** setting is displayed in the table.

- **Hide Extra Sample Names**

  When data is displayed in Vertical Orientation, the sample names are repeated for each row of data that the sample is associated with. If **Hide Extra Sample Names** is selected, then the sample name displays only once in the first of the rows it is associated with.

Chimerism Bin Table

If a peak is detected in at least one sample, the **Report Style** pane option **Bin Table** is selected, it reports the presence or absence of a peak at that position for the rest of the samples in the dataset.

Figure 186 Allele Report Settings

Features Options

- **Abide By Panel**

  When selected, the 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**

  Enter values to indicate the presence of a peak at the position (*Positive*), the absence of a peak at the position (Negative), and a Check or Undetermined Quality rank at the position (Suspected).

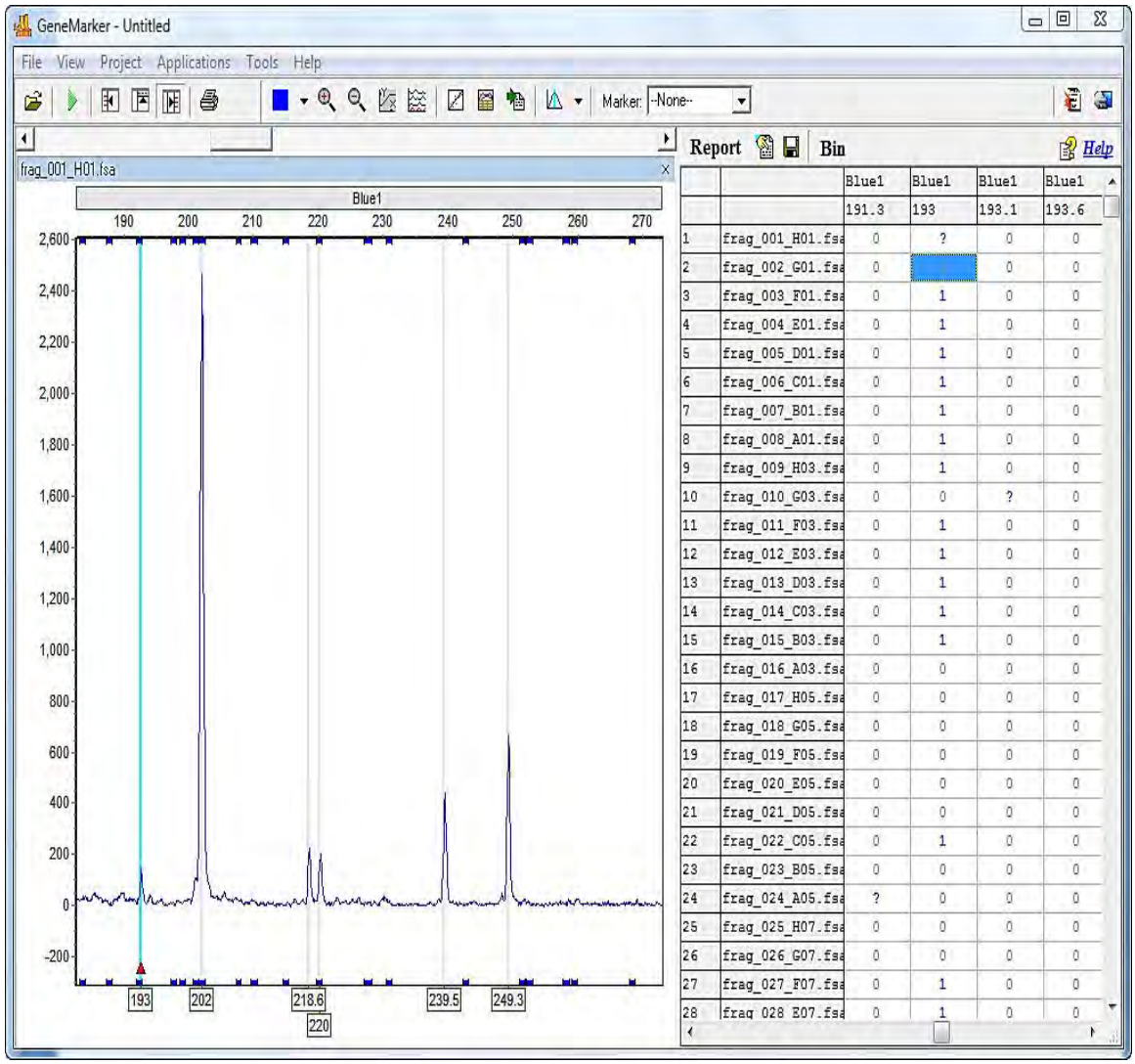
- **Show Intensity (Raw)**

  Displays the peak intensity (RFU) value for all Positive and Suspected peak positions. A “0” value is given to Negative positions. Selecting **Raw** shows the peak intensity values for all positions, including Negative positions.

- **Show Peak Area**

  Displays the peak area value for all Positive and Suspected peak positions. Dollar signs “$” separate values if more than one display option is selected.

Figure 187 Binary Peak Table Option



GeneMarker Dialog Orientation

- **Horizontal**

  Sample names appear on the left in rows and Markers appear at the top in columns.

- **Vertical**

  Sample names appear in the far-left column in rows. Markers and Alleles, in order of base pair size, appear at the top in columns.

- **Show Only Uncertain Alleles**

  When selected, displays only the peaks with Quality ranks of Check (yellow) and Undetermined (red).

- **Show Rejected Low Score Alleles**

  When selected, the peaks with peak scores below the Run Wizard Additional Settings Allele Evaluation Peak Score **Reject** setting is displayed in the table.

- **Hide Extra Sample Names**

  When data is displayed in Vertical Orientation, the sample names are repeated for each row of data that the sample is associated with. If **Hide Extra Sample Names** is selected, then the sample name only appears once in the first of the rows it is associated with.

Additional Functions Allele Editing Options

The Bin Table Report Style offers additional options when a cell in the table is right clicked.

- **Insert a Peak at this Bin Site**

  To indicate the presence of a peak at a position when it is labeled with a Negative Type Symbol, right-click the cell and select **Insert a Peak at this Bin Site**. The Negative Type Symbol changes to a Positive or Suspected Type Symbol depending on the Quality rank of the peak. (**Hot Key = INS**)

- **Delete**

  To indicate the absence of a peak at a position that is labeled with a Positive or Suspect Type Symbol, right-click the peak cell and select **Delete**. The Type Symbol changes to Negative. (**Hot Key = DEL**)

- **Confirm**

  To indicate the peak present at the position is truly a peak, right-click the peak cell and select **Confirm Peaks**. Only peaks centered within a Panel Bin change from Suspect Type Symbol to Positive Type Symbol when confirmed. Once a peak is confirmed, it cannot be unconfirmed, only deleted. (**Hot Key = CTRL+M**)

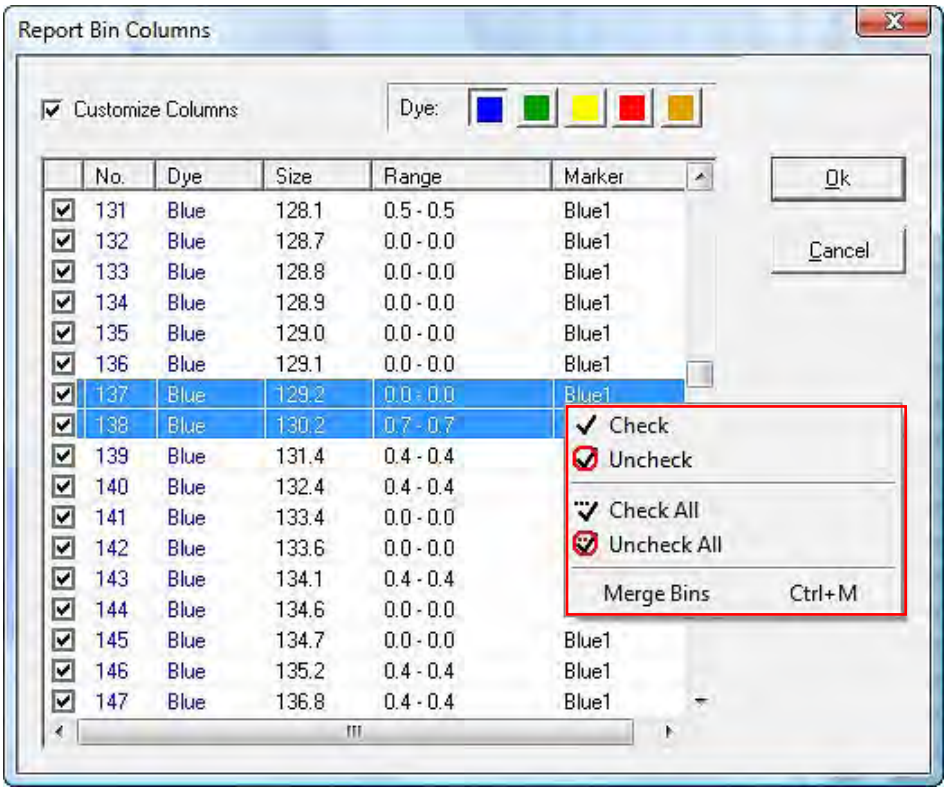
- **Delete Bin Columns**

  To eliminate an entire column in the Report Table, left-click any cell within the column then right-click the cell and select **Delete Bin Columns**. When **Vertical Orientation** is selected, the Report Table rows which contain the Bin information are deleted (not the columns that contain the sample information).

- **Binning Options**

  To adjust which Bins are displayed and to merge Bins in the Report Table, click the **Bin** icon in the toolbar of the Report Table. The **Report Bin Columns** dialog displays.

  Figure 188 Report Bib Columns



- **Display Bins**

  By default, all Bins are selected with a checkmark at the beginning of the row. Individually deselect Bins for exclusion from the Report Table by single left-clicking the checkmark dialog.

  a. To deselect all, right-click any cell in the **Report Bin Columns** dialog and select **Uncheck All**.

  b. To deselect only a few Bins, left click a cell to highlight the row then hold **CTRL** or **SHIFT** key and select additional rows.

  c. Next, right-click and select **Check** or **Uncheck** to include or exclude the Bins, respectively. Click **OK** in the **Report Bin Columns** dialog when finished and only the Bins with checkmarks are displayed in the Report Table.

- **Merge Bins**

  a. To make two (2) or more Bins become one Bin, single left-click a row to highlight it.

  b. Hold down **SHIFT** key to select additional rows.

c. Right-click the highlighted rows and select **Merge Bins**. (**Hot Key = CTRL+M**)

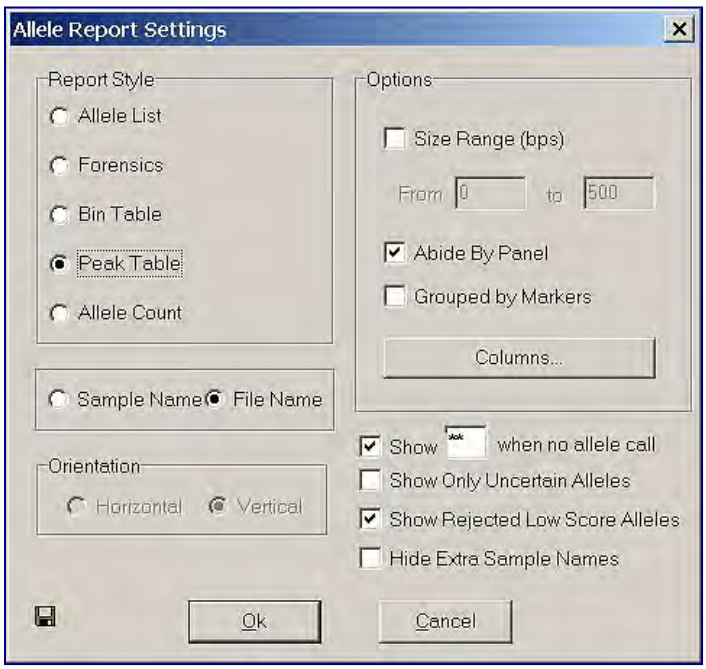
d. Click **OK** in the **Report Bin Columns** dialog when finished, and the selected Bins are averaged together. Only Bins immediately adjacent to one another may be selected for merging. Only the height and area for the first peak in the new merged Bin are reported.

Peak Table Reports

The Peak Table Report Style displays user-defined peak statistics. Sample names are displayed in the far-left column in rows and the Marker names are in the column adjacent to the sample names. In columns at the top of the table are the selected peak statistic information labels.

The column options available in the Peak Table Report Style are similar to the options available in the Peak Table that appears below the Electropherograms.

Figure 189 Allele Report Settings



See Chapter 4 Genotyping Main Analysis Overview for column option definitions.

Allele Report Features Options

- **Size Range (bps)**

  When selected, allows the user to define a specific base pair range. Only the peaks within the range is displayed within the Report Table.

- **Abide By Panel**

  When selected, the table shows only called alleles within Panel Marker ranges. This option is only active when a Panel is applied to the data.

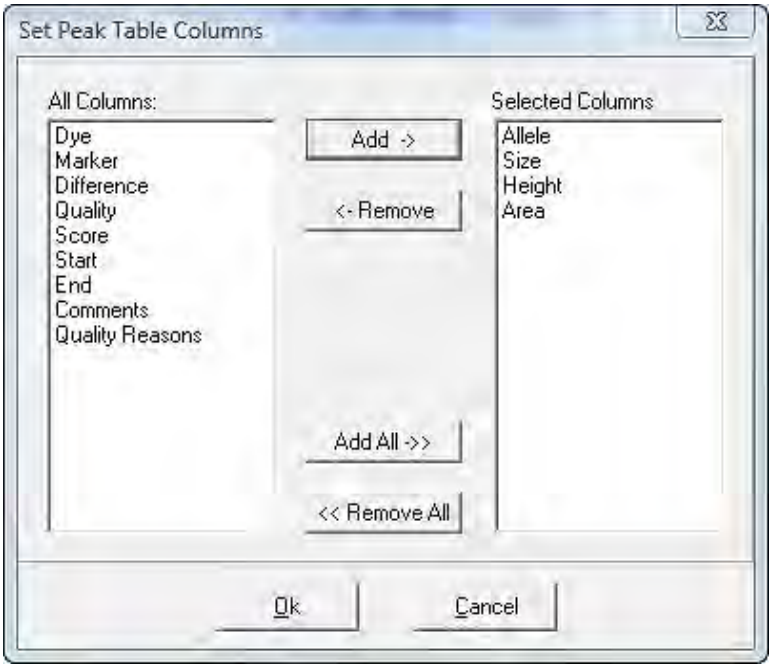
- **Grouped by Markers**

  When selected, alleles within the Marker are listed one after the other in the columns at the top of the table. When de-selected, each allele is represented by a row so that the Marker name may be listed several times according to the number of alleles in the Marker. This option is only active when a Panel is applied to the data.

Peak Table Columns

Click the **Columns** button to open the **Set Peak Table Columns** dialog. All column options are listed in the **All Columns** field on the left. The columns currently displayed in the Report Table are listed in the **Selected Columns** field on the right.

Figure 190 Set Peak Table Columns



- **Selecting Columns**
  a. Single left-click options in the **All Columns** field and click the **Add** button to add the column option to the **Selected Columns** field.
  b. Hold down **CTRL** or **SHIFT** key to select multiple options then click **Add**. Click the **Add All** button to move all the options in the **All Columns** field to the **Selected Columns** field.
- **De-selecting Columns**
  a. Locate the single left-click options in the **Selected Columns** field and click **Remove** to move the column option to the **All Columns** field.
  b. Hold down **CTRL** or **SHIFT** key to select multiple options then click **Remove**. Click the **Remove All** button to move all the options in the **Selected Columns** field to the **All Columns** field.

c. Click **OK** in the **Set Peak Table Columns** dialog and the **Allele Report Settings** dialog when finished. The options in the **Selected Column** field are displayed along the top of the table in columns.

See the **Peak Table Report** illustrated next.

Figure 191 Peak Table Report

	Sample	Marker	Allele#1	Size#1	Height#1	Area#1	Allele#2	Size#2	Height#2	Area#2
1	852_F05.SCF	D21S1437	115	115.1	469	3167	135	135.1	407	3003
		D21S11	242	242.3	847	6614				
		D13S628	319	318.6	1021	8787	327	327.0	1051	9178
		D13S634	397	397.2	1473	14559	401	401.1	1389	14114
		D18S535	483	482.8	2528	28522				
		D18S1002	120	119.9	605	3714	128	128.1	587	3612
		D18S391	178	177.9	1653	11898	182	181.9	1526	11004
		D13S742	266	265.7	800	5993	273	273.1	788	6032
		D18S386	351	350.8	776	6923	354	354.5	717	6315
		D13S305	446	446.2	648	7794	450	450.3	609	6589
		IFNAR	142	141.9	537	4052	151	150.9	584	4304
		D21S1411	313	312.5	440	3963	325	325.3	383	3335
2	993_F05.SCF	D21S1437	118	118.0	4790	39306	125	125.1	4906	40647
		D21S11	224	223.5	6237	51755	228	228.7	5804	47590
		D13S628	283	283.2	4711	35329	287	286.9	4341	32289
		D18S1002	156	156.2	7680	52108	160	160.5	7100	45671
		D13S742	257	256.7	2544	35036				
		D21S1411	278	278.5	4005	30945	282	282.3	3654	28542

- **Show Only Uncertain Alleles**

  When selected, displays only the peaks with Quality ranks of Check (yellow) and Undetermined (red).

- **Show Rejected Low Score Alleles**

  When selected, the peaks with peak scores below the Run Wizard Additional Settings **Allele Evaluation Peak Score Reject** setting are displayed in the table.

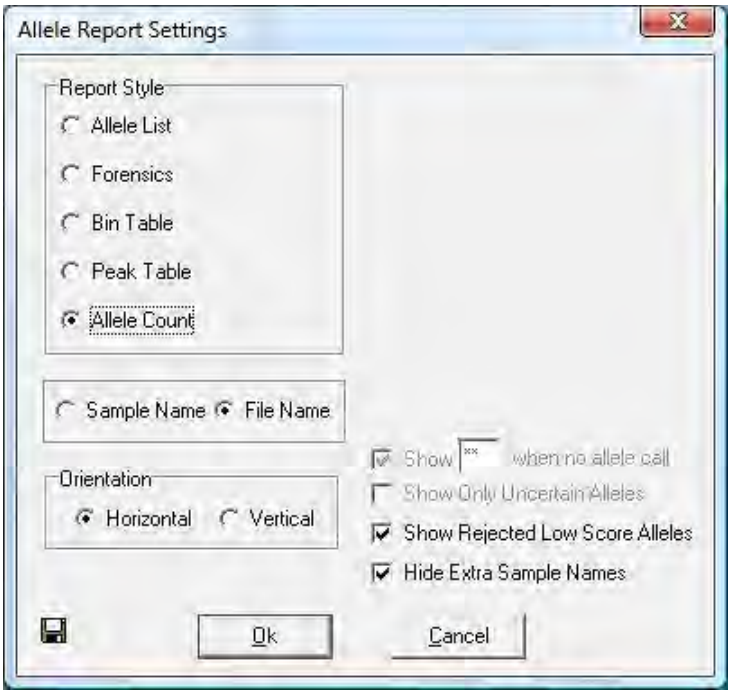
- **Hide Extra Sample Names**

  When data is displayed in Vertical Orientation, the sample names are repeated for each row of data that the sample is associated with. If **Hide Extra Sample Names** is selected, then the sample name only displays once in the first of the rows it is associated with.

Allele Count

The Allele Count Report Style displays the number of alleles present in the Panel Marker. Sample names are listed in rows in the left column and Markers are listed along the top row in columns. A Total Number column lists the number of peaks detected in the sample.

Figure 192 Allele Report Settings



Note: The **Allele Count** radial button requires that a Panel is applied to the data. See Chapter 6 Panel Editor.

Allele Count Features Orientation

- **Horizontal**

  Sample names appear on the left in rows and Markers appear at the top in columns.

- **Vertical**

  Markers appear on the left in rows and sample names appear at the top in columns.

  Figure 193 Samples Table

No.	Samples	D18S386	D13S305	IFNAR	D21S1411	Total Numbe
1	051_G03.SCF	2	1	2	1	22
2	052_A04.SCF	2	1	2	2	22
3	052_B04.SCF	2	1	2	2	22
4	052_H03.SCF	0	0	2	2	16
5	061_B05.SCF	2	2	2	2	23
6	062_C05.SCF	2	2	2	2	21
7	063_D05.SCF	2	2	1	1	21
8	064_E05.SCF	2	1	1	2	20
9	065_F05.SCF	1	2	2	2	22
10	066_G05.SCF	2	2	2	2	23
11	067_H05.SCF	2	2	2	2	21
12	068_A06.SCF	2	2	1	2	21
13	069_B06.SCF	2	2	1	2	23
14	539_H05.SCF	2	1	2	3	23

- **Show Rejected Low Score Alleles**

  When selected, the peaks with peak scores below the Run Wizard Additional Settings **Allele Evaluation Peak Score Reject** setting is displayed in the table.

- **Hide Extra Sample Names**

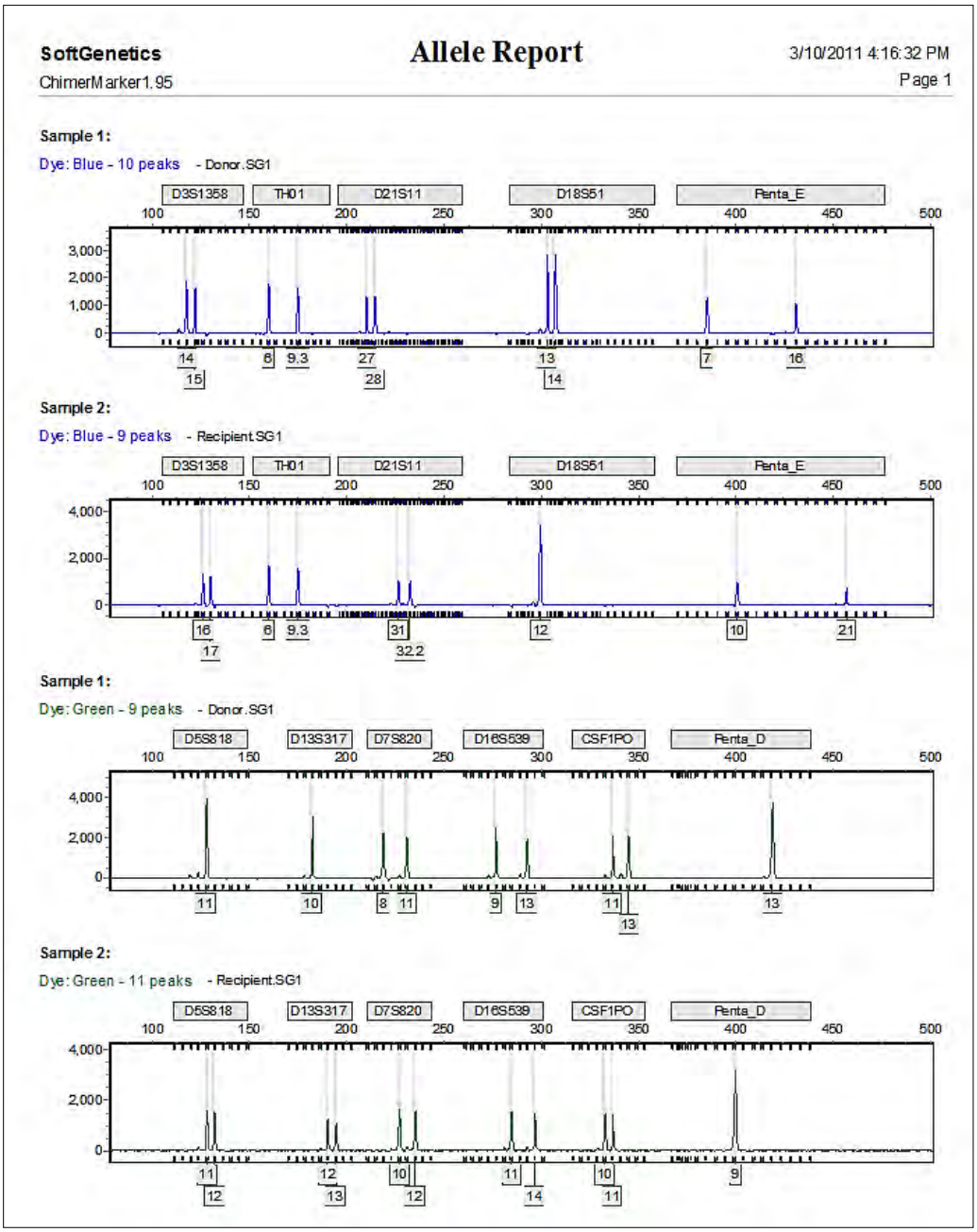
  This feature is not active for Allele Count Report Style.

Print Report

The ChimerMarker Print Report displays Electropherogram and/or Peak Table information for all or selected samples in a dataset. To access the Print Report, go to **Project** > **Print Report** or select the **Print Report** icon in the Main Analysis dialog.

The **Print Report** dialog options displays.

Figure 194 Allele Report of Single Source Sample



Select desired settings and click **Preview** to view the Print Report before printing or click **OK** to begin printing without previewing the report. The reference ladder (best match) used for each sample is listed on the allele report.

Note: The **View** > **Preference** > **Display Settings** options affect how the Print Report is displayed.

Report Content Options

The basic printing options allow the user to choose the **Print Type**, **Samples** to print, **Dyes** to include, and **Content** options. Each electropherogram is automatically labeled with its respective sample file name in the printed report. The **Advanced** button provides more options.

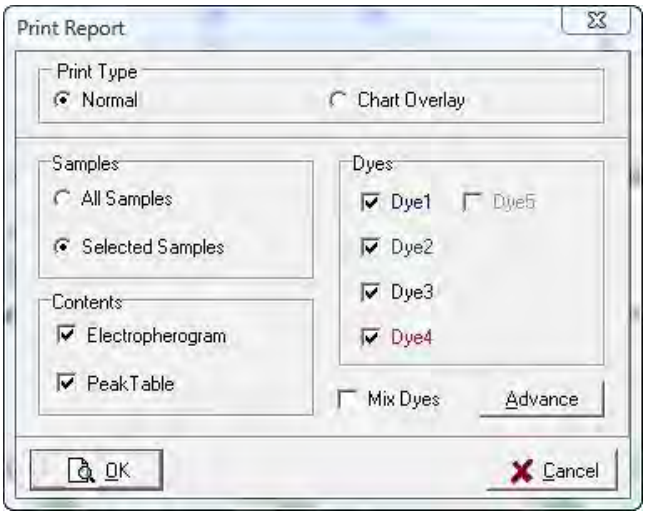
Print Type Pane

The Print Type pane contains the following settings:

- **Normal**

  In the **Print Report** dialog, select the **Print Type** pane > **Normal** radial button to enable all print options.

  Figure 195 Print Report Options



- **Chart Overlay**

  In the **Print Report** dialog, select the **Chart Overlay** radial button option to print only the Electropherogram with the report.

Samples Pane

The Samples pane contains the following settings:

- **All Samples**

  Prints all the samples in the project.

- **Selected Samples**

  Prints only those sample files that have been selected in the Main Analysis dialog Sample File Tree.

Contents Pane

The Contents pane contains the following settings:

- **Electropherogram**

  Prints the peak trace for each dye color and sample selected.

  Note: The zoom setting of the Electropherogram in the Main Analysis dialog is represented in the Print Report. Zoom out fully to include all peaks in the Print Report.

- **Peak Table**

  Prints the Peak Table for each dye color below the dye color's electropherogram trace.

  Note: If neither Electropherogram nor Peak Table were selected, the Print Report contains a list of each dye color selected for each sample selected, and the allele count within each dye color.

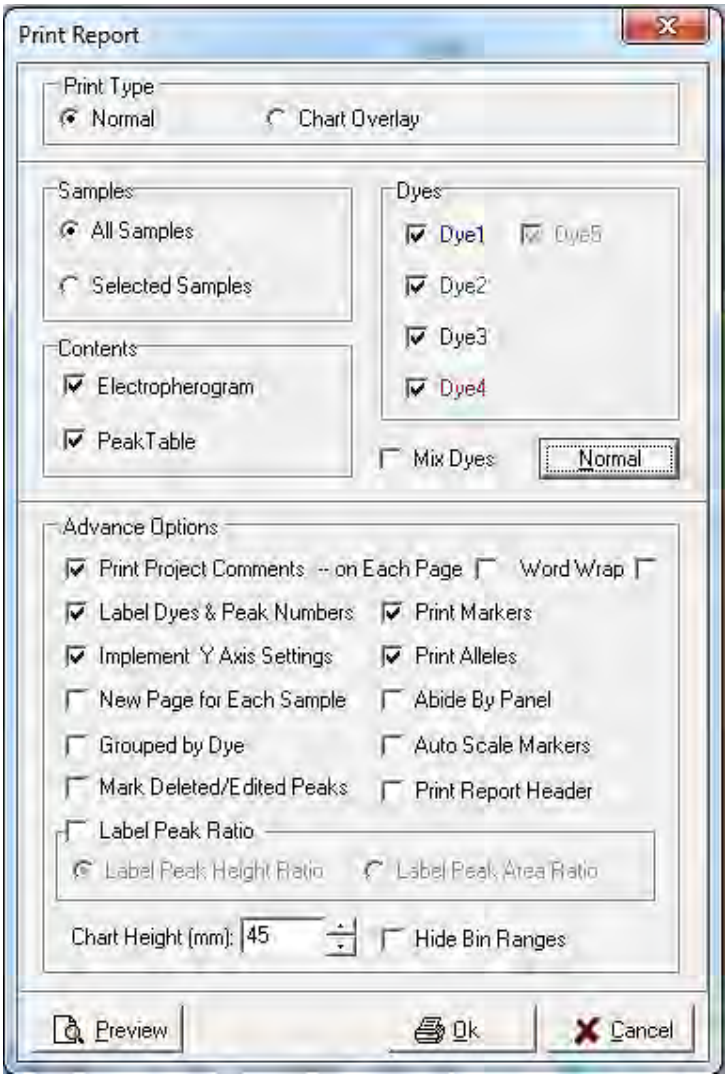
Dyes Pane

The Dyes pane contains the following settings:

- **Dye 1-6**: Click the checkbox to include the dye color in the Dyes.

The **Print Report Advanced Options** dialog displays.

Figure 196 Print Report Options



- **Mix Dyes**

  Prints all selected dye colors on one electropherogram.

Advance Options Pane

The **Advance Options** pane contains the following settings:

- **Print Project Comments**

  Includes the Project Comments at the top of the Print Report. Select the **Each Page** option to display the **Project Comments** on each page in the report.

- **Label Dyes & Peak Numbers**

  Labels dye color with number of peaks for each electropherogram.

- **Implement Y Axis Settings**

  Prints the report using the Y-axis settings the user selected in the Main Analysis dialog Set Axis icon.

- **Chart Height (mm)**

  Specify the size of the printed electropherograms (Minimum = 10mm, maximum = 100mm).

- **Print Markers**

  The Marker label bars appear above the electropherogram.

- **Print Alleles**

  The Allele Labels appear below the electropherogram.

- **Abide by Panel**

  Prints only alleles within a Panel. Alleles that are outside the Panel are not included in the printed report.

- **New Page for Each Sample**

  Prints a new page for each sample instead of continuing the same page as the previous sample.

- **Auto Scale Markers**

  When selected, the RFU intensities of low peaks are adjusted 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).

- **Grouped by Dye**

  Organizes the electropherograms in the Print Report such that samples are listed in order of dye color selected; for example, all samples in blue first, then all samples in green, etc.

- **Mark Deleted/Edited Peaks**

  Prints an x above a deleted peak and an E above an edited peak in the electropherogram.

- **Label Peak Ratio**

  Select this option to print the peak ratios on the electropherogram of the print report.

- **Chart Height**

  Use this feature to customize the size of the electropherogram in the print report.

Icons and Functions

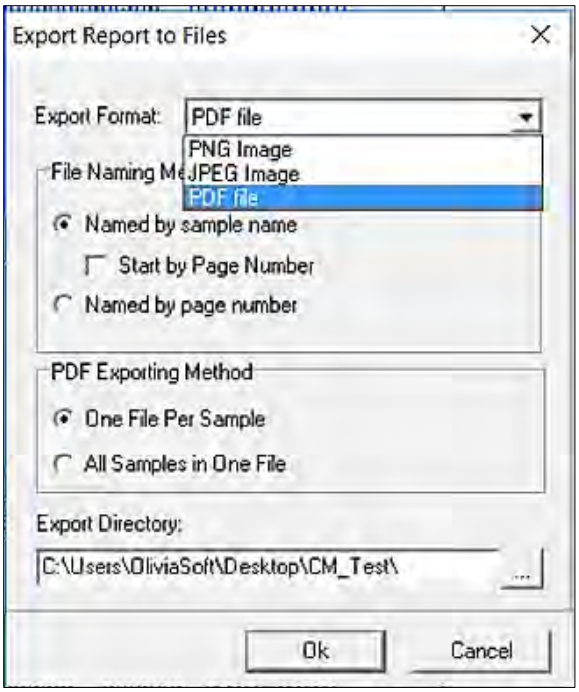
The following icons are available in the **Print Preview** dialog prior to printing the Print Report.

Table 14 Icons and Functions

Icon	Name	Function
	Print	Opens the **Print options** dialog. Select a printer, the print range and the number of copies.
	Export to File	Opens **the Export Report to Files** dialog, as illustrated next that provides the Export Format and the target Export Directory. See Figure 197. Save each page of the Print Report in JPEG, PNG, or PDF format. Select the directory to export the files to.

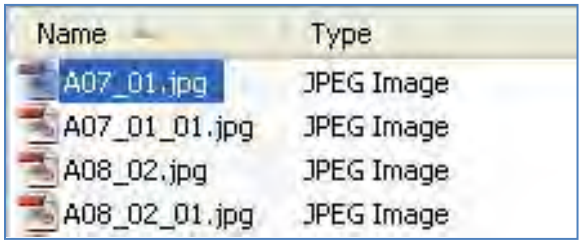
The **Export Report to Files** dialog provides the **Export Format** and the target **Export Directory**.

Figure 197 Export Report to Files



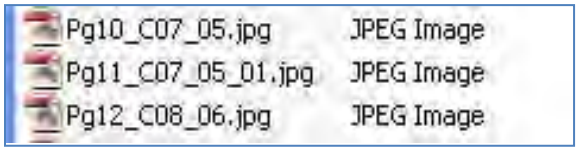
Named by sample name saves each PNG or JPEG under the sample name.

Figure 198 File Format and Sample Name



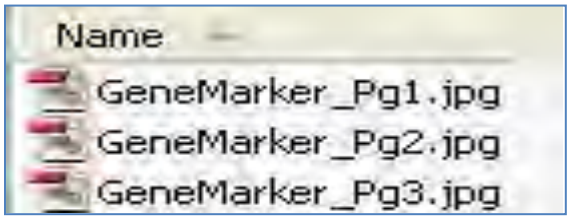
Start by Page Number combines the page number and the sample name for the saved file name.

Figure 199 Page Number and Sample Name



Named by Page Number saves each file by the page number within the report.

Figure 200 Page Number within Report



When PDF file is selected as the Export Format, the PDF is exported in two (2) ways.

- **One File per Sample:**

- **All Samples in One File*:* File is named using the Project name.**

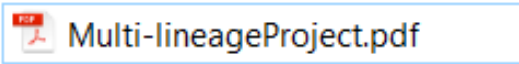
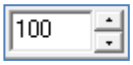


Table 15 Icons and Functions

Icon	Name	Function
	Page Setup	Opens the **Page Setup** dialog. Choose the paper size, margins and orientation (Portrait or Landscape).
	Content Options	Opens the **Print Report Options** dialog (see Figure 195). See the section above - Report Content Options.
	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 without losing information off the screen.
	Zoom Ratio	Enter percentage numbers to increase or decrease the zoom aspect of the Print Preview page.

Save Project

After a dataset is analyzed and edited, the project is saved as a SoftGenetics ChimerMarker Project (SGF). Project files contain the raw, unprocessed data files, the sample files after processing, the process parameters, and all edits. The project file does not contain any custom or modified Panels or Size Standards.

- To export a custom Panel, see Chapter 6 Panel Editor.
- To export a custom Size Standard, see Chapter 5 Fragment Sizing Standard.

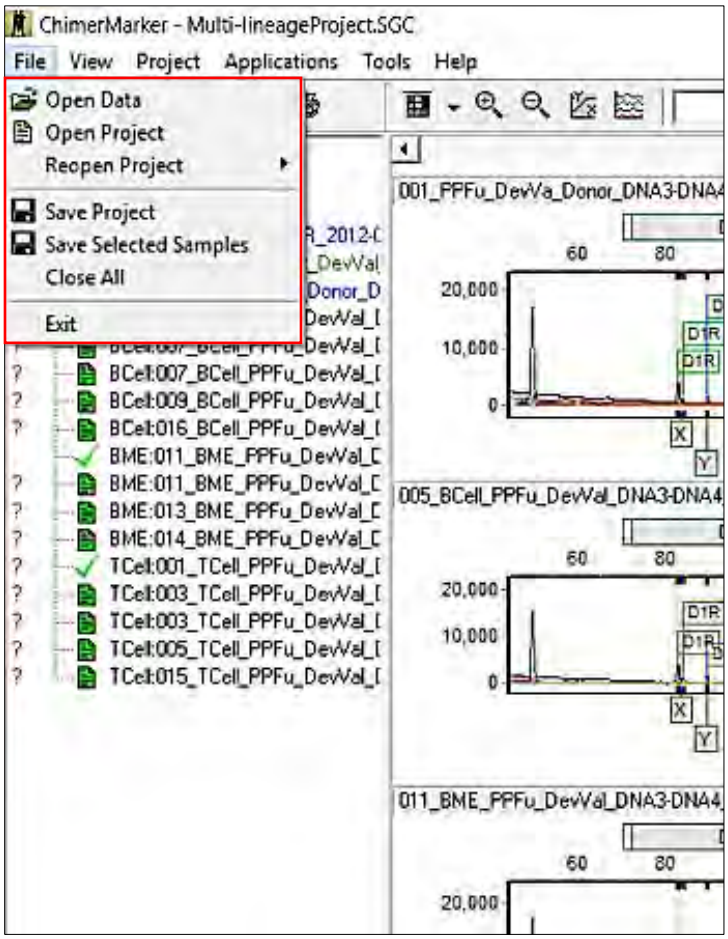
Note: Previous versions of ChimerMarker saved projects in SFP format. This format is opened using current ChimerMarker versions.

To save a project:

1. Go to **File** > **Save Project** in the Main Analysis dialog.

   The **Save Project** dialog displays. Select a directory and enter a project name.
2. Click **Save Project**.

Figure 201 ChimerMarker Save Project Option



To save a sub-set project, containing the files selected in the Main Analysis dialog, go to **File** > **Save Selected Samples**. The **Save Project** dialog displays as illustrated above in Figure 201. Select a directory and enter a project name. Click **Save Project**.

To re-open the project, follow the next steps:

1. Navigate to the **File** > **Open Project** in the Main Analysis dialog.

   The last folder accessed by ChimerMarker displays.

2. Navigate to the directory containing the SGF (or SFP) file and click **Open**.

   Additionally, the last four (4) projects opened by ChimerMarker display when the **File** > **Reopen Project** fly-out menu is selected.

3. Click a project from the fly-out menu and it is uploaded to ChimerMarker.

Chapter 8 Maternal Cell Contamination Analysis

Maternal Cell Contamination (MCC) Analysis

Overview

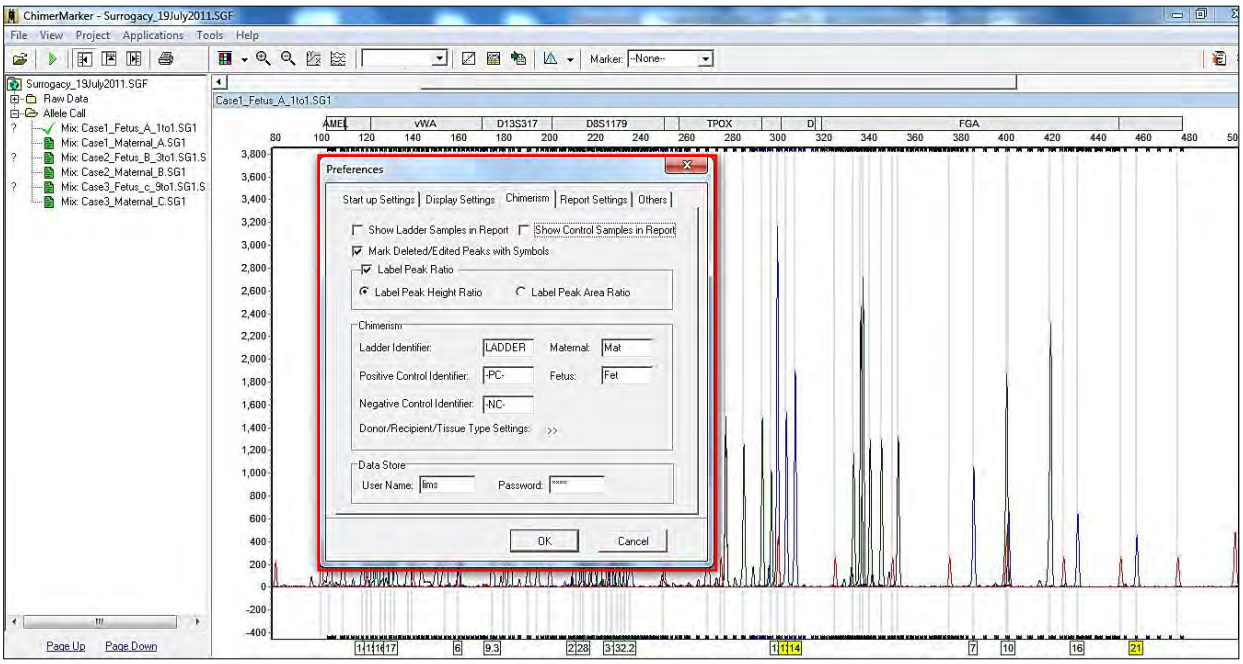
Follow the steps from Chapter 3 Genotyping General Procedure for importing of raw sample data. The initial step for Maternal Cell Contamination analysis is genotyping of all samples imported into ChimerMarker. After reviewing and confirming all allele calls, the MCC Analysis tool are accessed under the **Application** tab in the main menu toolbar.

Procedure

Genotyping of All Sample Data

Follow the steps for importing and processing data from Chapter 3 Genotyping General Procedure. Review the results in the Main Analysis dialog.

Figure 202 ChimerMarker Preferences

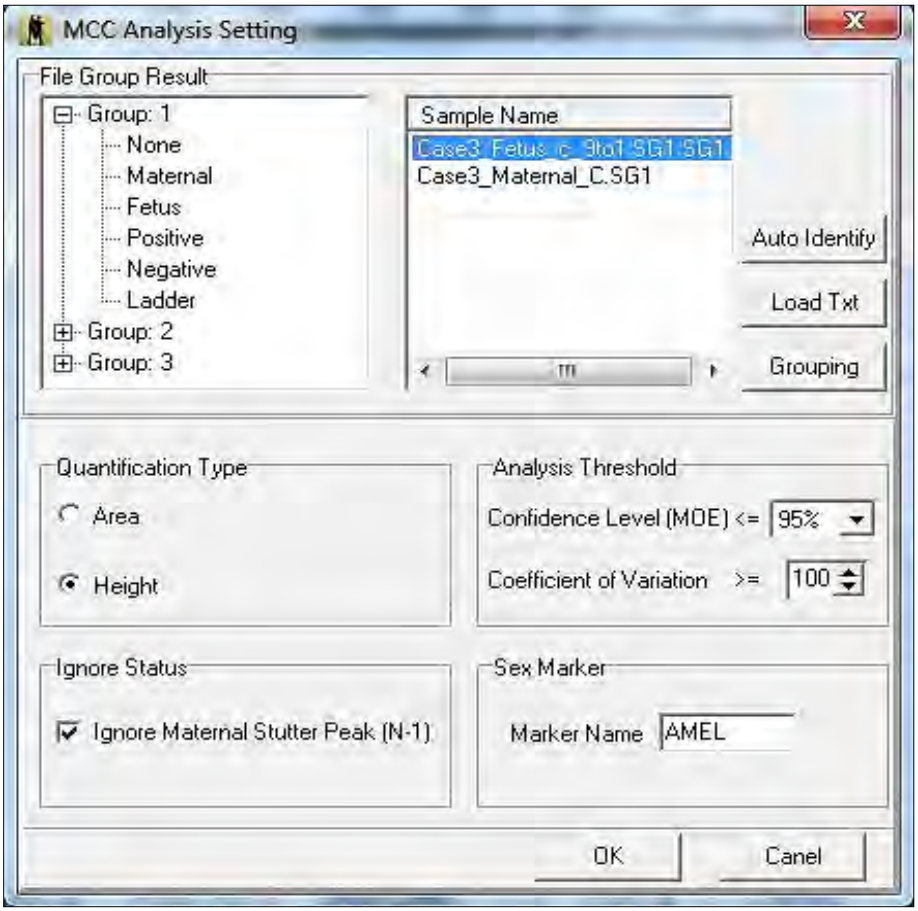


After reviewing the results and making any desired edits (see Chapter 3 Genotyping General Procedure), go to **View-> Preference-> Chimerism** tab to specify identifiers for maternal and fetus sample.

Maternal Cell Contamination Analysis

After genotyping and specifying the identifiers for the maternal and fetus samples, go to **Application-> MCC analysis**, as illustrated next

Figure 203 MCC Analysis Setting Sample Name



- **Auto Identify**

  Automatically groups fetus sample and maternal sample using identifiers specified in **View** > **Preference**-> **Chimerism** tab.

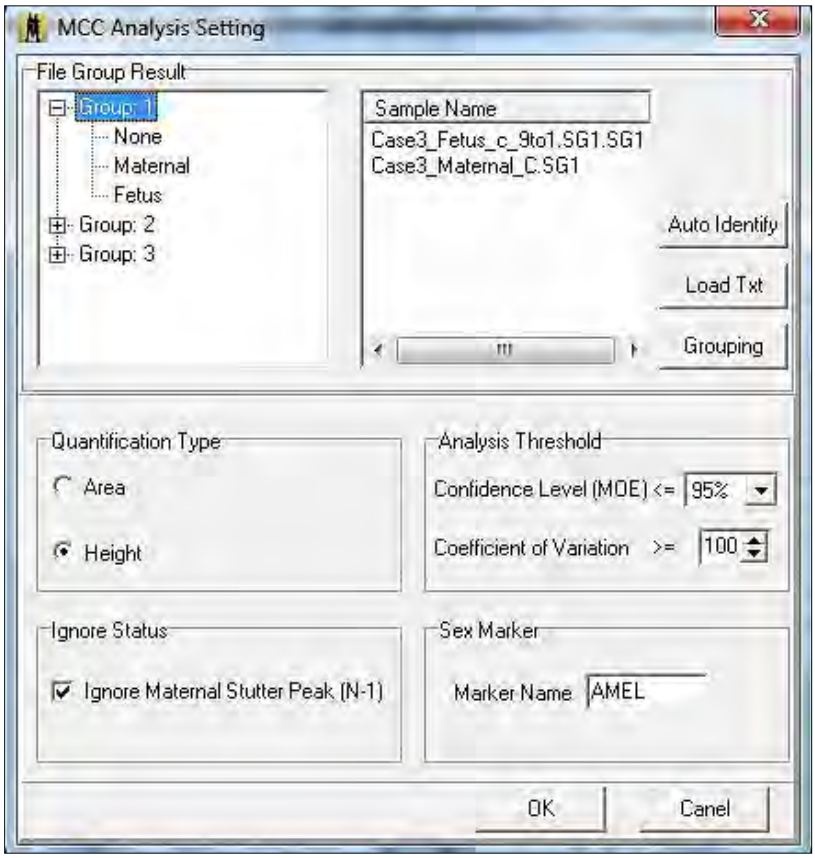
- **Load Txt**

  Groups samples using a text file created from Excel (tab delimited txt file) or other word editing programs. Refer to Chapter 7 Reports and Printing for more information.

- **Grouping**

  Brings up a dialog that is used to group maternal and fetus samples into different groups or patients.

Figure 204 MCC Analysis Setting Group 1



- **Quantification Type**
  - Area: uses area of allele for %MCC calculations.
  - Height: uses height of allele for %MCC calculations.
- **Analysis Threshold**

  Set desired threshold for analysis.
- **Ignore Status**

  If selected, any maternal allele detected in the stutter position of fetus sample is ignored.
- **Sex Marker**

  Specify marker name that is used for sex identification.

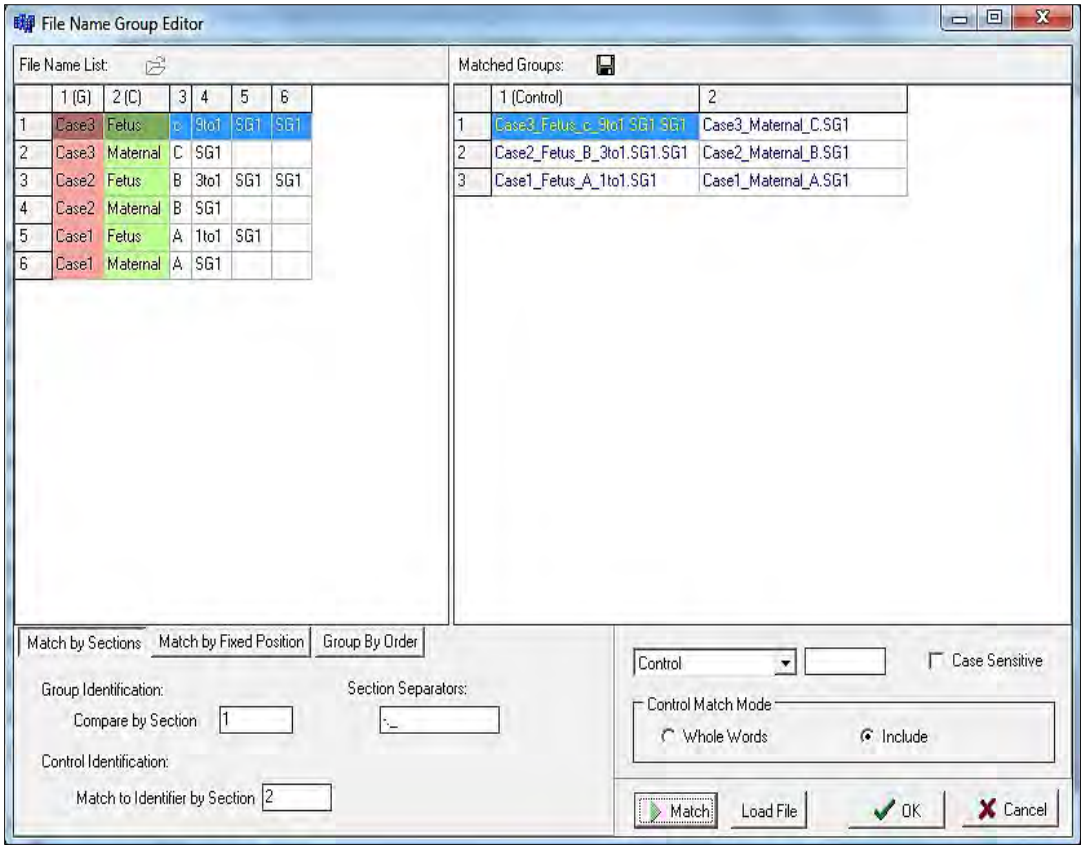
Grouping Options

Match by Section, Fixed Position, or Order.

Match by Section

- **Group Identification**: specify which column to group each set of samples/individual patients.

  Figure 205 File Name Group Editor Match by Sections tab



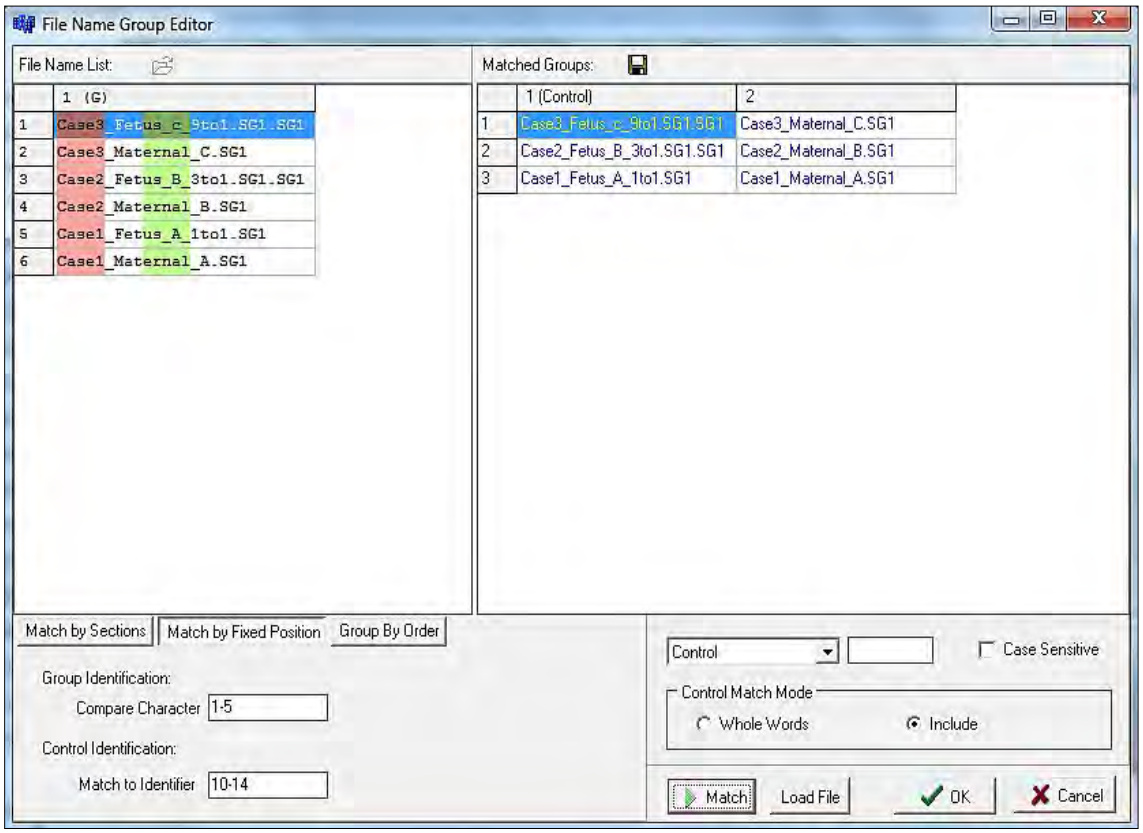
- **Control identification**

  Specify which column the identifiers specified in the **View** > **Preference** > **Chimerism** tab are found.

Match by Fixed Position tab

- **Group Identification**: specify characters location to group each set of samples/individual patients.

  Figure 206 File Name Group Editor Match by Fixed Position tab



- **Control Identification**: specify characters location that the identifiers specified in the **View** > **Preference** > **Chimerism** tab are found
- **Control**

  Specify identifiers to set as control (usually maternal sample).
- **Match**

  Group samples and control according to selected method.
- **Load File**

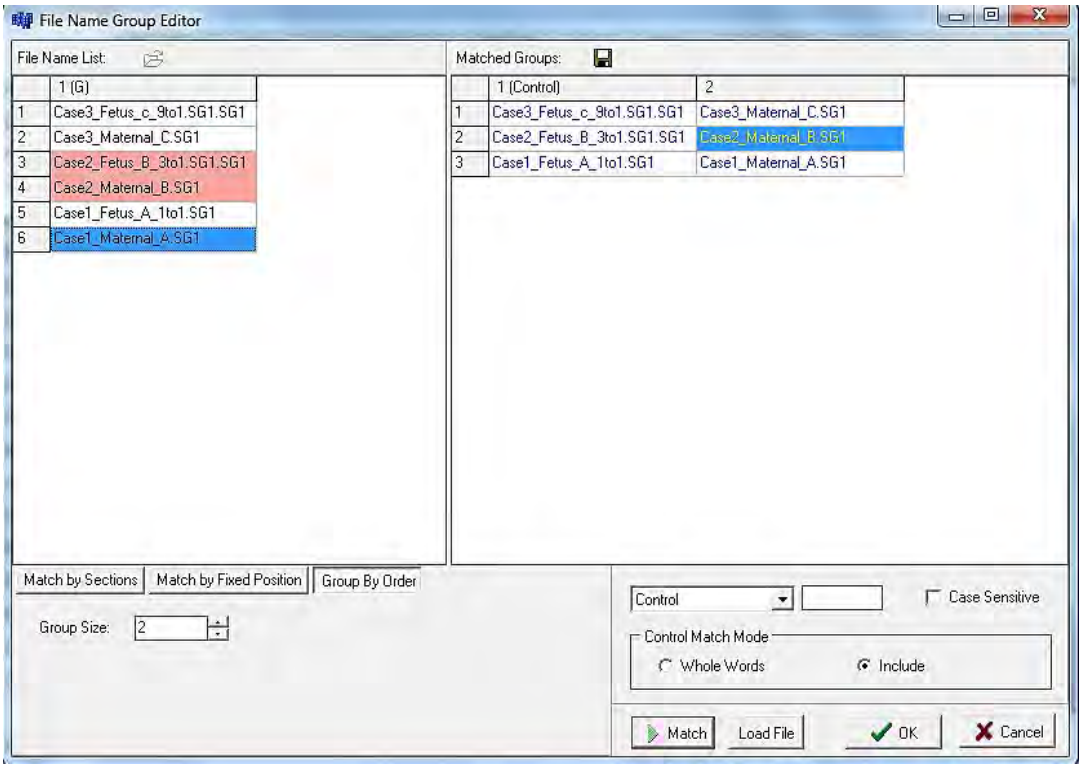
  Load grouping file from text.

Group by Order tab

- **Group Size**

  Select the number of samples to group by order.

  Figure 207 File Name Group Editor Group by Order Match



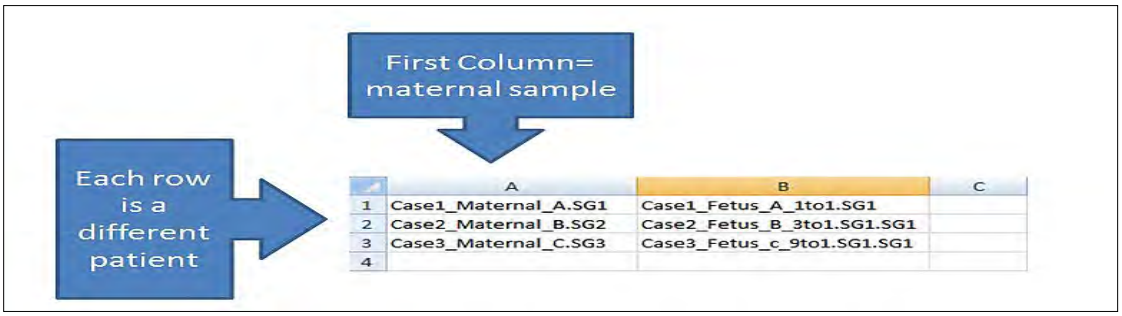
Grouping by Text File Rules

- Sample name in text file must be identical to sample name loaded in project.
- Each row corresponds to an individual group/patient.
- First column is recognized as maternal sample.
- Each sample name within a row is separated by a tab.

  Note: If using Excel to create group information, the file must be saved as a tab delimited text file. The following illustrations shows an example of grouping information created in Excel and Notepad.

Excel Example:

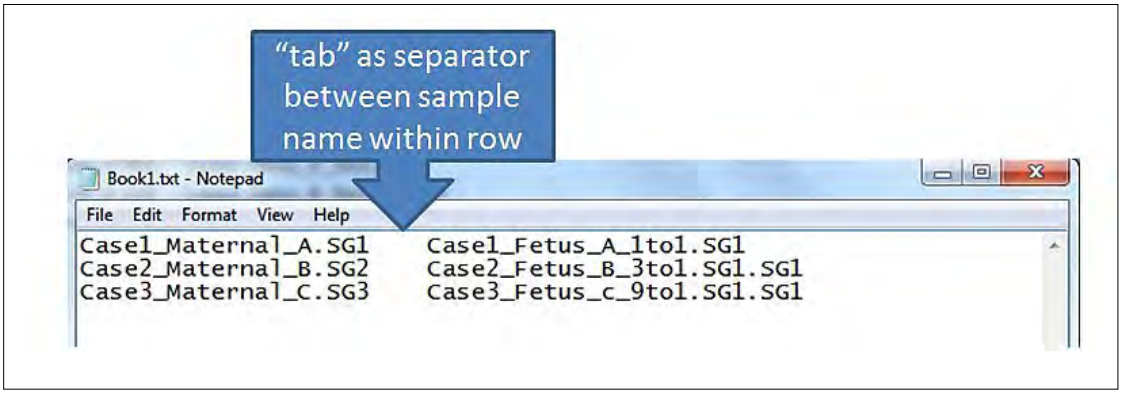
Figure 208 Excel Rows and Columns Example



Note: After creating the file, go to **File** > **select "Save As"** > **"Save as type"** > **select Text (Tab delimited) (*.txt)**.

Text File Example:

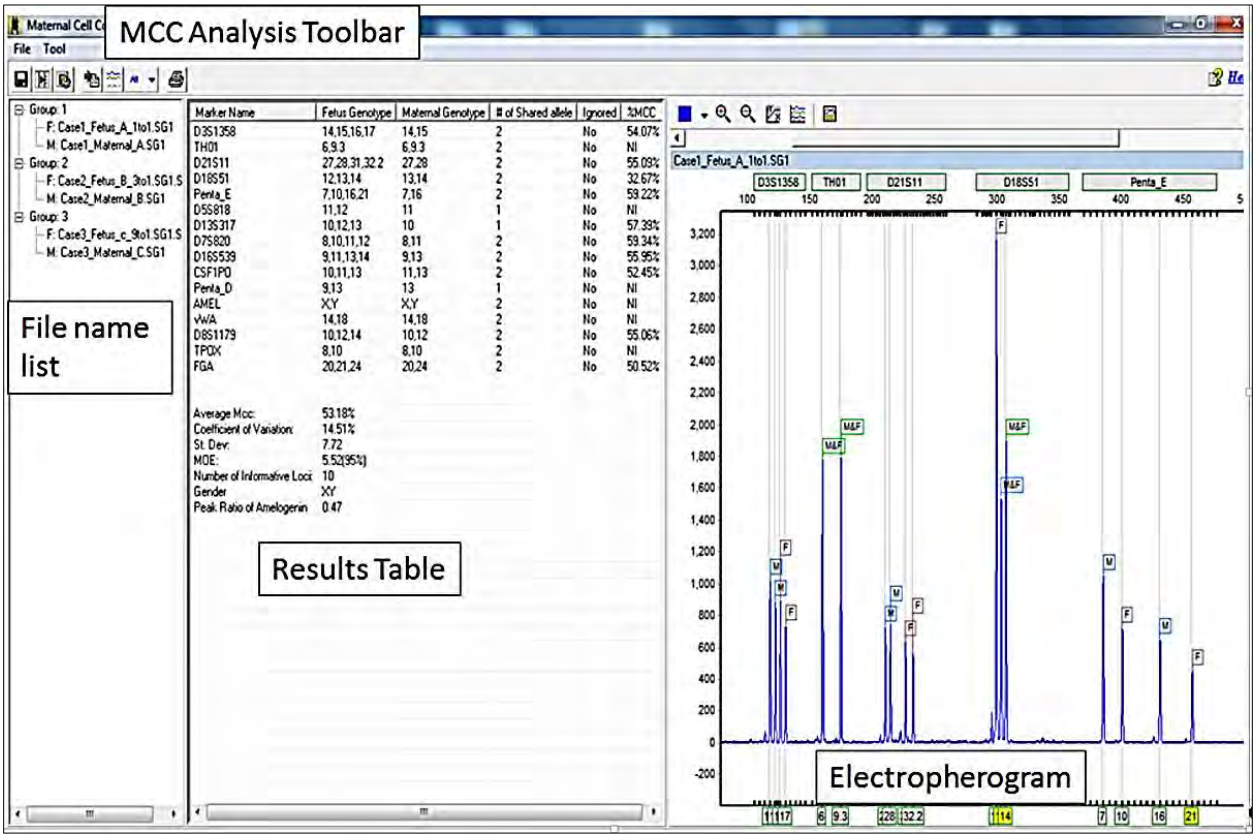
Figure 209 Excel Tab-Delimited File Example



Results

The automated MCC calculations are displayed in the center column. To view the results for a given file, click on the file name in the list at the left. Any markers that are ignored are highlighted in red in the electropherogram.

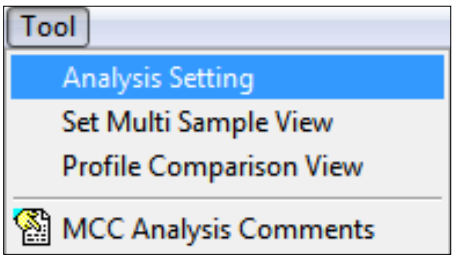
Figure 210 Automated MCC Calculations



Tools and Icons

The tools and icons are displayed in the **Tool** dialog detailed next.

Figure 211 Tool Dialog



- Analysis Setting

  Reopens **MCC Analysis Settings** dialog.

- **Set Multi Sample View**

  Select multiple samples to view electropherograms.

- **Profile Comparison View**

  Views selected traces and level of 2D or 3D offset.

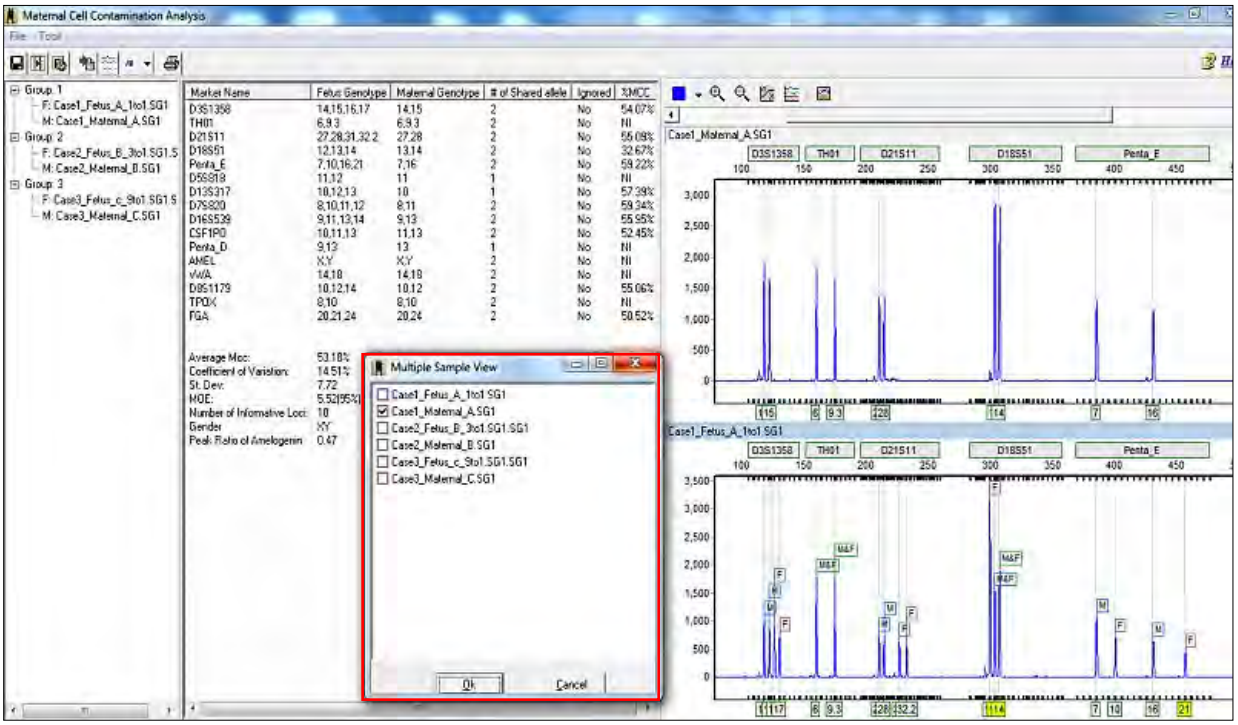
- **MCC Analysis Comments**

  Record any case notes to be saved and printed with the final report (saves up to 6400 characters).

Examples Screens of Tool Selections

Use the **Multi Sample View** dialog to display electropherograms of several samples.

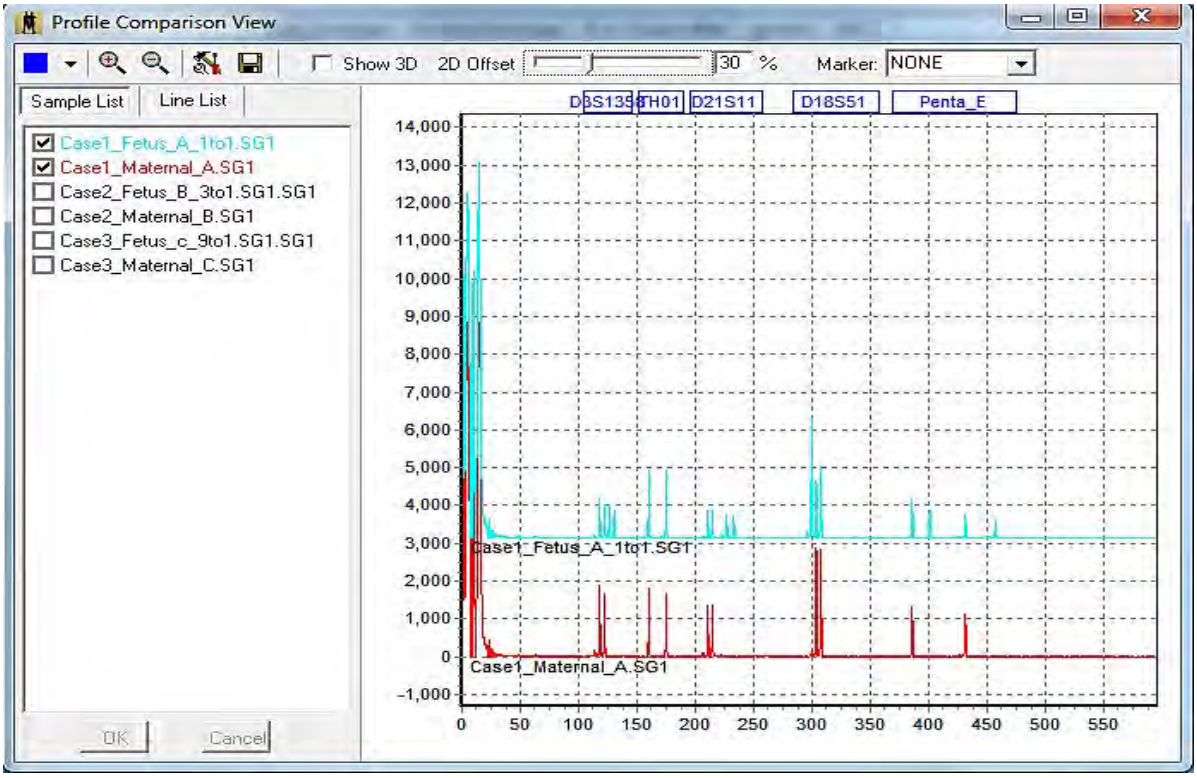
Figure 212 Maternal Cell Multiple Sample View Dialog



Profile Comparison View with 2D and 3D Options

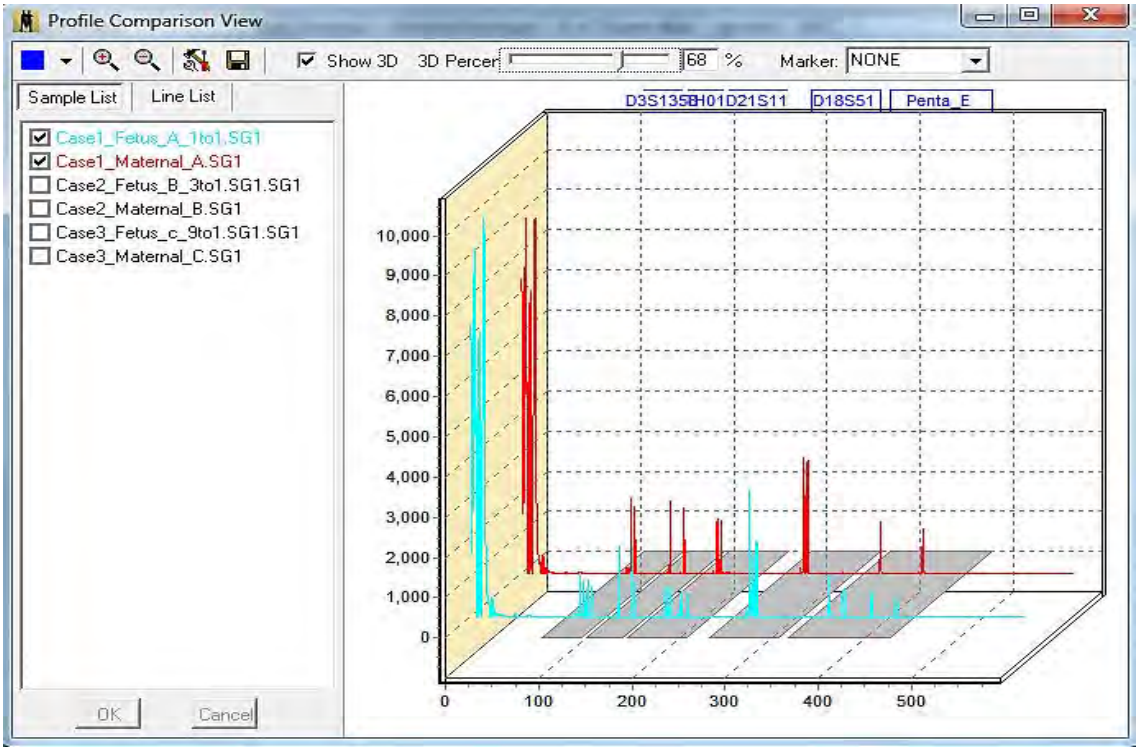
The profile comparison view with the 2D option is illustrated next.

Figure 213 Profile Comparison View 2D Option



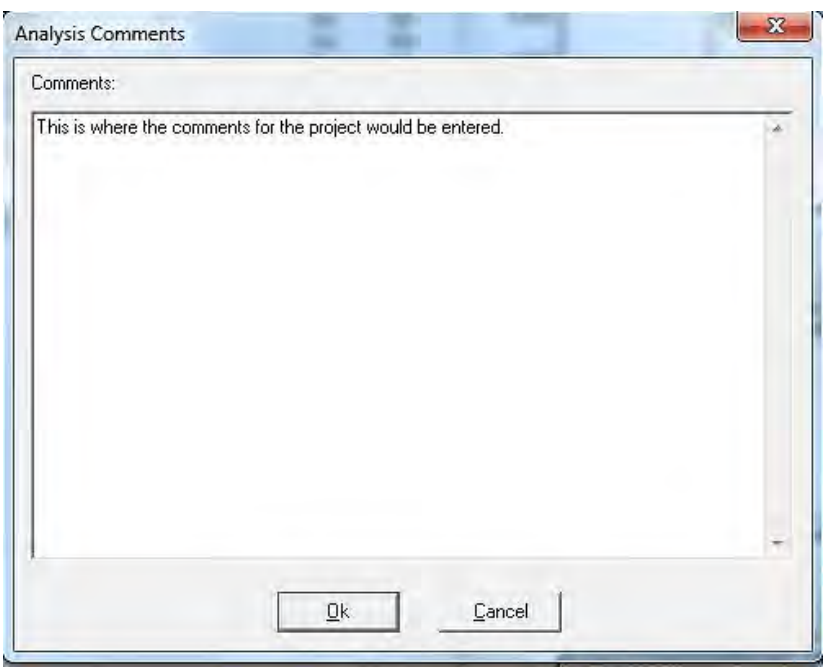
The profile comparison view with the 3D option is illustrated next.

Figure 214 Profile Comparison View 3D Option



The **MCC Analysis Comments** saved with project and displayed in comprehensive report.

Figure 215 MCC Analysis Comments



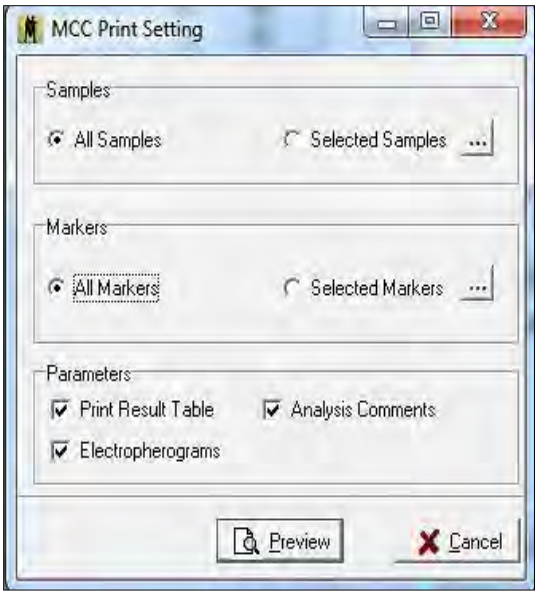
The next table details the icons and their functions for tools.

Table 16 Icons and Function

Icon	Name	Function
	Save	**Save** the report table in .xls or .txt file format.
	Show or Hide	Toggle this icon to show or hide the electropherograms.
	Analysis Parameter	Relaunches the Chimerism Analysis Settings Dialog.
	Multiple Sample	View alternative path to display electropherograms of multiple samples.
	Profile Comparison	Alternative path to display profile comparison view
	Column Headings	Select any or all available column headings for the report table.
	Printing Options	Preview – to review comprehensive research report or print directly from Chimerism Analysis screen.

The **MCC Print Settings** dialog is illustrated next.

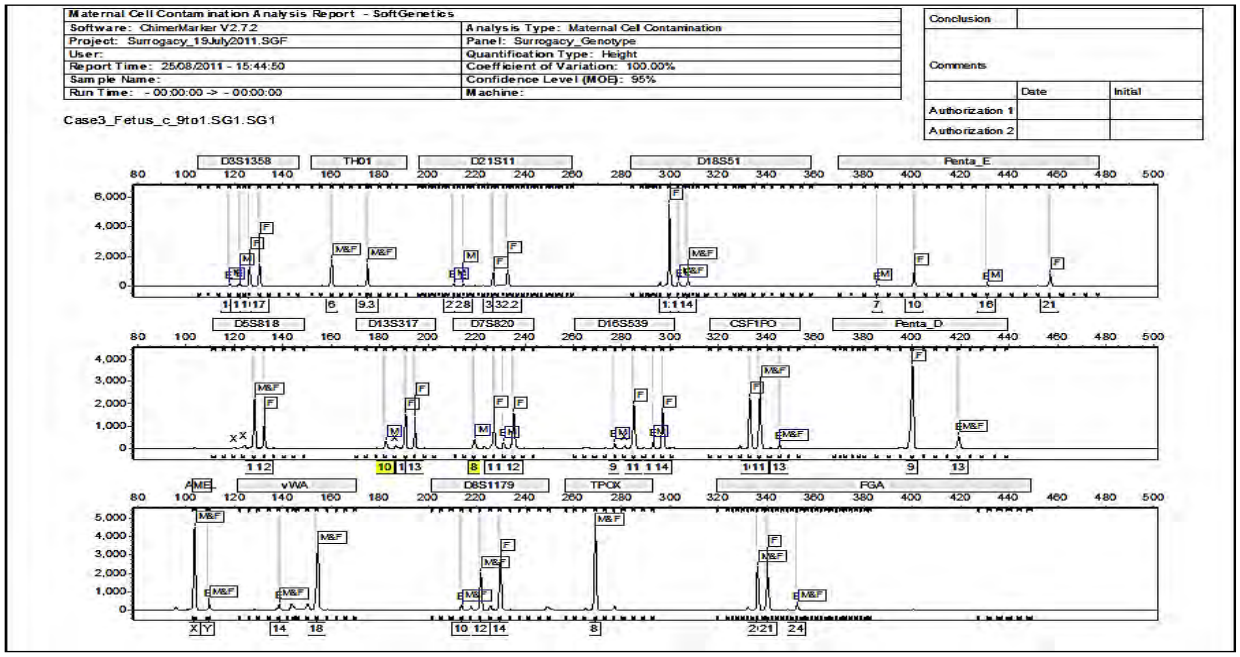
Figure 216 MCC Print Settings



Comprehensive Reports

First page of report includes header, electropherograms (traces) and report table.

Figure 217 Comprehensive Reports First Page



- The report header uses information from user management, signature dialog, and the analysis settings to specify the technician, project, time, date, and parameters for electronic record keeping.
- The electropherograms (traces) show each dye-color separately with labels for M (Maternal) and F (Fetus). Results displayed include quality control metrics: coefficient of variation, standard deviation, % MCC, and the total average %MCC. NI represents uninformative loci (all peaks are shared) and are not included in the chimerism calculations or indicates parameters could not be calculated based on data.

ChimerMarker software also contains the functionality to allow the analyst to manually exclude loci from calculations if needed.

The second page also includes the header, signature dialog, and the results table that was displayed in the MCC analysis results dialog.

Figure 218 Comprehensive Reports Second Page

Maternal Cell Contamination Analysis Report - SoftGenetics	
Software: ChimerMarker V2.7.2	Analysis Type: Maternal Cell Contamination
Project: Surrogacy_19July2011.SGF	Panel: Surrogacy_Genotype
User:	Quantification Type: Height
Report Time: 25/08/2011 - 15:44:50	Coefficient of Variation: 100.00%
Sample Name:	Confidence Level (MOE): 95%
Run Time: - 00:00:00 -> - 00:00:00	Machine:

Conclusion		
Comments		
	Date	Initial
Authorization 1		
Authorization 2		

Case3_Fetus_c_9to1.SG1.SG1

Marker Name	Fetus Genotype	Maternal Genotype	# of Shared allele	%MCC	Ignored
D3S1358	14,15,16,17	14,15	2	13.22%	No
TH01	6,9.3	6,9.3	2	NI	No
D21S11	27,28,31,32.2	27,28	2	14.78%	No
D18S51	12,13,14	13,14	2	5.88%	No
Penta_E	7,10,16,21	7,16	2	8.43%	No
D5S818	11,12	11	1	NI	No
D13S317	10,12,13	10	1	9.78%	No
D7S820	8,10,11,12	8,11	2	17.05%	No
D16S539	9,11,13,14	9,13	2	13.95%	No
CSF1PO	10,11,13	11,13	2	6.57%	No
Penta_D	9,13	13	1	NI	No
AMEL	X,Y	X,Y	2	NI	No
vWA	14,18	14,18	2	NI	No
D8S1179	10,12,14	10,12	2	8.49%	No
TPOX	8	8,10	1	NI	No
FGA	20,21,24	20,24	2	12.21%	No

Average MCC: 11.04%
St. Dev: 3.74
Coefficient of Variance: 33.93%
MOE 2.68 (95%)
Number of Informative Loci: 10
Gender: XY
Peak Ratio of Amelogenin: 0.09

Identical Genotype Alert Message

If the Maternal and Fetal samples have the same genotype, the MCC application alerts the user when the fetal sample is selected. An **IG** is displayed in front of the fetal sample as a red font message and displayed in the report.

Figure 219 Maternal Cell Contamination Analysis IG Message

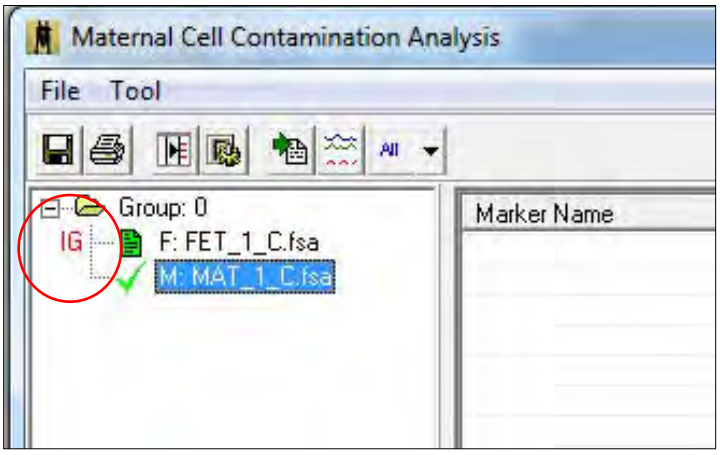
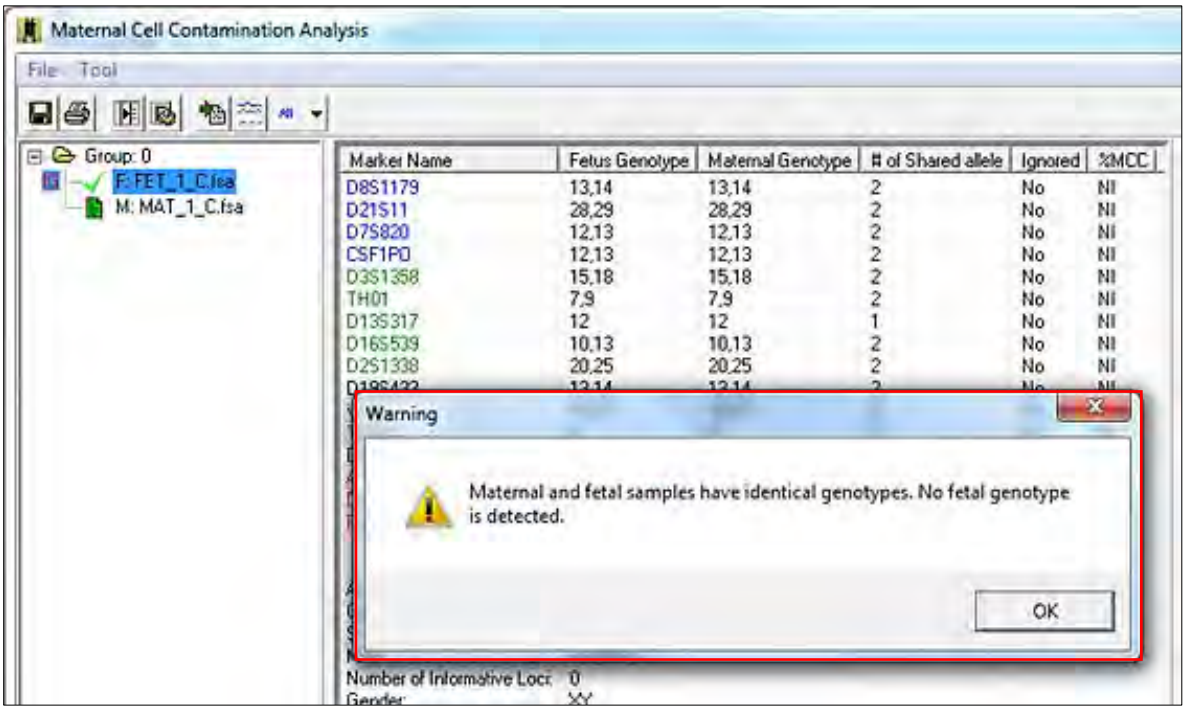
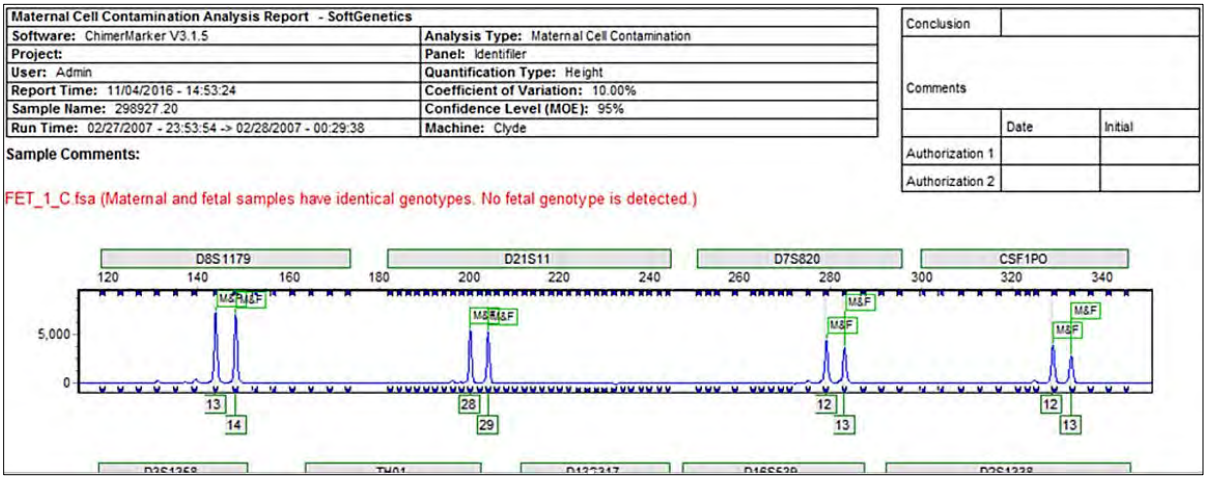


Figure 220 Maternal Cell Contamination Analysis Identical Geno Message



The **Maternal Cell Contamination Analysis Report** is illustrated next.

Figure 221 Maternal Cell Contamination Analysis Report



Chapter 9 Additional Tools

Automated Control Concordance

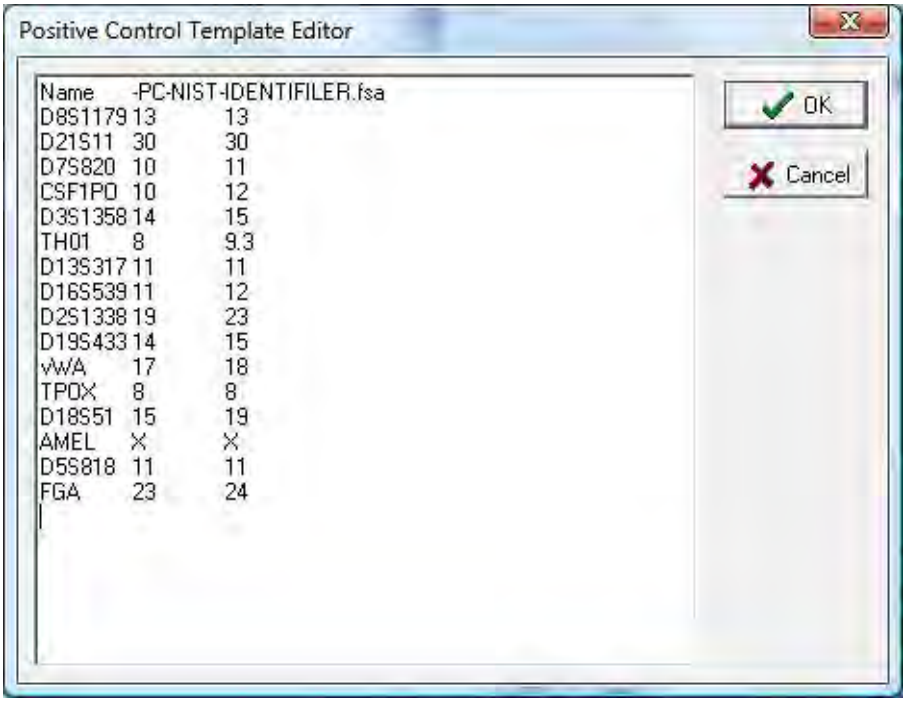
This section includes details about the automated control concordance.

Positive Control Template Editor

The positive control template editor is detailed next.

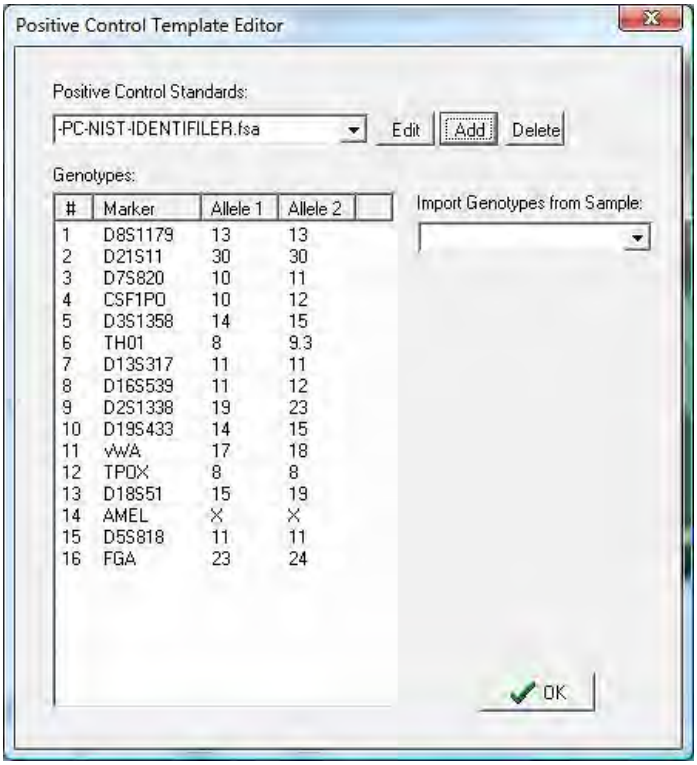
1. Navigate to **Tools** > **Positive Control Template Editor** to launch the dialog.

   Figure 222 Positive Control Template Editor Launch



2. Import Genotypes from samples using the dropdown menu.
3. Select from functions to **Add**, **Edit**, or **Delete** new positive control samples files.

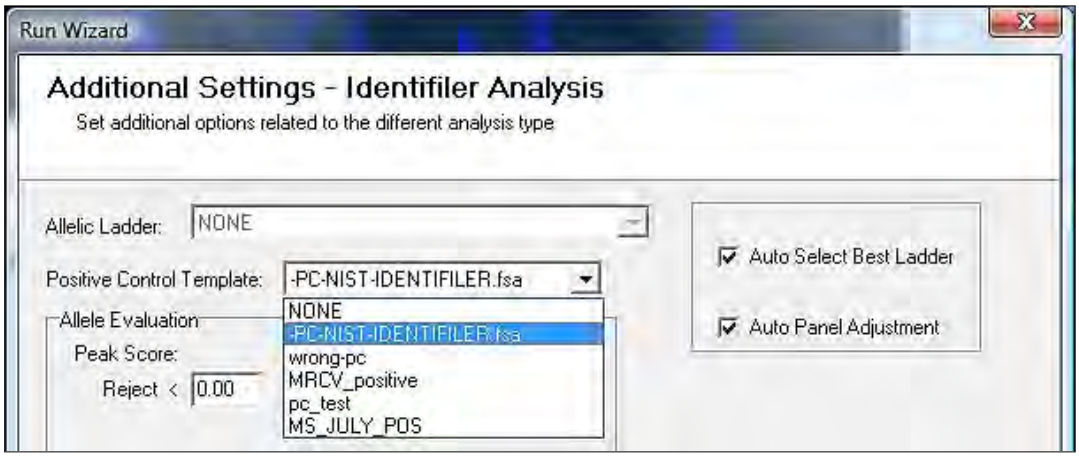
Figure 223 Positive Control Template Editor



#	Marker	Allele 1	Allele 2
1	D8S1179	13	13
2	D21S11	30	30
3	D7S820	10	11
4	CSF1PO	10	12
5	D3S1358	14	15
6	TH01	8	9.3
7	D13S317	11	11
8	D16S539	11	12
9	D2S1338	19	23
10	D19S433	14	15
11	vWA	17	18
12	TPOX	8	8
13	D18S51	15	19
14	AMEL	X	X
15	D5S818	11	11
16	FGA	23	24

4. Select the appropriate positive control file from the dropdown menu in the Run Wizard. A summary message is located at the bottom of the Main Analysis dialog. For example, if a project has one (1) positive control file that is in agreement with the positive control template, the message is PC error 0/1.

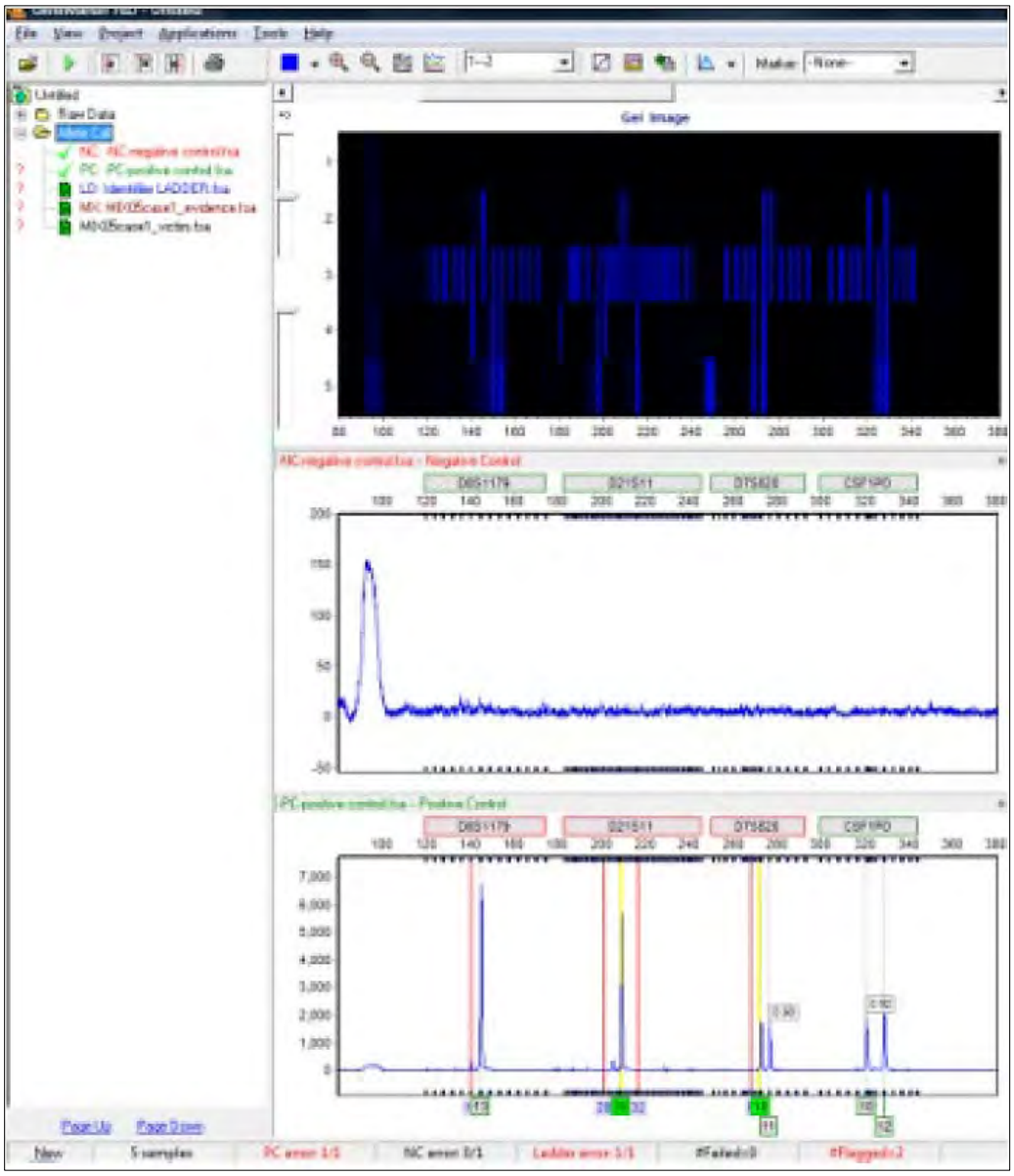
Figure 224 Run Wizard Additional Settings Identifier Analysis



5. If the positive control samples are not in agreement with the template the message is PC error 1/1 and red lines indicate the peaks in error.

   An example of Positive sample not in concordance with positive control template is illustrated next.

Figure 225 Sample Not in Concordance with Template



Negative Control Concordance

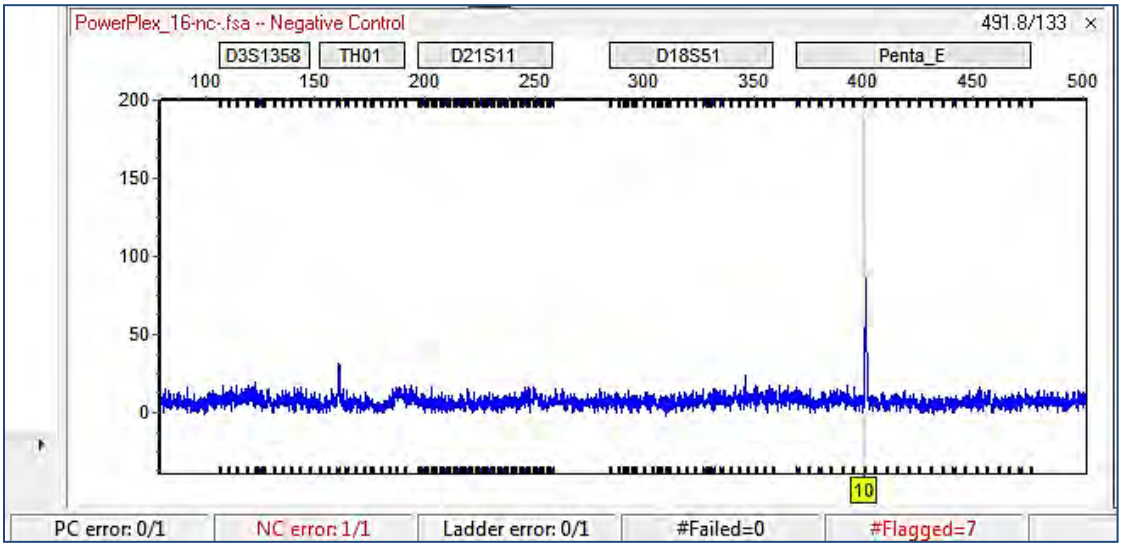
Negative control concordance is detailed next.

1. Use **View** > **Preferences** > **Chimerism** to select the file name recognition.

   GeneMarker automatically changes font of negative control file name to red in the Filename Tree.

2. Any negative control sample that has peak(s) results in a negative control (NC error) in the Project Summary Bar.

Figure 226 Project Summary Bar



Filename Group Editor

Navigate to **Project** > **Apply Sample Grouping** or **Tools** > **File Name Group Tool**.

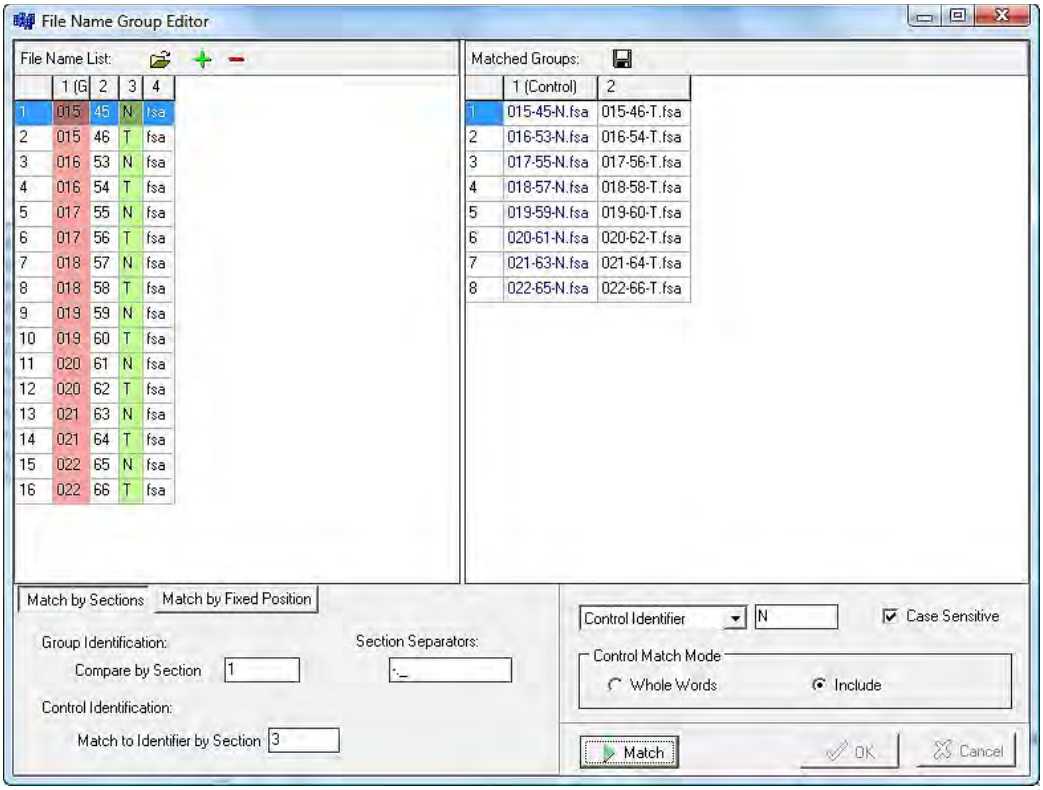
The Filename Group Editor is used to group family members or other related samples in the dataset based on their filenames for simplified analysis.

Group for Simplified Analysis Procedure

1. Select **Project > Apply Sample Grouping**.

The **File Name Group Editor** dialog displays.

Figure 227 File Name Group Editor



2. Click the **Load Files** icon and select all files to pair, if the dataset samples do not automatically appear in the **File Name List** field.
3. Choose **Match by Sections** or **Match by Fixed Position**.
4. Enter values for the **Group Identification** and **Control Identification** fields.
5. Enter a **Control Identifier** value and click **Match**.

   The samples from the **File Name List** are paired into groups in the **Matched Groups** dialog.
6. When the samples are grouped correctly, click **OK** or click the **Save Groups to File** icon to save the grouping information as a tab-delimited text file.
7. The **File Name Group Editor** dialog closes and the grouping information displays next to the sample filenames in the Sample File Tree in the Main Analysis dialog (if **Project** > **Apply Sample Grouping** was selected).
8. To navigate by group in the **Sample File Tree**, hold down the CTRL key and move the **Page Up/Down** keys. Sample groups are opened consecutively.

Icons and Functions

The icons and functions are detailed in the next table.

Table 17 Icons and Functions File Name Group Editor

Icon	Name	Function
	Load Files	Opens a directory dialog where raw data files are located and uploaded to the **Filename Group Editor**.
	Add Files	Opens a directory dialog where additional raw data files are uploaded into the **Filename List** field.
	Remove Files	Removes any files selected in the Filename List. Select multiple files to remove by holding down the SHIFT key and selecting additional samples.
	Save Groups to File	Saves the filenames of the samples paired in the **Matched Groups** field. Samples identified as Controls is in the first column of the Matched Groups tab-delimited text file.

Match by Sections

Automatically separates the sample filenames into groups based on the specified Section Separators.

- **Group Identification**

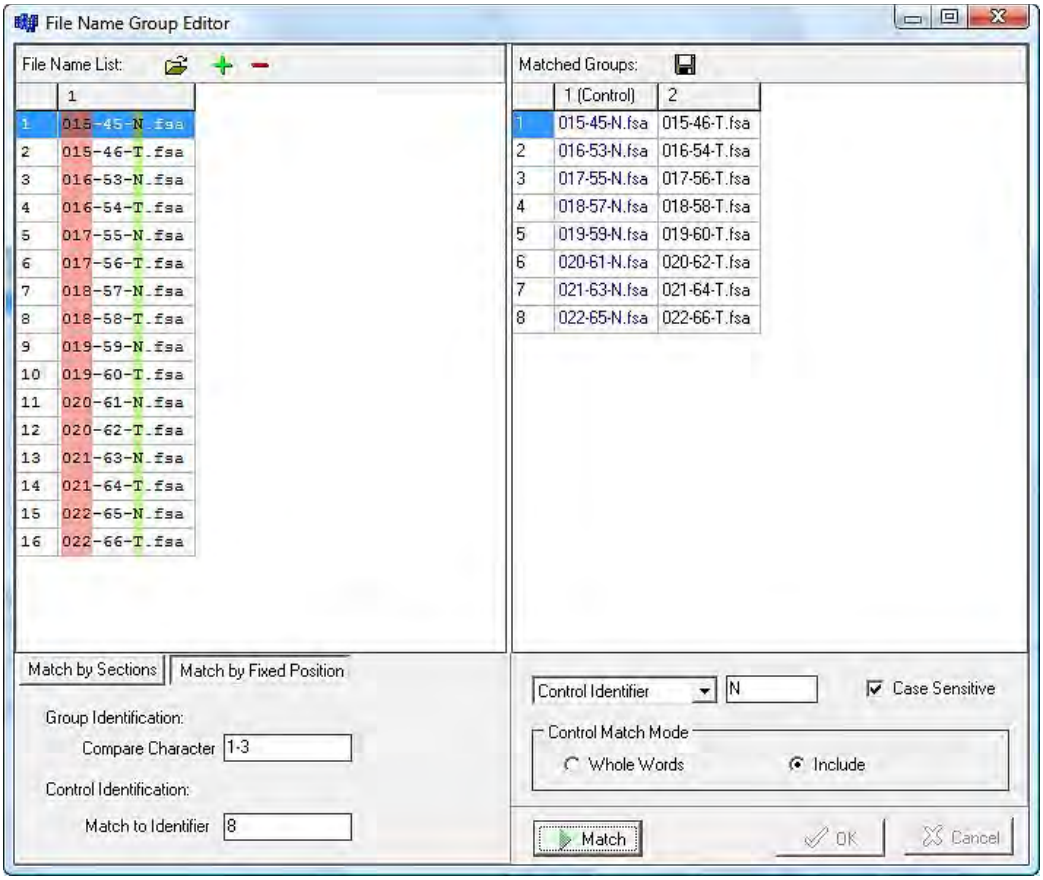
  Identifies how to match the filenames into groups based on the section entered the **Compare by Section** field. The section of the filename specified is highlighted red in the **File Name List**.

- **Control Identification**

  Identifies which section of the filename contains the reference vs. sample information based on the section number entered in the **Match to Identifier by Section** field.

The section of the filename specified is highlighted green in the **File Name List**.

Figure 228 File Name Group Editor



Match by Fixed Position

Allows the user to manually identify the characters of the filename for grouping the samples. Section Separators like "_ , -" are counted as individual characters.

- **Group Identification**

  Enter the number of the beginning and ending character to identify how to group the samples. The section of the filename specified is highlighted red in the File Name List.

- **Control Identification**

  Enter the number of the beginning and ending character to identify which part of the filename contains the control identifier. The section of the filename specified is highlighted green in the File Name List.

Control Identifier and Control Match Mode

Enter the character from the Control Identification section (highlighted green) that describes the control or reference sample. Example: N = normal or R = reference. Select Case Sensitive if the Control Identifier is identified by upper- or lower-case letters.

Choose either **Whole Words** or **Include**.

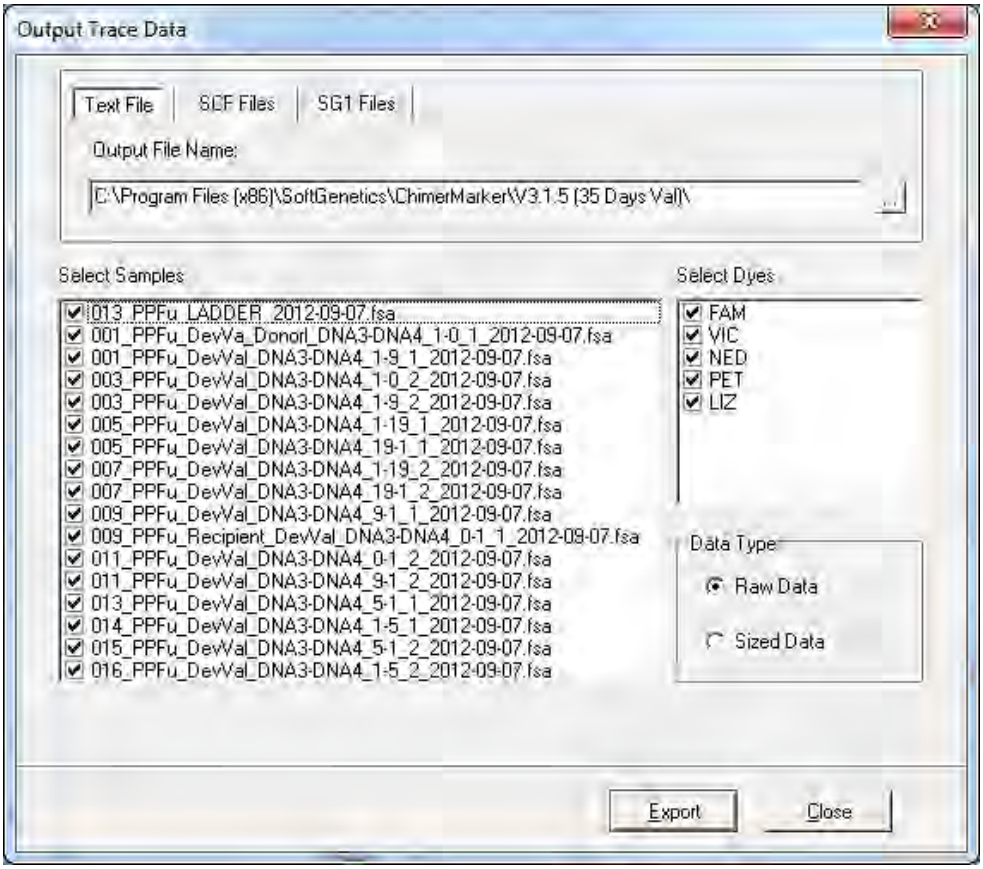
- Select **Whole Words** if the characters entered into the **Control Identifier** field need to match exactly.
- Select **Include** if the characters in the **Control Identifier** field are only identified in the filename, for example, are not an exact match.

Output Trace Data

Navigate to **Tools** > **Output Trace Data**.

The **Output Trace Data** tool exports raw or sized data of uploaded sample files as Text (.txt), SCF (.scf) files, or SG1 (.sg1) files.

Figure 229 Output Trace Data



Output Trace Data Procedure

1. Select whether to export the data as text, scf, or sg1 files.
2. Choose the directory and folder to save the exported data to in the **Output File Name** field.
3. Select the samples to include in the output file from the **Select Samples** field.
4. Select which dye color data to export from the **Select Dyes** field.
5. Select whether to export raw or sized data from the **Data Type** options.

6. Click **Export** to export the data to the specified folder.

Convert TXT to Binary

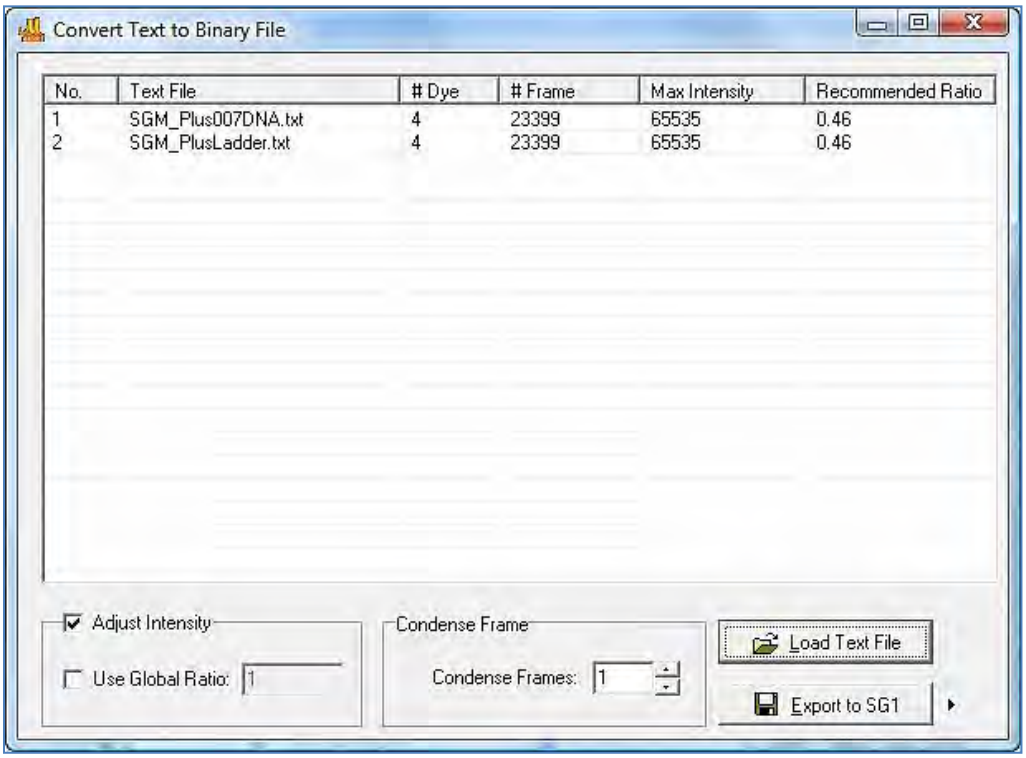
Navigate to **Tools** > **Convert Text to Binary Files**.

The Convert Text to Binary tool allows the user to upload trace data information in Text (.txt) file format for conversion into a four-color SCF file or a five-color SG1 file. The SCF and SG1 files can then be read by ChimerMarker and translated into chromatograms. This tool is useful for institutions developing their own fragment analysis instruments.

Procedure

1. Click the **Load Text File** button and select **Text** (.txt) files to convert.

   Figure 230 Convert Text to Binary File

   Once files are uploaded, they display in the **Text File** field.

   The software automatically calculates a **Recommended Ratio** for the user to condense the number of frames in a single trace.

2. Enter a condense frames by XX number in the **Condense Frames** field.
3. Click **Export to SG1** if exporting a five-color trace, click **Export to SCF** if exporting a four-color trace.

Export Electropherogram

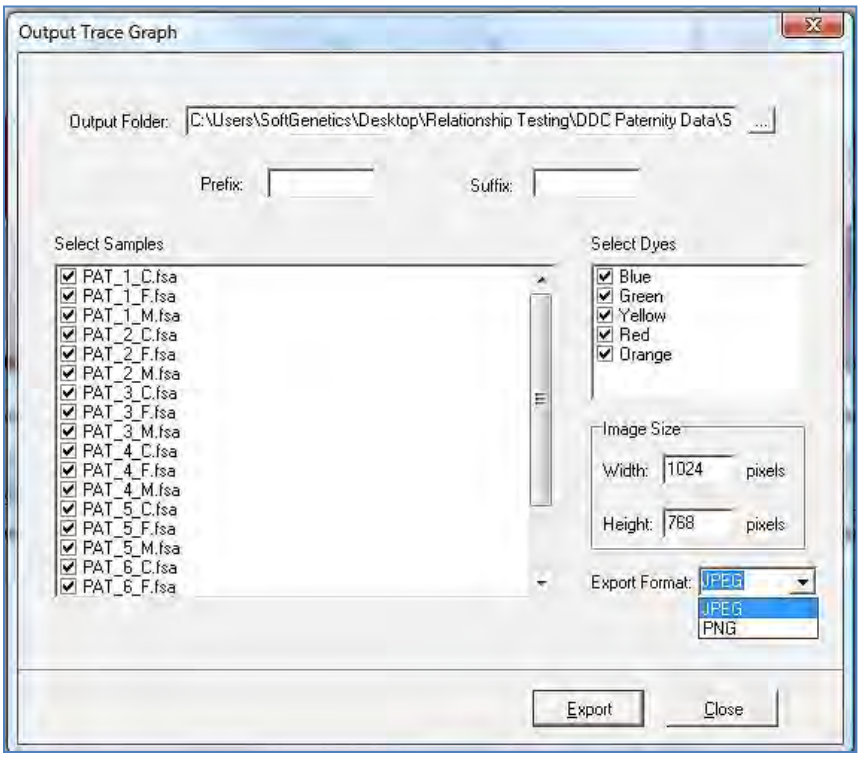
Navigate to **Tools** > **Export Electropherogram**.

The Export Electropherogram tool allows the user to export the trace images to a specified folder.

Export Trace Images Procedure

1. Use a dropdown menu to specify the output folder.
2. Specify the prefix and suffix for the exported file name. The full file name is Prefix+Sample name+"_"+Dye name+Suffix+Extension name.
3. Select samples, Dyes, and Image Size.
4. Use a dropdown menu to specify the export format. Both JPEG and PNG are available. PNG is recommended.

Figure 231 Output Trace Graph

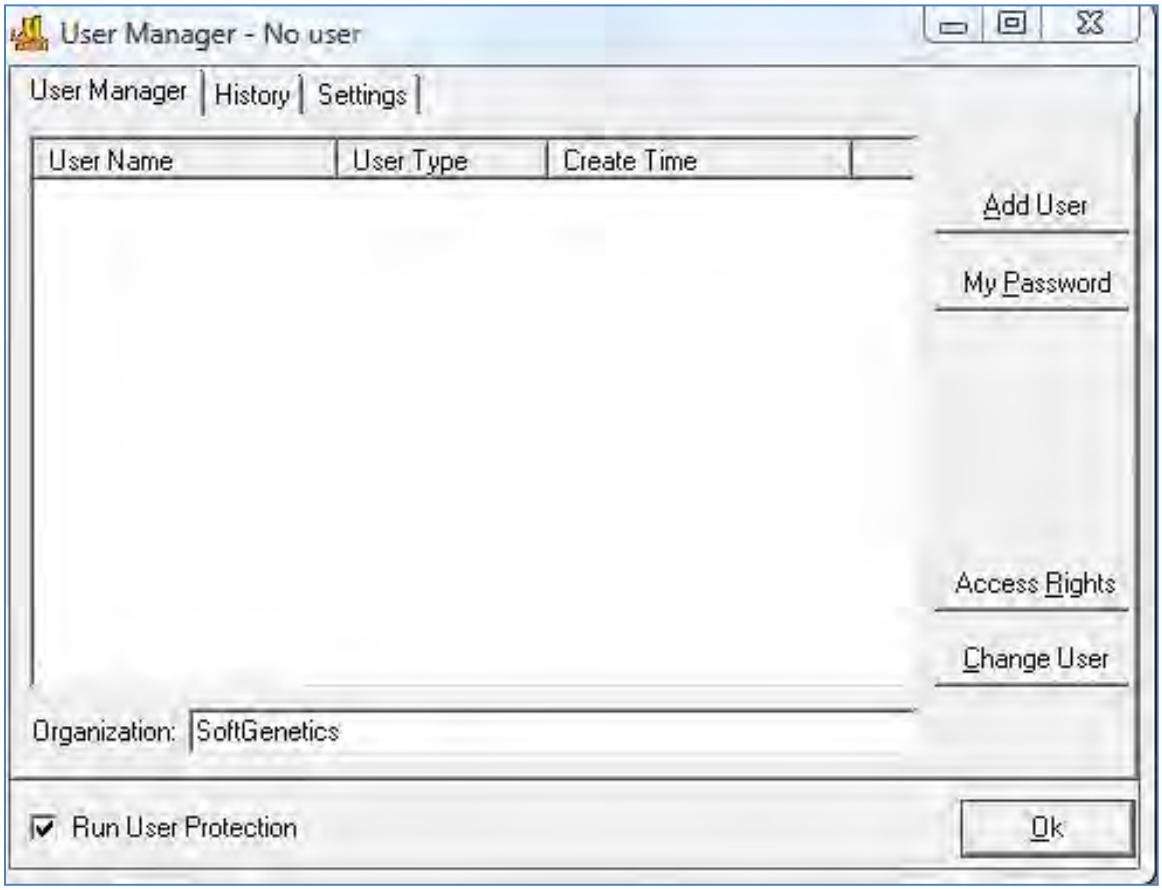


Chapter 10 User Management

Overview

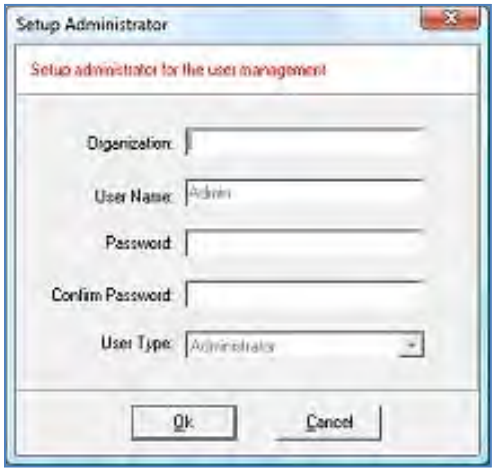
User management may be implemented after installation of ChimerMarker. The administrator activates User Management from the **Help** drop-down menu. User management provides control of user access rights and automatically generates an audit trail of all edits.

Figure 232 User Manager No user



Select **Add User** to display the **Setup Administrator** dialog.

Figure 233 Setup Administrator



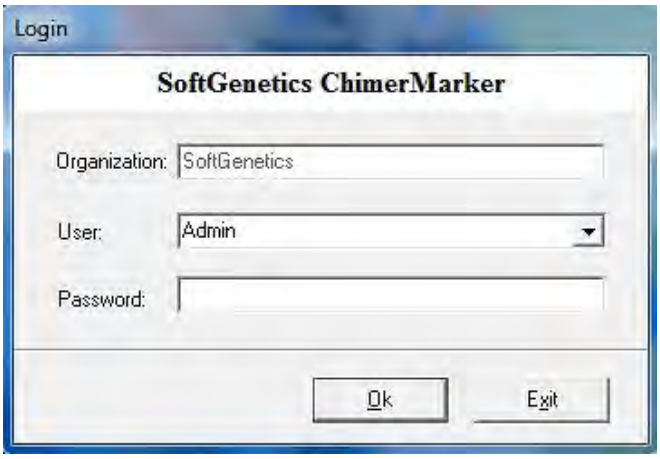
Setup User Procedure

Follow the next steps to setup a user.

1. Select **Help** > **User Management**.

   The **Login** dialog displays, as illustrated in Figure 234.
2. Click **Run User Protection** to activate the setup Administrator.
3. Enter **Organization** Name, an Administrator username and password.

   Figure 234 Login

4. Click **OK.**

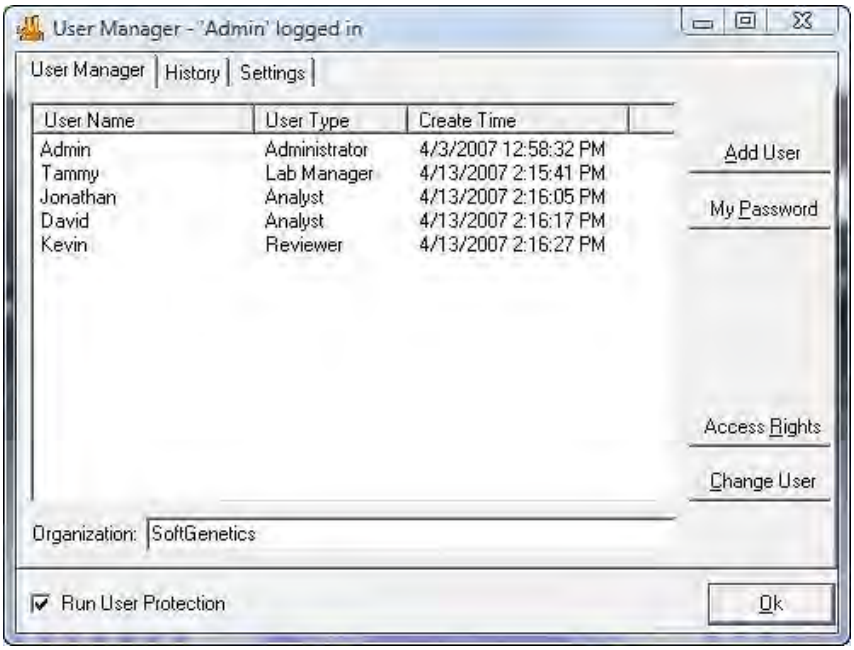
   You are now logged in as the Administrator. The **User Manager** dialog displays. Launches the **Add User** dialog where a new username and password are input.

This is also where the user type is chosen. Delete a user by right clicking the username and selecting **Delete User**.

User Manager

In the **User Manager** dialog, the **User Manager** tab displays user information and contains options for creating and deleting users.

Figure 235 User Manager - Admin Logged In



- **User Name** pane and other details.

  Displays all users by name, type and creation date.
- **Organization**

  In the **Organization** field enter the organization name.
- **Add User**

  Select the button to add additional users.

  Note: Only the Administrator can add and delete users.
- **My Password**

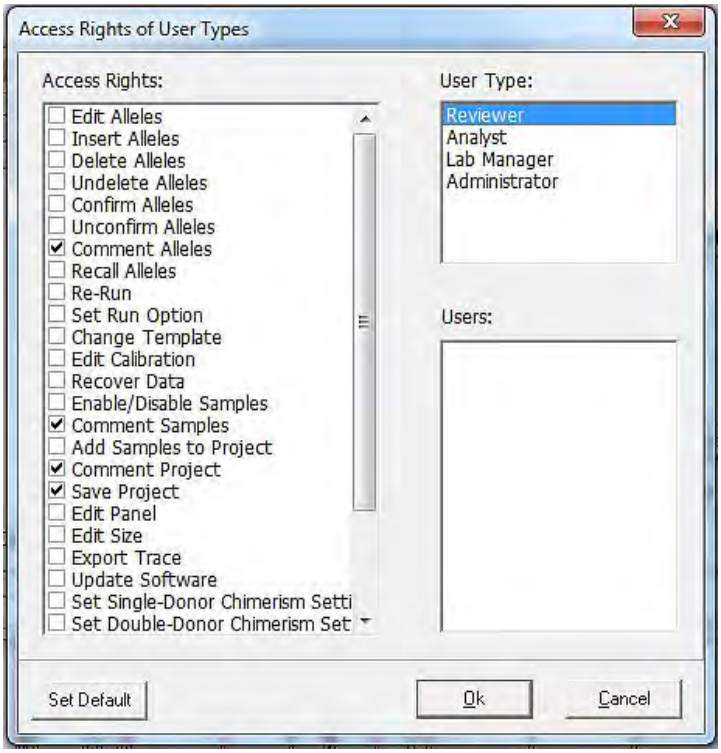
  Launches the **Change Password** dialog where the user that is logged in can enter a new password. The new password must be entered twice to ensure accuracy.
- **Access Rights**

  Select to set up user type access permissions.
- **Change User**

  Prompts for a confirmation of action then launches the **Login** dialog.

Launches the **Access Rights of User Types** dialog where the different access rights available to each user type are identified.

Figure 236 Access Rights of User Types



Click the **Set Default** button to return the Access Rights for the User Types selected back to factory defaults.

- **Run User Protection** check dialog.

  When selected, users are prompted to log on with a user name and password. When deselected, any person can launch ChimerMarker without a username and password. Select **Run User Protection** and click **OK** to exit.

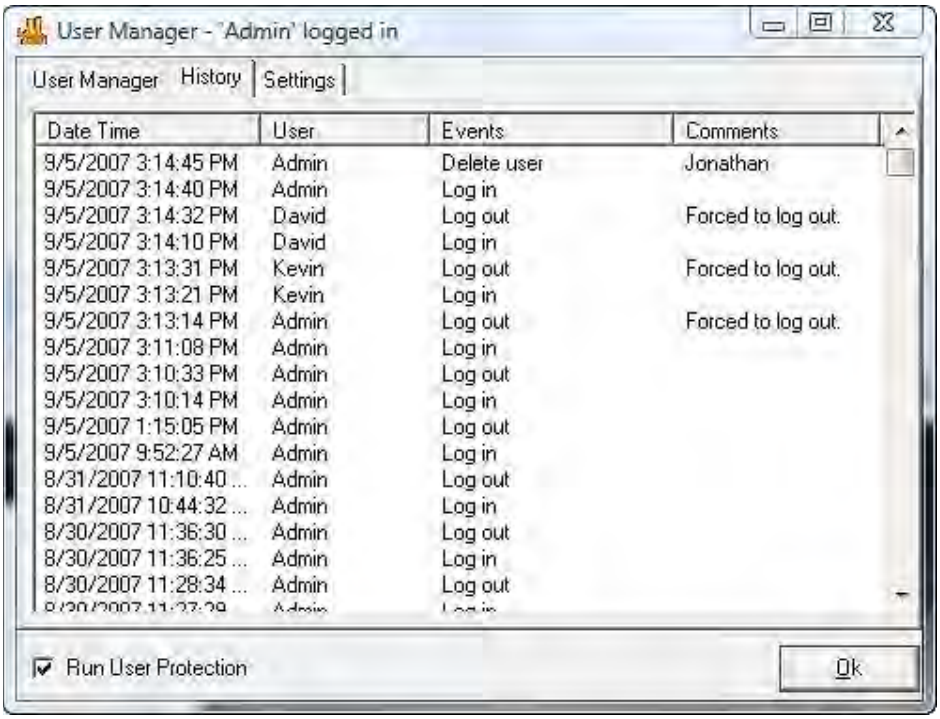
  Note: Only the Administrator can change Access Rights for a User Type.

  A login is required to open ChimerMarker after the User Manager is activated.

User Manager History

The **User Manager History** tab monitors user activity associated with the user manager function.

Figure 237 User Manager History Tab



- **Date/Time Column**

  Records the computer's date and time for the activity.

- **User Column**

  Identifies the username of the person that performed the activity.

- **Events Column**

  Records the user manager activity that was performed.

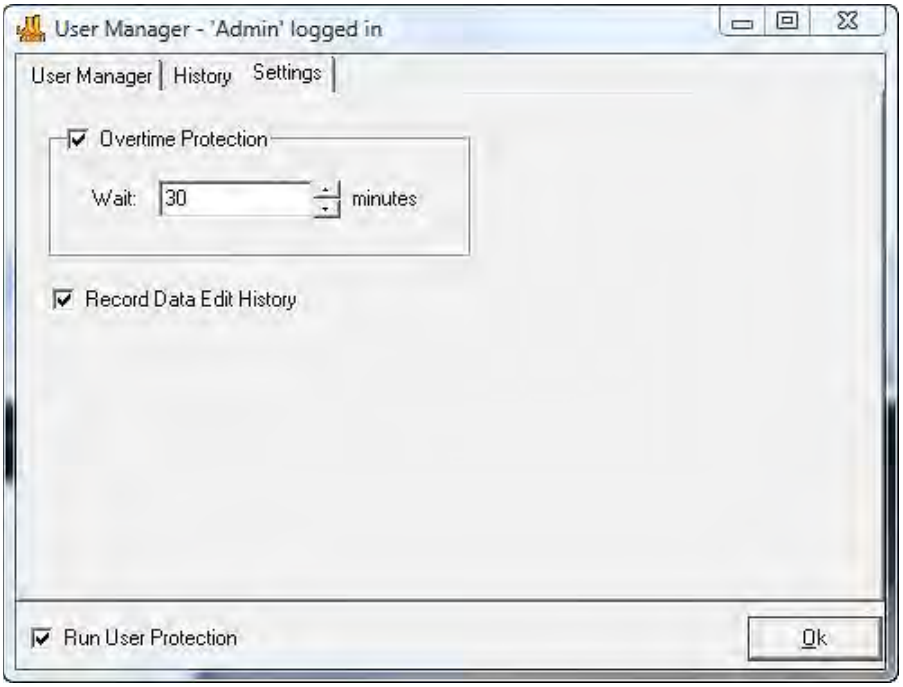
- **Comments Colum**

  Gives additional information for the event that was performed. For example, if a user is added, then the username of the person that was added is recorded under Comments.

User Manager Settings

The **User Manager Settings** tab contains additional options for the User Management function.

Figure 238 User Manager Settings Tab



When selected, ChimerMarker logs out the user after the specified 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 username and password).

- **Record Data Edit History**

  When selected, any changes made to the allele calls of the project are saved in the **Edit History** log. See the Edit History section next for more information.

  When **Record Data Edit History** is selected in the **User Manager Settings** dialog (see User Manager Settings section above), any change to allele calls in the analysis is recorded. Changes are also recovered in the Edit History feature.

Allele Call Procedure

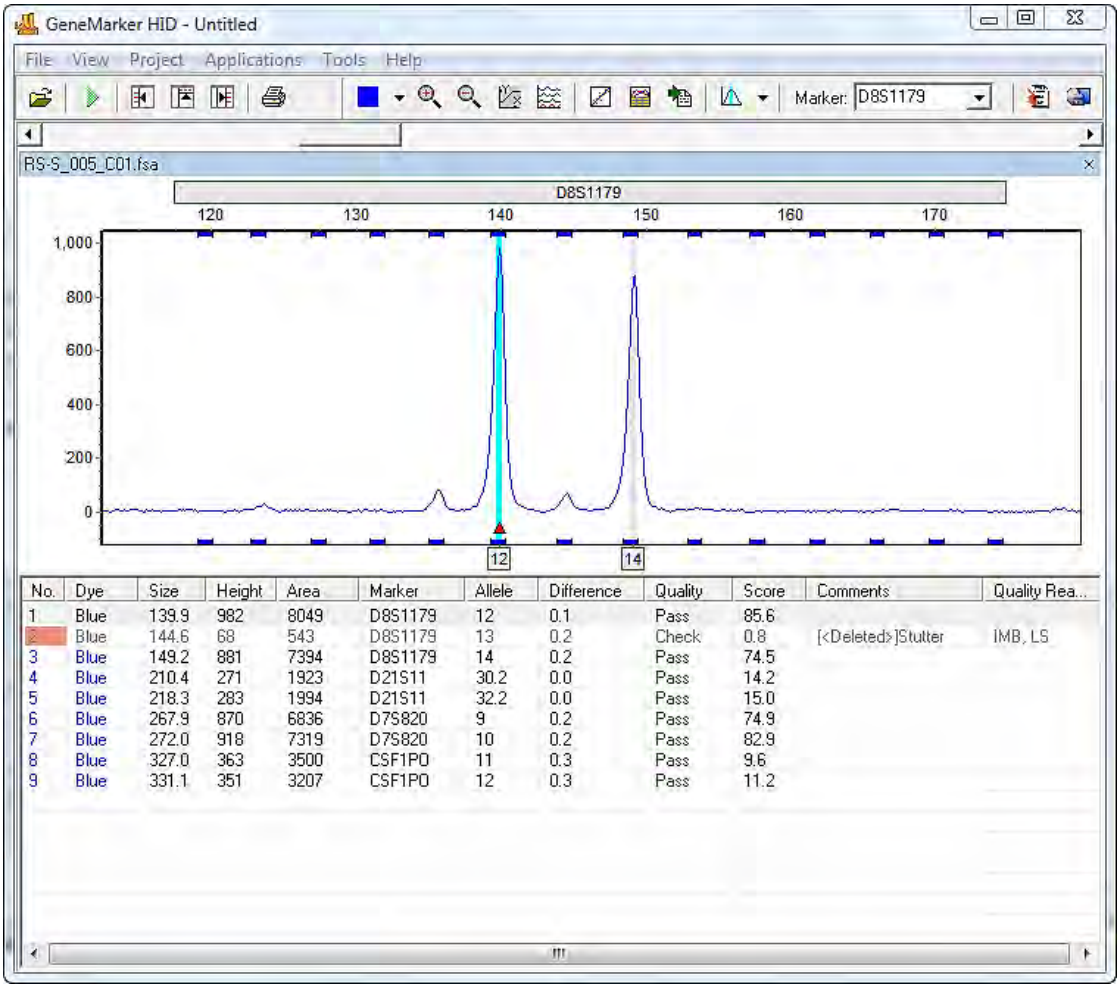
1. Click the **Show Chart/Table** icon in the Main Analysis dialog.

   The Peak Table displays below the sample electropherogram.

2. Make changes to allele calls by right-clicking any cell in that allele's row in the Peak Table or right-click the grey vertical bar at the center of the peak in the electropherogram.

3. Choose to **Edit Allele**, **Edit Comments**, **Add/Delete Allele,** and **Confirm**. See Chapter 4 Genotyping Main Analysis Overview for details.

Figure 239 GeneMarker HID

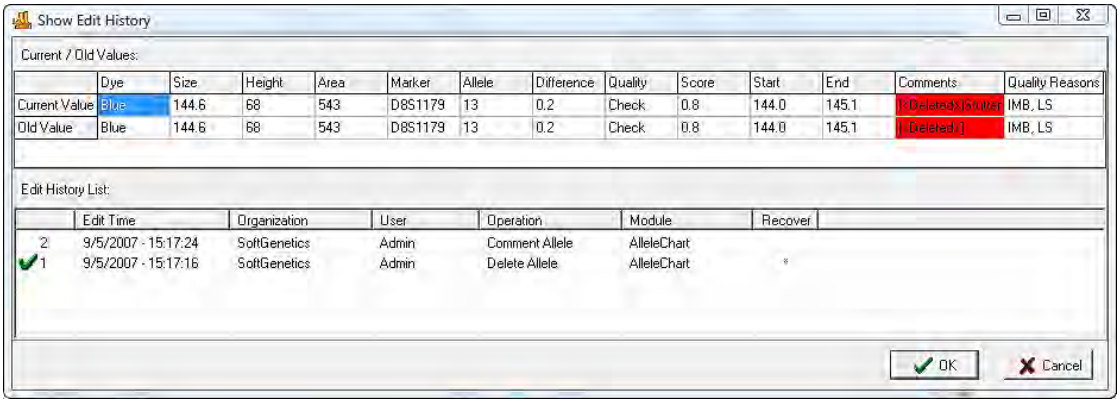


Once a change has been made to the allele call, notice the pink shading in the **No**. column of the Peak Table. This indicates a change was made to that allele.

4. Right-click any changed allele and select **View History**.

   The **Show Edit History** dialog displays.

Figure 240 Show Edit History



5. Select a change from the **Edit History List** to view changes in the **Current/Old Values** table. Changes are highlighted in red.
6. To recover a change, right-click the row in the **Edit History List** and select **Recover Old Value**. A star displays in the **Recover** column.
7. Click **OK** and click **Yes** when the warning prompts a confirmation.

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