SOFTGENETICS®

ChimeRMarker™

Automated Chimerism Analysis Software

User Manual

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Software PowerTools for Genetics Analysis
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February 2016

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Chapter 1 Installing ChimerMarker

Computer System Requirements

ChimerMarker software has been tested and validated for various computer systems. The minimum system requirements are:

Windows® PC
OS: Windows® 7, Windows® 8, 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 will only recognize PC file formats. To convert Macintosh file formats to PC file formats, please download the ABI PRISM® 3100 Genetic Analyzer Conversion Utilities to convert Mac files to PC files at:
http://www.appliedbiosystems.com/support/software/3100/conversion.cfm

Validation Version

The validation or trial version of ChimerMarker can be installed on as many computers as you wish. The trial period expires 35 days after installation of the software.

Installation

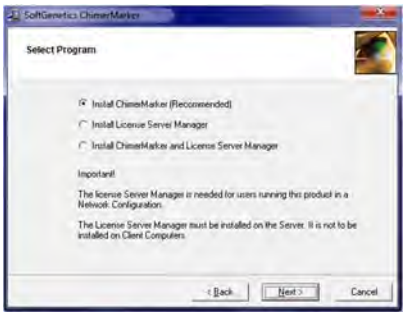
1. Insert the *SoftGenetics CD* into the CD-ROM drive. If your computer is not set to automatically open a CD, navigate to the optical or CD-ROM drive on the computer and open the directory.
2. Double-click the ChimerMarker Setup executable file (EXE)
3. The *Installation Wizard* will launch
4. Click the **Next** button in the *Welcome* window
5. 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* window
6. Select "Install ChimerMarker (Recommended)" in the *Select Program* window and click Next
7. Click **Next** in the *Destination Location* window to install ChimerMarker in the default folder. Click the **Browse** button to choose a different installation directory

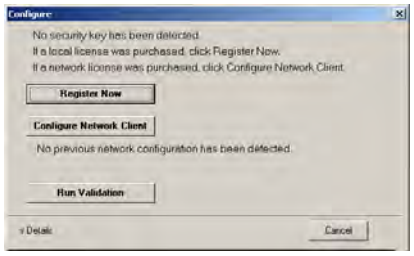
NOTE: The default *Destination Location* for the ChimerMarker program is C:\ProgramFiles\SoftGenetics\ChimerMarker\"version number"

8. Click **Next** in the *Select Program Manager Group* window to accept the default *Program Manager Group*

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

9. Click **Next** in the *Start Installation* window to install ChimerMarker
10. Click **Finish** in the *Installation Complete* window
11. The *Installation Wizard* will close
12. Eject the *SoftGenetics CD*
13. Launch ChimerMarker by double-clicking the **ChimerMarker** desktop icon <u>OR</u> open the **Start** menu and navigate to *SoftGenetics → ChimerMarker, the version that was just installed → ChimerMarker program*





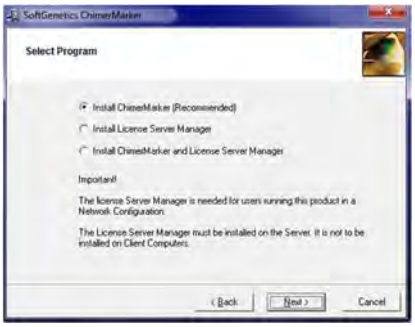
14. The *Configure* window will appear. Click **Run Validation** to launch the software
15. If the **Run Validation** button is grayed-out this indicates the 35-day trial period has expired.

Local-licensing Option

ChimerMarker v2.8.1 software and above supports text-based registration for the local-licensing option.

Text-based registration does not require a USB device, dongle, or key. This text-based registration ID is registered to one specific PC--the license will be 'locked' to this one PC. If the license needs to be transferred to a different PC, registration for that one license/PC must be inactivated first before the software can be registered to a new PC.

Installation



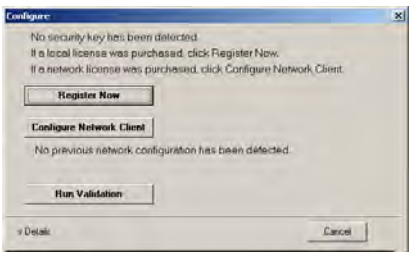
1. Insert the *SoftGenetics CD* into the optical or CD-ROM drive. If your computer is not set to automatically open a CD, navigate to the optical or CD-ROM drive on the computer and open the directory.
2. Double-click the ChimerMarker Setup executable file (EXE)
3. The *Installation Wizard* will launch
4. Click the **Next** button in the *Welcome* window
5. 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* window
6. Select "Install ChimerMarker (Recommended)" in the *Select Program* window and click **Next**
7. Click **Next** in the *Destination Location* window to install ChimerMarker in the default folder. Click the **Browse** button to choose a different installation directory

NOTE: The default *Destination Location* for the ChimerMarker program is C:\ProgramFiles\SoftGenetics\ChimerMarker\ver#

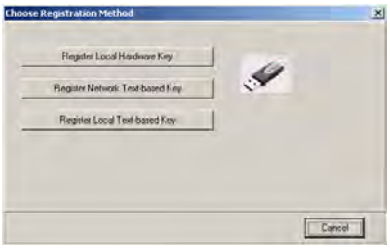
8. Click **Next** in the *Select Program Manager Group* window to accept the default *Program Manager Group*

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

9. Click **Next** in the *Start Installation* window to install ChimerMarker
10. Click **Finish** in the *Installation Complete* window
11. The *Installation Wizard* will close
12. Eject the *SoftGenetics CD*
13. Launch ChimerMarker by double-clicking the **ChimerMarker** desktop icon <u>OR</u> open the **Start** menu and navigate to *SoftGenetics → ChimerMarker, the version that was just installed → ChimerMarker program*
14. The *Configure/Registration* window will appear. Click **Register Now** to register the local license



15. Select local-licensing registration method from the *Choose Registration Method* dialog box:
    a. Click **Register <u>Local</u> <u>Text-based</u> Key**



Registration

1. The *Register Local Text-based Key* window appears for the Register Local Text-based Key method.
2. If the computer ChimerMarker is being installed on 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**.

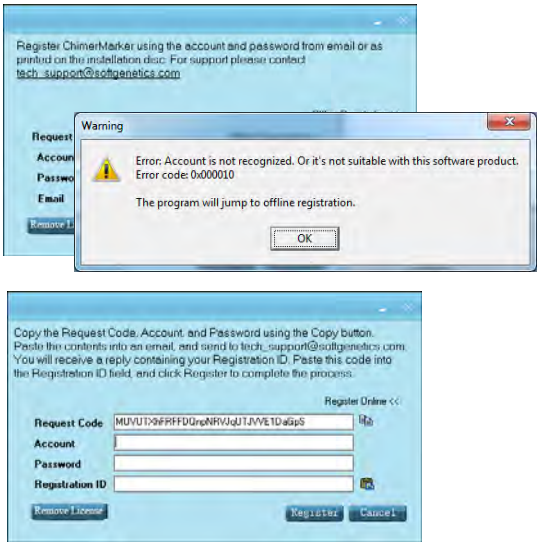
Online Registration

A. Locate the **Account** and **Password** on the *SoftGenetics CD*.
B. Enter your *Account*, *Password,* and *e-mail address* information in the appropriate fields
C. The *Request Code* information is automatically generated by ChimerMarker
D. Click **Register**
E. Your software will be registered automatically. A confirmation e-mail will be sent to you once registration is complete.

NOTE: Some characters can commonly be misread. If you get an error trying to register, check for number "1" and lower case letter "L" or number "0" and upper case letter "O" confusion.

F. Launch ChimerMarker and begin analysis

Offline Registration



A. Click the Register Offline option. The license message displays the offline registration procedure, "Email the User ID, Account, and Password to tech_support@softgenetics.com by clicking the Copy button then pasting into an email. SoftGenetics will send a reply with the appropriate Registration ID. Paste this ID into the Registration ID field. Click Register to complete the registration process." Click **OK**
B. Enter your *Account* and *Password* information from the *SoftGenetics CD* and use the **Copy** button to copy this information along with the *Request Code* string and paste into the body of an e-mail
C. Send the e-mail to tech_support@softgenetics.com
D. The *Registration ID* will be sent to you (via e-mail) within one business day
E. Copy and paste the entire *Registration ID* from the e-mail into the *Register ID* field
F. Click **Register**
G. Launch ChimerMarker and begin analysis

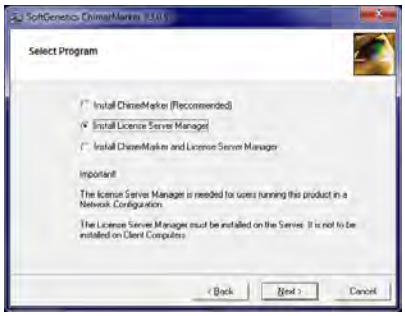
Network-licensing Option

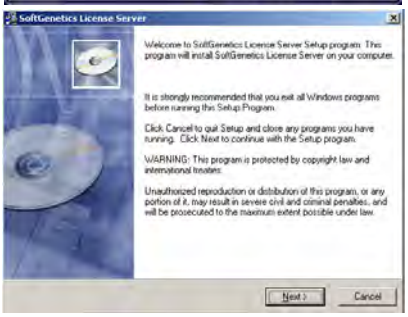
Installing License Server Manager and ChimerMarker Software

The network-licensing option of ChimerMarker can be 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 will require restarting the system to complete installation. Please save all work and close all applications before installing LSM.

Install License Server Manager

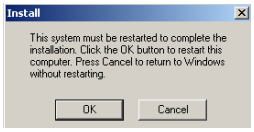




1. Insert the *SoftGenetics CD* into the optical or CD-ROM drive. If your computer is not set to automatically open a CD, navigate to the optical or CD-ROM drive on the computer and open the directory.
2. Double-click the ChimerMarker Setup executable file (EXE)
3. The *Installation Wizard* will launch
4. Click the **Next** button in the *Welcome* window
5. 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* window
6. Select "Install License Server Manager" in the *Select Program* window and click **Next**
7. Click **Next** in the *Destination Location* window, **Next** in the *Select Program Manager Group* window, and Next in the *Start Installation* window to enter the LSM installation wizard
8. Click the **Next** button in the *Welcome* window
9. 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* window
10. Click **Next** in the *Destination Location* window to install LSM in the default folder. Click 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

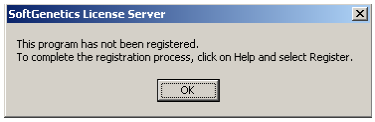
11. Click **Next** in the *Start Installation* window to install License Server Manager
12. Select the Launch License Server Manager option and click **Finish**
13. Click **OK** in the *Install* window to restart the system.
14. The *Installation Wizard* will close and the system will restart
15. Eject the *SoftGenetics CD*



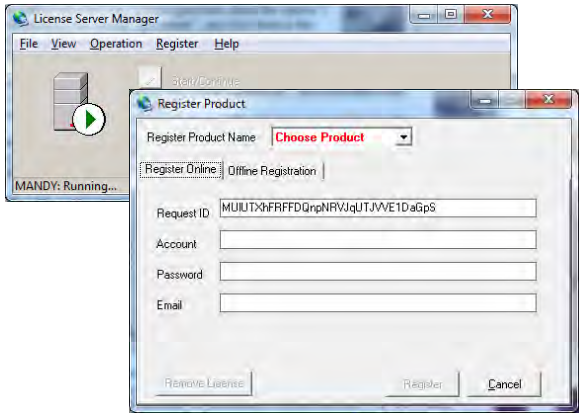
Register License Server Manager for ChimerMarker Usage

1. Open License Server from the System or Icon Tray by clicking the LSM icon

NOTE: A red star indicates the License server is not running. The icon with a white star indicates the License Server is running properly.

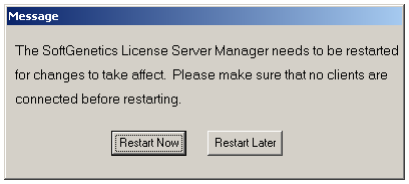


2. Click **OK** in the dialog box to proceed with registering License Server from the License Server Manager console.
3. Select Register from the main toolbar to activate the Register Product window
4. Select **ChimerMarker** from the *Register Product Name* drop-down menu
5. If the computer License Server is being 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**.



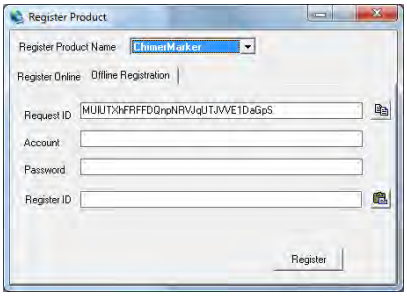
Online Registration

A. Locate the **Account** and **Password** on the *SoftGenetics CD*
B. Enter your *Account*, *Password,* and *E-mail address* information in the appropriate fields
C. The *Request Code* information is automatically generated by License Server
D. Click **Register**
E. Your software will be registered automatically. A confirmation e-mail will be sent to you once registration is complete



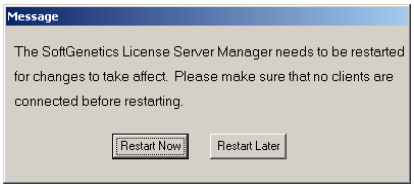
NOTE: Some characters can commonly be misread. If you get an error trying to register, check for number "1" and lower case letter "L" or number "0" and upper case letter "O" confusion.

F. Restart License Server to apply the registration information
G. Install ChimerMarker software and configure network client



Offline Registration

H. Copy and paste the entire *Request ID* string from the Register Online dialog, and type your *Account* and *Password* information from the *SoftGenetics CD* into the body of an e-mail
I. Send the -email to tech_support@softgenetics.com
J. The *Register ID* character string will be sent to you via e-mail within one business day
K. Copy and paste the *Registration ID* from the e-mail into the *Register ID* field of the Offline Registration tab. Please be sure to copy the entire registration ID character string
L. Click **Register**
M. Restart License Server to apply the registration information
N. Install ChimerMarker software and configure network client



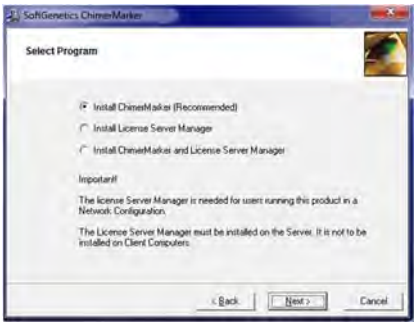
Installing ChimerMarker Software

Install ChimerMarker software on client computers

16. Insert the *SoftGenetics CD* into the optical or CD-ROM drive. If your computer is not set to automatically open a CD, navigate to the optical or CD-ROM drive on the computer and open the directory
17. Double-click the ChimerMarker Setup executable file (EXE)
18. The *Installation Wizard* will launch
19. Click the **Next** button in the *Welcome* window
20. 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* window
21. Select "Install ChimerMarker (Recommended)" in the *Select Program* window and click Next
22. Click **Next** in the *Destination Location* window to install ChimerMarker in the default folder. Click the **Browse** button to choose a different installation directory

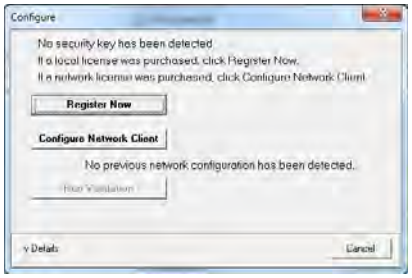
NOTE: The default *Destination Location* for the ChimerMarker program is C:\ProgramFiles\SoftGenetics\ChimerMarker\ver#

23. Click **Next** in the *Select Program Manager Group* window to accept the default *Program Manager Group*

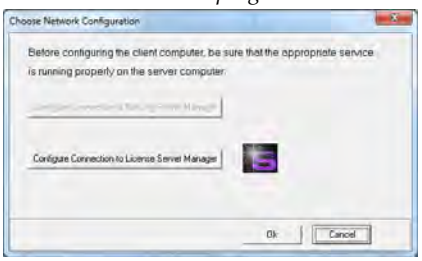


NOTE: Changing the *Program Manager Group* default may affect program operability. It is recommended to accept the default.

24. Click **Next** in the *Start Installation* window to install ChimerMarker
25. Click **Finish** in the *Installation Complete* window



26. The *Installation Wizard* will close
27. Eject the *SoftGenetics CD*
28. Launch ChimerMarker by double-clicking the **ChimerMarker** desktop icon *OR* open the **Start** menu and navigate to *SoftGenetics → ChimerMarker, the version that was just installed → ChimerMarker program*
29. The *Configure/Registration* window will appear. Click **Configure Network Client** to configure the client software to contact License Server Manager
30. Click **Configure Connection to License Server Manager** from the *Choose Network Configuration* dialog box
31. Input 'Server Name' or 'Server IP Address' of the License Server Manager server
32. Click Configure and ChimerMarker software will automatically open if connection is properly established and a license is available



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

Overview

ChimerMarker™, automated chimerism analysis software, integrates speed and accuracy with a biologist-friendly interface. The software can be used to monitor chimerism level in both allogeneic and autologous stem cells transplant (SCT) or hematopoietic stem cells transplant (HSCT), bone marrow transplant (BMT, post bone marrow engraftment), cord and peripheral blood stem cells transplant (PBSCT) samples. The program provides accurate, rapid genotyping and chimerism analysis; automatically identifies donor and recipient peaks in post-BMT samples, calculates percent chimerism and quality metrics for single donor or double donor cases, and has multi-lineage capabilities for chimerism analysis of T-cells, B-cells, and other cell type populations. ChimerMarker includes functions for comparison of samples at different time points to conduct longitudinal studies for monitoring each individual and a comprehensive chimerism analysis report. The chimerism analysis performs repetitive calculations (using published methods). ChimerMarker is compatible with ABI®PRISM, Beckman-CEQ™, and MegaBACE® genetic analyzers, and custom primers or commercially available human identification chemistries from ThermoFisher (AB), Promega, and Qiagen for STR genotyping, including GlobalFiler™ 6-Dye™, Identifiler®, Minifiler®, PowerPlex®16, PowerPlex®18D, PowerPlex®21, PowerPlex®ESI/ESX, PowerPlex®Fusion, GenePrint®24, PowerPlex®Fusion 6C, Investigator®24Plex, and Investigator®ESSplex Plus. Chimerism analysis is completely linked to the main analysis screen, removing the error-prone step of data transfer from genotyping software to chimerism analysis software.

Procedure

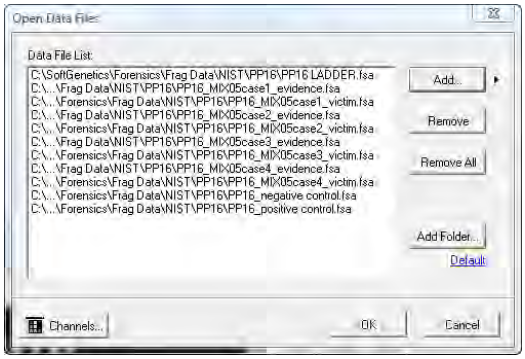
Import Data Files

After installing ChimerMarker software you are 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
Spectrumedix - .smd
Generic - .scf, sg1

Procedure

1. Launch **ChimerMarker**
2. Click **Open Data**
3. The *Open Data Files* box will appear
4. Click **Add** button
5. The *Open* dialog will appear
6. Navigate to directory containing raw data files
7. Select all files by **CTRL+A** or use **CTRL** and/or **SHIFT** keys to select individual samples
8. Click **Open** button in the *Open* dialog
9. The files selected will appear in the *Data File List* field
10. Click **OK** button in the *Open Data Files* box and the samples will be uploaded to ChimerMarker



Features

There are several features available in the *Open Data Files* box to make data upload easier.

Add…
Used to locate and select raw data files for upload. Click the arrow button next to the **Add** button to see the four most recently accessed directories.

Remove
Used to remove samples from the *Data File List*. Highlight the sample to remove by single left-clicking it in the *Data File List* then click **Remove**.

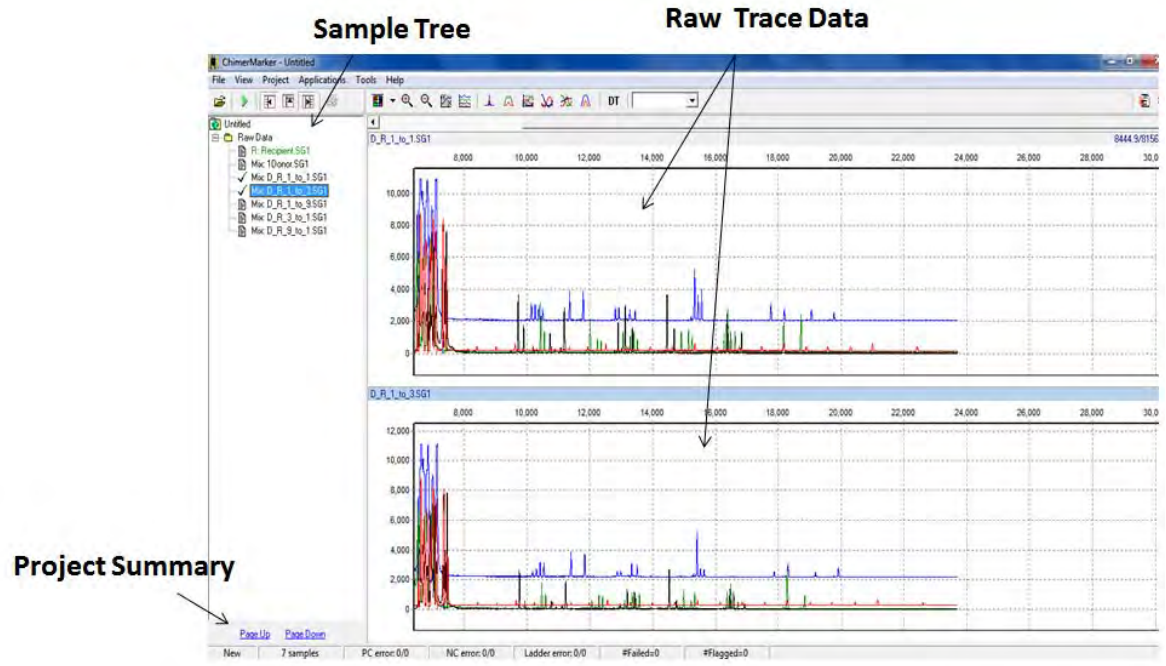
Remove All
Removes all sample files from the *Data File List* field.

Add Folder…
Click **Add Folder** to upload raw data files from a specific folder in the file directory tree. Click the *Default* hyperlink to choose a folder to which ChimerMarker will always open when the **Add** or **Add Folder** buttons are clicked.

Channels
Opens the *Set Channels* dialog with 4, 5, 6-color tab options and allows the user to choose from ABI, MegaBACE, and Beckman-Coulter standard dye color orders. The user can also manually enter 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 box.

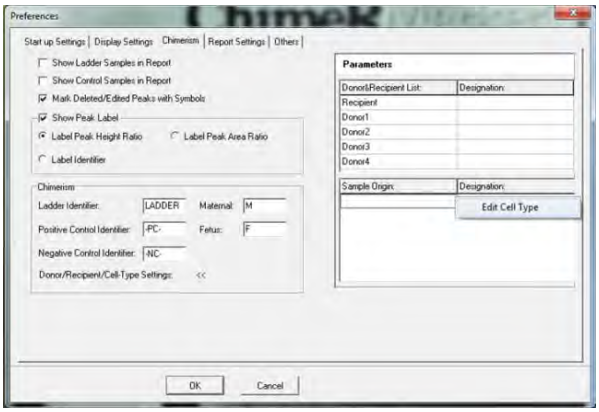
Raw Data Analysis

Once the raw data files are uploaded, the *Raw Data Main Analysis* window 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* window with fragment mobility from right to left. The largest size fragments are on the far right of the trace.



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 can be set automatically when file naming systems are used (see figure below) or by right mouse click on the sample name and select set sample type.

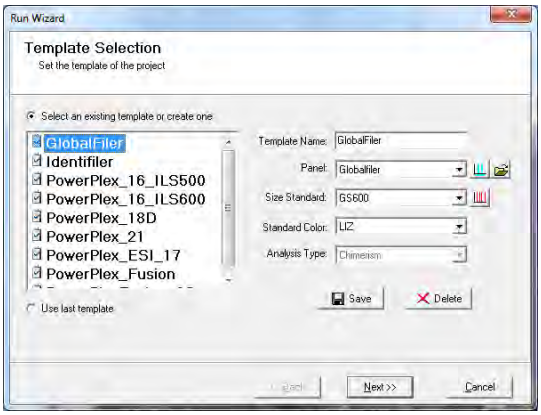


Analysis Preferences, Multi-Lineage Analysis

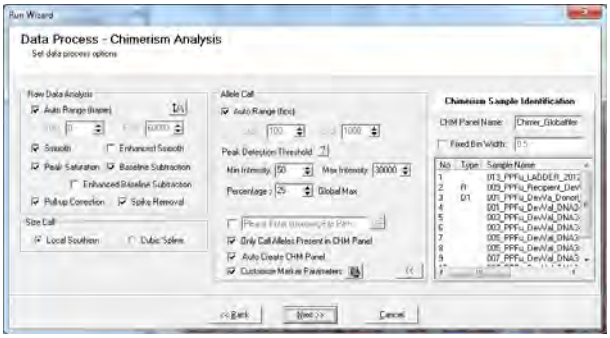
The program preferences include labeling peak ratios by height or area; with multi-lineage capabilities for chimerism analysis of T-cells, B-cells, and other cell type populations. By doing multi-lineage assessment, valuable information about the current graft status of the host can be seen. In addition,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

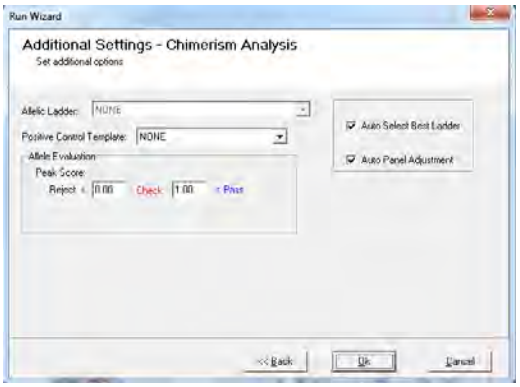
1. Run Wizard Template Selection:
   - Select from the pre-loaded run templates for many commercially available human identification kits, or select custom parameters and save the template using the save icon.
   - Panel→ Select from drop-down menu
   - Size Standard→ Select from drop-down menu
   - Standard Color→ Select from drop-down menu



2. Data Processing:
   - Select "Auto Create CHM Panel" to have ChimerMarker automatically creates a Chimertyping panel and applies it (recommended). Deselect to manually create Chimertyping panel (see Alternative Chimerism Method below)
   - Default settings for Raw data analysis, Size Call and Allele Call are recommended for most data sets.
   - Select "Customize Marker Parameters" to specify the marker parameters for the Chimertyping Panel.



3. If an allelic ladder sample was run with the samples amplified with a commercially available kit, select Auto Select Best Ladder and Auto Panel Adjustment. If no allelic ladder sample was used, deselect these options.



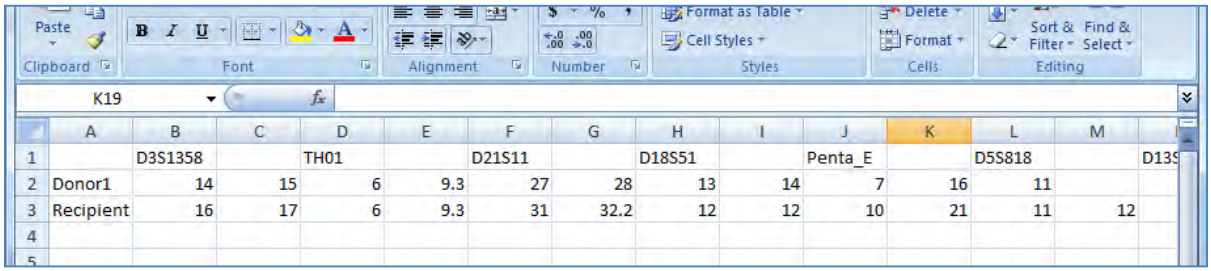
NOTE: If data has high noise-to-signal ration please use the Alternative Method Procedure presented at the end of this section.

If Pre-transplant CE data files are not available, Import Donor and Recipient Genotypes as a Text File:

If samples files (.fsa, .hid, etc.) aren't available for the recipient and donor(s), the user may upload the genotypes in a tab-delimited text file. ChimerMarker supports upload of either the allele calls, or allele sizes. The text file requires specific formatting described below. It is recommended that you construct your file in Excel or another spreadsheet program, and then save the file as a tab delimited text-file. The file may then be uploaded to ChimerMarker using the text-input tool.

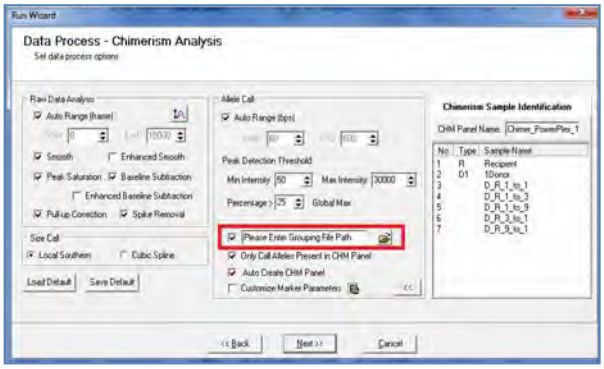
1) Creating the Text File Using Excel:

1. Your file should use the excel format displayed below.
2. Your file may contain Allele Sizes OR Allele Calls, not a mixture of both.
3. Marker names should be written exactly as they appear in your panel. They are case sensitive.
4. For homozygous markers, enter each allele only once (see D5S818 below).
5. Do not put a space between the word Donor and 1 or 2.
6. You must include every marker that will be in your project panel. If no calls are available for a given marker, you can leave the donor and recipient cells blank, (but keep the marker name).
7. Save your Excel sheet as a tab-delimited text-file (.txt).

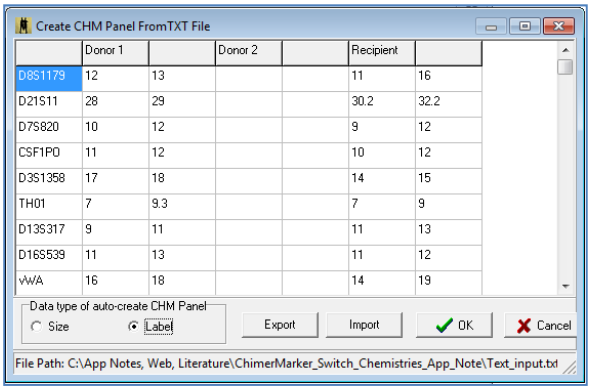


	A	B	C	D	E	F	G	H	I	J	K	L	M
1		D3S1358		TH01		D21S11		D18S51		Penta_E		D5S818	
2	Donor1	14	15	6	9.3	27	28	13	14	7	16	11	
3	Recipient	16	17	6	9.3	31	32.2	12	12	10	21	11	12
4													
5													

2) Importing Text File into ChimerMarker:



1. Select appropriate genotyping panel and size standard in the first window of the run wizard
2. Select the option to import text file as shown in the figure.
3. Click the Folder icon to open the text-input tool.
4. Click Import, and navigate to your text file.
5. Select Size or Label to indicate whether your text file contains allele sizes, or allele labels.
6. Click OK to proceed with your analysis.



	Donor 1		Donor 2		Recipient	
D8S1179	12	13			11	16
D21S11	28	29			30.2	32.2
D7S820	10	12			9	12
CSF1PO	11	12			10	12
D3S1358	17	18			14	15
TH01	7	9.3			7	9
D13S317	9	11			11	13
D16S539	11	13			11	12
vWA	16	18			14	19

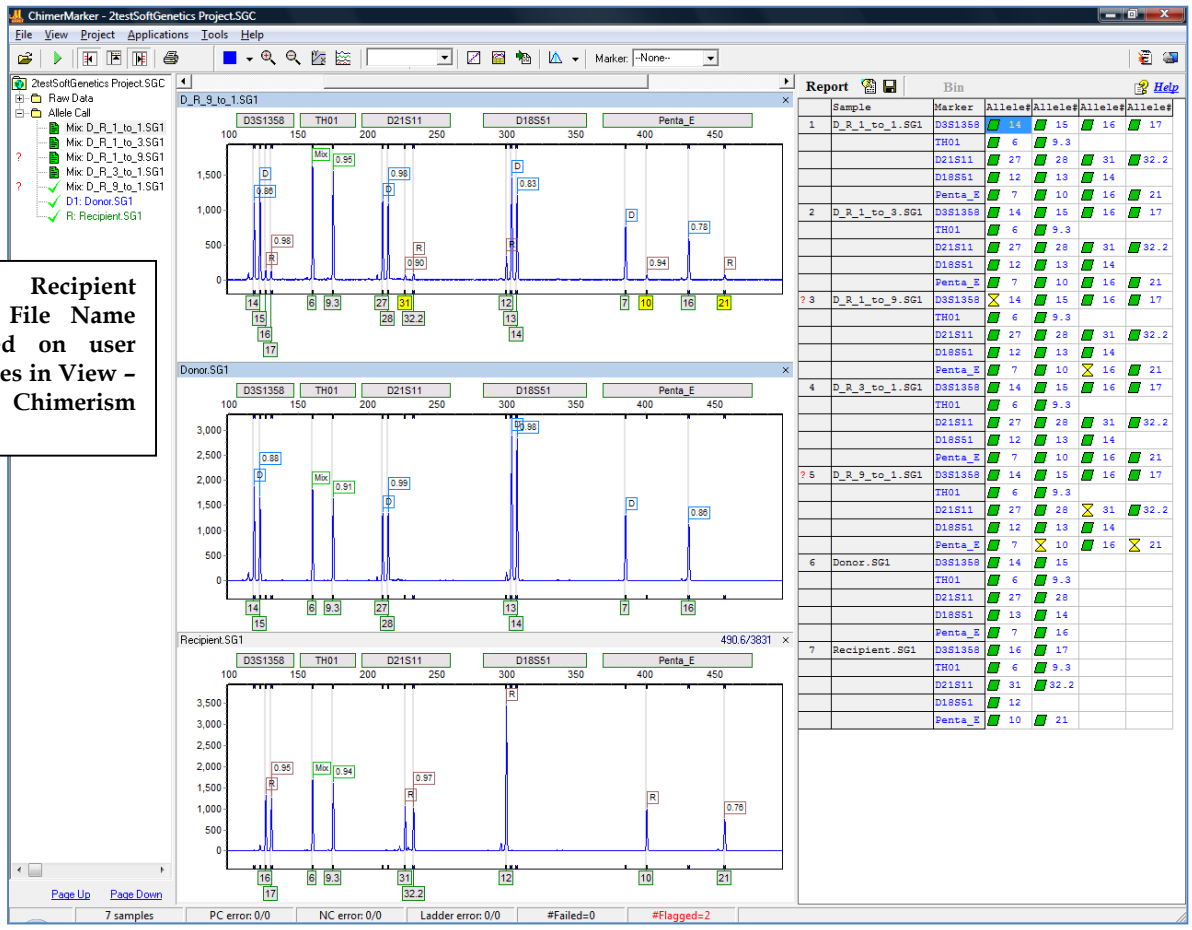
Additional Details:

The user may also manually edit text files using the text input tool. After a text file has been uploaded, simply click on a cell and type to modify the file.

When you are finished editing, click Export to save your edits. If you edit a file using the text input tool, you must save the changes before proceeding with data processing.

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 please refer to the Size Calibration chapter. Allele calls are displayed below each peak. If there are any red OL (off ladder) allele peaks, please see the Panel Editor chapter for information on panel alignment and calibration.

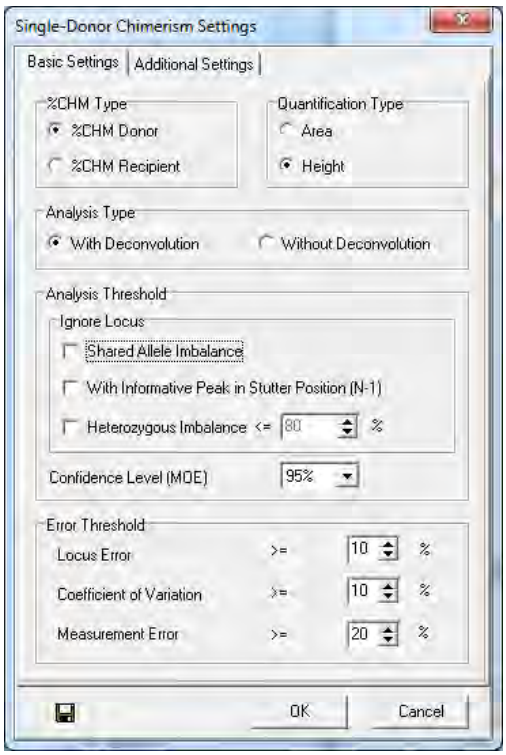


Donor and Recipient indicated in File Name Tree – based on user specified names in View – Preferences – Chimerism Tab

Post-Transplant Sample: ChimerMarker will differentiate and label 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 single source sample(s) 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.

Any edits must be made in the main analysis window and saved in the chimerism project (.SGC) file. This maintains integrity of the analysis going forward into the Chimerism Application and Calculations.



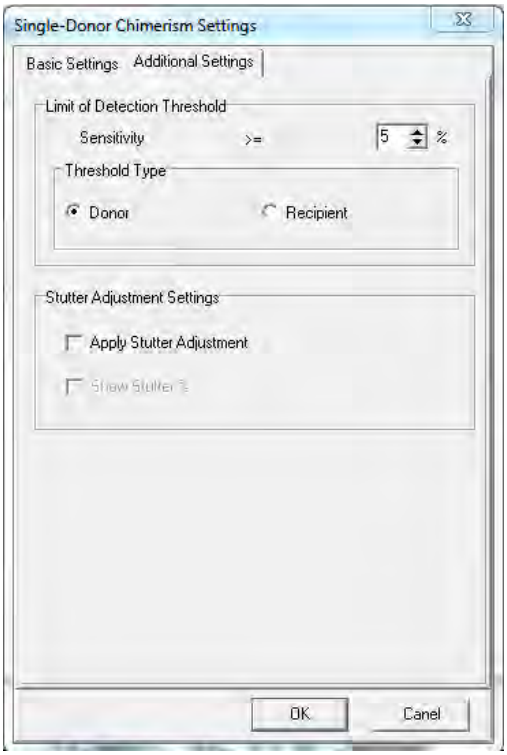
Automated Chimerism Detection and Quantification

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 dialog boxes for Single Donor Chimerism Settings and Double Donor Chimerism Settings provide flexibility of analysis options. Details of the equations used for all calculations are provided at the end of this chapter in the section on Chimerism Equations.

1. Select %Chimerism Type
   a. %CHM Donor or
   b. %CHM Recipient

2. Select Quantification Type
   a. Peak Area or
   b. Peak Height

3. Select Analysis Type
   a. With Deconvolution or
   b. Without Deconvolution



4. The Ignore Locus section provides three additional conditions under which markers should be ignored.
   a. Shared Allele Imbalance: If this option is selected, the marker will be ignored if a single source peak in the marker has higher RFU than a shared peak.
   b. With Informative Peak in Stutter Position (N-1): The marker will be ignored if a donor, recipient, or shared peak is in a stutter position. If selected, the stutter adjustment option will be disabled.
   c. Heterozygous Imbalance: Ignores a locus if the smaller of two donor or recipient peaks is shorter than the specified percent times the height of the larger of the two peaks.

5. Set the desired Error Threshold for Locus Error, Coefficient of Variation and Measurement Error

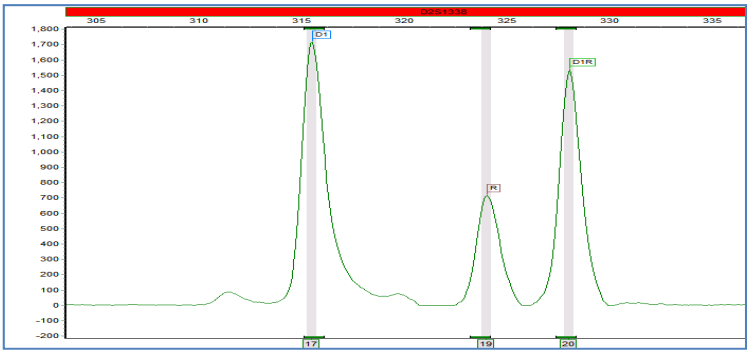
6. Specify the percent for Limit of Detection Threshold. Any sensitivity above the threshold % will be flagged red. Also specify the Threshold type, donor or recipient. It is best to use the same type as the %CHM Type selected in the Basic Settings.

7. Apply Stutter Adjustment will subtract marker stutter specific values, specified in the panel, in the stutter alleles. The stutter % value for each markers can be displayed if "Show Stutter %" is selected along with "Apply Stutter Adjustment"

8. Click the save icon (bottom left corner) to save your selections as the default settings.

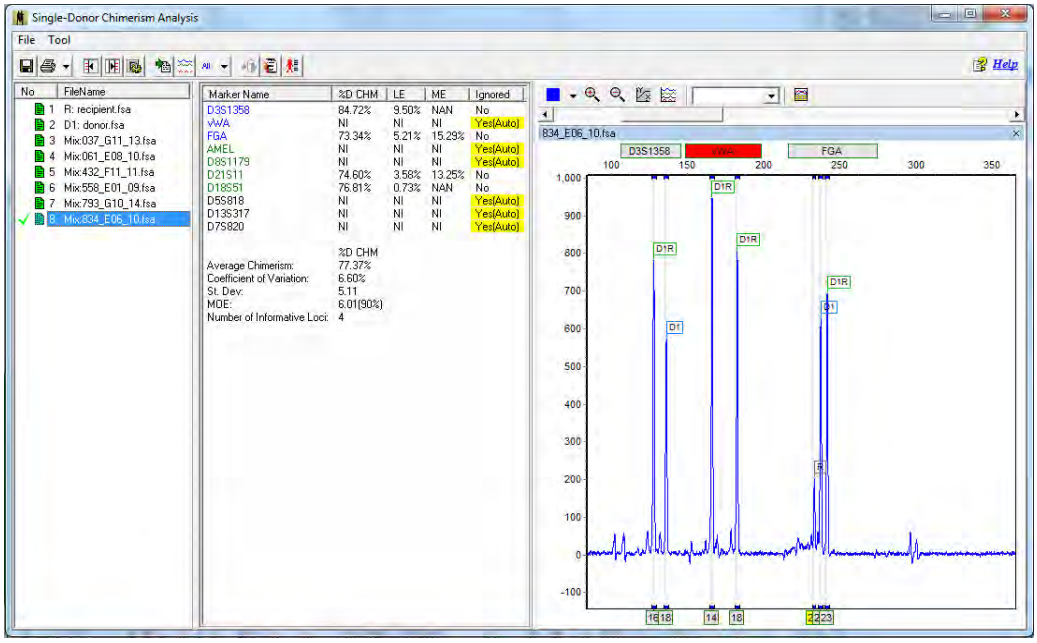
9. Select OK to review the results of chimerism calculations.

An example of SAI for locus D2S1338 is displayed here. Quantification is suspect when PCR bias results in the smaller fragment from one individual is more efficiently amplified than the larger fragment that is shared between donor and recipient.



REVIEW RESULTS

The automated chimerism calculations are displayed in the center table. To view the results for a given file, click on the file name in the list at the left. Loci that do not meet the selected chimerism analysis settings are Ignored and highlighted in yellow in the report table. To manually ignore (disable) a locus, right click on the cell in the Ignore column and select *Ignore*. Additionally, multiple loci can be ignored at once by holding the shift or ctrl key down, selecting the desired loci, then right-click and select *Ignore markers*. A marker can be ignored in all samples by right-clicking and selecting *Ignore marker in all Samples*. The locus label of any uninformative loci are red in the electropherogram.



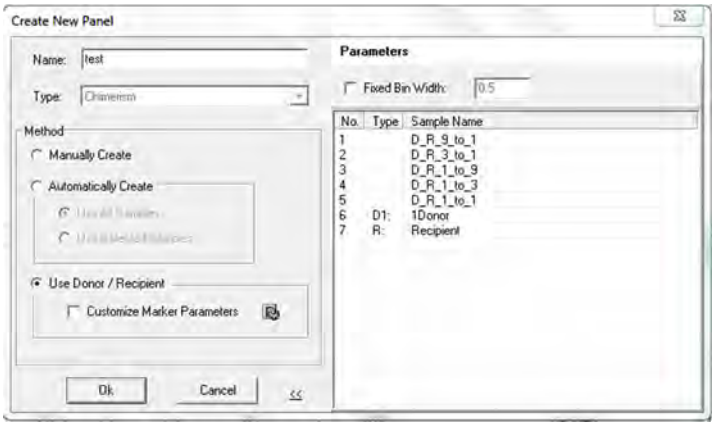
NI: Non-Informative-Marker is Non-Informative based on peak pattern and parameters chosen in analysis settings.
NAN: No Available Number-No results can be calculated for the following peak pattern.
ME: Measurement Error
LE: Locus Error
MOE: Margin of Error

Alternative Method Procedure

The alternative workflow is designed for problematic data – such as for files that have extensive pull up peaks or shifts in migration from capillary electrophoresis variation over time. Optimally, these issues are resolved by maintenance to the CE system; however, the alternative workflow in ChimerMarker provides the flexibility to allow accurate chimerism detection and monitoring in some sub-optimal data sets. Initial analysis starts with genotyping (determining size and allele calls) of the single source donor and recipient sample for each case. Please follow the steps in Chapter 3 Genotyping Details. After genotyping the original single source samples the case specific chimerism panel is constructed as described in Chapter 6, Panel Editor. Return to the main analysis screen and activate the Run Wizard. Use the chimerism panel for the case under analysis and re-analyze the data with 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 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

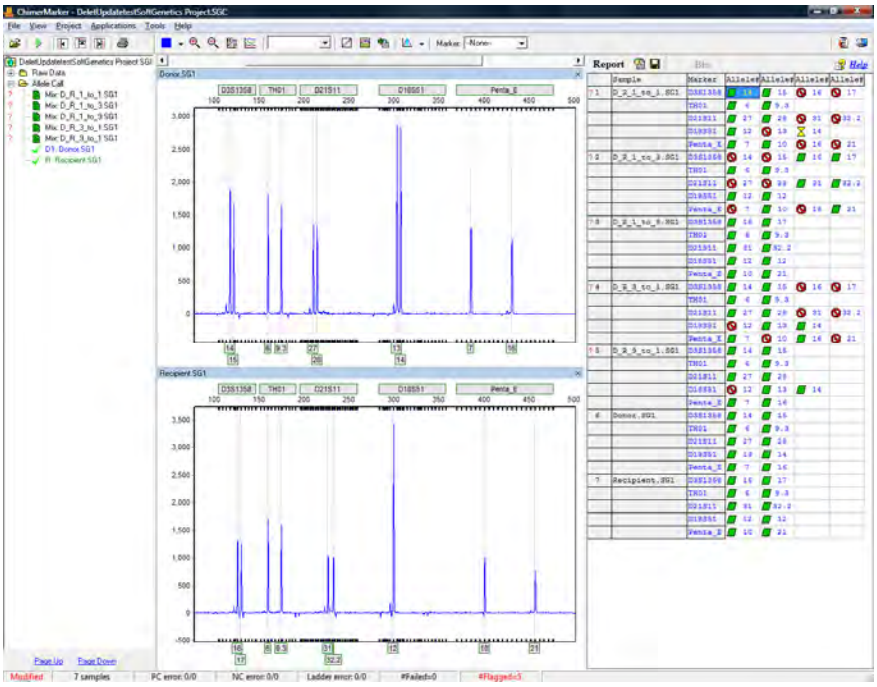
ChimerMarker uses the allele calls of the donor(s) and recipient pre-transplant samples to automatically detect and label peak origin in all post-transplant samples (donor, recipient or shared). This information can be set automatically when file naming systems are used (see figure below) or by right mouse click on the sample name and select set sample type.



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 can be 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.

Genotype the single source samples

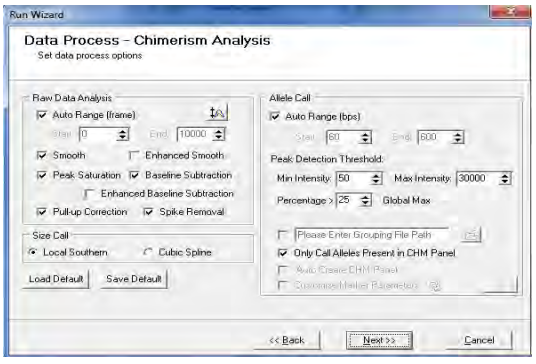
Follow the steps for importing and processing data from Chapter 3 and 4 Genotyping General Procedure and Genotyping Main Analysis Review. Review the results in the main analysis.



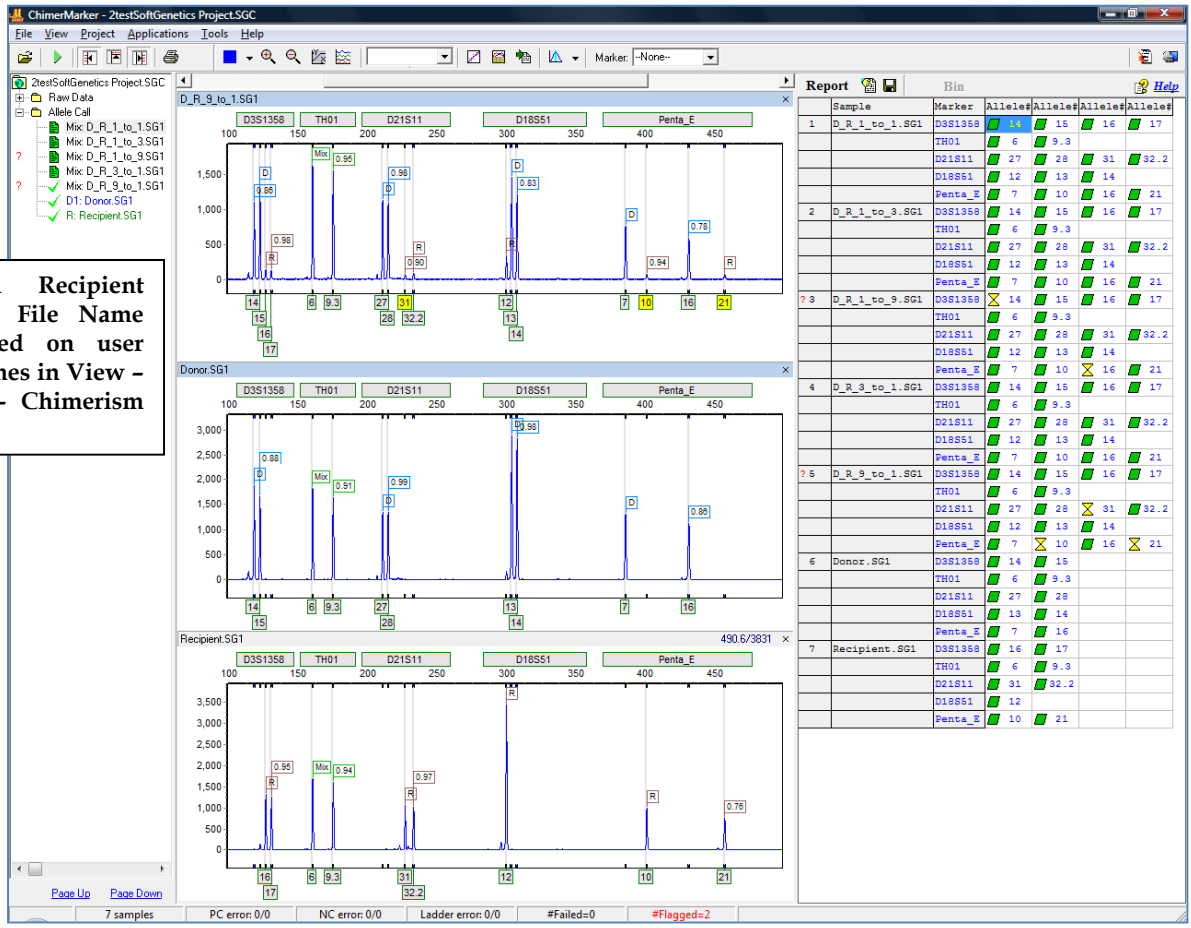
After reviewing the results and making any desired edits (Chapter 4, Genotyping Main Analysis), Use Tools → Panel Editor and construct the Chimertyping panel as described in Chapter 6, Panel Editor.

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.

1. Launch the Run Wizard (Project drop-down or green arrow icon)
2. Select the Chimertyping panel from the dropdown menu
3. Click Next
4. Select Only Call Alleles Present in CHM Panel to filter out extraneous peaks. Adjust other parameters if needed to proceed with genotyping analysis.
5. Click Next and OK
6. Review the results in the electropherograms and linked report table



Review Results



Donor and Recipient indicated in File Name Tree – based on user specified names in View – Preferences – Chimerism Tab

Post-Transplant Sample: ChimerMarker will differentiate and label 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 single source sample(s) 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.

Any edits must be made in the main analysis window and saved in the chimerism project (.SGC) file. This maintains integrity of the analysis going forward into the Chimerism Application and Calculations.

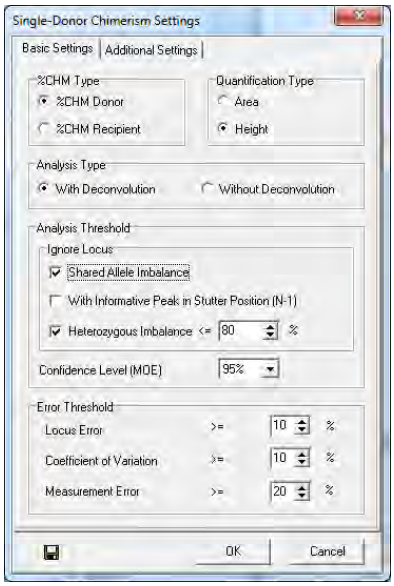
Automated Chimerism Calculations

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 dialog boxes for Single Donor Chimerism Settings and Double Donor Chimerism Settings provides 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 Equations.

1. Select %Chimerism Type
   a. %CHM Donor or
   b. %CHM Recipient

2. Select Quantification Type
   a. Peak Area or
   b. Peak Height

3. Select Analysis Type
   a. With Deconvolution or
   b. Without Deconvolution



4. The Ignore Locus section provides three additional conditions under which markers should be ignored.
   a. Shared Allele Imbalance: If this option is selected, the marker will be ignored if a single source peak in the marker has higher RFU than a shared peak.
   b. With Informative Peak in Stutter Position (N-1): The marker will be ignored if a donor, recipient, or shared peak is in a stutter position. If selected, the stutter adjustment option will be disabled.
   c. Heterozygous Imbalance: Ignores a locus if a the smaller of two donor or recipient peaks is shorter than the specified percent times the height of the larger of the two peaks.

5. Set the desired Error Threshold for Locus Error, Coefficient of Variation and Measurement Error

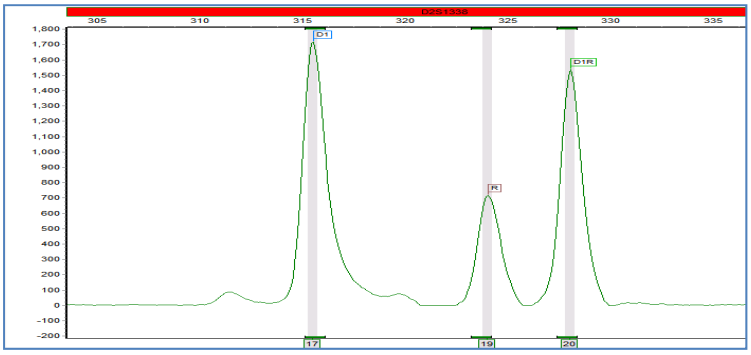
6. Specify the percent for Limit of Detection Threshold. Any sensitivity above the threshold % will be flagged red. Also specify the Threshold type, donor or recipient. It is best to use the same type as the %CHM Type selected in the Basic Settings.

7. Apply Stutter Adjustment will subtract marker stutter specific values, specified in the panel, in the stutter alleles. The stutter % value for each markers can be displayed if "Show Stutter %" is selected along with "Apply Stutter Adjustment"

8. Click the save icon (bottom left corner) to save your choices as the default settings.

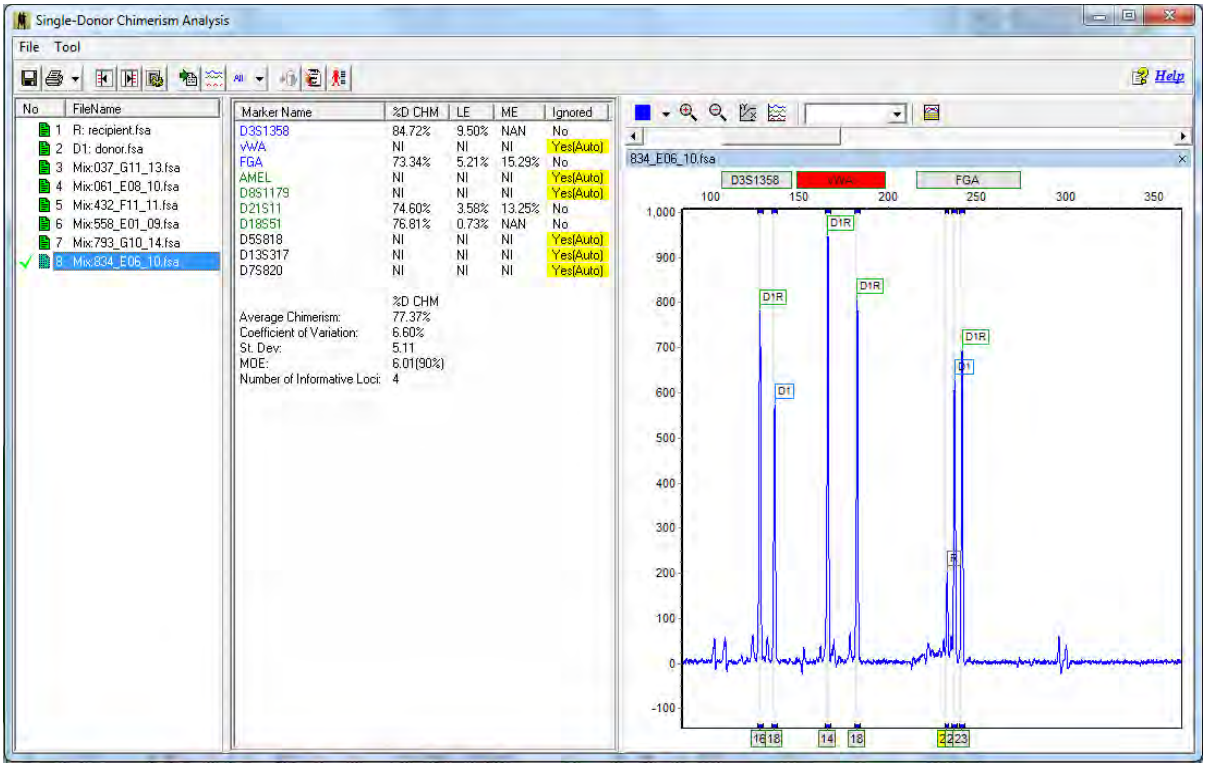
9. Select OK to review the results of chimerism calculations.

An example of SAI for locus D2S1338 is displayed here. Quantification is suspect when PCR bias results in the smaller fragment from one individual is more efficiently amplified than the larger fragment that is shared between donor and recipient.



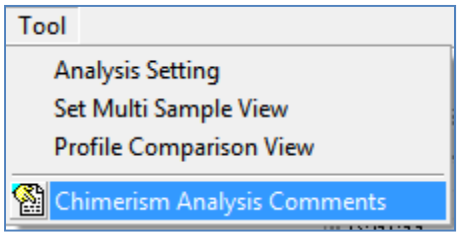
REVIEW RESULTS

The automated chimerism calculations are displayed in the center table. To view the results for a given file, click on the file name in the list at the left.



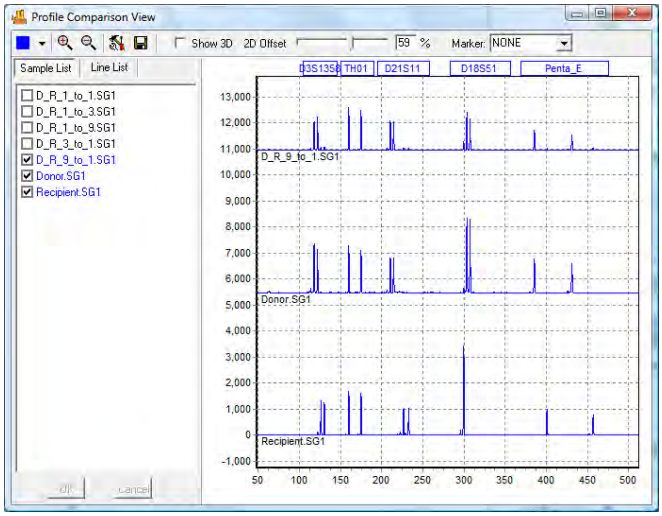
NI: Non-Informative-Marker is Non-Informative based on peak pattern and parameters chosen in analysis settings.
NAN: No Available Number-No results can be calculated for the following peak pattern.
ME: Measurement Error
LE: Locus Error
MOE: Margin of Error

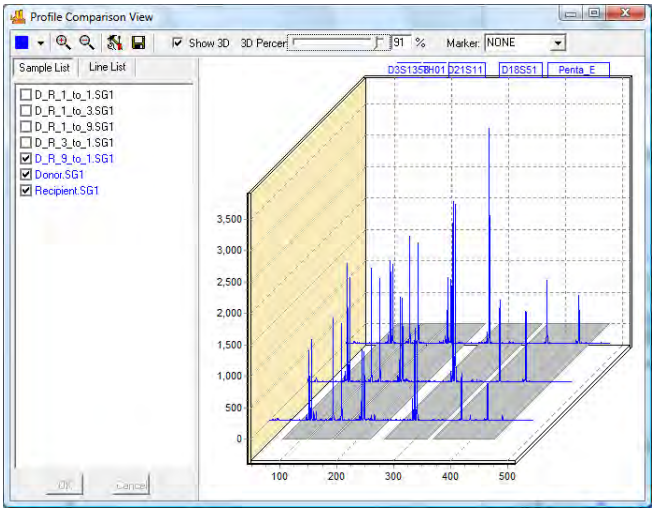
Tools and Icons



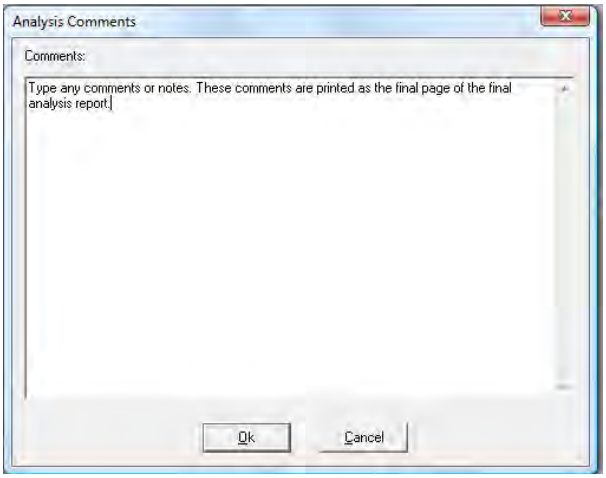
Analysis Settings – Reopens the Chimerism Settings Dialog Box
Set Multiple Sample View – to view traces of selected files, selected files will remain selected if the Chimerism application is closed and re-opened

Profile Comparison View – to view selected traces and level of 2D or 3D offset





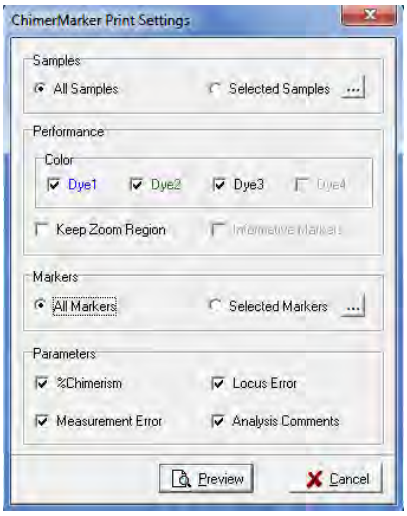
Chimerism Analysis Comments – to record any case notes to be saved and printed with the final report (saves up to 6400 characters).



Save the report table in .xls or .txt file format

Show or Hide – toggle this icon to show or hide the electropherograms

Analysis Parameter icon to relaunch the Chimerism Analysis Settings Box



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

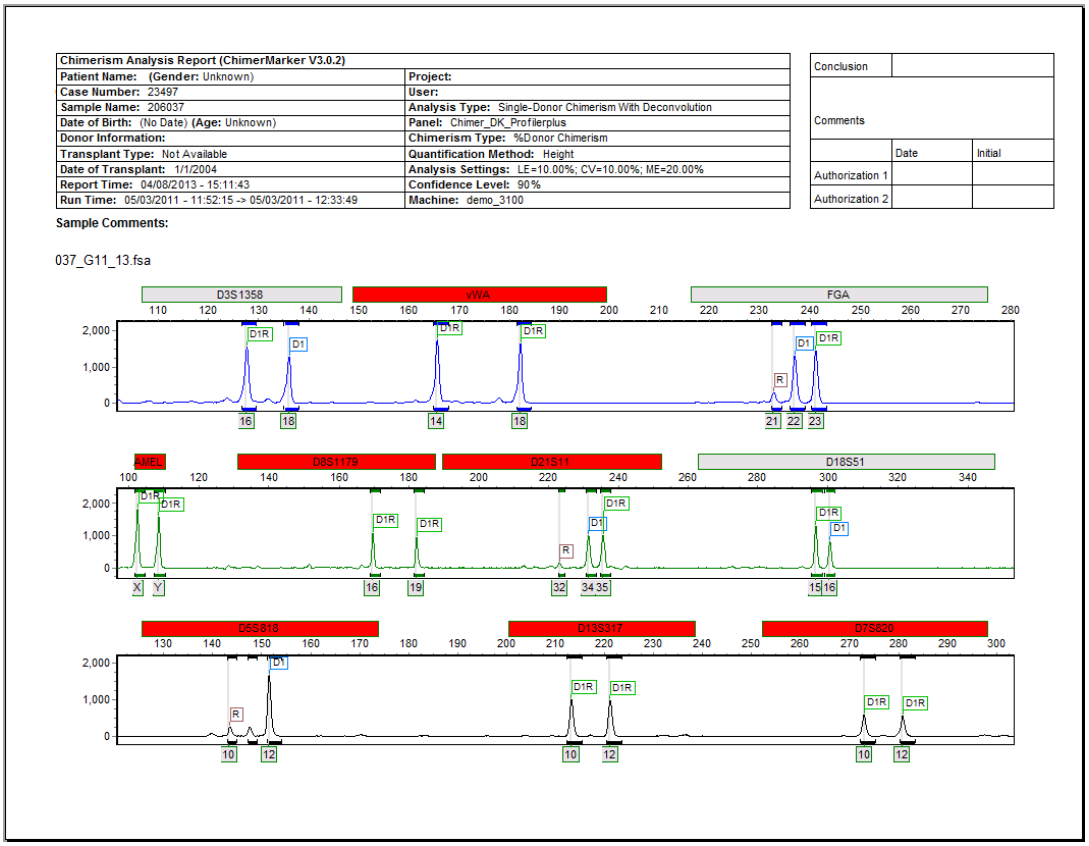
Printing Options – Preview – to review comprehensive research report or print directly from Chimerism Analysis screen

Longitudinal Report – Activates the Longitudinal Report Options to monitor Chimerism trend

Help Icon - quick reference and description to abbreviations and statistical parameters used in chimerism results window.

Comprehensive Research Report

Single Donor Report (page 1)



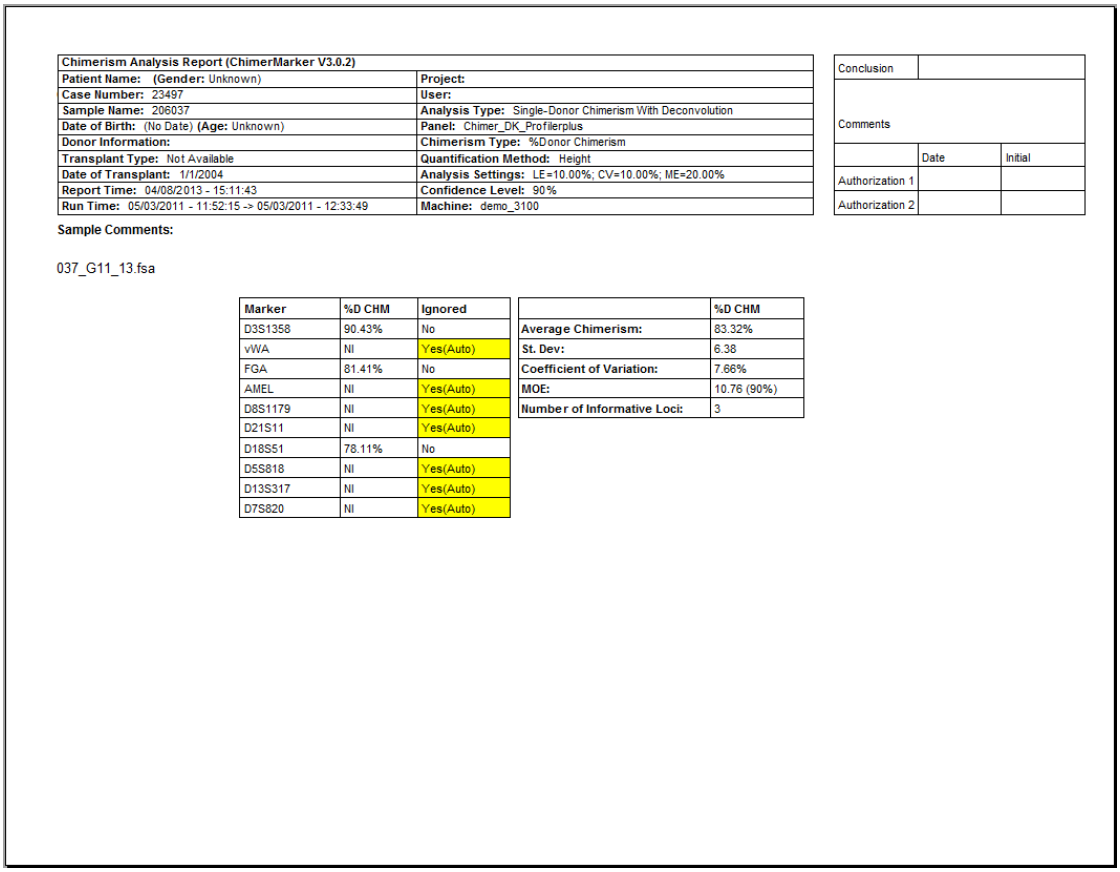
Chimerism Analysis Report (ChimerMarker V3.0.2)	
Patient Name: (Gender: Unknown)	**Project:**
Case Number: 23497	**User:**
Sample Name: 206037	**Analysis Type:** Single-Donor Chimerism With Deconvolution
Date of Birth: (No Date) (Age: Unknown)	**Panel:** Chimer_DK_Profilerplus
Donor Information:	**Chimerism 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)



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

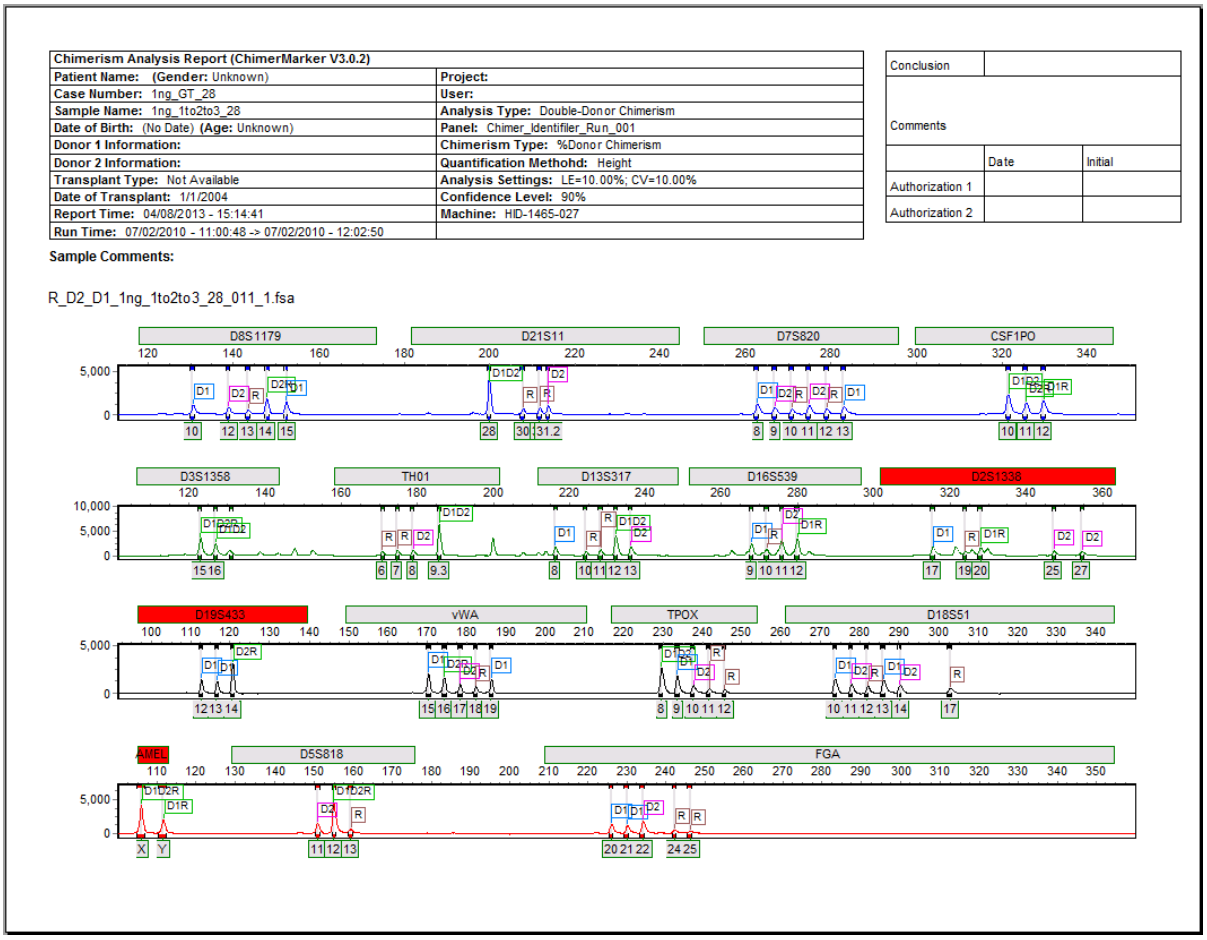
Comprehensive report for each sample at a given time point in the study; includes header, electropherograms (traces) and report table. The report header uses information from user management and the analysis settings to specify the technician, project, time, date, and parameters for electronic record 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) 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 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 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



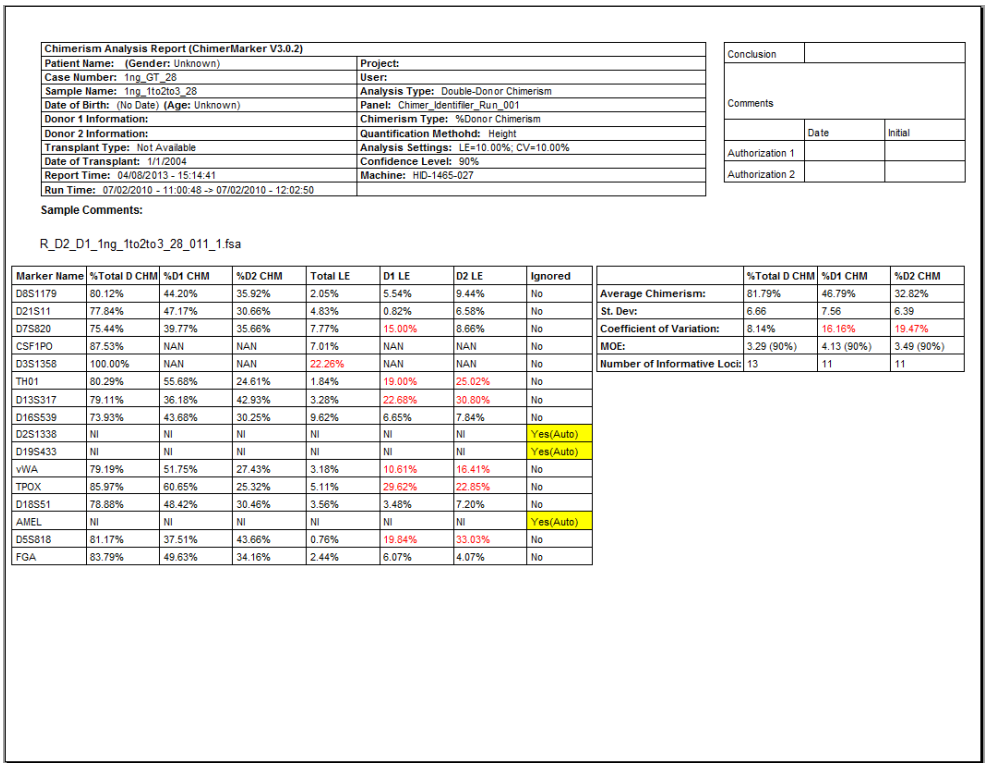
Chimerism Analysis Report (ChimerMarker V3.0.2)	
Patient Name: (Gender: Unknown)	**Project:**
Case Number: 1ng_GT_28	**User:**
Sample Name: 1ng_1to2to3_28	**Analysis Type:** Double-Donor Chimerism
Date of Birth: (No Date) (Age: Unknown)	**Panel:** Chimer_Identifiler_Run_001
Donor 1 Information:	**Chimerism 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

Page 2: Results Table



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:

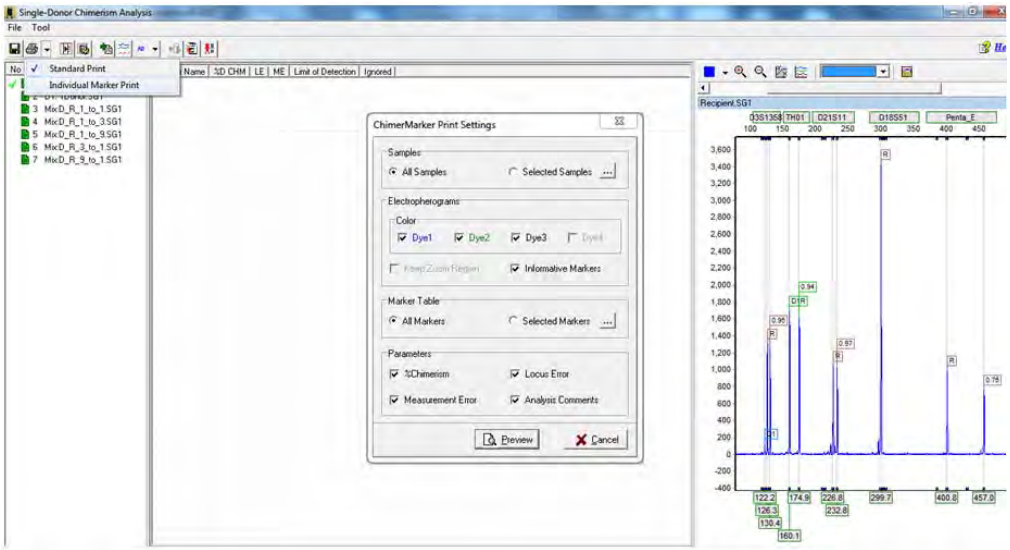
R_D2_D1_1ng_1to2to3_28_011_1.fsa

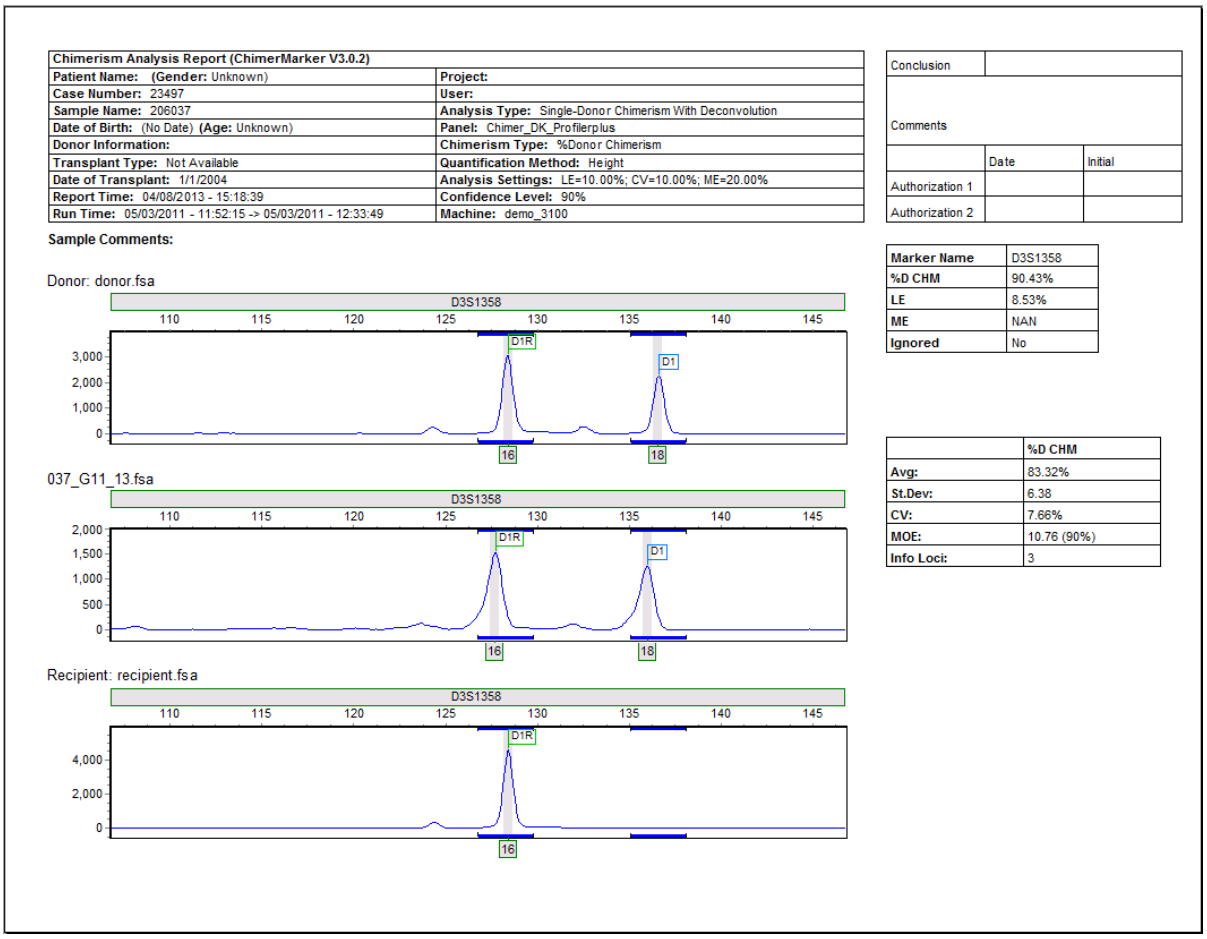
Marker Name	%Total D CHM	%D1 CHM	%D2 CHM	Total LE	D1 LE	D2 LE	Ignored
D8S1179	80.12%	44.20%	35.92%	2.05%	5.54%	9.44%	No
D21S11	77.84%	47.17%	30.66%	4.83%	0.82%	6.58%	No
D7S820	75.44%	39.77%	35.66%	7.77%	15.00%	8.66%	No
CSF1PO	87.53%	NAN	NAN	7.01%	NAN	NAN	No
D3S1358	100.00%	NAN	NAN	22.26%	NAN	NAN	No
TH01	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 can be 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.





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 will be grouped with the appropriate marker from Donor and Recipient sample for comparison. Both Marker result and 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 Monitoring / Detection of Malignant Relapse

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.

Procedure

1. Open a saved project or start a new project following the Chimerism Analysis procedures in Chapter 2.
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


Print Report: Print the longitudinal report as zoomed view or global view


Save Report: Save report in PNG, JPG, BMP, or PDF format. Options to save whole page or table only.


Display Settings: Change the display settings on the Longitudinal Report, including how 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.

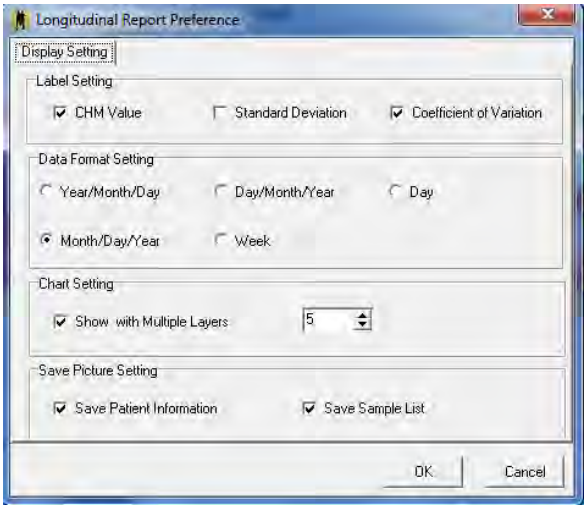

Graph Display: Switch from bar graph to line graph and vice versa.


Show/Hide Table: Toggle between displaying graph and table or only the graph. Table contains all samples and stats of the samples.


Refresh: Update the graphical display.

"*Show 3D*": Will display the graph in a 3-D format

Display Settings:



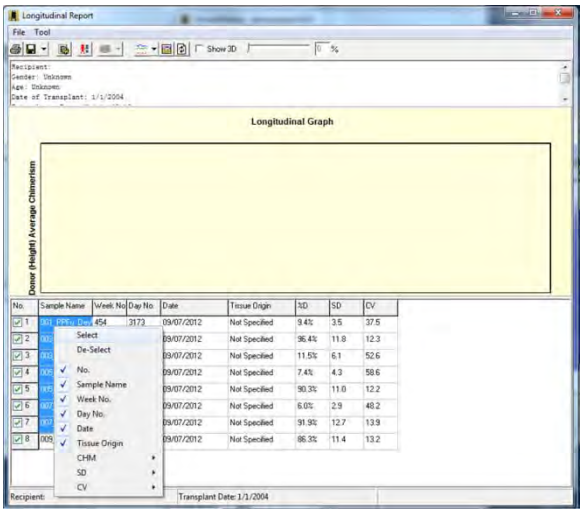
Label Settings: Select the statistics that will be displayed on top of the bar or line graph for each sample.

Date Format Setting: Select the date display that will be shown on the X-axis of the graph.

Chart Setting: Specify number of layers to display statistics label.

Save Picture Setting: Specify if patient information and sample list should be saved with image.

Add/Remove Samples:

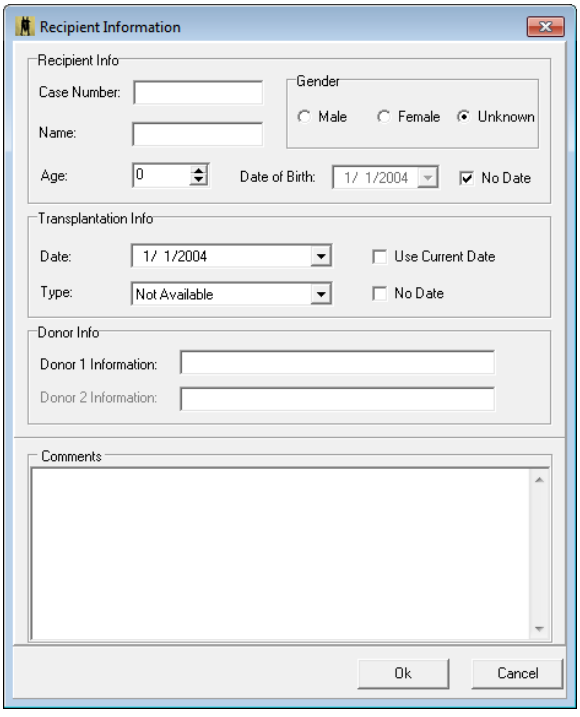


Multiple samples can be selected at once by left-clicking on a sample and highlighting other samples. Once the desired samples are selected, right-click and choose "Select". Use the *Refresh* icon to update the graphical display.

Date: Specify dates for each sample.

Tissue Origin: Choose the desired Tissue Origin from the drop-down options or manually enter. The cell type information added in View-> Preferences-> Chimerism will be displayed in the drop-down.
NOTE: Up to 10 tissue types can be monitored on a single graph.

Recipient Information:



Note: the following entries are included in print report headers.

Case Number: If applicable, input the case number for this sample or project.

Recipient Name: Specify Patient Name/ID. This will appear as header on graph.

Gender: Specify patient sex.

Age: Specify patient age. NOTE: Age will be 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.
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 report.

Donor Information: Use this section to briefly describe your donors, (e.g. donor sex, relation to patient, and so on).

Comments: Add comments to the project if needed. Any comments added will be printed with the report.

Print Settings for Double Donor:

CHM Type: Select type of donor chimerism to display.

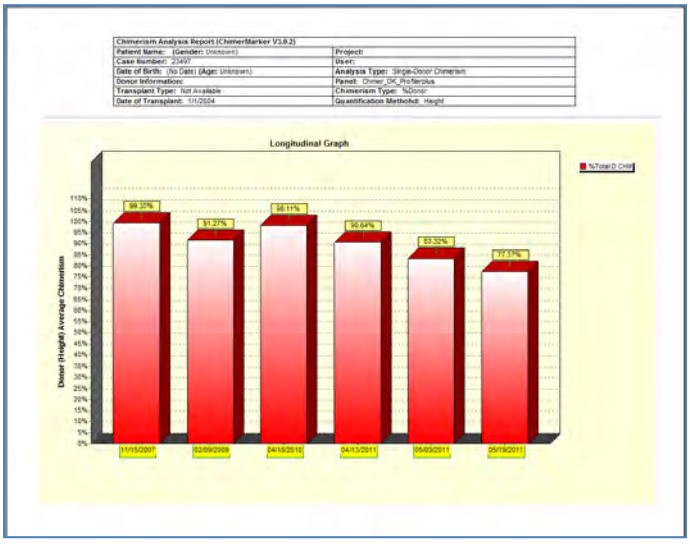
Print Settings: Select print settings for the graph.
NOTE: "Keep Show Setting," if selected will print the report as zoomed in the graphical view.

Chart Type: Select the type of chart to print.

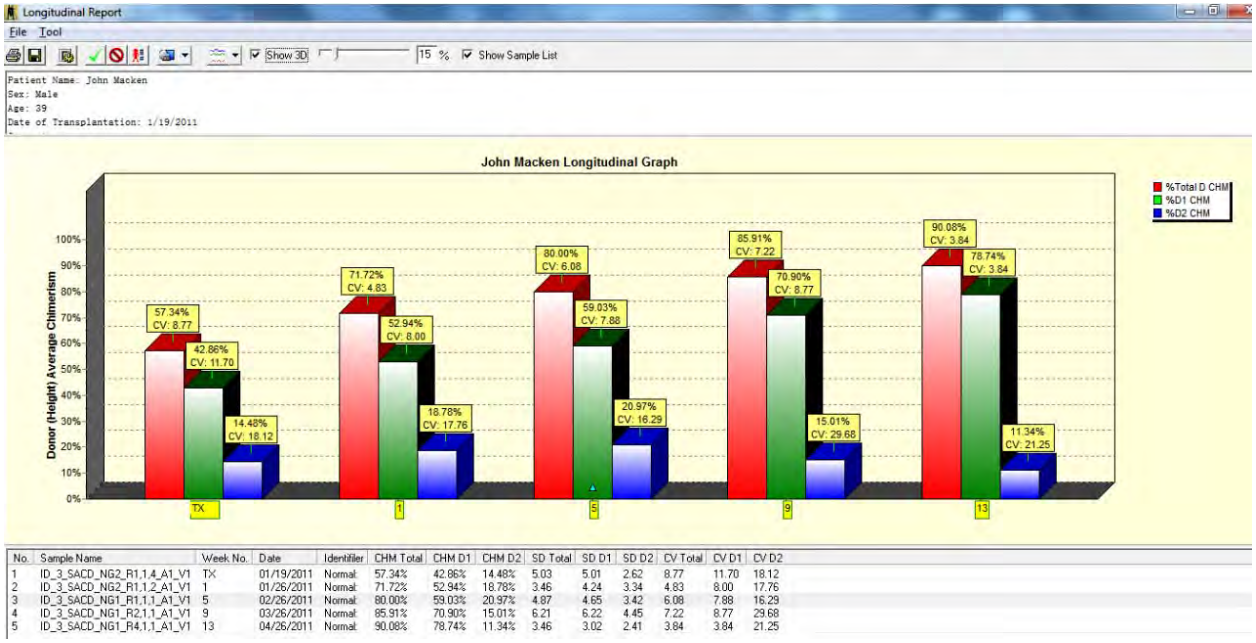
"*Preview*" will show a print preview of the settings selected.

What to Expect

The Longitudinal Report contains a header with User Identification from User Management (see Chapter 9 – User Management) and Analysis Parameters, 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.



Single donor Example with quality control metrics in the bar graph and sample date displayed as the month/day/year.



No.	Sample Name	Week No.	Date	Identifiler	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

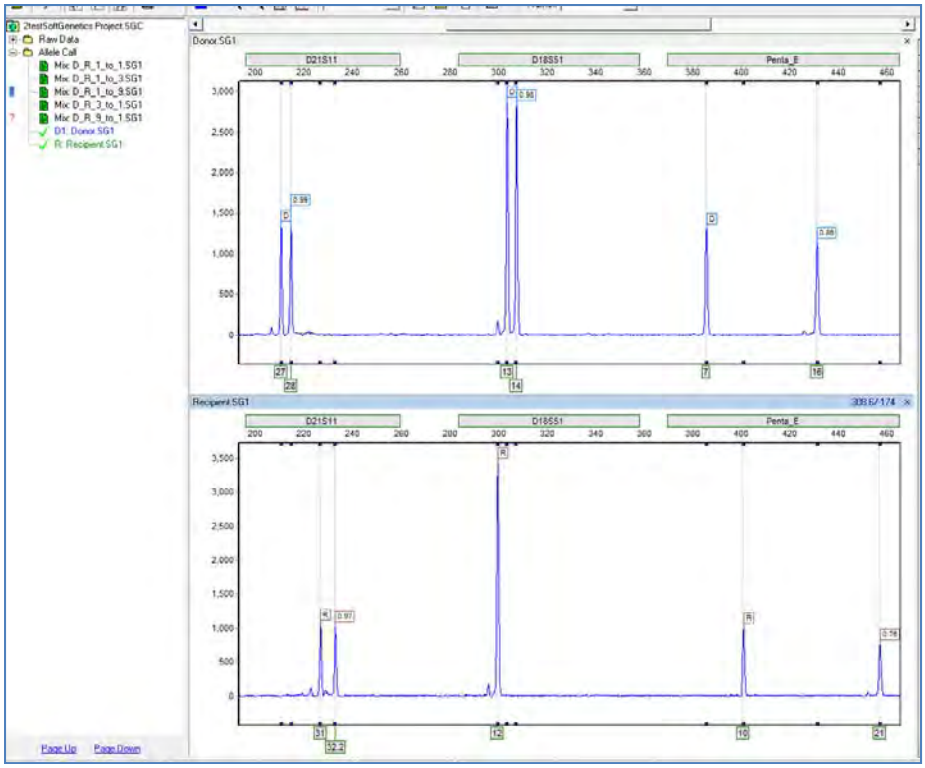
This Double donor monitoring report displays the trend of engraftment with an increase in overall chimerism and Donor 1 stability and a decrease in Donor 2 chimerism percentage. In this example the analyst entered Tx to indicate sample at transplant and then the weeks post-transplant for the sequential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

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 will be graphed in a unique color. Up 10 different tissue types can be displayed on a single graph.

Chimerism Calculations

Metrics for variation in amplification --- measure of variation from sources other than chimeras (LCN, amplification variation --- PCR conditions, PCR bias, random events, CE conditions…)



Example of variation PCR bias: Alleles in loci that have a smaller size range are able to be amplified more than alleles in loci of larger size range in the same amount of time. One of the causes of heterozygous imbalance is PCR bias – the amplification of the smaller fragment more times than the larger one. In the example above there is almost no amplification difference between the two 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 fragments being amplified more than the larger fragment. This is a source of variation (heterozygous imbalance) due to PCR process. In this example, both D21S11 and Penta_E are acceptable candidate loci 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, less random variation.

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 percentage.

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 take into account 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 deviation (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 multiply 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:

Homozygous: [(T)/Total DNA in Marker]* 100

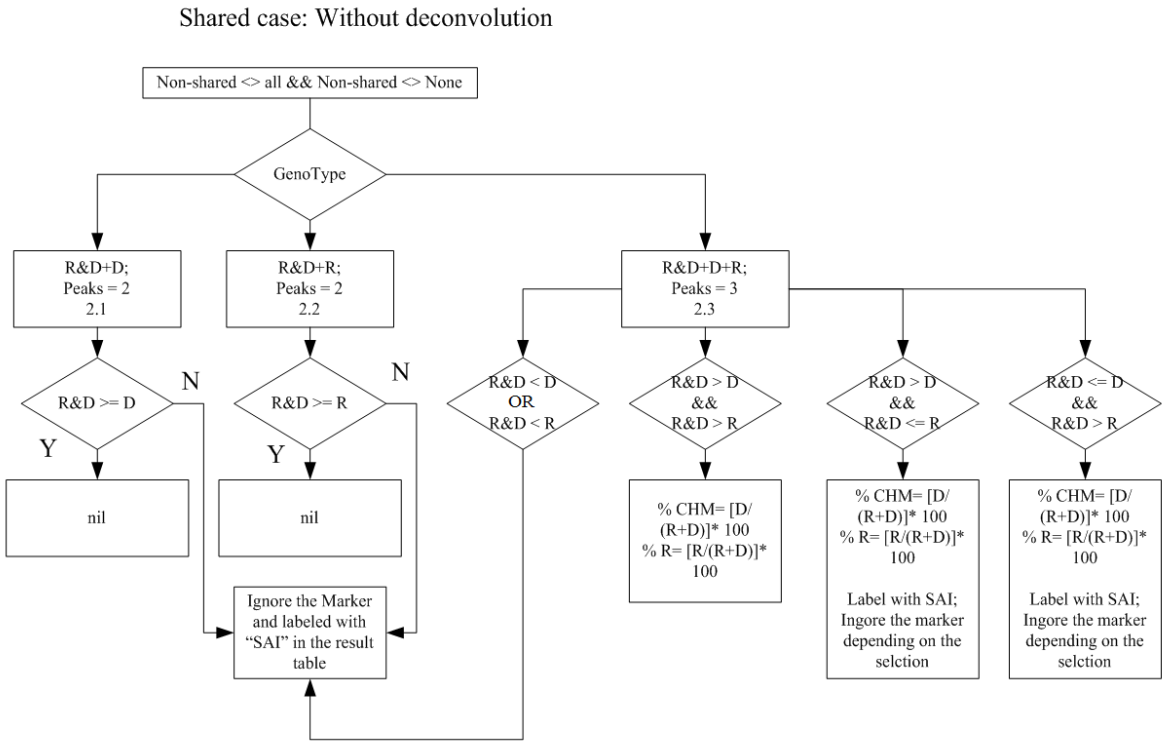
Heterozygous:[(T*2)/ Total DNA in Marker]*100

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

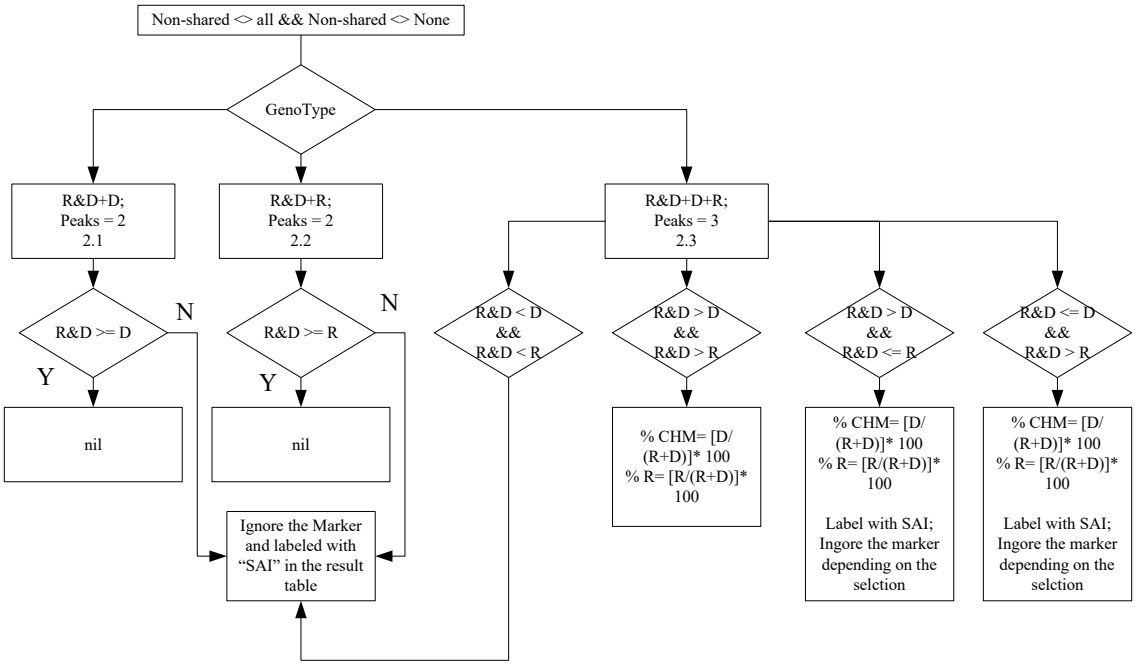
Stutter Adjustment:
X= Stutter % specified in panel
A=Stutter contribution
Y= DNA of minor allele not affected by stutter

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:

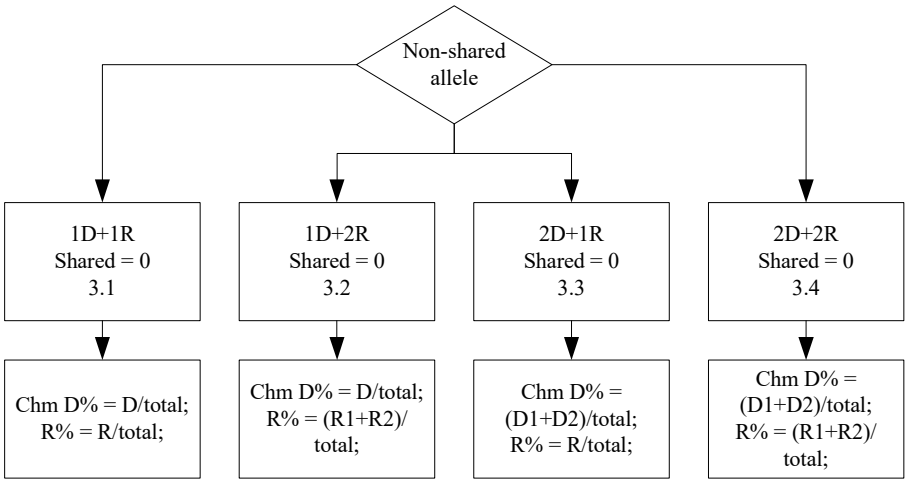
Decision Pathway for Single Donor Chimerism Calculations



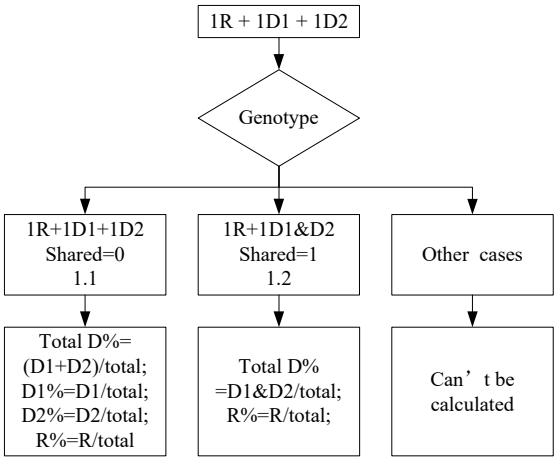
Shared case: Without deconvolution



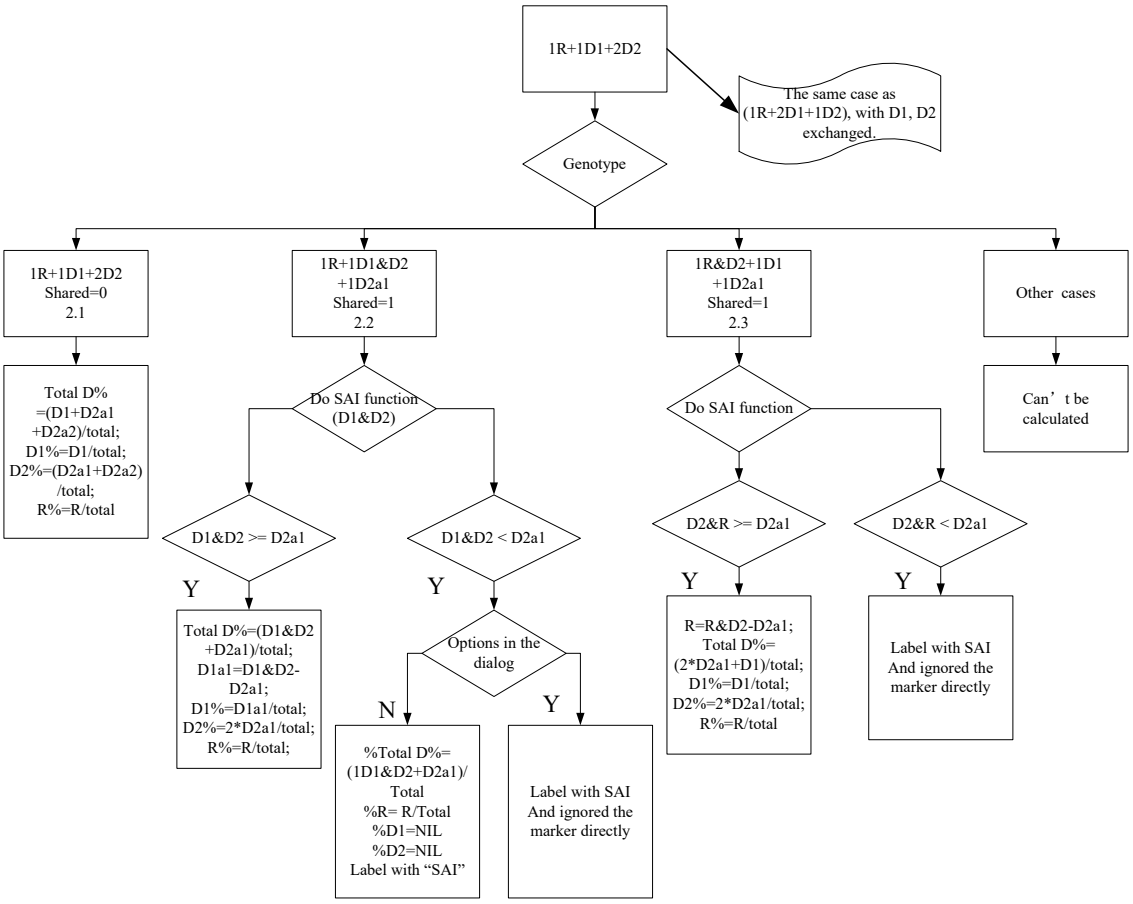
Non-shared case: With/without deconvolution



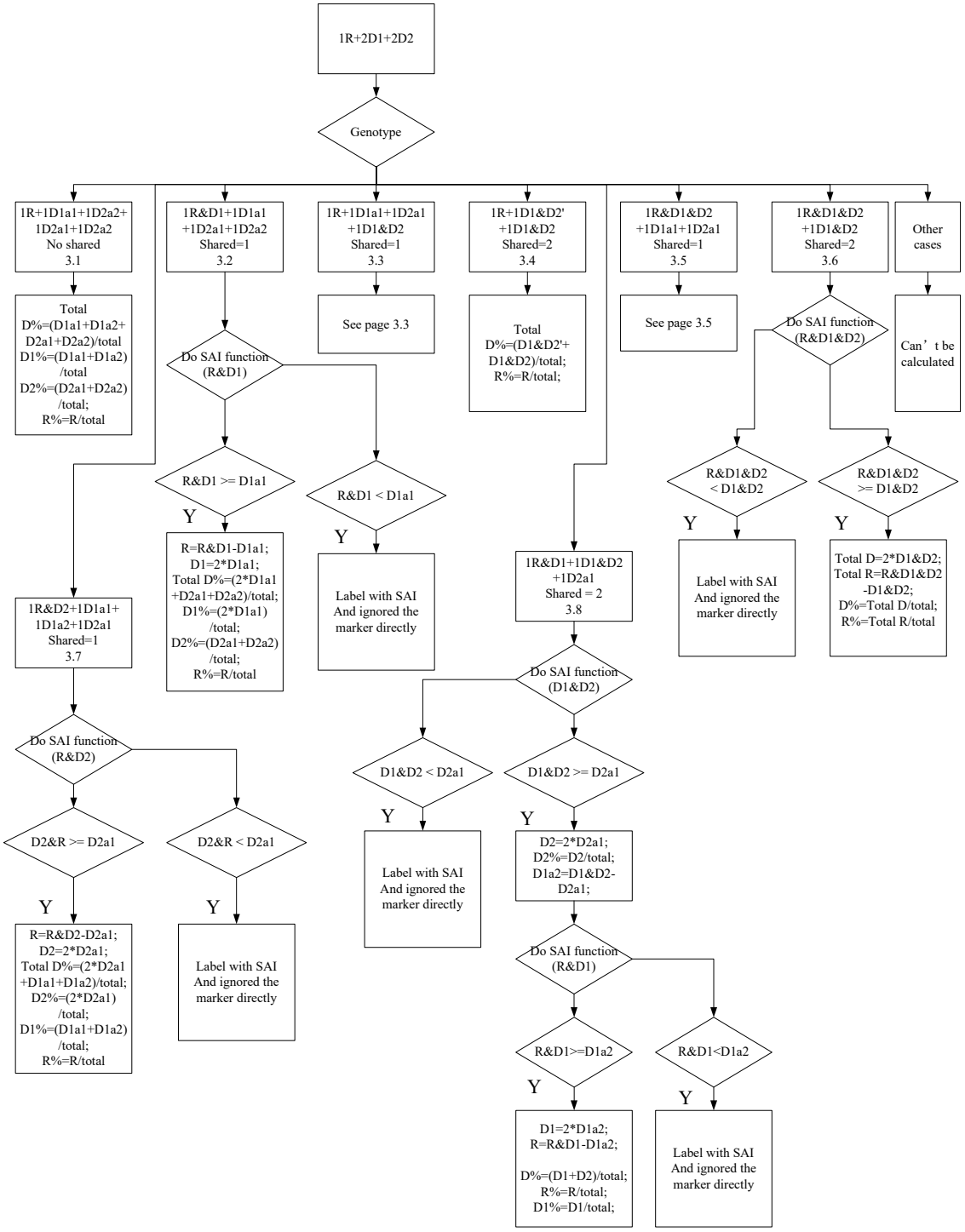
Decision Pathway for Double Donor Chimerism Calculations



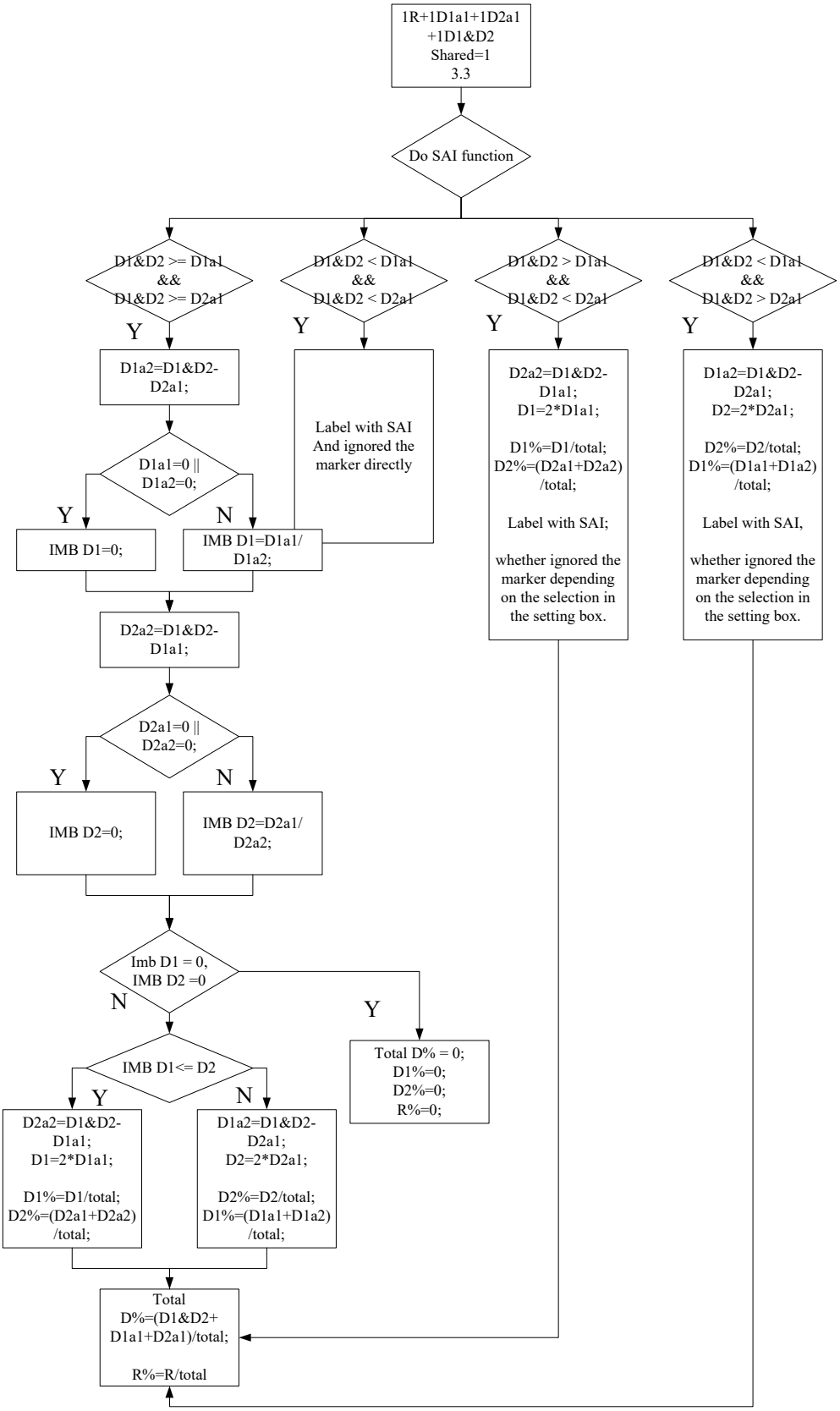
Case 1.0



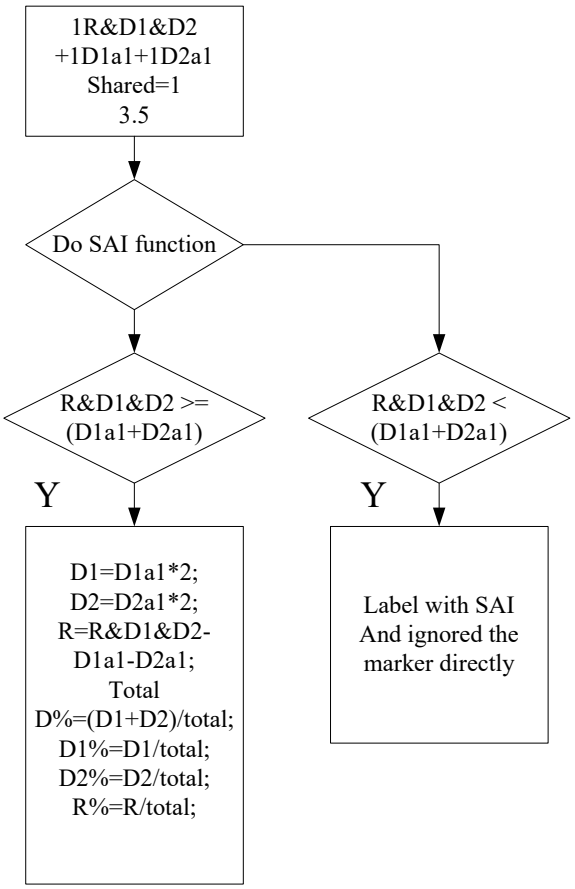
Case 2.0


1R+2D1+2D2
Genotype
1R+1D1a1+1D2a2+ 1D2a1+1D2a2 No shared 3.1
1R&D1+1D1a1 +1D2a1+1D2a2 Shared=1 3.2
1R+1D1a1+1D2a1 +1D1&D2 Shared=1 3.3
1R+1D1&D2' +1D1&D2 Shared=2 3.4
1R&D1&D2 +1D1a1+1D2a1 Shared=1 3.5
1R&D1&D2 +1D1&D2 Shared=2 3.6
Other cases
Total D%=(D1a1+D1a2+ D2a1+D2a2)/total D1%=(D1a1+D1a2) /total D2%=(D2a1+D2a2) /total; R%=R/total
Do SAI function (R&D1)
See page 3.3
Total D%=(D1&D2'+ D1&D2)/total; R%=R/total;
See page 3.5
Do SAI function (R&D1&D2)
Can't be calculated
R&D1 >= D1a1
R&D1 < D1a1
R&D1&D2 < D1&D2
R&D1&D2 >= D1&D2
Y
R=R&D1-D1a1; D1=2*D1a1; Total D%=(2*D1a1 +D2a1+D2a2)/total; D1%=(2*D1a1) /total; D2%=(D2a1+D2a2) /total; R%=R/total
Label with SAI And ignored the marker directly
Label with SAI And ignored the marker directly
Total D=2*D1&D2; Total R=R&D1&D2 -D1&D2; D%=Total D/total; R%=Total R/total
1R&D2+1D1a1+ 1D1a2+1D2a1 Shared=1 3.7
1R&D1+1D1&D2 +1D2a1 Shared = 2 3.8
Do SAI function (R&D2)
Do SAI function (D1&D2)
D2&R >= D2a1
D2&R < D2a1
D1&D2 < D2a1
D1&D2 >= D2a1
R=R&D2-D2a1; D2=2*D2a1; Total D%=(2*D2a1 +D1a1+D1a2)/total; D2%=(2*D2a1) /total; D1%=(D1a1+D1a2) /total; R%=R/total
Label with SAI And ignored the marker directly
Label with SAI And ignored the marker directly
D2=2*D2a1; D2%=D2/total; D1a2=D1&D2-D2a1;
Do SAI function (R&D1)
R&D1>=D1a2
R&D1<D1a2
D1=2*D1a2; R=R&D1-D1a2; D%=(D1+D2)/total; R%=R/total; D1%=D1/total;
Label with SAI And ignored the marker directly

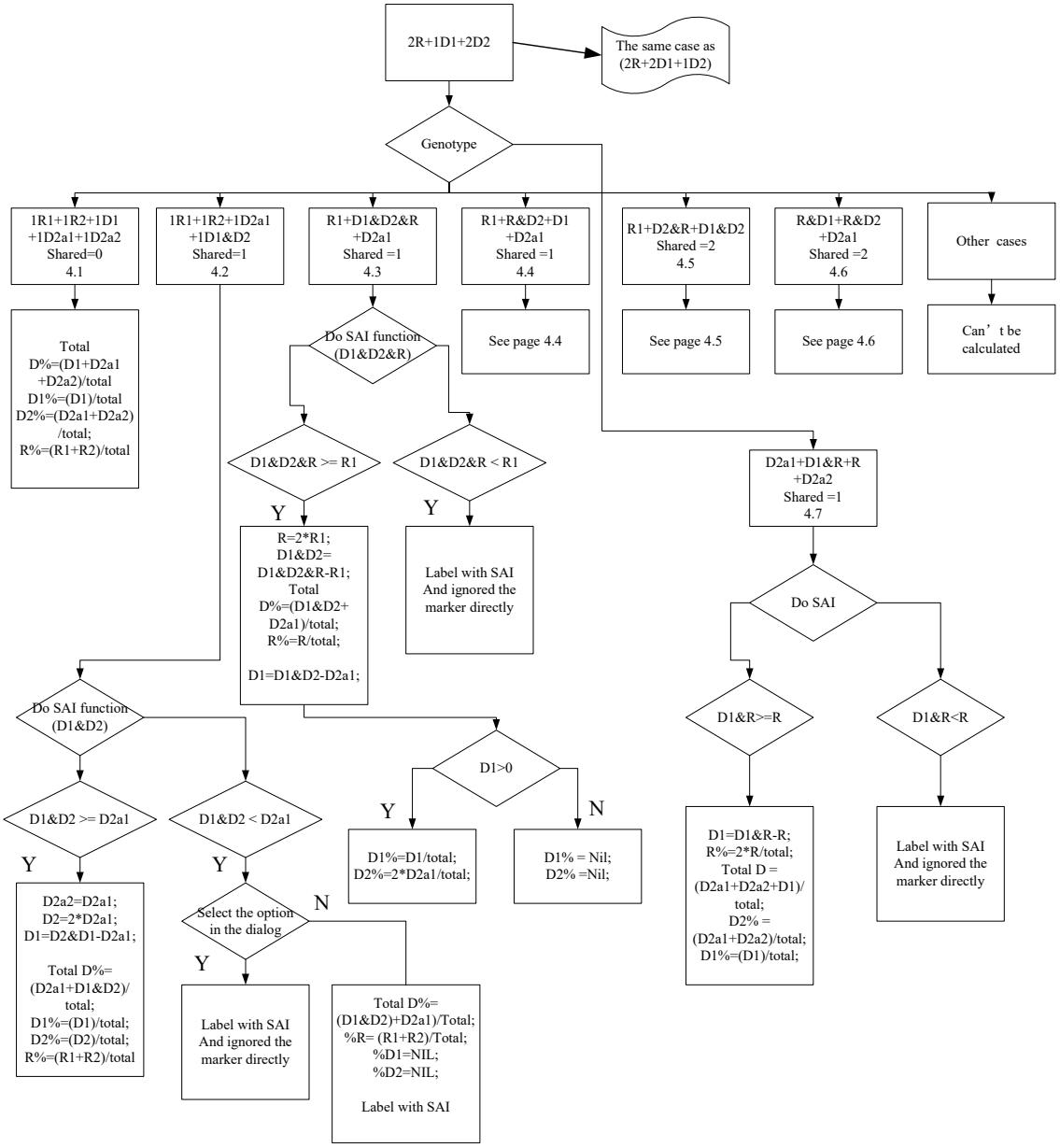
Case 3.0


1R+1D1a1+1D2a1
+1D1&D2
Shared=1
3.3
Do SAI function
D1&D2 >= D1a1
&&
D1&D2 >= D2a1
D1&D2 < D1a1
&&
D1&D2 < D2a1
D1&D2 > D1a1
&&
D1&D2 < D2a1
D1&D2 < D1a1
&&
D1&D2 > D2a1
Y
Y
Y
Y
D1a2=D1&D2-
D2a1;
Label with SAI
And ignored the
marker directly
D2a2=D1&D2-
D1a1;
D1=2*D1a1;
D1%=D1/total;
D2%=(D2a1+D2a2)
/total;
Label with SAI;
whether ignored the
marker depending
on the selection in
the setting box.
D1a2=D1&D2-
D2a1;
D2=2*D2a1;
D2%=D2/total;
D1%=(D1a1+D1a2)
/total;
Label with SAI,
whether ignored the
marker depending
on the selection in
the setting box.
D1a1=0 ||
D1a2=0;
Y
N
IMB D1=0;
IMB D1=D1a1/
D1a2;
D2a2=D1&D2-
D1a1;
D2a1=0 ||
D2a2=0;
Y
N
IMB D2=0;
IMB D2=D2a1/
D2a2;
Imb D1 = 0,
IMB D2 =0
N
Y
Total D% = 0;
D1%=0;
D2%=0;
R%=0;
IMB D1<= D2
Y
N
D2a2=D1&D2-
D1a1;
D1=2*D1a1;
D1%=D1/total;
D2%=(D2a1+D2a2)
/total;
D1a2=D1&D2-
D2a1;
D2=2*D2a1;
D2%=D2/total;
D1%=(D1a1+D1a2)
/total;
Total
D%=(D1&D2+
D1a1+D2a1)/total;
R%=R/total

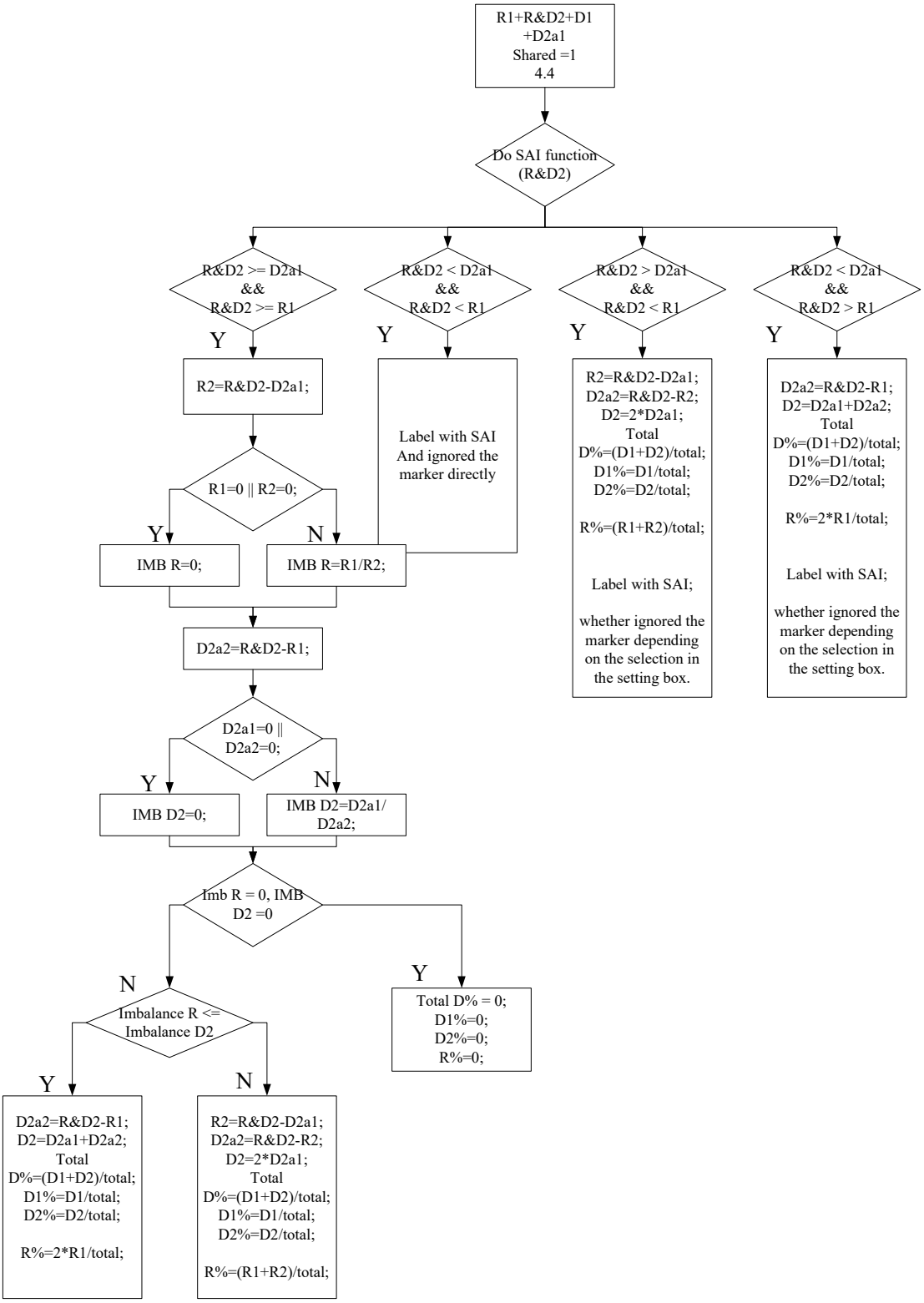
Case 3.3


1R&D1&D2
+1D1a1+1D2a1
Shared=1
3.5
Do SAI function
R&D1&D2 >=
(D1a1+D2a1)
R&D1&D2 <
(D1a1+D2a1)
Y
Y
D1=D1a1*2;
D2=D2a1*2;
R=R&D1&D2-
D1a1-D2a1;
Total
D%=(D1+D2)/total;
D1%=D1/total;
D2%=D2/total;
R%=R/total;
Label with SAI
And ignored the
marker directly

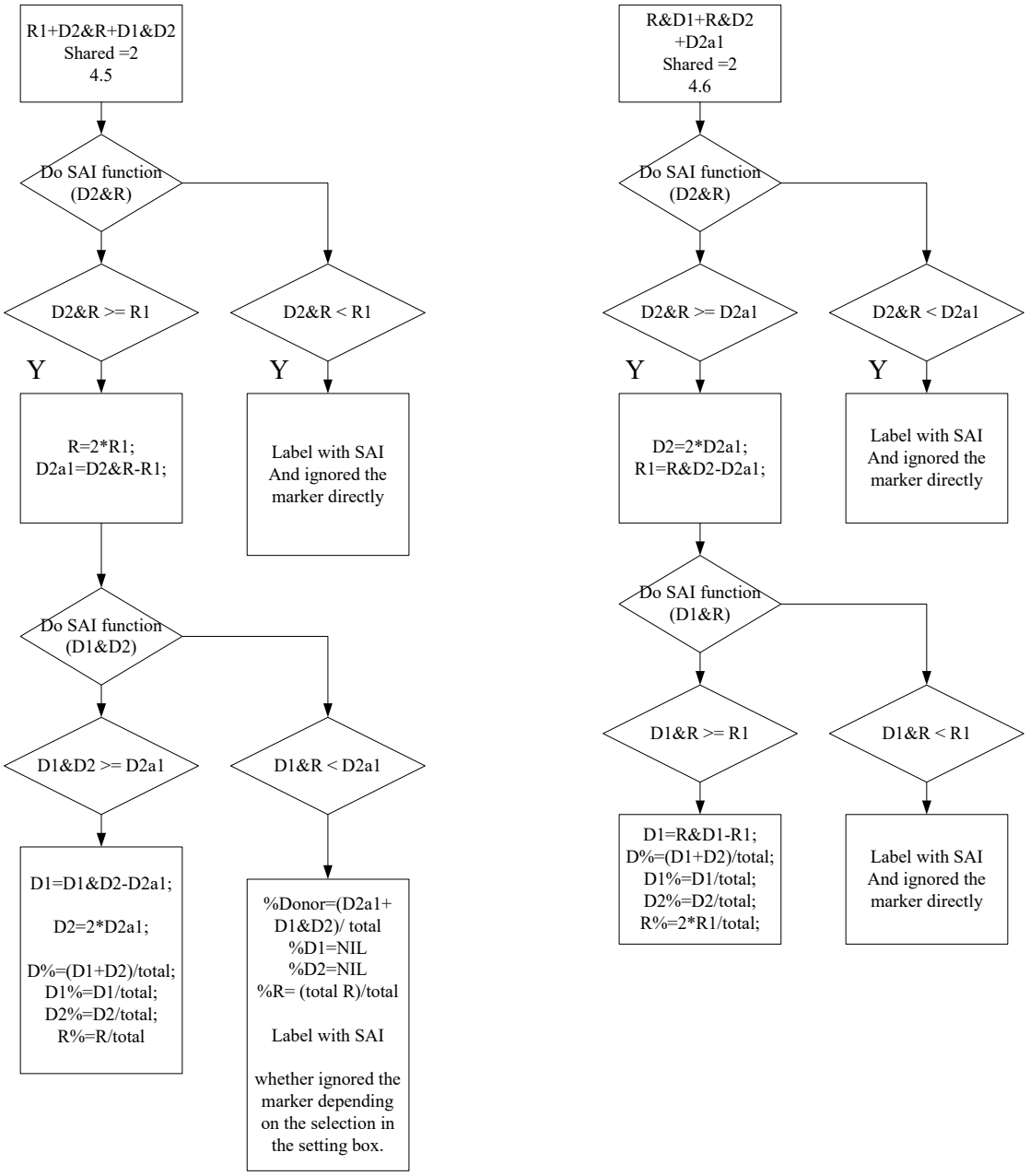
Case 3.5


2R+1D1+2D2
The same case as (2R+2D1+1D2)
Genotype
1R1+1R2+1D1 +1D2a1+1D2a2 Shared=0 4.1
1R1+1R2+1D2a1 +1D1&D2 Shared=1 4.2
R1+D1&D2&R +D2a1 Shared =1 4.3
R1+R&D2+D1 +D2a1 Shared =1 4.4
R1+D2&R+D1&D2 Shared =2 4.5
R&D1+R&D2 +D2a1 Shared =2 4.6
Other cases
Total D%=(D1+D2a1 +D2a2)/total D1%=(D1)/total D2%=(D2a1+D2a2) /total; R%=(R1+R2)/total
Do SAI function (D1&D2&R)
See page 4.4
See page 4.5
See page 4.6
Can’t be calculated
D1&D2&R >= R1
D1&D2&R < R1
D2a1+D1&R+R +D2a2 Shared =1 4.7
Y
Y
R=2*R1; D1&D2= D1&D2&R-R1; Total D%=(D1&D2+ D2a1)/total; R%=R/total;
D1=D1&D2-D2a1;
Label with SAI And ignored the marker directly
Do SAI
Do SAI function (D1&D2)
D1&R>=R
D1&R<R
D1>0
D1&D2 >= D2a1
D1&D2 < D2a1
Y
N
Y
Y
D1%=D1/total; D2%=2*D2a1/total;
D1% = Nil; D2% =Nil;
D1=D1&R-R; R%=2*R/total; Total D = (D2a1+D2a2+D1)/ total; D2% = (D2a1+D2a2)/total; D1%=(D1)/total;
Label with SAI And ignored the marker directly
D2a2=D2a1; D2=2*D2a1; D1=D2&D1-D2a1;
Total D%= (D2a1+D1&D2)/ total; D1%=(D1)/total; D2%=(D2)/total; R%=(R1+R2)/total
Select the option in the dialog
N
Y
Label with SAI And ignored the marker directly
Total D%= (D1&D2)+D2a1)/Total; %R= (R1+R2)/Total; %D1=NIL; %D2=NIL;
Label with SAI

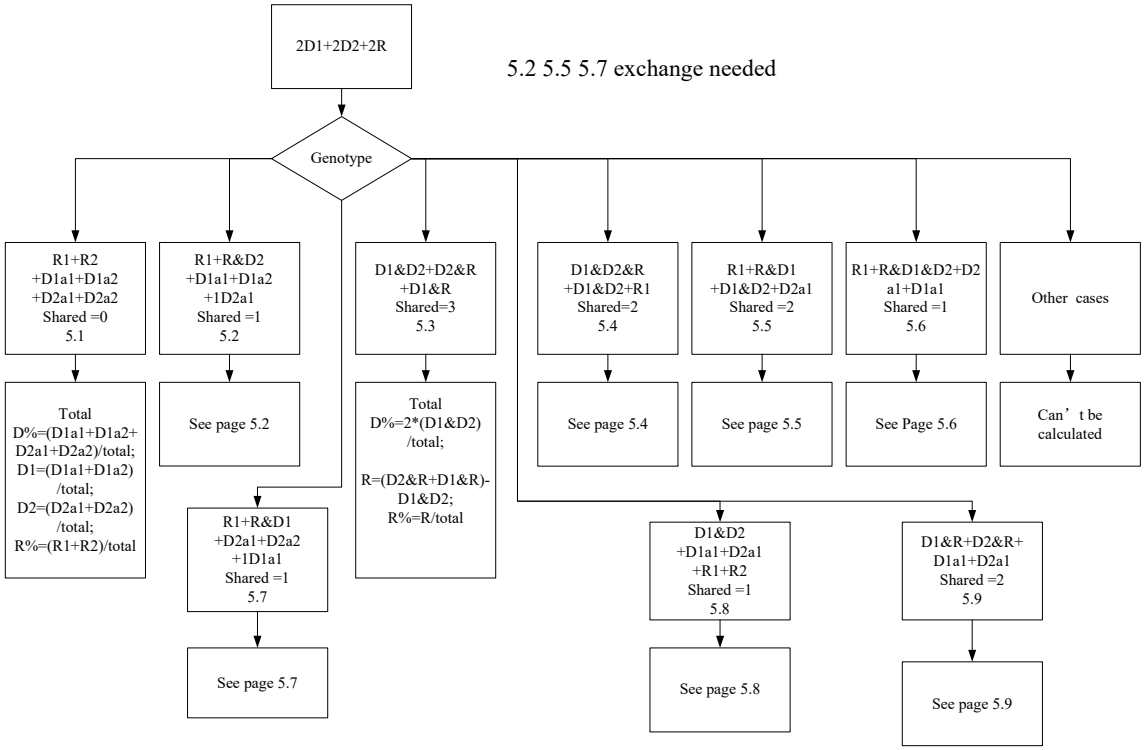
Case 4.0


R1+R&D2+D1
+D2a1
Shared =1
4.4
Do SAI function
(R&D2)
R&D2 >= D2a1
&&
R&D2 >= R1
R&D2 < D2a1
&&
R&D2 < R1
R&D2 > D2a1
&&
R&D2 < R1
R&D2 < D2a1
&&
R&D2 > R1
Y
Y
Y
Y
R2=R&D2-D2a1;
Label with SAI
And ignored the
marker directly
R2=R&D2-D2a1;
D2a2=R&D2-R2;
D2=2*D2a1;
Total
D%=(D1+D2)/total;
D1%=D1/total;
D2%=D2/total;
R%=(R1+R2)/total;
Label with SAI;
whether ignored the
marker depending
on the selection in
the setting box.
D2a2=R&D2-R1;
D2=D2a1+D2a2;
Total
D%=(D1+D2)/total;
D1%=D1/total;
D2%=D2/total;
R%=2*R1/total;
Label with SAI;
whether ignored the
marker depending
on the selection in
the setting box.
R1=0 || R2=0;
Y
N
IMB R=0;
IMB R=R1/R2;
D2a2=R&D2-R1;
D2a1=0 ||
D2a2=0;
Y
N
IMB D2=0;
IMB D2=D2a1/
D2a2;
Imb R = 0, IMB
D2 =0
N
Y
Imbalance R <=
Imbalance D2
Total D% = 0;
D1%=0;
D2%=0;
R%=0;
Y
N
D2a2=R&D2-R1;
D2=D2a1+D2a2;
Total
D%=(D1+D2)/total;
D1%=D1/total;
D2%=D2/total;
R%=2*R1/total;
R2=R&D2-D2a1;
D2a2=R&D2-R2;
D2=2*D2a1;
Total
D%=(D1+D2)/total;
D1%=D1/total;
D2%=D2/total;
R%=(R1+R2)/total;

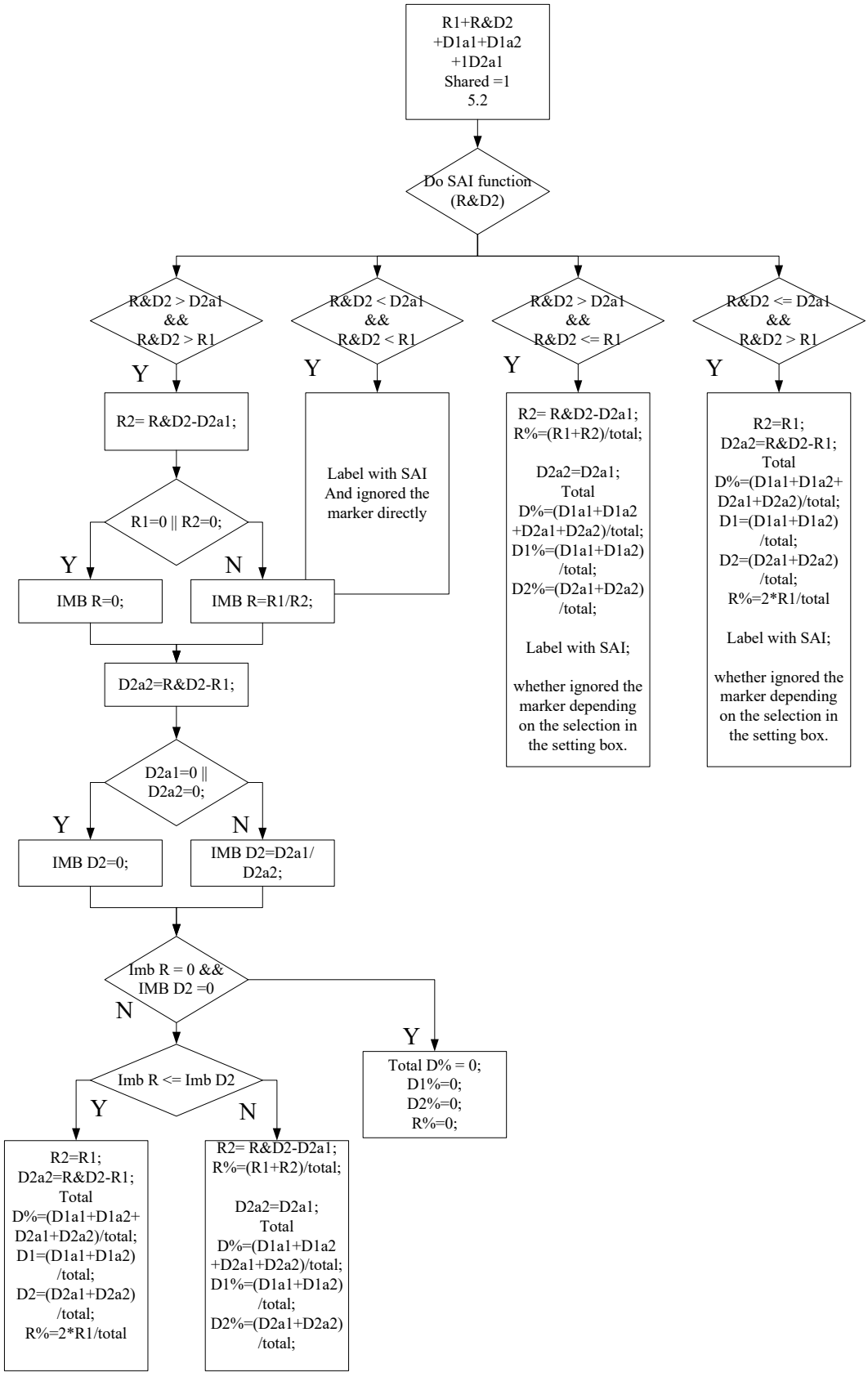
Case 4.4



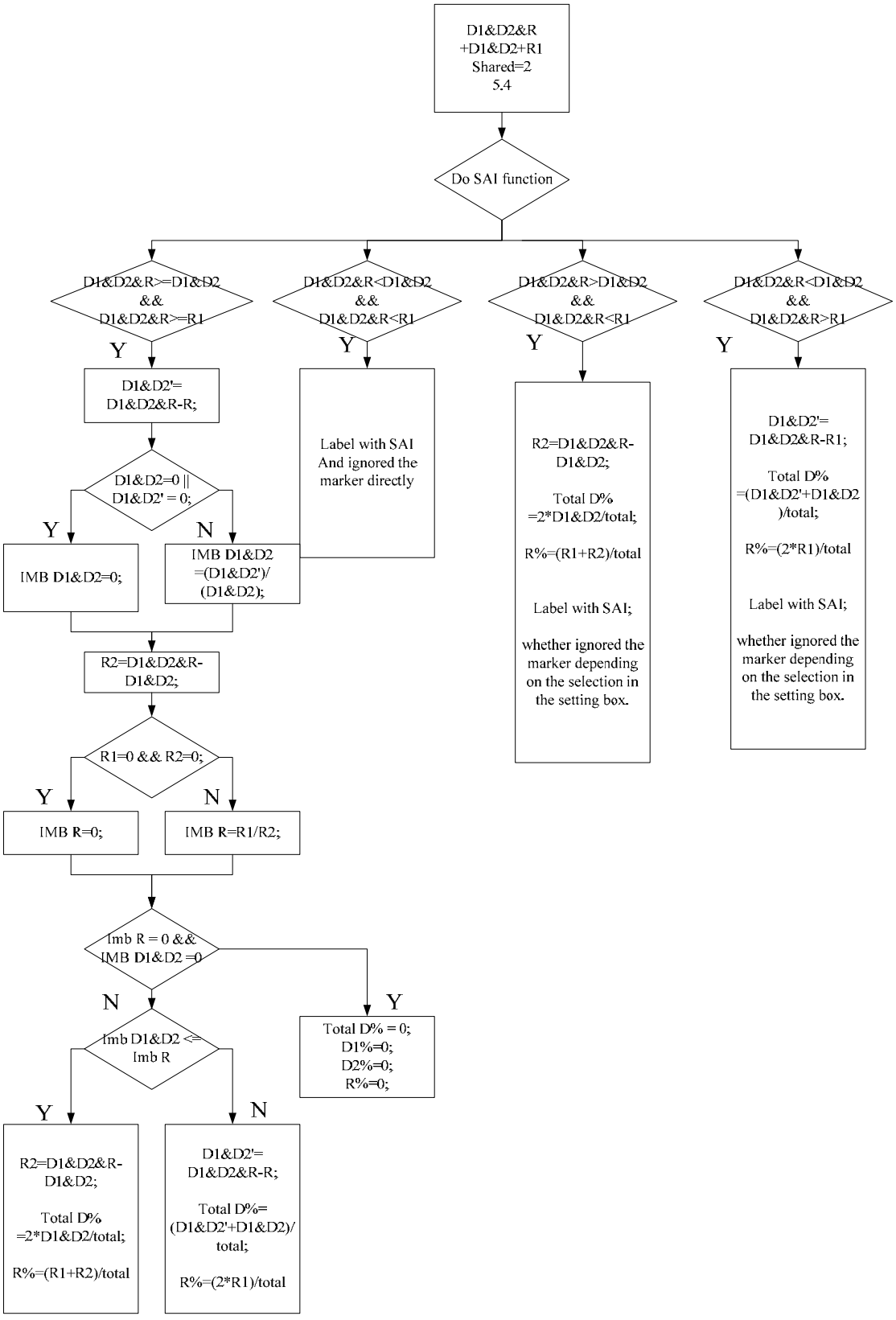
Case 4.5 and Case 4.6


2D1+2D2+2R
5.2 5.5 5.7 exchange needed
Genotype
R1+R2 +D1a1+D1a2 +D2a1+D2a2 Shared =0 5.1
R1+R&D2 +D1a1+D1a2 +1D2a1 Shared =1 5.2
D1&D2+D2&R +D1&R Shared=3 5.3
D1&D2&R +D1&D2+R1 Shared=2 5.4
R1+R&D1 +D1&D2+D2a1 Shared =2 5.5
R1+R&D1&D2+D2 a1+D1a1 Shared =1 5.6
Other cases
Total D%=(D1a1+D1a2+ D2a1+D2a2)/total; D1=(D1a1+D1a2) /total; D2=(D2a1+D2a2) /total; R%=(R1+R2)/total
See page 5.2
Total D%=2*(D1&D2) /total; R=(D2&R+D1&R)-D1&D2; R%=R/total
See page 5.4
See page 5.5
See Page 5.6
Can’t be calculated
R1+R&D1 +D2a1+D2a2 +1D1a1 Shared =1 5.7
D1&D2 +D1a1+D2a1 +R1+R2 Shared =1 5.8
D1&R+D2&R+ D1a1+D2a1 Shared =2 5.9
See page 5.7
See page 5.8
See page 5.9

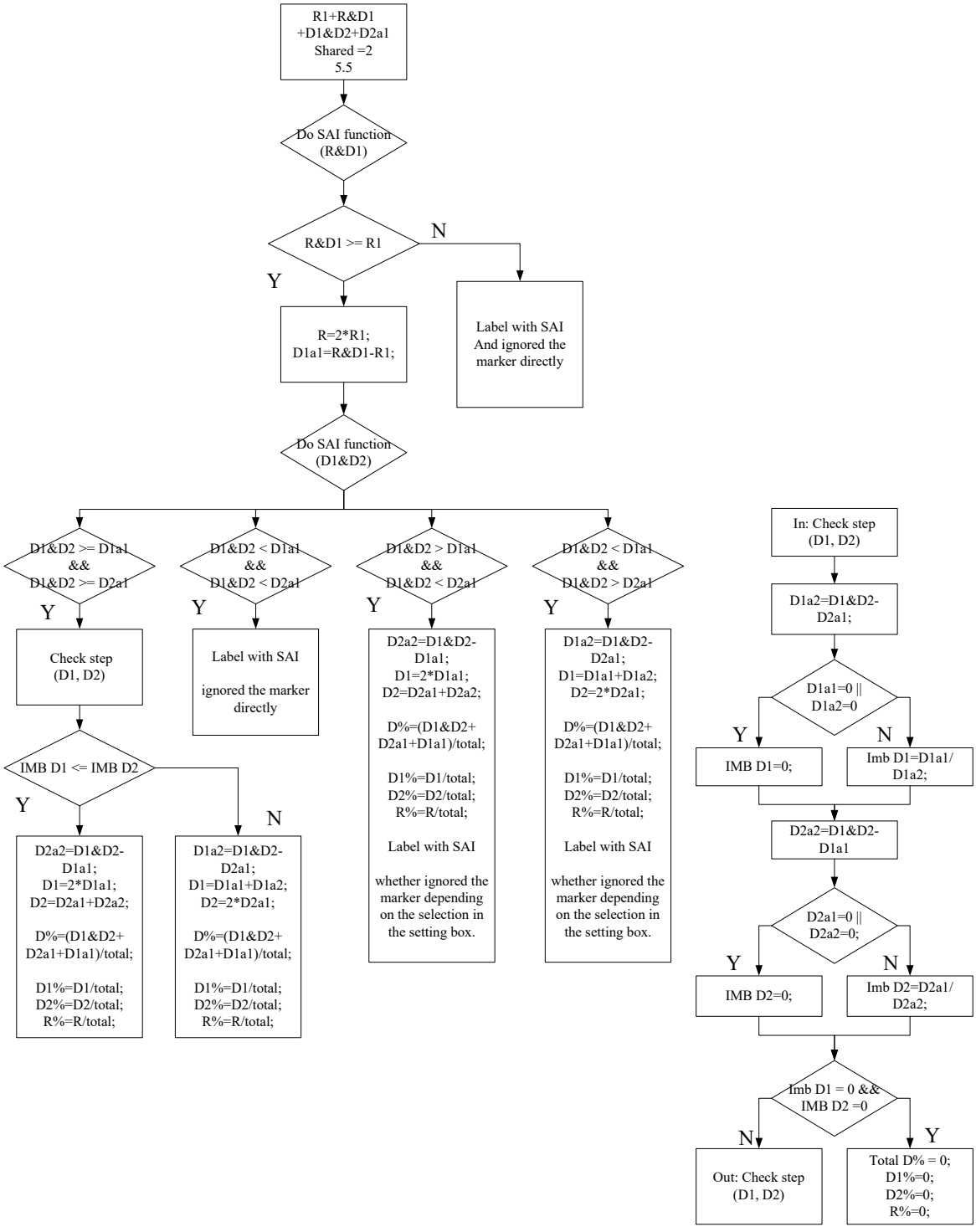
Case 5.0


R1+R&D2
+D1a1+D1a2
+1D2a1
Shared =1
5.2
Do SAI function
(R&D2)
R&D2 > D2a1
&&
R&D2 > R1
Y
R2= R&D2-D2a1;
R1=0 || R2=0;
Y
IMB R=0;
N
IMB R=R1/R2;
D2a2=R&D2-R1;
D2a1=0 ||
D2a2=0;
Y
IMB D2=0;
N
IMB D2=D2a1/
D2a2;
Imb R = 0 &&
IMB D2 =0
N
Imb R <= Imb D2
Y
R2=R1;
D2a2=R&D2-R1;
Total
D%=(D1a1+D1a2+
D2a1+D2a2)/total;
D1=(D1a1+D1a2)
/total;
D2=(D2a1+D2a2)
/total;
R%=2*R1/total
N
R2= R&D2-D2a1;
R%=(R1+R2)/total;
D2a2=D2a1;
Total
D%=(D1a1+D1a2
+D2a1+D2a2)/total;
D1%=(D1a1+D1a2)
/total;
D2%=(D2a1+D2a2)
/total;
Y
Total D% = 0;
D1%=0;
D2%=0;
R%=0;
R&D2 < D2a1
&&
R&D2 < R1
Y
Label with SAI
And ignored the
marker directly
R&D2 > D2a1
&&
R&D2 <= R1
Y
R2= R&D2-D2a1;
R%=(R1+R2)/total;
D2a2=D2a1;
Total
D%=(D1a1+D1a2
+D2a1+D2a2)/total;
D1%=(D1a1+D1a2)
/total;
D2%=(D2a1+D2a2)
/total;
Label with SAI;
whether ignored the
marker depending
on the selection in
the setting box.
R&D2 <= D2a1
&&
R&D2 > R1
Y
R2=R1;
D2a2=R&D2-R1;
Total
D%=(D1a1+D1a2+
D2a1+D2a2)/total;
D1=(D1a1+D1a2)
/total;
D2=(D2a1+D2a2)
/total;
R%=2*R1/total
Label with SAI;
whether ignored the
marker depending
on the selection in
the setting box.

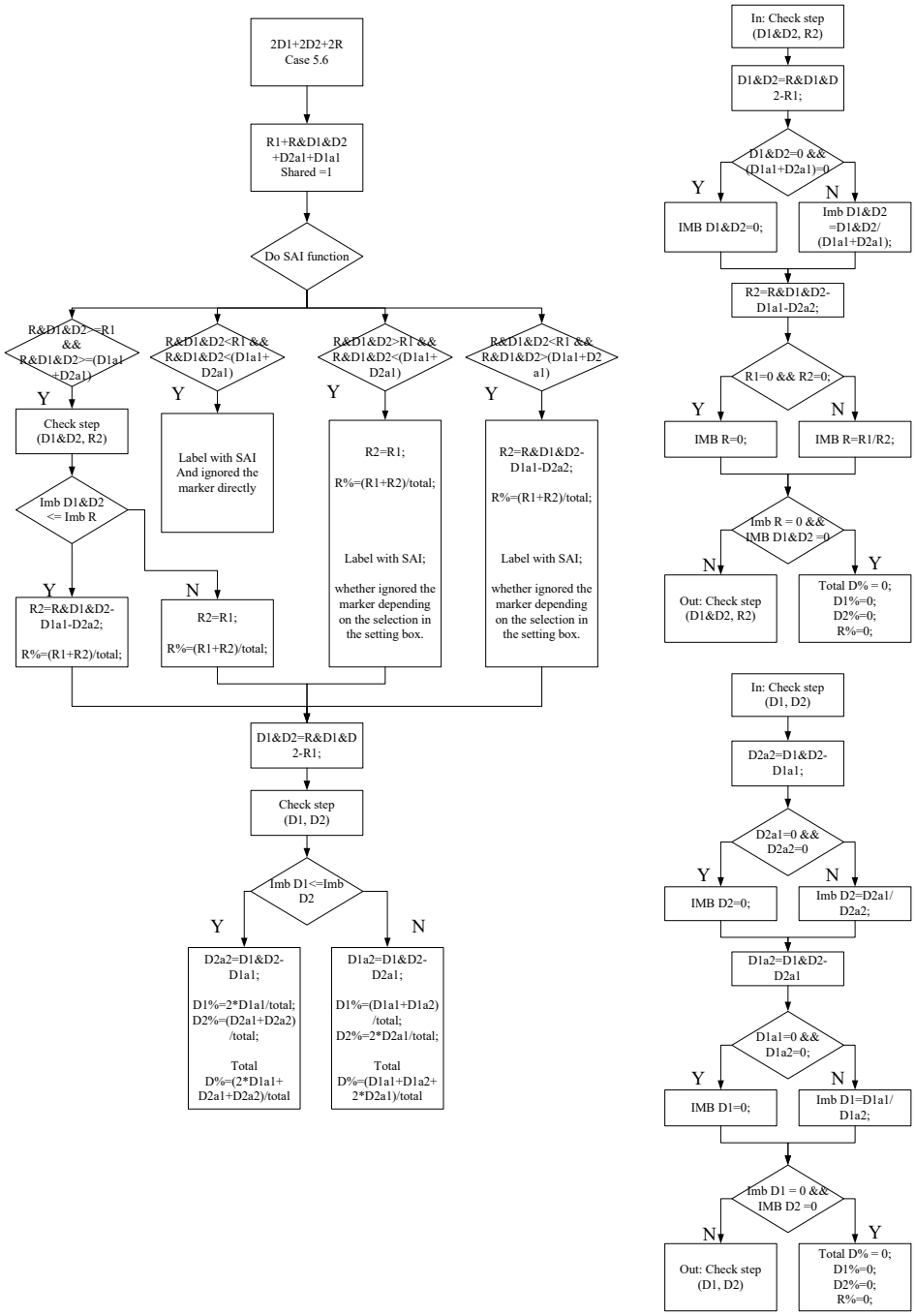
Case 5.2


D1&D2&R
+D1&D2+R1
Shared=2
5.4
Do SAI function
D1&D2&R>=D1&D2
&&
D1&D2&R>=R1
D1&D2&R<D1&D2
&&
D1&D2&R<R1
D1&D2&R>D1&D2
&&
D1&D2&R<R1
D1&D2&R<D1&D2
&&
D1&D2&R>R1
Y
Y
Y
Y
D1&D2'=
D1&D2&R-R;
Label with SAI
And ignored the
marker directly
R2=D1&D2&R-
D1&D2;
Total D%
=2*D1&D2/total;
R%=(R1+R2)/total
Label with SAI;
whether ignored the
marker depending
on the selection in
the setting box.
D1&D2'=
D1&D2&R-R1;
Total D%
=(D1&D2'+D1&D2
)/total;
R%=(2*R1)/total
Label with SAI;
whether ignored the
marker depending
on the selection in
the setting box.
D1&D2=0 ||
D1&D2' = 0;
Y
N
IMB D1&D2=0;
IMB D1&D2
=(D1&D2')/
(D1&D2);
R2=D1&D2&R-
D1&D2;
R1=0 && R2=0;
Y
N
IMB R=0;
IMB R=R1/R2;
Imb R = 0 &&
IMB D1&D2 =0
N
Y
Imb D1&D2 <=
Imb R
Total D% = 0;
D1%=0;
D2%=0;
R%=0;
Y
N
R2=D1&D2&R-
D1&D2;
Total D%
=2*D1&D2/total;
R%=(R1+R2)/total
D1&D2'=
D1&D2&R-R;
Total D%=
(D1&D2'+D1&D2)/
total;
R%=(2*R1)/total

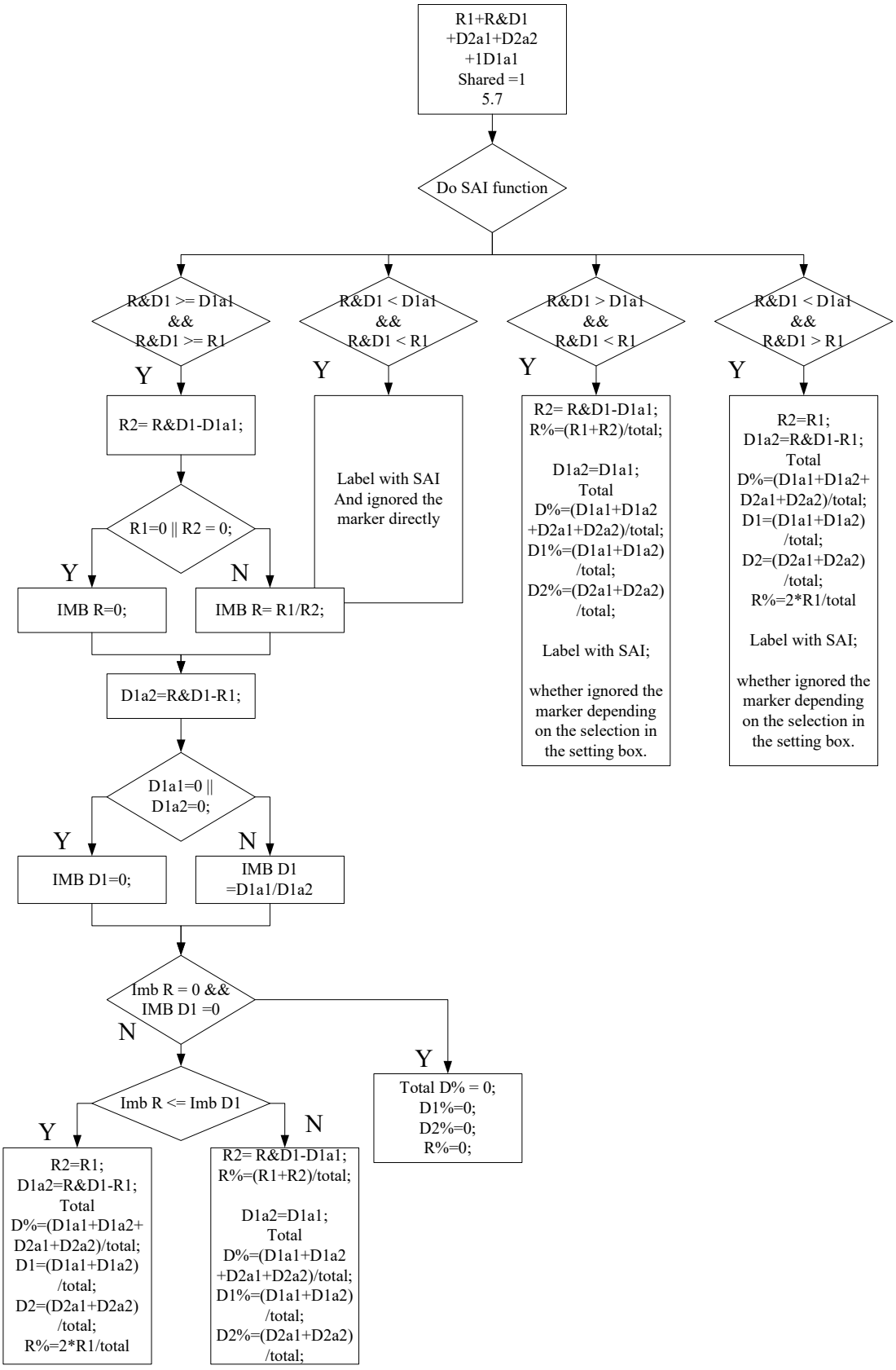
Case 5.4


R1+R&D1 +D1&D2+D2a1 Shared =2 5.5
Do SAI function (R&D1)
R&D1 >= R1
N
Y
Label with SAI And ignored the marker directly
R=2*R1; D1a1=R&D1-R1;
Do SAI function (D1&D2)
D1&D2 >= D1a1 && D1&D2 >= D2a1
D1&D2 < D1a1 && D1&D2 < D2a1
D1&D2 > D1a1 && D1&D2 < D2a1
D1&D2 < D1a1 && D1&D2 > D2a1
Check step (D1, D2)
Label with SAI ignored the marker directly
D2a2=D1&D2-D1a1; D1=2*D1a1; D2=D2a1+D2a2; D%=(D1&D2+D2a1+D1a1)/total; D1%=D1/total; D2%=D2/total; R%=R/total; Label with SAI whether ignored the marker depending on the selection in the setting box.
D1a2=D1&D2-D2a1; D1=D1a1+D1a2; D2=2*D2a1; D%=(D1&D2+D2a1+D1a1)/total; D1%=D1/total; D2%=D2/total; R%=R/total; Label with SAI whether ignored the marker depending on the selection in the setting box.
IMB D1 <= IMB D2
D2a2=D1&D2-D1a1; D1=2*D1a1; D2=D2a1+D2a2; D%=(D1&D2+D2a1+D1a1)/total; D1%=D1/total; D2%=D2/total; R%=R/total;
D1a2=D1&D2-D2a1; D1=D1a1+D1a2; D2=2*D2a1; D%=(D1&D2+D2a1+D1a1)/total; D1%=D1/total; D2%=D2/total; R%=R/total;
In: Check step (D1, D2)
D1a2=D1&D2-D2a1;
D1a1=0 || D1a2=0
IMB D1=0;
Imb D1=D1a1/D1a2;
D2a2=D1&D2-D1a1
D2a1=0 || D2a2=0;
IMB D2=0;
Imb D2=D2a1/D2a2;
Imb D1 = 0 && IMB D2 =0
Out: Check step (D1, D2)
Total D% = 0; D1%=0; D2%=0; R%=0;

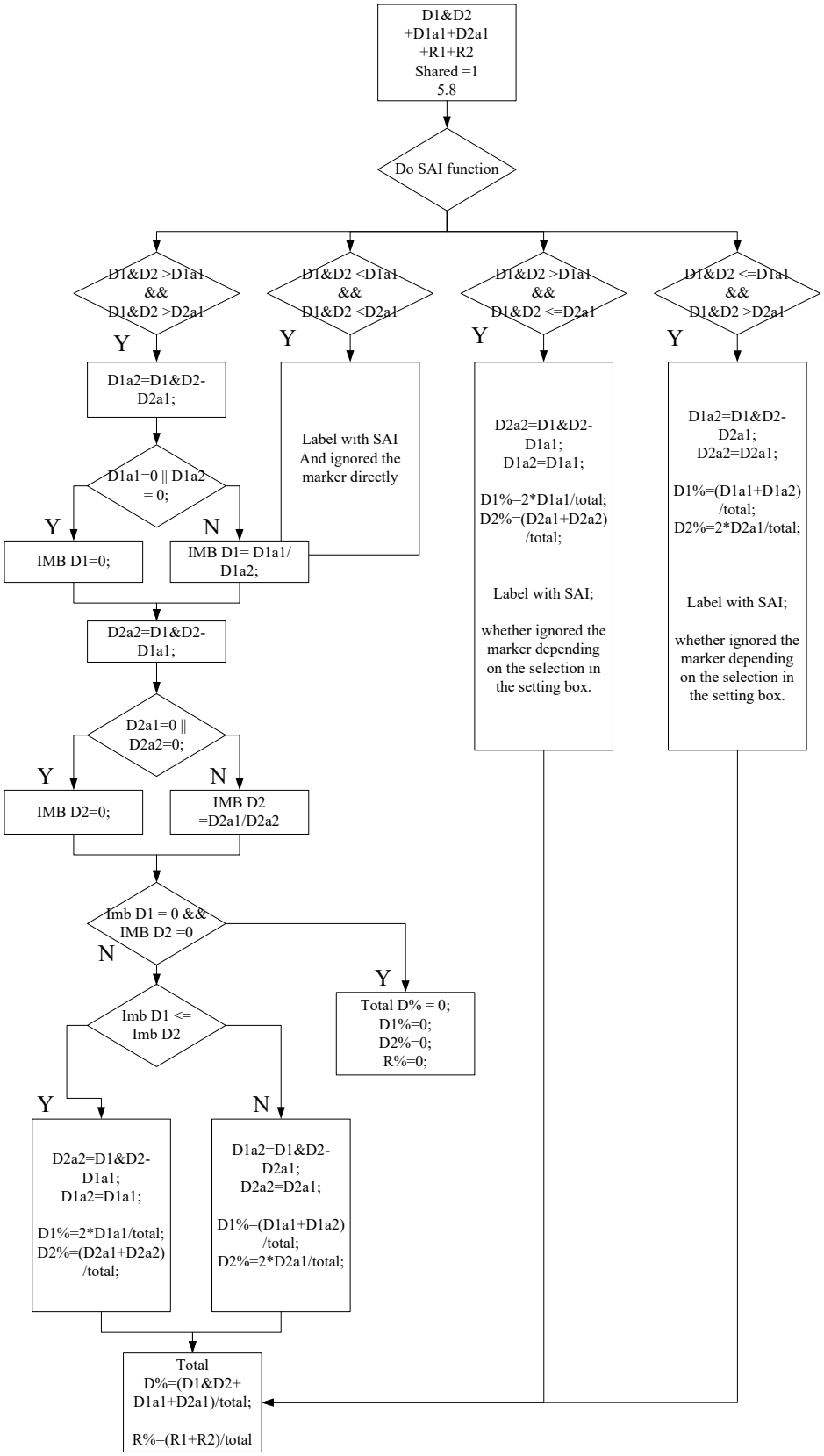
Case 5.5


2D1+2D2+2R
Case 5.6
R1+R&D1&D2
+D2a1+D1a1
Shared =1
Do SAI function
R&D1&D2>=R1
&&
R&D1&D2>=(D1a1
+D2a1)
R&D1&D2<R1 &&
R&D1&D2<(D1a1+
D2a1)
R&D1&D2>R1 &&
R&D1&D2<(D1a1+
D2a1)
R&D1&D2<R1 &&
R&D1&D2>(D1a1+D2
a1)
Y
Check step
(D1&D2, R2)
Label with SAI
And ignored the
marker directly
R2=R1;
R%=(R1+R2)/total;
Label with SAI;
whether ignored the
marker depending
on the selection in
the setting box.
R2=R&D1&D2-
D1a1-D2a2;
R%=(R1+R2)/total;
Label with SAI;
whether ignored the
marker depending
on the selection in
the setting box.
Imb D1&D2
<= Imb R
N
R2=R&D1&D2-
D1a1-D2a2;
R%=(R1+R2)/total;
R2=R1;
R%=(R1+R2)/total;
D1&D2=R&D1&D
2-R1;
Check step
(D1, D2)
Imb D1<=Imb
D2
D2a2=D1&D2-
D1a1;
D1%=2*D1a1/total;
D2%=(D2a1+D2a2)
/total;
Total
D%=(2*D1a1+
D2a1+D2a2)/total
D1a2=D1&D2-
D2a1;
D1%=(D1a1+D1a2)
/total;
D2%=2*D2a1/total;
Total
D%=(D1a1+D1a2+
2*D2a1)/total
In: Check step
(D1&D2, R2)
D1&D2=R&D1&D
2-R1;
D1&D2=0 &&
(D1a1+D2a1)=0
IMB D1&D2=0;
Imb D1&D2
=D1&D2/
(D1a1+D2a1);
R2=R&D1&D2-
D1a1-D2a2;
R1=0 && R2=0;
IMB R=0;
IMB R=R1/R2;
Imb R = 0 &&
IMB D1&D2 =0
Out: Check step
(D1&D2, R2)
Total D% = 0;
D1%=0;
D2%=0;
R%=0;
In: Check step
(D1, D2)
D2a2=D1&D2-
D1a1;
D2a1=0 &&
D2a2=0
IMB D2=0;
Imb D2=D2a1/
D2a2;
D1a2=D1&D2-
D2a1
D1a1=0 &&
D1a2=0;
IMB D1=0;
Imb D1=D1a1/
D1a2;
Imb D1 = 0 &&
IMB D2 =0
Out: Check step
(D1, D2)
Total D% = 0;
D1%=0;
D2%=0;
R%=0;

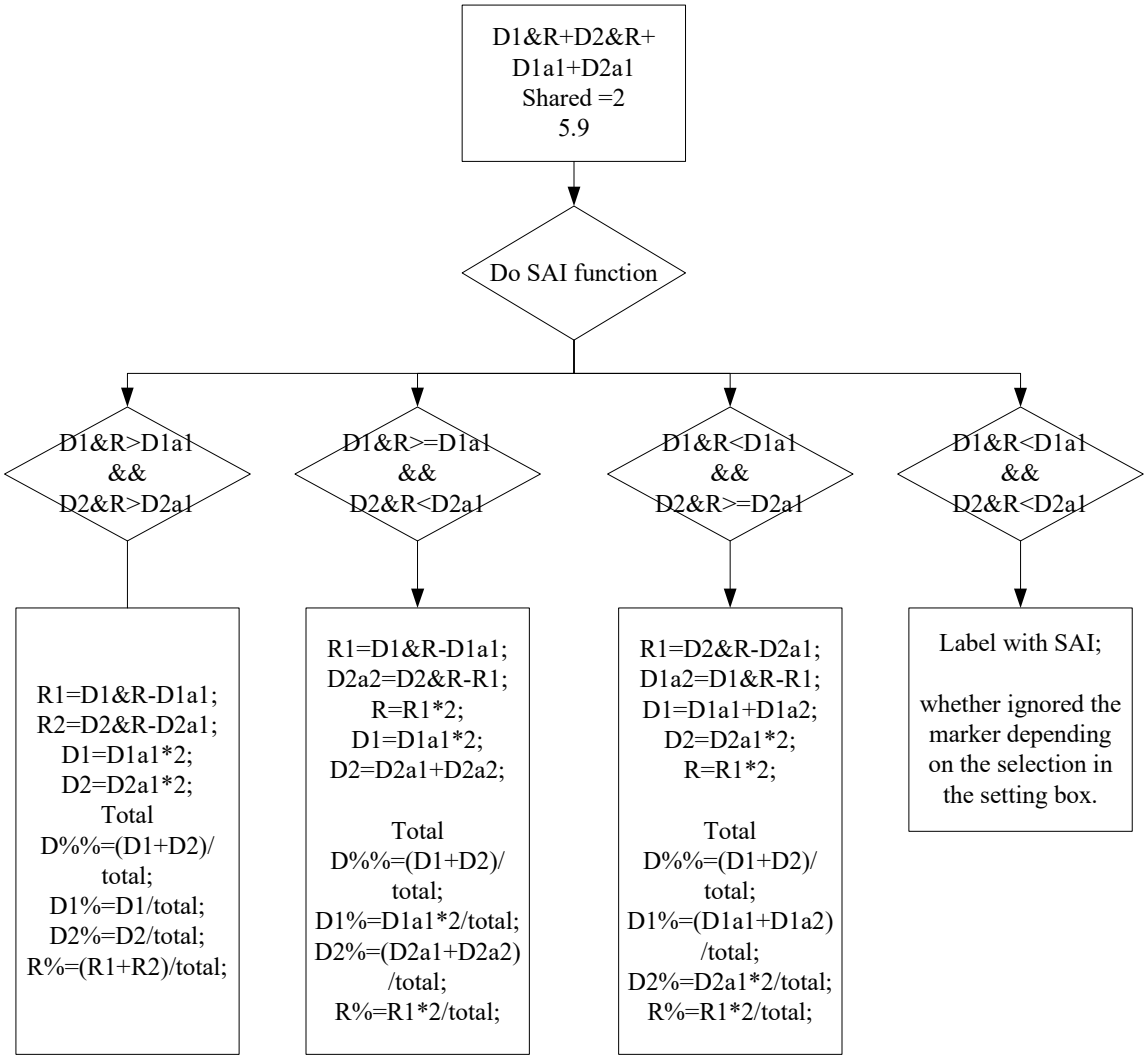
Case 5.6


R1+R&D1
+D2a1+D2a2
+1D1a1
Shared =1
5.7
Do SAI function
R&D1 >= D1a1
&&
R&D1 >= R1
Y
R2= R&D1-D1a1;
R1=0 || R2 = 0;
Y
IMB R=0;
N
IMB R= R1/R2;
D1a2=R&D1-R1;
D1a1=0 ||
D1a2=0;
Y
IMB D1=0;
N
IMB D1
=D1a1/D1a2
Imb R = 0 &&
IMB D1 =0
N
Imb R <= Imb D1
Y
R2=R1;
D1a2=R&D1-R1;
Total
D%=(D1a1+D1a2+
D2a1+D2a2)/total;
D1=(D1a1+D1a2)
/total;
D2=(D2a1+D2a2)
/total;
R%=2*R1/total
N
R2= R&D1-D1a1;
R%=(R1+R2)/total;
D1a2=D1a1;
Total
D%=(D1a1+D1a2
+D2a1+D2a2)/total;
D1%=(D1a1+D1a2)
/total;
D2%=(D2a1+D2a2)
/total;
Y
Total D% = 0;
D1%=0;
D2%=0;
R%=0;
R&D1 < D1a1
&&
R&D1 < R1
Y
Label with SAI
And ignored the
marker directly
R&D1 > D1a1
&&
R&D1 < R1
Y
R2= R&D1-D1a1;
R%=(R1+R2)/total;
D1a2=D1a1;
Total
D%=(D1a1+D1a2
+D2a1+D2a2)/total;
D1%=(D1a1+D1a2)
/total;
D2%=(D2a1+D2a2)
/total;
Label with SAI;
whether ignored the
marker depending
on the selection in
the setting box.
R&D1 < D1a1
&&
R&D1 > R1
Y
R2=R1;
D1a2=R&D1-R1;
Total
D%=(D1a1+D1a2+
D2a1+D2a2)/total;
D1=(D1a1+D1a2)
/total;
D2=(D2a1+D2a2)
/total;
R%=2*R1/total
Label with SAI;
whether ignored the
marker depending
on the selection in
the setting box.

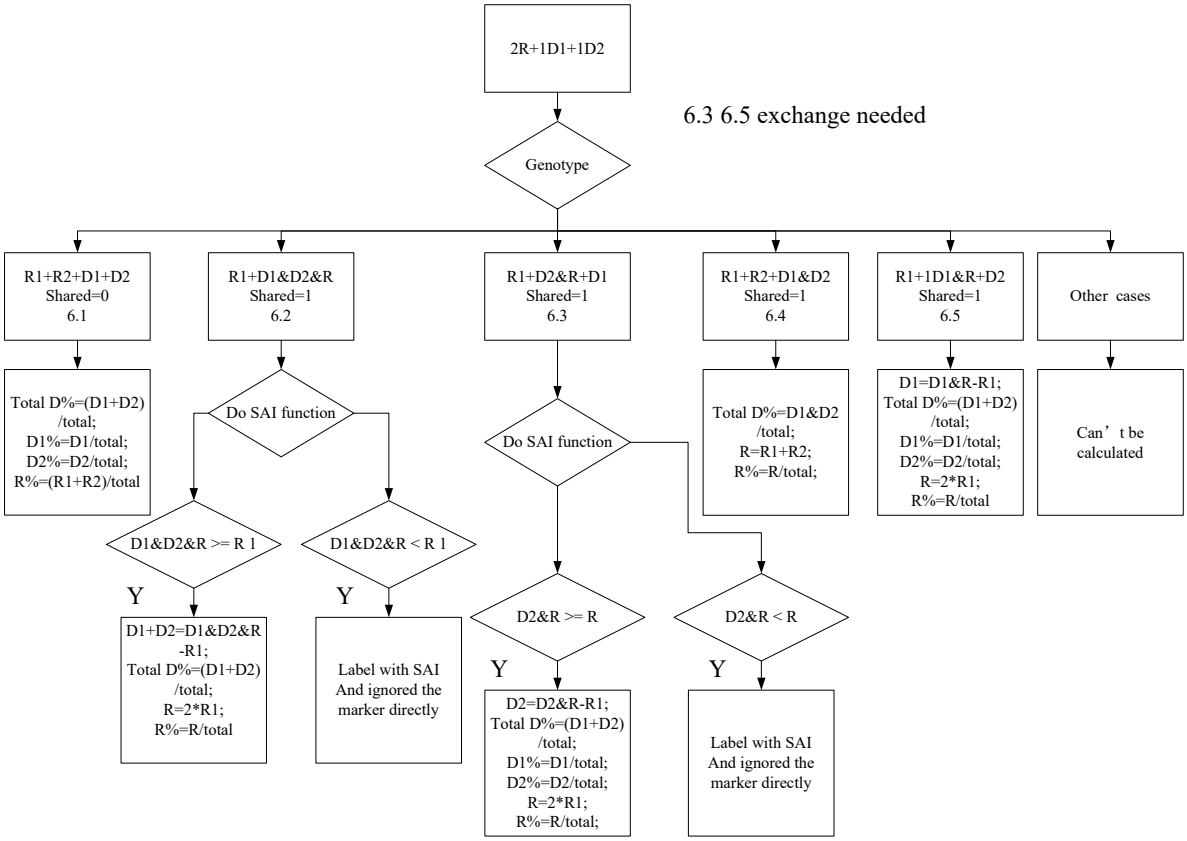
Case 5.7


D1&D2 +D1a1+D2a1 +R1+R2 Shared =1 5.8
Do SAI function
D1&D2 >D1a1 && D1&D2 >D2a1
D1&D2 <D1a1 && D1&D2 <D2a1
D1&D2 >D1a1 && D1&D2 <=D2a1
D1&D2 <=D1a1 && D1&D2 >D2a1
Y
D1a2=D1&D2-D2a1;
Label with SAI And ignored the marker directly
D2a2=D1&D2-D1a1; D1a2=D1a1; D1%=2*D1a1/total; D2%=(D2a1+D2a2) /total; Label with SAI; whether ignored the marker depending on the selection in the setting box.
D1a2=D1&D2-D2a1; D2a2=D2a1; D1%=(D1a1+D1a2) /total; D2%=2*D2a1/total; Label with SAI; whether ignored the marker depending on the selection in the setting box.
D1a1=0 || D1a2 = 0;
N
IMB D1=0;
IMB D1= D1a1/ D1a2;
D2a2=D1&D2-D1a1;
D2a1=0 || D2a2=0;
IMB D2=0;
IMB D2 =D2a1/D2a2
Imb D1 = 0 && IMB D2 =0
Total D% = 0; D1%=0; D2%=0; R%=0;
Imb D1 <= Imb D2
D2a2=D1&D2-D1a1; D1a2=D1a1; D1%=2*D1a1/total; D2%=(D2a1+D2a2) /total;
D1a2=D1&D2-D2a1; D2a2=D2a1; D1%=(D1a1+D1a2) /total; D2%=2*D2a1/total;
Total D%=(D1&D2+ D1a1+D2a1)/total; R%=(R1+R2)/total

Case 5.8


D1&R+D2&R+
D1a1+D2a1
Shared =2
5.9
Do SAI function
D1&R>D1a1
&&
D2&R>D2a1
D1&R>=D1a1
&&
D2&R<D2a1
D1&R<D1a1
&&
D2&R>=D2a1
D1&R<D1a1
&&
D2&R<D2a1
R1=D1&R-D1a1;
R2=D2&R-D2a1;
D1=D1a1*2;
D2=D2a1*2;
Total
D%%=(D1+D2)/
total;
D1%=D1/total;
D2%=D2/total;
R%=(R1+R2)/total;
R1=D1&R-D1a1;
D2a2=D2&R-R1;
R=R1*2;
D1=D1a1*2;
D2=D2a1+D2a2;
Total
D%%=(D1+D2)/
total;
D1%=D1a1*2/total;
D2%=(D2a1+D2a2)
/total;
R%=R1*2/total;
R1=D2&R-D2a1;
D1a2=D1&R-R1;
D1=D1a1+D1a2;
D2=D2a1*2;
R=R1*2;
Total
D%%=(D1+D2)/
total;
D1%=(D1a1+D1a2)
/total;
D2%=D2a1*2/total;
R%=R1*2/total;
Label with SAI;
whether ignored the
marker depending
on the selection in
the setting box.

Case 5.9


2R+1D1+1D2
6.3 6.5 exchange needed
Genotype
R1+R2+D1+D2
Shared=0
6.1
R1+D1&D2&R
Shared=1
6.2
R1+D2&R+D1
Shared=1
6.3
R1+R2+D1&D2
Shared=1
6.4
R1+1D1&R+D2
Shared=1
6.5
Other cases
Total D%=(D1+D2)
/total;
D1%=D1/total;
D2%=D2/total;
R%=(R1+R2)/total
Do SAI function
Do SAI function
Total D%=D1&D2
/total;
R=R1+R2;
R%=R/total;
D1=D1&R-R1;
Total D%=(D1+D2)
/total;
D1%=D1/total;
D2%=D2/total;
R=2*R1;
R%=R/total
Can’t be
calculated
D1&D2&R >= R 1
D1&D2&R < R 1
Y
Y
D2&R >= R
D2&R < R
Y
Y
D1+D2=D1&D2&R
-R1;
Total D%=(D1+D2)
/total;
R=2*R1;
R%=R/total
Label with SAI
And ignored the
marker directly
D2=D2&R-R1;
Total D%=(D1+D2)
/total;
D1%=D1/total;
D2%=D2/total;
R=2*R1;
R%=R/total;
Label with SAI
And ignored the
marker directly

Case 6.0

Chapter 3 Genotyping General Procedure

Overview

Chapters 3 and 4 provide a detailed description of importing data, all raw data and analysis icons, capillary electrophoresis review and details for genotyping. For streamlined workflow for chimerism detection, quantification and monitoring please go to the Chimerism Analysis Chapter.

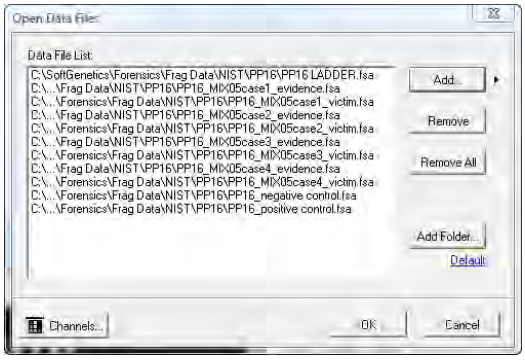
Import Data Files

After installing ChimerMarker software you are 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, .abi, ab1, .hid
MegaBACE - .rsd
Beckman-Coulter - .esd
Spectrumedix - .smd
Generic - .scf, sg1

Procedure

2. Launch **ChimerMarker**
3. Click **Open Data**
4. The *Open Data Files* box will appear
5. Click **Add** button
6. The *Open* dialog will appear
7. Navigate to directory containing raw data files
8. Select all files by **CTRL+A** or use **CTRL** and/or **SHIFT** keys to select individual samples
9. Click **Open** button in the *Open* dialog
10. The files selected will appear in the *Data File List* field
11. Click **OK** button in the *Open Data Files* box and the samples will be uploaded to ChimerMarker



Features

There are several features available in the *Open Data Files* box to make data upload easier.

Add…
Used to locate and select raw data files for upload. Click the arrow button next to the **Add** button to see the four most recently accessed directories.

Remove
Used to remove samples from the *Data File List*. Highlight the sample to remove by single left-clicking it in the *Data File List* then click **Remove**.

Remove All
Removes all sample files from the *Data File List* field.

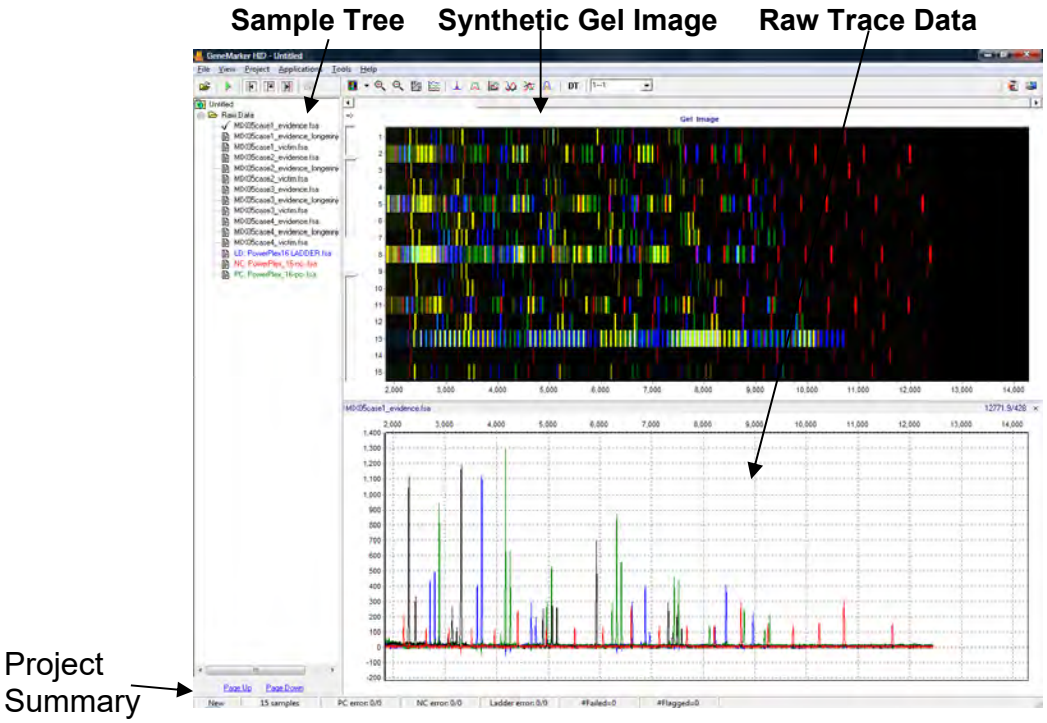
Add Folder…
Click **Add Folder** to upload raw data files from a specific folder in the file directory tree. Click the *Default* hyperlink to choose a folder to which ChimerMarker will always open when the **Add** or **Add Folder** buttons are clicked.

Channels
Opens the *Set Channels* dialog with 4, 5, 6-color tab options and allows the user to choose from ABI, MegaBACE, and Beckman-Coulter standard dye color orders. The user can also manually enter 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 box.

Raw Data Analysis

Once the raw data files are uploaded, the *Raw Data Main Analysis* window 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* window with fragment mobility from right to left. The largest size fragments are on the far right of the trace.

Raw Data Analysis Window



Main Toolbar Icons

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 will be 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 will be 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 recommend to de-select *Pull-up Correction* in the Run Wizard when using this function.

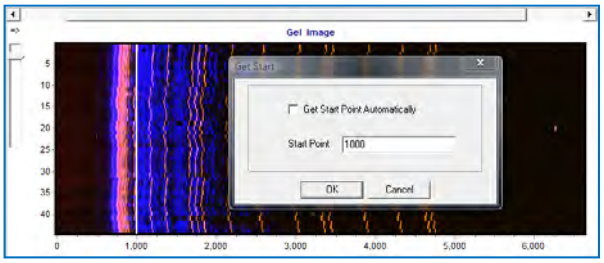
DT **2nd Derivative Trace:** This feature reduces high background noise and sharpens peaks. Baseline fluctuation caused from dye blobs or the DNA template in PCR can also be reduced with this function. It is recommended to de-select *Spike Removal* in the Run Wizard when this function has been activated.

What to Expect

The raw data correction icons can be selected individually in the *Raw Data Analysis* window. The images below demonstrate how the data will look before (left image) and after (right image) the parameter is applied.

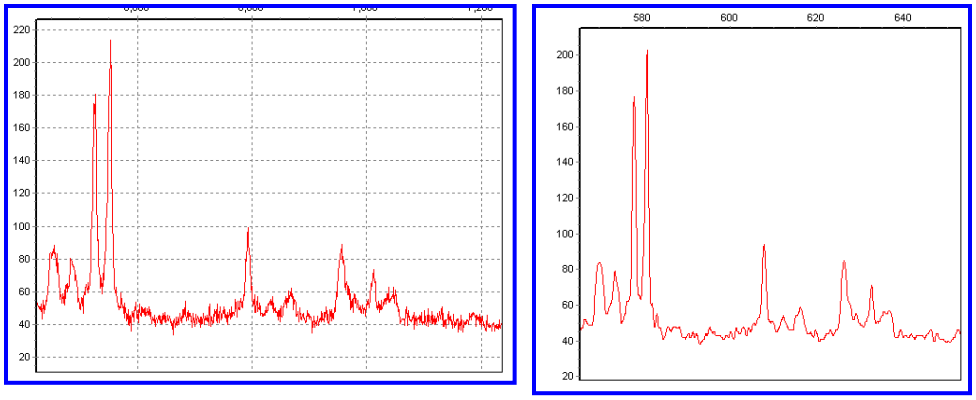
Range

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**



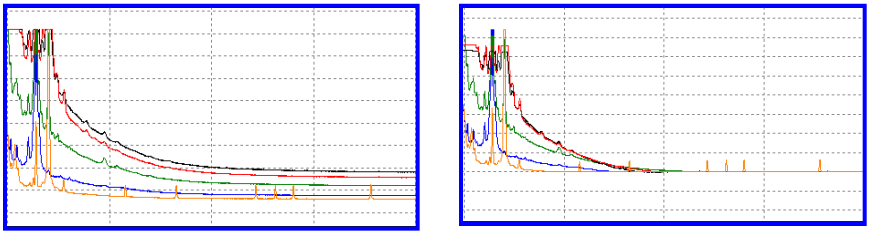
Smooth

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



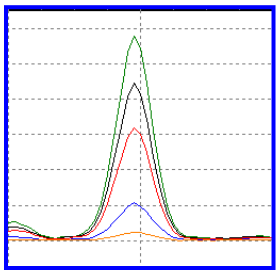
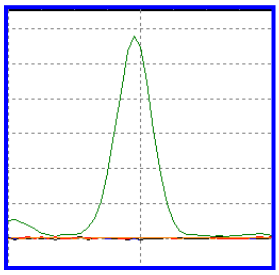
Baseline Subtraction

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



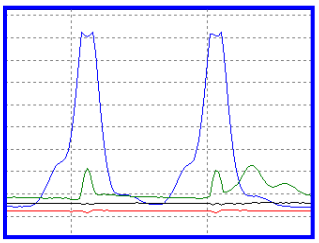
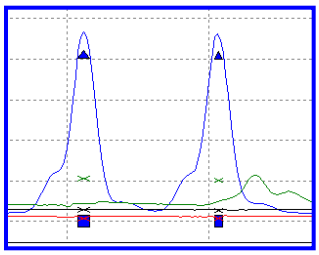
Pullup Correction

Ax=B
A being the major coefficient
Input matrix or use single dye adjustment up to 0.20 for small corrections
When **Manual Pullup** correction is chosen, a .txt or .mtx matrix file can be uploaded and used to deconvolute dye colors.
NOTE: De-select automatic *Pullup Correction* in the Run Wizard *Data Process* box if a manual matrix correction has been applied.

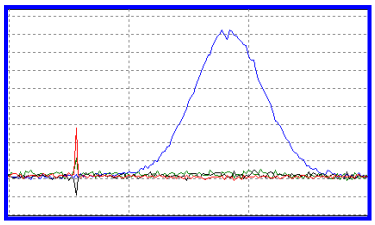
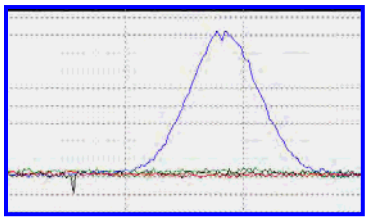
Saturated Peak Correction

ABI instrument saturated peaks are typically >8000 RFU
The top of a saturated peak looks split
A small pullup peak may be present under the saturated peak
ChimerMarker takes the small pullup peak and adds it to the split in the saturated peak

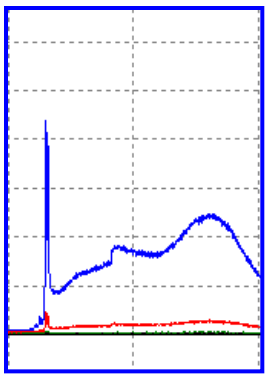



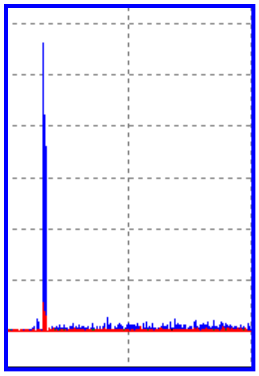
Spike Removal

Caused by overheating of camera chip, voltage spike, etc
Spikes usually only 1-2 frames wide; peaks usually 5-10 frames wide
Create a first derivative trace of the raw data
Spikes are the 1st DT outliers (3-5 sigma)

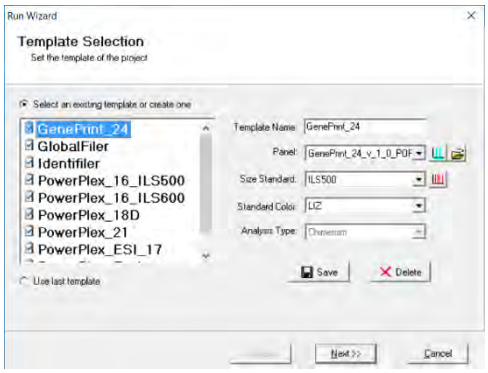
Second Derivative Trace
(A1-A2)-(A2-A3) = A1+A3-2(A2)
Use when you have a fat base to your peaks (ex. Dye blob under peak, etc)
NOTE: Do not use 2nd DT with *Spike Removal* because real peaks look like spikes.





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

Procedure

1. Click the **Run Project** icon in the toolbar.
2. The Run Wizard *Template Selection* dialog box will appear.
3. 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.
4. Click **Next** when finished.

Icons and Functions

Template Name

Select from existing pre-made templates or create your own by entering a *Template Name* and clicking the **Save** button. When a template is saved, only the settings in the *Template Selection* box of Run Wizard will be saved.

To create a new template, click *Select an existing template or create one*. A template can also be selected from the list of available templates in the left section of the window and then saved for future use by clicking the **Save** button.

If you do not want to use a template, select the appropriate size standard, standard color, and type of analysis; *Use last template* will automatically be selected.

Panel

ChimerMarker comes preloaded with many common kit Panels including Promega's PowerPlex kits and additional Panels can be imported by selecting the *Open Files* icon next to the *Panel* field. A custom Panel can be created in the *Panel Editor* tool. **See Chapter 6 Panel Editor.**

Panel Editor: A Panel can be selected from any available from the drop-down menu or can be viewed and selected by clicking the *Panel Editor* icon.

Import a Panel: If a Panel cannot be found in the *Panel Editor* tool, it can be 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 can be created by selecting the *Size Template Editor* icon next to the *Size Standard* field. **See Chapter 5 Fragment Sizing Standards.**

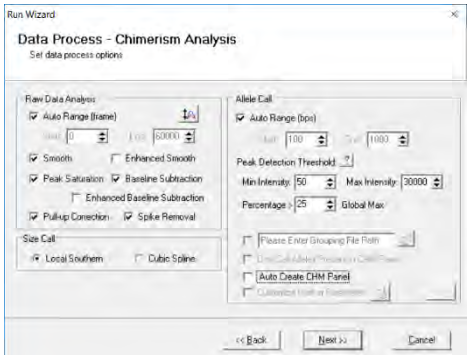
Size Template Editor: This allows the user to check sample files against a selected size standard, modify and save the size standard for future use, or create a new size standard.

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

Procedure

5. The *Data Process* window of Run Wizard appears.
6. Select the appropriate analysis settings in the *Data Process* window and click **Next** to continue.

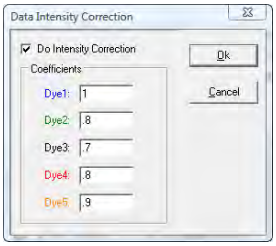
Icons and Functions

Raw Data Analysis

Auto Range (frame)

The range in camera frames will automatically find 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.



Intensity Coefficients: Allows for manual correction of excessive bleed-through peaks; best used for experiments with one-color analysis. Allows for manual correction of low RFU by using an number greater than 1 to increase the RFU

Smooth

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

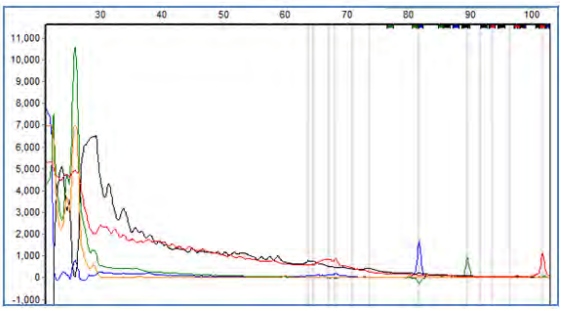
NOTE: Saturated and Saturated Repaired peaks are not recommended for any quantitative calculations.

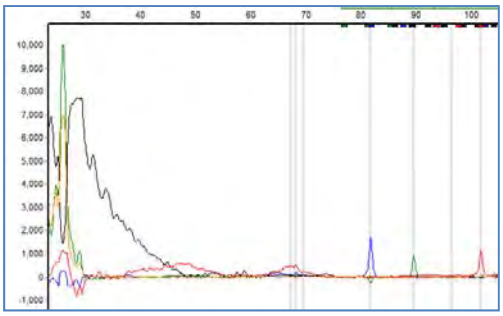
Baseline Subtraction

This function removes the baseline completely so that the Y-axis will be 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

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





In situations where there is an extended ion front in the mini-STR range Enhanced Baseline Subtraction should be 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* window.

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* has been applied.

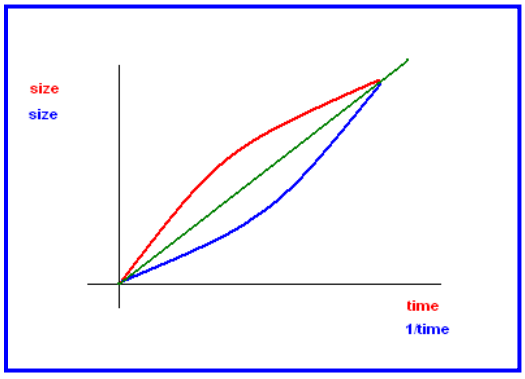
Size Call

ChimerMarker offers two sizing methods:

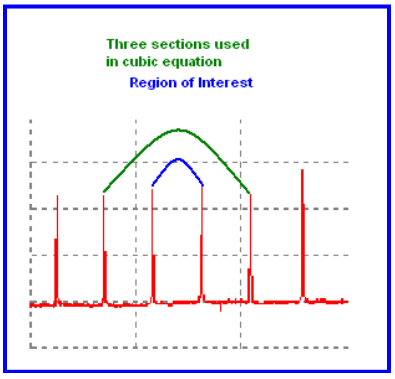
Local Southern
Used in most genotyping software applications and is recommended for most analyses. This method is based on the idea that smaller size fragments run faster. Plot a size v. time graph and overlay a size v. 1/time graph to determine linear trace. (Southern, E.M. "Measurement of DNA Length 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



Allele Call

The *Allele Call* section allows the user to set allele calling range, detection thresholds and filters.

Auto Range
The software will identify 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 will not be 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**.

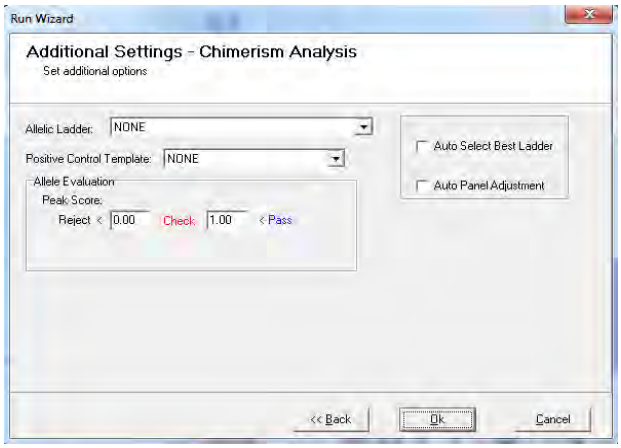
Min Intensity
Minimum RFU threshold of peak height used for peak detection. Peaks below this value will not be called.

Max Intensity
Maximum RFU threshold of peak height. Peaks above this value will be 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 will not be 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 particular analysis type.

Save Default
Saves any settings defined by the user that is different from the default. These settings can be recalled for consistency of analysis on similar data sets.



Run Wizard Additional Settings

Procedure

7. The Run Wizard *Additional Settings* box appears
8. Select an Allelic Ladder and adjust the Peak Score parameters.
9. Click **OK**
10. The *Data Processing* box appears
11. The data is sized, peaks are filtered, and the Panel is applied
12. Click **OK** when the *Data Processing* box is finished.

Functions

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

Allele Evaluation

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

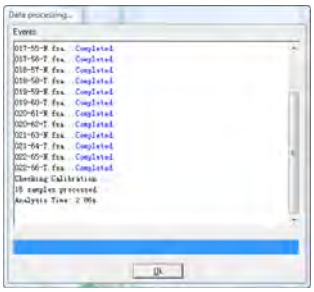
Auto Select Best Ladder
ChimerMarker identifies ladder samples in the dataset as defined in the *View → Preference → Chimerism → Ladder Identifier* field. Ladder samples are then compared to the chosen Panel. Each ladder that is within the range of the selected panel will pass and appears in bold font in the Sample File Tree.. Auto Select Best Ladder will analyze each sample file with the passing ladder that best matches that sample. The print report provides the file name of reference ladder used for each sample.

Auto Panel Adjustment
When selected, the Markers and Bins of the chosen Panel will be 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 can be manually adjusted in the *Panel Editor* by first clicking the *Major Adjustment of Panel* icon then the *Minor Adjustment of Panel* icon.

Adjust Analysis Parameters

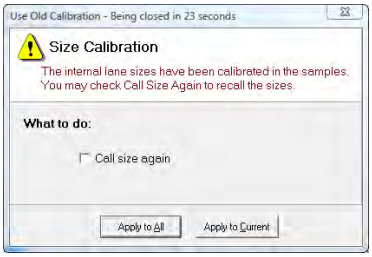


After the clicking **OK** in the Run Wizard *Additional Settings* box, the *Data Processing* box appears. The raw data is being processed and sized, then the filtering parameters are applied, and finally a Panel is applied (if selected). Click **OK** in the *Data Processing* box when analysis is complete.

Review the results in the *Main Analysis* window. **See Chapter 4 Main Analysis Overview.** If you notice many false positive peak calls, you may need to adjust the analysis parameters. There are three options for adjusting the analysis parameters as discussed below.

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

Re-analyze with Run Wizard



To re-analyze with the Run Wizard tool, simply click the **Run Project** icon in the main toolbar. The Run Wizard will launch and the most recently selected parameters will be displayed. Adjust parameters as necessary and click **OK** in the Run Wizard *Additional Settings* box. The *Use Old Calibration* box will appear with the option to **Call Size Again**. Only select *Call Size Again* 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. Click the **Apply to All** button. The *Data Processing* box will appear again and the data will be re-analyzed with the new parameters.

Re-analyze with Auto Run

To re-analyze with Auto Run, first select *Project* → *Options*. The *Project Options Settings* box will appear. This box offers all the same parameters settings as are available in the Run Wizard. Use the tabs to view the *Template Selection*, *Data Process*, and *Additional Settings* boxes. Click **OK** when finished. Next, select *Project* → *Auto Run*. The data will be re-analyzed with the new parameters.

NOTE: The *Additional Settings Allele Evaluation Peak Score* parameters can be changed in the *Project Options Settings* box and will be applied to the data without having to re-analyze the data with Run Wizard or Auto Run.

Re-analyze Individual Samples

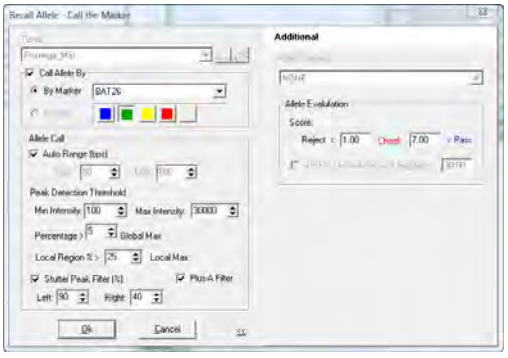
To re-analyze an individual sample, dye color, or marker, click the **Call Allele** icon in the main toolbar. The arrow next to the icon opens the drop-down menu with additional options. Click an option from the drop-down and the *Recall Allele* box will appear. Adjust parameters as necessary and click **OK**. The new parameters will be applied.

All Samples
Applies the new parameter settings to all samples in the dataset – 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.



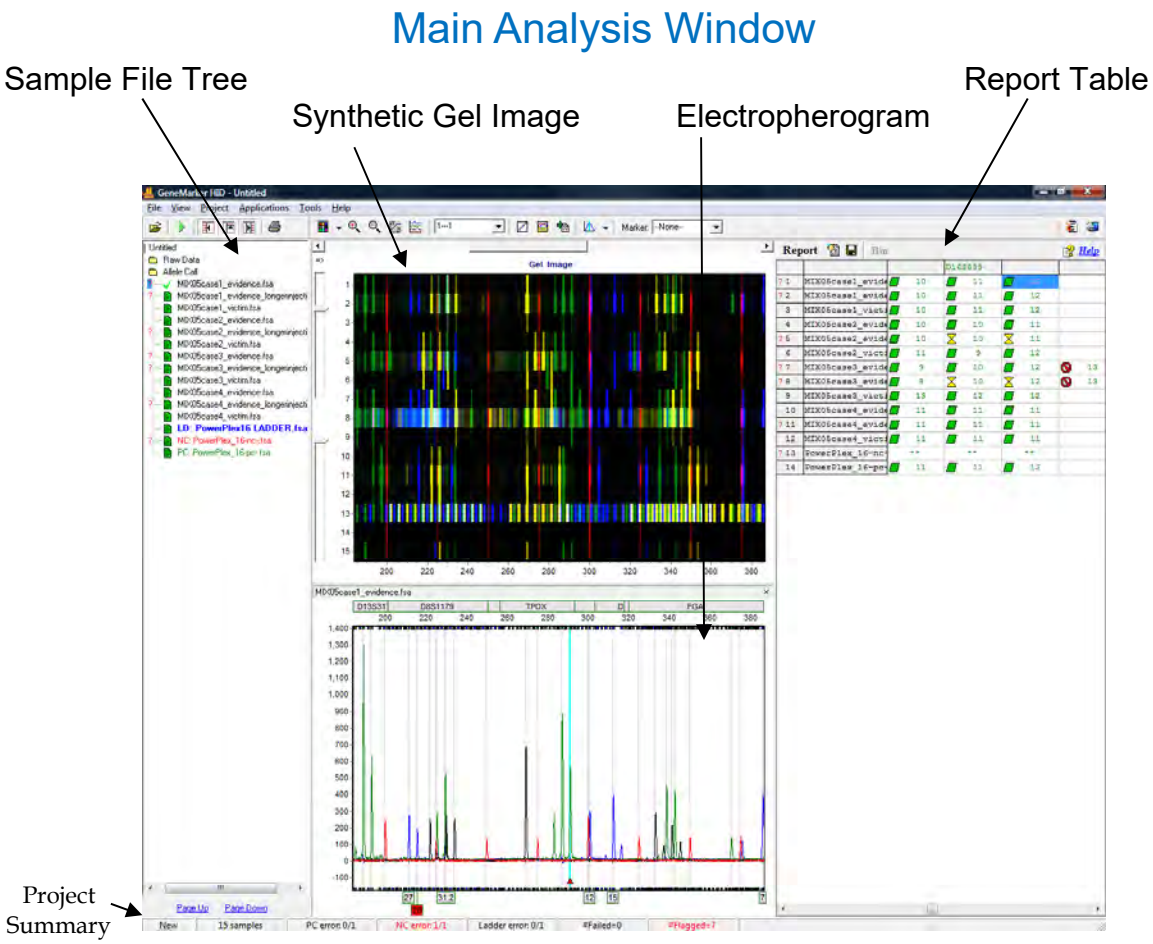
Call the Marker
Applies the new parameter settings to the marker selected in the *Recall Allele* → *Call Allele by Marker* field

Chapter 4 Genotyping Main Analysis Overview

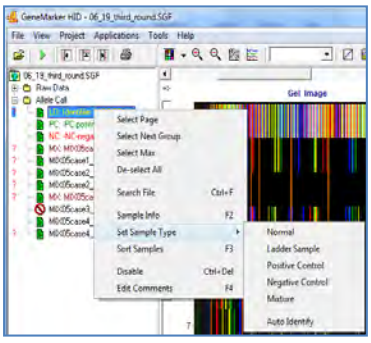
Main Analysis Window

The main window of ChimerMarker has an easy to use layout. The sample files are displayed on the left, the *Synthetic Gel Image* is displayed at the top, *Electropherograms* appear below the gel image, and the *Report Table* is on the right side of the window.

To resize the frames in the *Main Analysis* window, simply place the cursor over the partitions that separate the *Synthetic Gel Image/Electropherogram/Sample File Tree/Report Table*. The cursor will change to a two-headed arrow bisected by two vertical lines. Hold down the left mouse button and drag the gray vertical line in the direction you wish. To open and close the frames, use the **Show/Hide** icons in the main toolbar.



Sample File Tree



The *Sample File Tree* of the main analysis window contains two 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 will appear in the *Raw Data Analysis* window. **See Chapter 3 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 appears in the *Main Analysis* window with all sizing information and allele call filtering 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 Standards.**

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

Sorting Options

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 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* box. Select First, Second, and Third Order sorting from the drop-down menu options Sample Type, File Name, Lane Number, Well ID, Size Score, File Name (Section), and Run Date. You can specify the key word for index that is separated by the "split symbol". 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

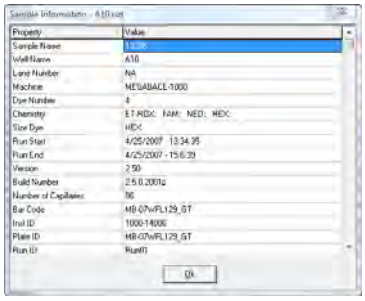
Search File

Opens the *File Search* box. Enter any part of a filename to search for the sample in the list. Click the **Search** button. Left-click and use **CTRL** or **SHIFT** key to highlight samples then click the **Open Selected** button. The electropherograms of the selected samples will open in the *Main Analysis* window. **Hot Key = CTRL+F**

Sample Information

Sample Info

Opens the *Sample Information* box. A list of *Properties* appears and includes information like; Sample Name, Well ID, Lane Number, Instrument Name, and Chemistry. The list of *Properties* varies depending on the file type. **Hot Key = F2**



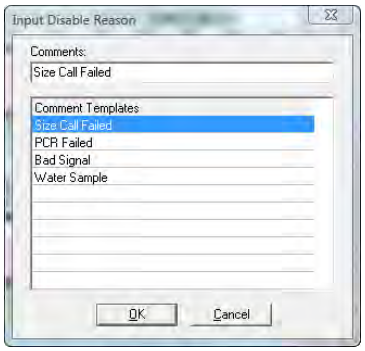
Edit Comments

Opens the *Edit Comments* box. Enter information in the *Comments* field. The last ten comments will be stored and can be subsequently selected for future samples. The *Sample Comments* will appear on the *Print Report*. **See Chapter 7 Reports and Printing.** **Hot Key = F4**

Disable Samples

Disable Sample

Opens the *Input Disable Reason* box and marks the sample with a red strike-through. A disabled sample cannot be selected for display in the *Main Analysis* window and will not appear in the *Report Table* if *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* box will appear. Click the **Add** button to select additional samples to add to the project and click **OK**. The added samples will be sized and the allele calls will be filtered according to the parameters set in the Run Wizard.

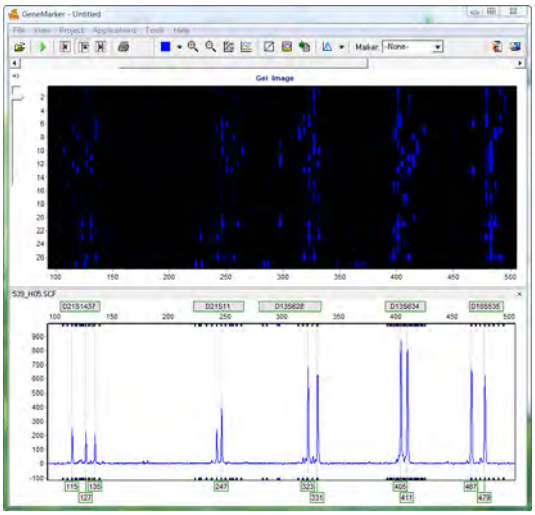
Sample Grouping

From the main toolbar, select *Project* → *Apply Sample Grouping*. The *File Name Group Editor* tool will appear (**See Chapter 9 Additional Tools**). Select *Group* and *Control* identifiers and click **Match**. Click **OK** to apply the matched groups. Group numbers will appear next to the filenames in the *Sample File Tree*. Use the **Select Next Group** right-click menu option *OR* **CTRL+PageUp/Down** to open samples in a group. 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* window. Both display the fragment information in a visual form. When ChimerMarker is initially launched, all dye colors are displayed in the *Synthetic Gel Image* and *Electropherogram* at once. 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 box from upper left to lower right around the area you would like to zoom in on. To zoom back out, hold down the left mouse button and drag a box 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 window also allows the user to manually set the x and y axis with the **Set Axis** icon.

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.

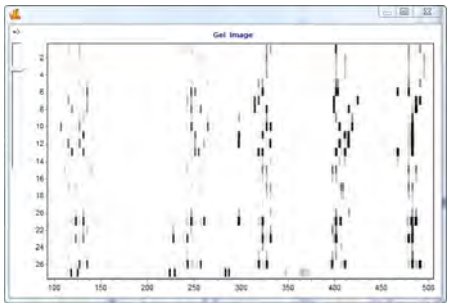
Show in Window

Opens a separate window containing the *Synthetic Gel Image*. The separate window can be maximized 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.



Grey-Scale

Go to *View* → *Preference* → *Display Settings* → *Gel Image*. Select **Gray for Single Dye** to change the single dye *Synthetic Gel Image* to black and white when only a single dye color is selected (when multiple dye colors are selected the fragments will appear in their respective colors). 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* window 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 box. The RFU range (y-axis) is variable and will re-adjust 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* will appear above the electropherogram indicating locus ranges. *Bin* ranges appear as dye-colored brackets above and below the sample trace. *Allele Labels* appear 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 will only indicate the base pair size of the peak.

Peak Table

The *Peak Table* can be displayed below the *Electropherogram* by clicking the **Show Chart/Table** icon in the main toolbar. Right-click in the *Peak Table* and select **Show Columns**. The *Show Columns* fly-out appears 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* box (**See Chapter 3 General Procedure**) and/or software editing of the original raw data, such as correction of saturated peaks (SAT Repaired).

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 and three 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* box.

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* box.

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* box

HI = High Intensity
Peak intensity approaches and/or exceeds the maximum peak intensity filter as set in the *Run Wizard Data Process* box.

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* box.

IHE = Inconclusive Heterozygous
Peak intensity is within the heterozygous inconclusive range set for this locus in the *Panel Editor Edit Panel* box.

IHO = Inconclusive Homozygous
Peak intensity is within the homozygous inconclusive range set for this locus in the *Panel Editor Edit Panel* box.

Save Peak Table
Click the **Save Peak Table** icon in the main toolbar to export the *Peak Table* information currently being displayed in Excel (.xls) or tab-delimited Text (.txt) format. All samples peak information for only the dye colors selected will be 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 would like to add an allele and select *Insert Allele*. The base pair size or bin name will be applied in the *Allele Label* and the peak specifications will be calculated and displayed in the *Peak Table*.

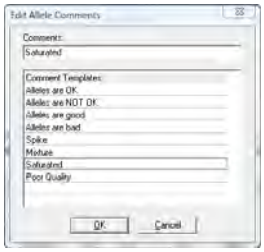
Delete/Undelete Allele
Right-click at the vertical grey bar indicating the center of the called peak or the peak cell in the *Peak Table* and select *Delete* (**Hot Key = DEL**). To call the allele again, right-click the peak and select *Undelete* (**Hot Key = SHIFT+DEL**).

Confirm/Unconfirm Allele
If a peak is given a low quality score, it will receive 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 will be marked *Pass* (green) and receive 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/Unconfirm All
Confirm All and *Unconfirm All* options perform the same actions as the *Confirm/Unconfirm* allele except that the Quality ranking for all peaks in that dye color for that sample will be affected.

Edit Allele
Right-click an allele in the *Electropherogram* or *Peak Table* and select *Edit Allele*. The *Edit Allele* box appears. Add or change the values in the *Allele* and/or *Size* field. The *Allele* field will be blank if no Panel has been 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* box appears. Select a comment from the *Comments Template* list or enter a new comment in the *Comments* field. Click **OK** and the comment will appear in the *Comments* column of the *Peak Table*. Only one user edited comment can be added to a peak. Comments automatically generated by the software cannot be removed. Additional user comments will simply be added next to the software comment.



View History
Opens the *Show Edit History* window. Shows a record of all manual edits performed on the peak. The *Show Edit History* window 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* window *Report Table* contains additional information about sample peaks. **See Chapter 7 Reports and Printing**.

Navigation

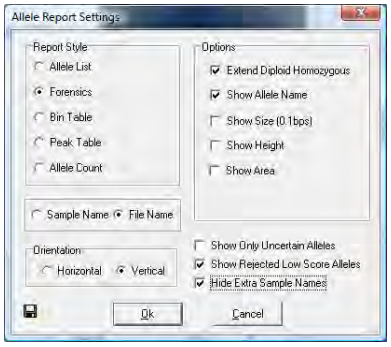
The *Report Table* is linked to the other frames in the *Main Analysis* window. Double-click on the desired allele *OR* use the **Arrow keys** to move to the cell of interest and hit **Enter** key *OR* use **Alt + Arrow keys** to move to different cells and zoom in on the peak in the *Electropherogram*.

Select multiple cells by holding down **SHIFT** key *OR* hold left mouse button and drag over desired cells.

The rules by which the *Report Table* and other frames in the *Main Analysis* window 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* box will appear. Select different *Report Styles* to see additional options. After selecting *Report Style* options, click the **Save as Default** icon in the bottom left corner of the *Allele Report Settings* box. Your options will be saved and will be recalled the next time you select that *Report Style*. 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 will be sorted to the top of the table. If *Descending* is chosen, then the lower quality peaks will be 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 will be 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 will be removed from the *Report Table*.

Confirm Peaks

If a peak is given a low quality score, it will receive 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 will be 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.

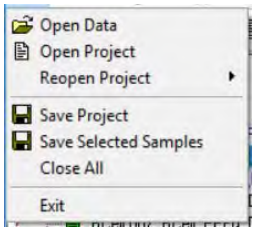
To export only selected cells in the report table, first select the cells by left-mouse drag across the cell range or hold **SHIFT** key and select cells. Right-click on the highlighted cells and select *Copy* (**Hot Key = CTRL+C**). The information is saved to the Windows clipboard and can be pasted into any common word processor or spreadsheet program like Microsoft Excel. The row and column headers for those cells will be copied with the highlighted cell information.

Menu Options

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

File Menu

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



Open Data

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

Open Project

Opens a folder search window where the user can select to open previously saved SoftGenetics *ChimerMarker* project files (.sgf, .sfp)

Re-Open Project

Saves the last four projects that were opened by *ChimerMarker* and allows the user to launch any one of those four 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 projects for long term monitoring cases.

Close All

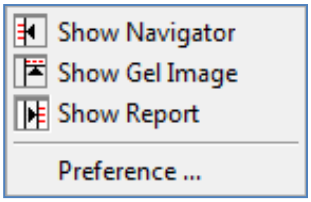
Closes a project without exiting the program.
NOTE: It is recommended to select *Close All* before exiting the program.

Exit

Closes the *ChimerMarker* program.

View Menu

The *View* menu contains options for how the data is displayed in the *Main Analysis* window.

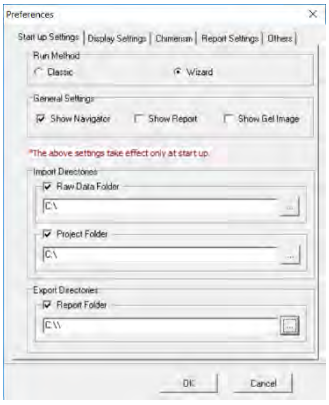


Show Navigator/ Gel Image/ Report

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

Preference…

Activates the three-tab *Preferences* box.



Start up Settings

The *Start up Settings* tab, effective only at start up, allows you to select the *Run Method* and *General Settings*.

Run Method

Classic: Appropriate for experienced users. The user will move 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 will guide 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* will automatically be displayed in the *Main Analysis* window after data processing.

Show Report: When selected, the *Report Table* will automatically be displayed in the *Main Analysis* window after data processing.
Show Gel Image: When selected, the *Synthetic Gel Image* will automatically be displayed in the *Main Analysis* window after data processing.

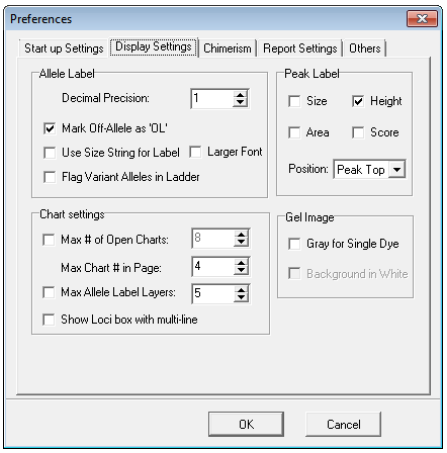
Import Directories
Raw Data Folder: Input the path to the directory the program should open to when importing files.
Project Folder: Input the path to the directory the program should open to when importing projects.

Export Directories
Report Folder: Input the path to the directory the program should save reports to.

Display Settings

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

Allele Label



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. This increased font size will carry over to the *Print Report*.
Flag Variant Alleles in Ladder: Variant alleles called in the ladder sample will be highlighted with a vertical yellow bar.

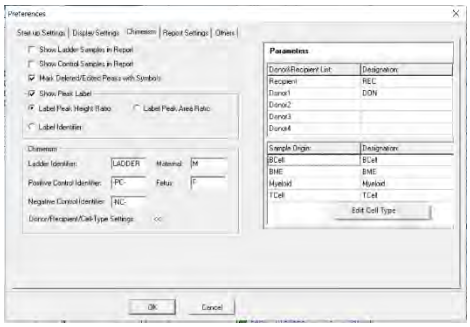
Chart Settings
Max # of Open Charts: Select the maximum number of samples you would like to display as an electropherogram at one time (Max = 96). Use the *Sample File Tree* right-click option *Select Max* to open the number of samples specified.
Max Chart # in Page: Select the maximum number of sample electropherograms you would like displayed in the *Main Analysis* window 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 you must zoom in to clearly read neighboring allele labels and affects how the print report will be displayed.
Show Loci with multiline: Select this option to display the names of all markers above the electropherogram when two or more dyes are displayed.

Peak Label
Choose up to four labels (size, height, area, score) to display as a flag next to individual peaks in the electropherogram.
Position: 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 will be displayed in color regardless if 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 will be white and the band fragments will be 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 can be 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



Enter the File identifiers used by your laboratory. The program uses these portions in the file name to differentiate between allelic ladder, positive and negative controls, Donor, recipient and tissue type. For example, using these designators: 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

7. RECIPIENT_BCELL_
8. RECIPIENT_BME_
9. 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. The Sample Origin and Identifiers can be edited by right-clicking in the list and select "Edit Cell Type". A text editor dialog will appear where the Identifiers can be 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 will appear in the *Report Table*.

Mark Deleted/Edited Peaks with Symbols: when selected samples that are deleted are marked with an χ 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 will automatically scan the dataset filenames for the *Ladder Identifier* values and subsequently label the Ladder samples with an "LD:" and display the sample filename in blue font in the *Sample File Tree*. 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* box. 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.

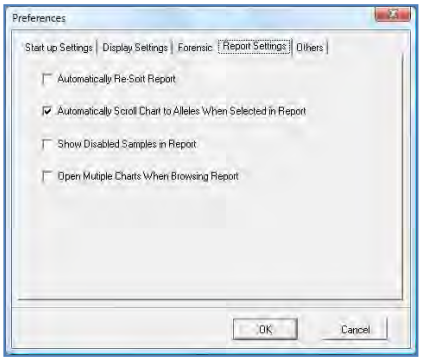
Positive Control Identifier: Enter common filename nomenclature for Positive Control samples in the dataset (must be in all capital letters). Upon first analysis, ChimerMarker will automatically scan the dataset filenames for the *Positive Control Identifier* values and subsequently label the Positive Control samples with a "PC:" and display the sample filename in green font in the *Sample File Tree*. 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 will automatically scan the dataset filenames for the *Negative Control Identifier* values and subsequently label the Negative Control samples with an "NC:" and display the sample filename in red font in the *Sample File Tree*. 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 will automatically scan the dataset filenames for the Maternal identifier values and group 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 will automatically scan the dataset filenames for the Fetus identifier values and group the samples accordingly in the MCC application.

Report Settings



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

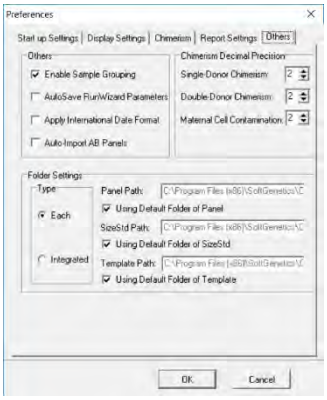
Automatically Re-Sort Report: Check this option if you would like ChimerMarker to automatically re-sort the report every time you modify alleles. Un-check this feature if you want the report to remain sorted until you choose to re-sort.

Automatically Scroll Charts to Alleles When Selected in Report: You may choose whether to scroll to alleles in the trace when selecting the allele in the report. Leave this feature on to have the software automatically call up alleles in the trace when you double-click on them in the report.

Show Disabled Samples in Report: ChimerMarker identifies samples that failed during electrophoresis or size calling. The default setting excludes the disabled samples from the report. The option may be selected to have failed or user-disabled samples to be identified in the report.

Open Multiple Charts When Browsing Report: Double clicking an entry in the allele report table will open a new electropherogram for each new sample selected.

Others



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

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 and can be recalled by selecting *Enable Sample Grouping*. **See Chapter 9 Additional Tools – 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 if you would like the dates of the Reports for Applications to be 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 can be 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 be made to automatically import panels, size standards, and/or templates from an alternative location instead. This is particularly useful for users with a network setup, with files stored at a shared location. The folder settings tool gives the user two options for changing the default folders

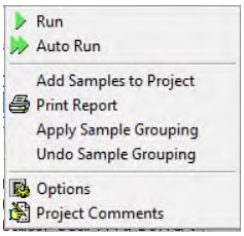
Each: When the "Each" radio button is selected (see figure above) the user may give new paths individually to the Panel, Size Standard, and Template folders. To use the default location installed with ChimerMarker for a given folder, simply check the "Using Default Folder" box.

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 check the "Using Default Folder" box 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 for how the data is processed and printed.



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. The same process action can also be accomplished by clicking the *Run* icon in the toolbar.

Auto Run

ChimerMarker will process data using the last set of parameters selected. If one or more of the parameters require changing to improve analysis, select *Project* → *Options*, 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* box will appear. Click **Add** to select individual files to the project and click **OK**. The raw data file will be 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* box which allows the user to group their samples by Sections, Fixed Positions, Order, Run Date, or Cell Type.
NOTE: Sample grouping can be activated in View-> Preferences-> Others-> Enable Sample Grouping.

Undo Sample Grouping

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

Print Report

Selecting *Print Report* launches the *Print Report Settings* box which allows the user to define display settings in the *Print Report*. The software permits printing of the sample electropherograms. You can choose to print all samples, selected samples, or print samples along with the allele table, if desired. **See Chapter 6 Reports and Printing.**

Options

Allows you to access and change parameters in the *Project Option Settings* window. This three-tab window contains settings identical to the Run Wizard. Adjust settings in the *Project Options Settings* box *before* selecting Auto Run. **See Chapter 3 General Procedure.**
NOTE: Auto Run does not need to be selected after adjusting the *Additional* → *Allele Evaluation* → *Peak Score* settings. The changes will automatically be applied when the *Project Option Settings* window is closed.

Project Comments

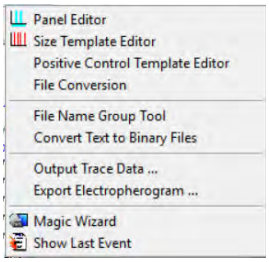
Allows the user to write free-form comments regarding the analysis. These comments are saved with the project file and can be displayed in the *Print Report*.

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 particular application. The types of Chimerism Analysis (single donor or double donor) may be selected 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.



Panel Editor

Provides a variety of tools to adjust, edit, and create control Panels. **See Chapter 6Panel 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 create a customized size standard. **See Chapter 5 Fragment Sizing Standards**.

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 Chapters 2 General Procedure and 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

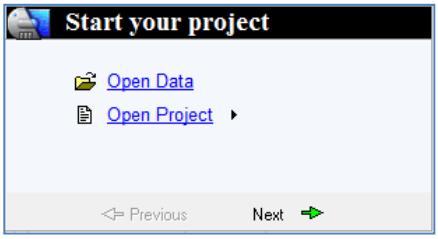
Provides the option to output the raw or sized trace data as a TXT or SCF file. Select the samples to include, dye colors, data type, and the directory to output the trace files. **See Chapter 9 Additional Tools.**

Export Electropherogram

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

Magic Wizard

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



Start Your Project

Allows the user to easily access the *Open Data* or *Open Project* upload windows. The user can also re-open the four previously opened projects by selecting the black arrow next to *Open Project.*

Run

Selecting *Run* launches the Run Wizard. Selecting *AutoRun* will process the data automatically with the process options currently selected. **See Chapter3 General Procedure.**

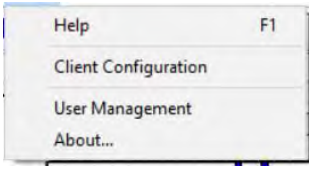
Report

Allows the user to *Save Project*, *Print Report,* or *Close Project*. Selecting *Print Report* will launch the *Print Report Settings* box. **See Chapter 7 Reports and Printing.**

Show Last Event

Opens the last active *Data Process* action.

Help Menu



Help
Launches a searchable version of this manual.

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

Open Data
Opens data input dialog box 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*.

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 box, 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 box, 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 can be selected from the drop-down menu.

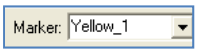
Allele Call Icons
These icons are only available after the raw data has been processed and the *Sample File Tree Allele Call* folder is selected.

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 the *Peak Table* as an Excel (.xls) file or tab-delimited Text (.txt) file.

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 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* dialog boxes.

Report Table Icons

The icons are located directly above the *Report Table*.

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.

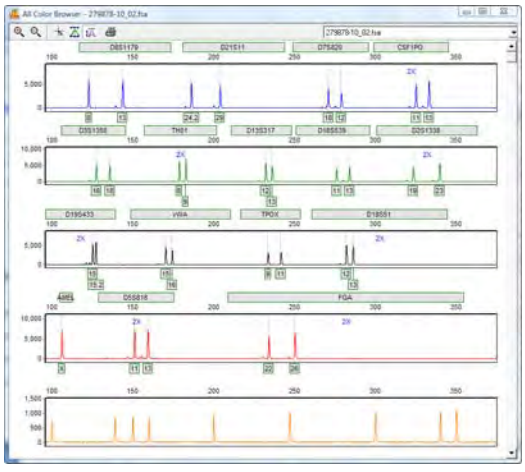
Customize Bin Column
Allows the user to select which bins to include/exclude in the *Report Table*.

Additional Analysis Options

In addition to the *Main Analysis* window, there are two other display options in which the sample data can be 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* window toolbar.



Navigation and peak editing options in the *All Color Browser* is similar to the *Main Analysis* window.

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

Icons and Functions

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 box. **See Chapter 7 Reports and Printing**.

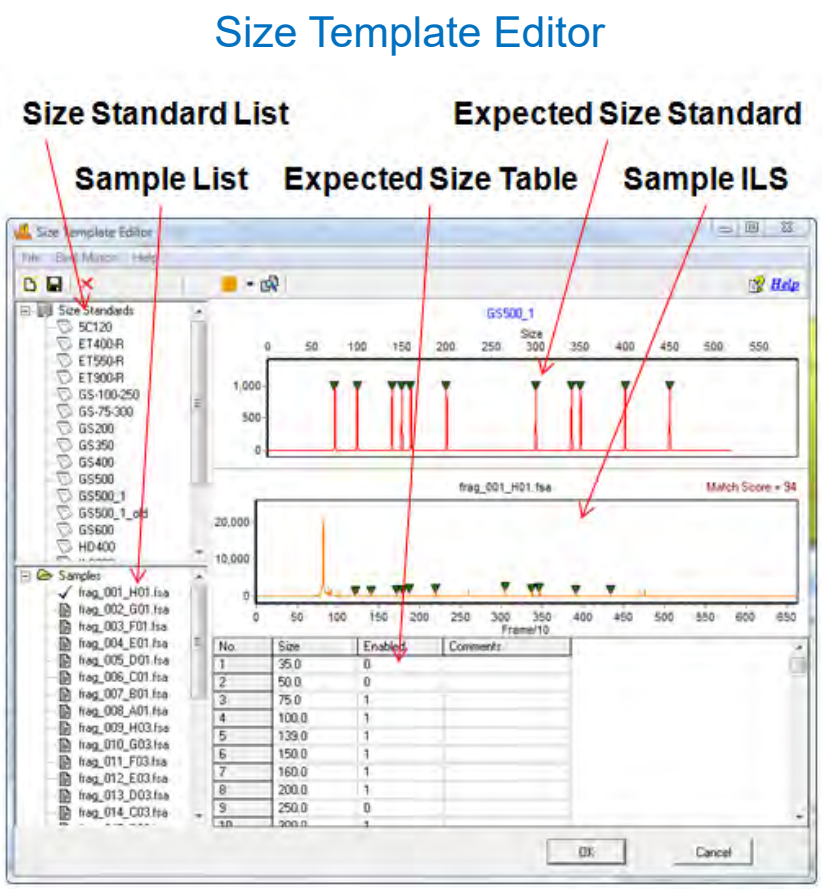
Chapter 5 Fragment Sizing Standards

Size Template Editor

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

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

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



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* will appear 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* window. Choose a directory folder and click **Save**. The Size Standard will be copied to the selected directory and will also remain in the *Size Standard List* and SoftGenetics ChimerMarker Size Standard directory. The Size Standard will be exported as an XML file which can be opened with 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 will be restored.

NOTE: If the user selects **Save** Size Standard and then answers "NO" to the "Size Standard has been changed, save changes?" the changes will remain in the *Expected Size Standard* and *Size Table* until the user chooses *Reload* or 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 will appear in the *Sample ILS* frame. Use the **Up/Down Arrow** keys to scroll through samples in the list.

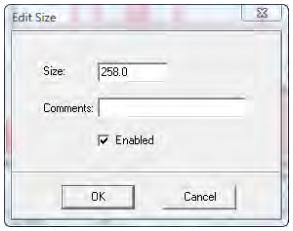
Expected Size Standard and Size Table

The *Expected Size Standard* frame displays, as a trace, all the known fragment peaks that are expected to appear in the *Sample ILS*. Single left-click a green triangle atop a peak to select the peak. The green triangle will turn 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 will appear with the following options.

Edit Size



The *Edit Size* box appears. 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" will appear in the *Expected Size Table*. Deselect this option to disable the Size in the Size Standard. Disabled sizes will be used for pattern recognition in the sample ILS but will not be 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*, you must click another cell in the *Size Table* before saving the Size Standard or the change will not take effect.

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* box will appear. ChimerMarker will automatically interpolate the value in the *Size* field if there are two or more Sizes present in the trace. Adjust as necessary and click **OK**. A green triangle will appear 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.

Delete Size

Select *Delete Size* to remove the Size completely from the Size Standard. Alternatively, the Size can be disabled by deselecting *Enabled* in the *Edit Size* box or by placing a "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* box will appear. Adjust as necessary and click **OK**. The green triangle will now appear atop the peak and also in the *Expected Size Standard*.

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 0.

Navigation in the *Sample ILS* frame is similar to the navigation options in the *Main Analysis* window. **See Chapter 4 Main Analysis Overview.**

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:

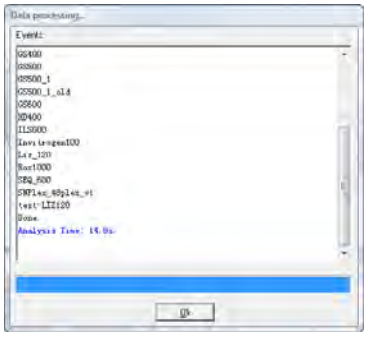
5C120	*GS500*
CEQ_400	*GS500_1*
CED_600	*GS600*
ET400-R	*GS1200*
ET550-R	*HD400*
ET900-R	*ILS500*
GS-100-250	*ILS600*
GS-75-300	*Liz_120*
GS200	*Rox1000*
GS350	*SEQ_600*
GS400	*SNPlex_48plex_v1*

Pre-Defined Size Standards

There are two 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 will then appear in the *Size Standard* field of Run Wizard *Template Selection* box and will be used to size the data.

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

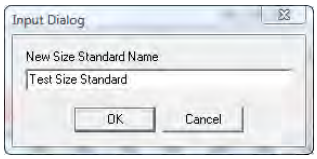
1. In *Size Template Editor*, select *BestMatch* → *Match All*
2. The *Data Processing* box appears
3. ChimerMarker cycles through all Size Standards
4. Click **OK** when *Data Process* is finished
5. The Size Standard with the best average *Match Score* across all samples in the dataset will be highlighted in the *Size Standard List* and appear in the *Expected Size Standard* frame



NOTE: *BestMatch* will not always choose the correct Size Standard. User inspection is required.

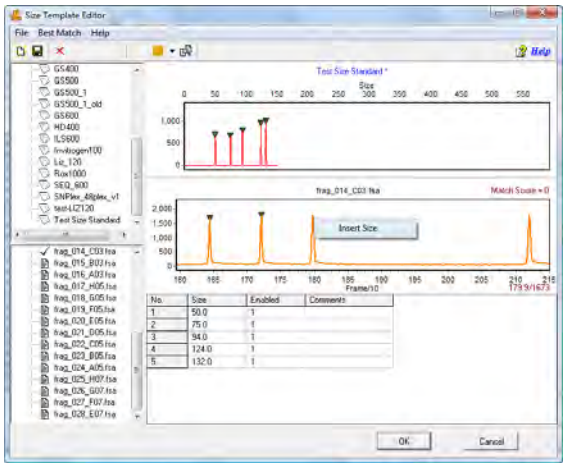
6. Once the Size Standard is chosen, click **OK** in the *Size Template Editor*
7. The selected Size Standard will then appear in the *Size Standard* field of Run Wizard *Template Selection* box and will be used to size the data.

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
2. The *Input Dialog* box appears
3. Enter a Size Standard name and click **OK**
4. The *Expected Size Standard* frame will be blank
5. Right-click at a known peak in the *Sample ILS* frame
6. Select *Insert Size*
7. The *Insert Size* box appears
8. Enter the base pair size of the peak in the *Size* field and click **OK**
9. A green triangle will appear atop the peak in the *Sample ILS* and a new peak will appear in the *Expected Size Standard* frame
10. Continue *Insert Size* operation for the rest of the peaks in the *Sample ILS*
11. ChimerMarker will interpolate Size values after two 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.

12. When the Size Standard is complete, select *File* → *Save Changes* *OR* click **Save Changes** icon
13. Click **OK** in *Size Template Editor*
14. Proceed with Run Wizard data analysis

Icons and Functions

The following are explanations of menu and icon options in *Size Template Editor*.

Menu Options

The *Size Template Editor* contains three menu options – *File, BestMatch*, and *Help*. The *File* menu allows the user to create, save, and export Size Standards. The *BestMatch* menu contains options for selecting a Size Standard. The *Help* menu shows navigation hints for *Size Template Editor*.

File Menu

New Size Standard

Opens the *Input Dialog* box with a field to enter a new Size Standard name. Follow the steps above – *Custom Size Standard Creation.*

Delete Current Size Standard

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

NOTE: This action is irreversible.

Save Changes

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

Save as New Size Standard

Opens the *Input Dialog* box 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.

Import Size Standard

Opens a Windows Explorer window 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.

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.

Import ABI Size Standard
Opens a Windows Explorer window to the same folder the sample files were uploaded from.

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.

Exit
Closes the *Size Standard Editor* tool. Be sure to save changes to the Size Standard before exiting.

BestMatch

Match Selected
When selected, the *Data Process* box 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.

Match All
When selected, the *Data Process* box 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*.

Help

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

Toolbar Icons

Size Template Editor
Found in the Run Wizard *Template Selection* box 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.

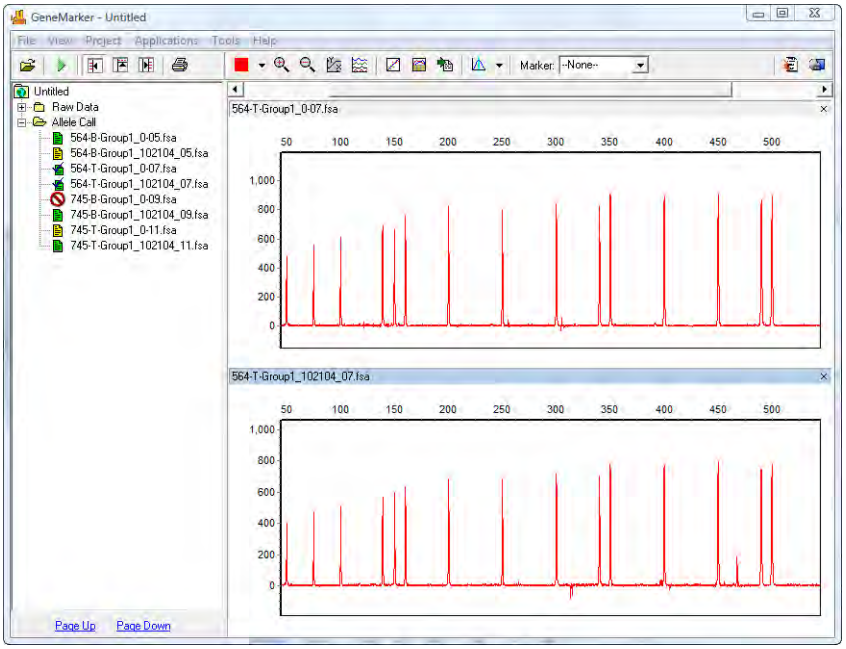
Size Match
Automatically places the green size marker triangles atop the peaks of the sample trace and matches it with the selected Size Standard.

What to Expect

Once the Size Standard is created, it can be applied to the dataset. **Save** the edited Size Standard in *Size Template Editor* then exit *Size Template Editor*. If the *Size Template Editor* was accessed via the Run Wizard *Template Selection* box icon then the selected Size Standard will appear in the *Size Standard* field. If the *Size Template Editor* was accessed via the *Tools* menu then click the **Run Process** icon in the *Main Analysis* toolbar. The Run Wizard will appear. Select the Size Standard from the *Size Standard* drop-down menu in the Run Wizard *Template Selection* box. Proceed through the other Run Wizard boxes and click **OK** when the *Data Process* window is complete. The Size Standard will be 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* window. 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 will be marked with a yellow sheet. If the size calling failed (the sample's ILS peaks could not be aligned with the Size Standard selected) then the sample will be 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.

Low Match Score and Failed Samples



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. Click the **Size Calibration Charts** icon in the main toolbar of the *Main Analysis* window. As mentioned previously, once a Size Standard has been applied to the dataset, *Size Match Score* indicators appear next to the filename in the *Main Analysis* window *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 was 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

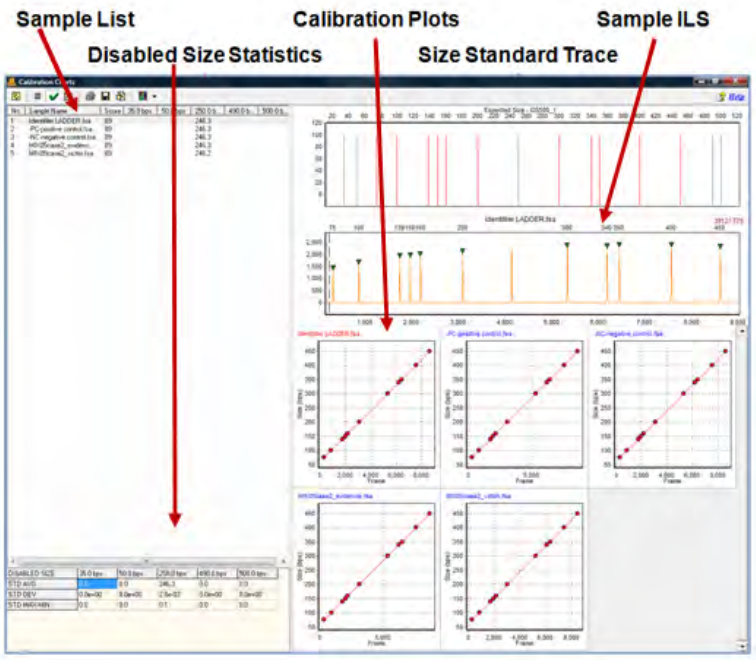
The *Sample List* includes filename, *Match Score*, and disabled peak information for each sample in the dataset. Sort the list by single left-clicking the column header. The list will re-sort in ascending or descending order based on the values in the column selected. Single left-click a sample to view 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 will appear "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 0 and the sample is considered to have failed size calling.

Disabled Size Columns
The Sizes that were disabled in the Size Standard (see *Size Template Editor* section above) will appear as column headers in the *Sample List*. If no Sizes were disabled then only the *Sample Name* and *Score* columns will appear 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 will reported.

Size Calibration Charts



Disabled Size Statistics
If Sizes were disabled in the Size Standard (see previous section – *Size Template Editor*), then the *Disabled Size Statistics* table will appear in the bottom left corner of the *Size Calibration Charts* window. 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 will be 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. 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. The peaks selected for size calling can be edited in the *Sample ILS* frame as described below.

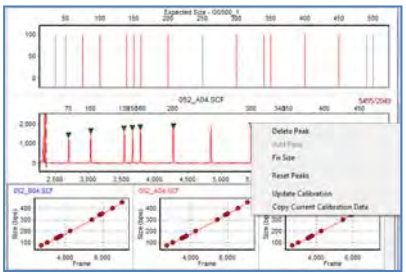
Navigation in the *Sample ILS* frame is similar to navigation options in the *Main Analysis* window. **See Chapter 4 Main Analysis Overview**.

Editing Size Call
Single left-click a green triangle peak indicator to select it. The triangle that is currently selected will be yellow. To move the green triangle, hold down the **CTRL** key and left-click and drag it to the desired

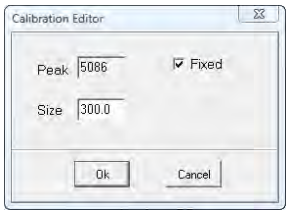
position. Right-click the green triangle peak indicator or right-click the top of an unmarked peak to see additional options.

Delete Peak
Removes the green triangle peak indicator from the *Sample ILS* and the peak will not be 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 will appear 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 will be 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* box appears. Enter the correct base pair size of the peak and click **OK**.
NOTE: Only Sizes that occur in the selected Size Standard can be entered in the *Calibration Editor Size* field.
The peak will be "fixed" at the specified base pair position and all green triangle peak indicators to the left and right of the "fixed" peak will be 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 thereby influencing the pattern recognition algorithm.
NOTE: *Fix Size* is not active for manually added peaks or peaks outside the Size Standard range.

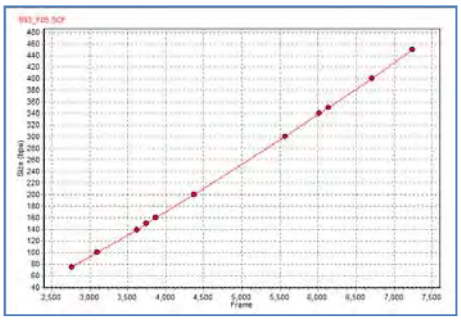
Reset Peaks
Select *Reset Peaks* to eliminate manually added peaks and/or extra green triangle peak indicators after *Fix Size*.
NOTE: Deleted peaks will not be recalled when *Reset Peaks* is selected.

Update Calibration
After editing peaks in the *Sample ILS*, select *Update Calibration*. The *Match Score* for the sample will be 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* window will be updated.

Copy Current Calibration Data
When selected, the frame position and base pair position of the green triangle peak indicators for the selected sample will be copied to the Windows clipboard and can be 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. 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 will 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 will appear red in the upper left corner of the *Calibration Plot*.

Procedure

After a Size Standard has been chosen and the data is processed by the Run Wizard, the *Size Calibration Charts* can be used to correct improperly sized samples.

1. Click the **Size Calibration Charts** icon in the main toolbar
2. The *Calibration Charts* window appears
3. Select a sample to edit in the *Sample List*
4. The sample's ILS appears in the *Sample ILS* frame
5. Right-click in the *Sample ILS* frame and chose *Add, Delete*, or *Fix Size* to correct size call
6. Right-click again and select *Update Calibration*
7. The changes will be implemented for the sample and the *Match Score* will be updated
8. When editing is finished, close *Size Calibration Charts*
9. The *Size Match Score* indicators in the *Sample File Tree* of the *Main Analysis* window will be updated

Icons and Functions

Toolbar Icons

Size Calibration
Found in the main toolbar of the *Main Analysis* window.

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.

Print
Provides Print Preview and Print of size calibration page, providing a physical record of size calibration for the project.

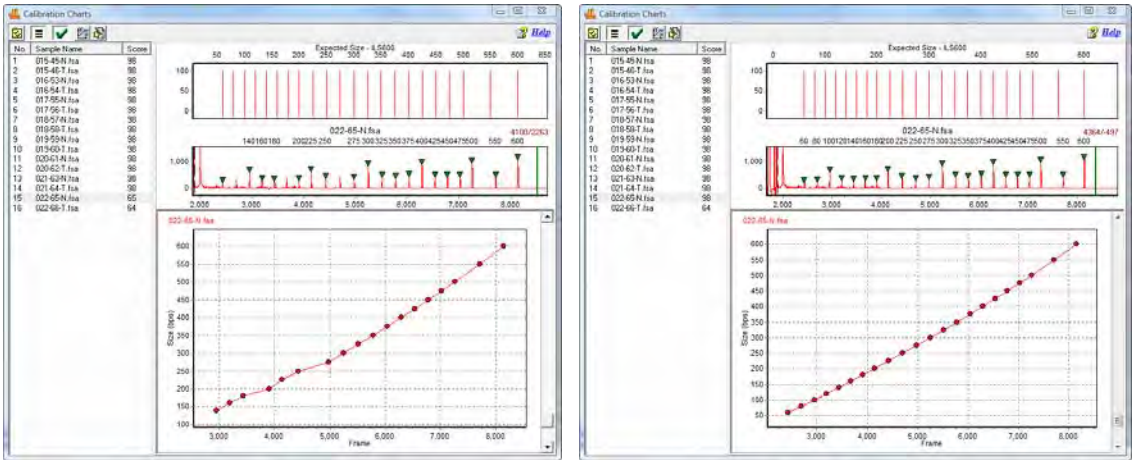
Save
Allows the user to save the size calibration pages as .png images.

Manual Calibration
Provides the option of manually entering standard peak sizes if many peaks have been modified. This window 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.

Before & After Editing Size Call



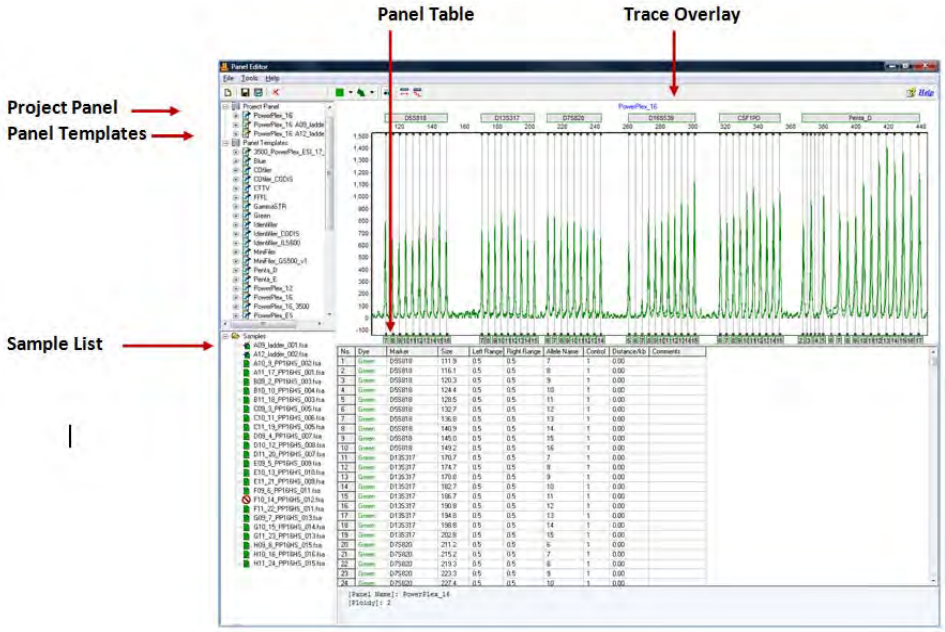
Chapter 6 Panel Editor

Overview

The *Panel Editor* can be accessed from the *Tools* menu in the *Main Analysis* window *OR* via the **Panel Editor** icon in the Run Wizard *Template Selection* box. 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 appear 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.

Panel Editor

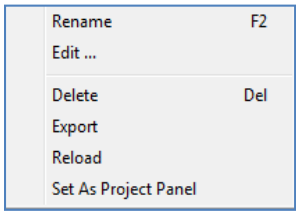


Project Panel

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 window 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.

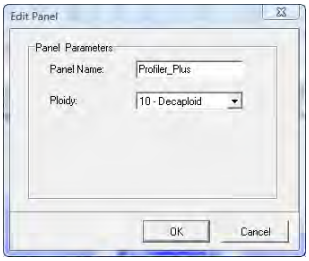
Panel Templates

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 displaythe 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 will appear with the following options.



Edit Panel
Opens the *Edit Panel* box. Editing the *Panel Name* field will change how the Panel is labeled in *Panel Editor*. Set the *Ploidy* from Monoploid (1) to Decaploid (10) to Unlimited. If the number of peaks within a Marker exceeds the *Ploidy* setting, the additional peaks will be 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* window. Choose a directory folder and click **Save**. The Panel will be copied to the selected directory and will also remain in the *Panel List* and SoftGenetics ChimerMarker Panel directory. The Panel will be exported as an XML file which can be opened with Internet Explorer, Microsoft Excel, or Notepad.

Reload Panel
Click *Reload* to undo editing changes to the Panel. The most recently saved Panel will be restored.
NOTE: If the user makes changes to the panel and then answers "NO" to the "The Panel has been changed, save changes to file?" the changes will remain in the *Overlay View* until the user chooses *Reload Panel* or 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 will be 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 appears in the *Size Calibration Charts* (**See Chapter 4 Fragment Sizing Standards**). Samples with higher scores will appear at the top of the list.

Overlay Trace

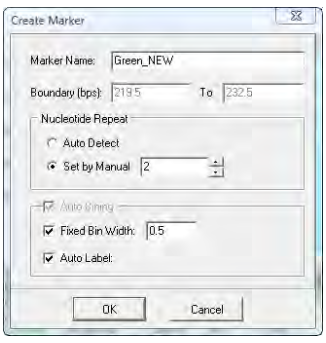
The *Overlay Trace* displays all selected samples in the *Sample List*. The Marker bars appear above the electropherogram and the Bins appear within the electropherogram as brackets at the top and bottom. The center of the Bin is indicated by the vertical grey bar in the electropherogram (only in *Panel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

Navigation in the *Overlay Trace* frame is similar to the navigation options in the *Main Analysis* window. **See Chapter 4 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 box will appear. Right-click in the hashed box and select *Create Marker*. The *Create Marker* box appears. Adjust parameters and click **OK**.

Marker Name: Edit the *Marker Name* field to change how the Marker will be labeled in the Panel.
Boundary: The base pair range of the Marker is defined by the range of the light-blue hashed box and is therefore inactive in the *Create Marker* box. To edit the *Boundary*, see *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 will place 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 will be 1.0 base pairs.

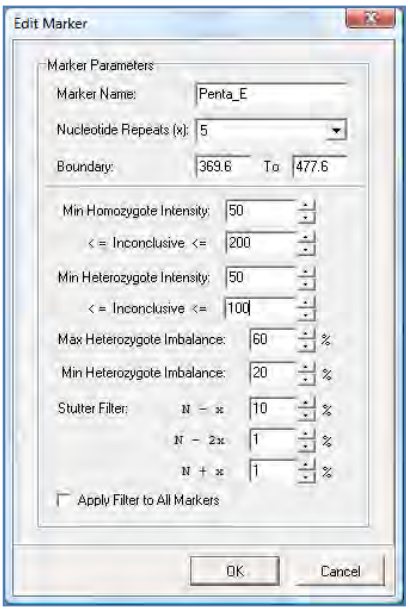
Auto Label: When deselected, the Bins are automatically labeled with the base pair size of the Bin position to the nearest tenth of a base pair. If selected, the base pair size is rounded up to a whole number value.

Edit Marker

Double-click the Marker bar *OR* right-click the Marker bar and select *Edit Marker*. The *Edit Marker* box appears. Adjust parameters and click **OK**.

NOTE: The *Edit Marker* box can also be accessed by right-clicking the Marker name in the *Panel List* and selecting *Edit*.

Marker Parameters



Marker Name: Edit the *Marker Name* field to change how the Marker will be 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* box appears. 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 will move automatically 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 will call 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 vaues are different from each other, a single peak above the(inconclusive heterozygous) *Min Heterozygote Intensity* but below *Min Homozygote Intensity* will be called and labeled with a *Check* Quality and *LO* Quality Reason if a second peak is detected above the *N-x Stutter Filter* value. The second peak will not be 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 will be 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 particular 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 will not be 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 can be 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 will not be 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 will be applied to all Markers in the Panel. To optimize the stutter filter, set each marker with stutter values determined during your lab validation procedures.

Edit Marker Bins

Right-click the Marker bar and select *Update Alleles*. The *Update Marker Alleles* box will appear. Adjust parameters and click **OK**.

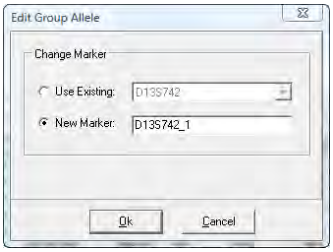
Nucleotide Repeat

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 will place 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 will be 1.0 base pairs.
Auto Label: When deselected, the Bins will be automatically labeled with the base pair size of the Bin position to the nearest tenth of a base pair. If selected, the number will be 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 box will appear. Right-click in the hashed box and select *Change Marker*. The *Edit Group Allele* box will appear. Select *New Marker* and a pre-defined name will appear. 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* box will appear. Adjust parameters and click **OK**.

Allele: Enter a name for the Bin. All peaks that appear within the Bin will display this value in the Allele Label in the *Main Analysis* window.
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 will be 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* will display the Allele Label below the *Electropherogram* trace of the Ladders in the *Main Analysis* window. Bins marked "0" will 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* box appears. Adjust parameters and click **OK**. See *Create Bin* section above for explanation of *Allele Editor* options.

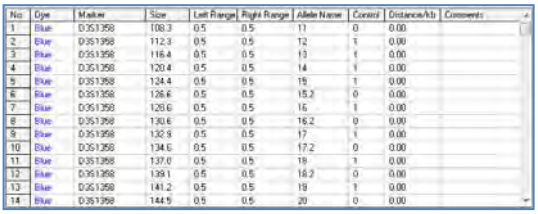
To move a bin, hold down **SHIFT** key and left-click and drag the vertical grey bar in the center of the Bin to the right or left. Let go of the **SHIFT** key and mouse button and the Bin will remain in place. To edit the range of a Bin in the *Overlay View*, click the **Trace Mode** icon to view the *Gel Image*. 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

Right-click the vertical grey bar in the center of the Bin in the *Overlay Trace*. Select *Delete Allele*. The Bin will be deleted from the Panel. To delete multiple Bins, hold down **CTRL** key and left-click and drag across peaks in the *Overlay View*. A light-blue hashed box will appear. Right-click in the hashed box and select *Delete Alleles*. The Bins highlighted by the hashed box will be removed from the Panel.

Panel Table

The *Panel Table* displays Marker and Bin information for the dye color displayed in the *Overlay Trace* frame. All columns except *Dye* and *Marker* can be edited 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.



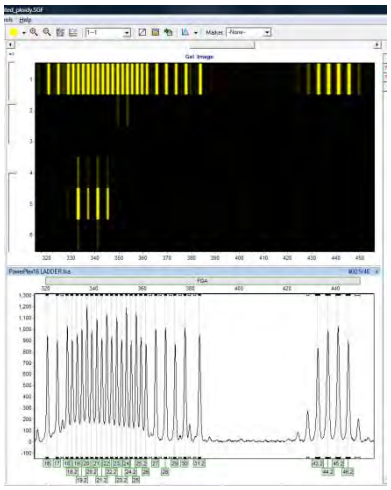
Dye
Indicates the dye color of the Bin.

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 will be labeled with the Allele Name.



Control
Bins marked with a "1" are considered major alleles in the Ladder sample and will be marked with an Allele Label and a darkened rectangle to mark the bin in the *Main Analysis* window. Bins marked with a "0" are minor peaks and will not receive an Allele Label, the bin will be marked with an clear rectangle in the *Main Analysis* window.

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.

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 identiy 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. Below is a discussion of how to use the pre-defined Panels or create a new Panel with ChimerMarker's *Panel Editor* tool.

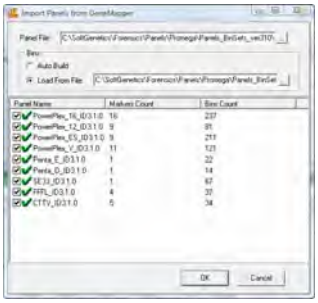
The Panels displayed by default include:

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 appear 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.
2. The *Import Panels from GeneMapper* box appears.
3. Click the access button next to the *Panel File* field. A Windows Explorer window will appear.
4. Navigate to the location of the Panels.txt file and click **Open**.
5. Next, click the access button next to the *Bins Load from File* field and locate the Bins.txt file.
6. Click **Open**.

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

7. Click **OK** in the *Import Panels from GeneMapper* box.
8. All Panels in the Panels.txt file will be uploaded into ChimerMarker.
9. Select a newly uploaded Panel from the *Panel List*.
10. Edit the Markers and Bins so that they align with the peaks in the dataset.
11. Save the edited Panel and close *Panel Editor*.
12. Click the **Run Project** icon in the *Main Analysis* window.
13. Select the Panel from the *Panel* field in the Run Wizard *Template Selection* box.

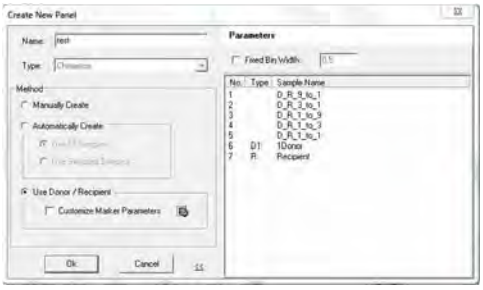
14. Proceed through Run Wizard and data analysis. **See Chapter 3 General Procedure and Chapter 4 Main Analysis Overview.**

Custom Panel Creation

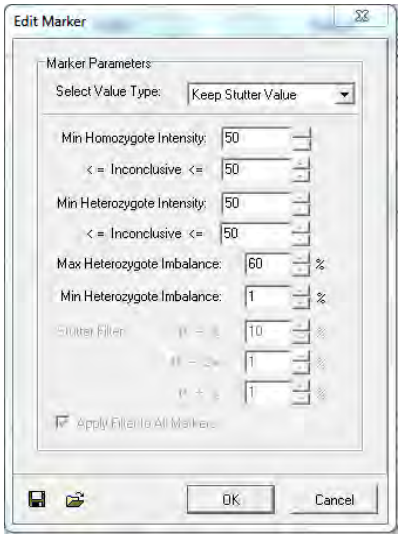
Follow the steps below to create a new Panel based on the dataset currently uploaded to ChimerMarker.

Automatic Panel Creation

1. In *Panel Editor*, select *File* → *Create New Panel* from the menu bar or click the **Create New Panel** icon.
2. The *Create New Panel* box appears.
3. Enter a name for the Panel in the *Name* field. This will be the Panel name that is displayed in the *Panel List*.
4. The *Type* will, by default, display the *Analysis Type* chosen initially in Run Wizard *Template Selection*. The only option in ChimerMarker is Chimerism*Type*.
5. Select *Automatically Create*
    a. *Use All Samples* will create a Panel based on an overlay of all the sample peaks in the dataset
    b. *Use Selected Samples* will create a Panel based only on the samples selected in the *Panel Editor Sample List*



6. Select User Donor/Recpient to use the donor and recipient allele calls to make a Chimertyping Panel.
7. If required, check the *Fixed Bin Width* option and enter a value for the left and right Bin ranges
8. Select Customize Marker Parameters to input specific parameters for the new panel. Select this option to keep stutter specific values for each marker.
9. When finished, click **OK**
10. The new Panel will be created and added to the **Panel List**



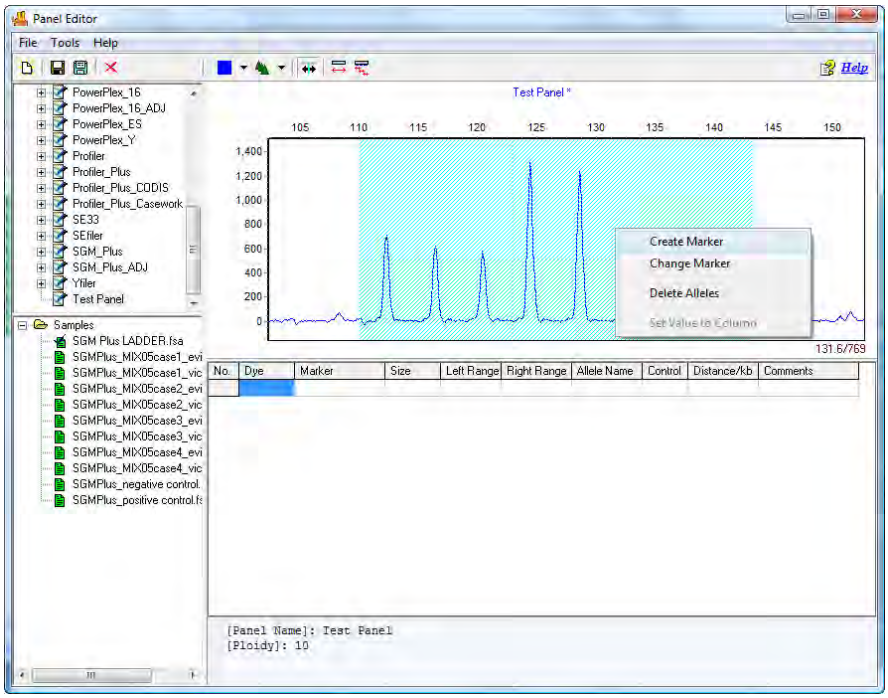
NOTE: New Panels are created based on the *Max & Average View Mode*. More intense peaks are given higher priority for Bin placement when peaks do not overlap perfectly.

11. Edit the Markers and Bins as described in the previous section – *Panel Editor Overview*.
12. Follow steps **10-14** above.

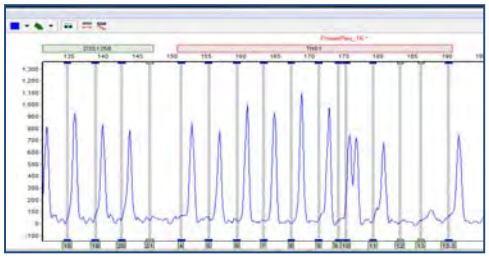
Manual Panel Creation

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**
6. The Panel name will appear in the *Panel List*; however, no Markers or Bins will be associated with the Panel
7. Follow the steps in the previous section – *Panel Editor Overview* – to create Markers and Bins

Create a Marker in Panel Editor



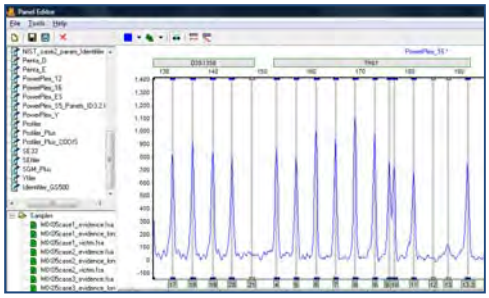
Adjusting Panels



It is common for panel alignment to be shifted due to variations in genetic analyzers or run conditions (such as temperature, injection time). Markers or bins can be manually aligned to the allele ladder using the shift and mouse key.

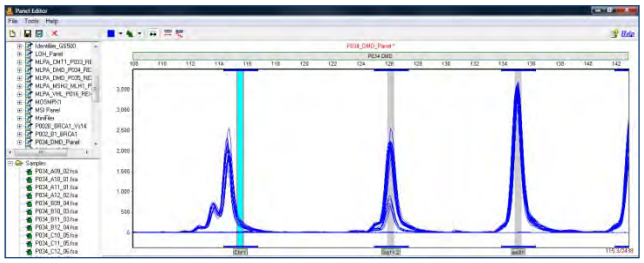
Align all of the bins within a marker

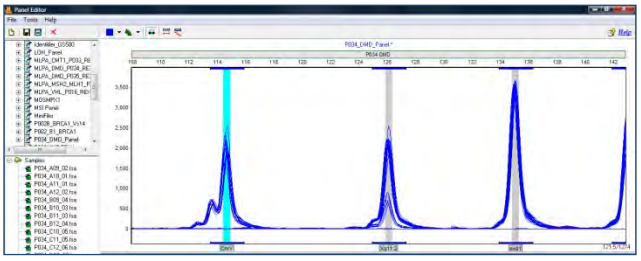
1. Hold down the shift key
2. At the same time place the mouse over the gray marker name bar at the top of the electropherogram
3. The marker rectangle will be outlined in red and the panel name will be in red font when the adjust feature is active
4. Drag the marker to align the bins with the peaks of the allelic ladder
5. Save the panel with signal information (the turquoise save icon) to enable the major panel adjust feature to work in future projects



Align an individual bin

1. Select the gray vertical bar of the bin with the mouse – the bar will turn blue
2. Hold down the shift key and click on the gray, vertical bar for the bin
3. The vertical bar will be outlined in red and the panel name will be 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

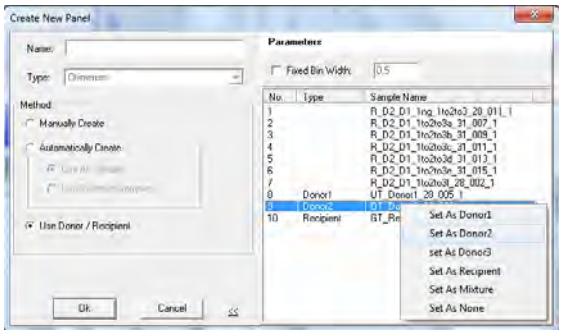




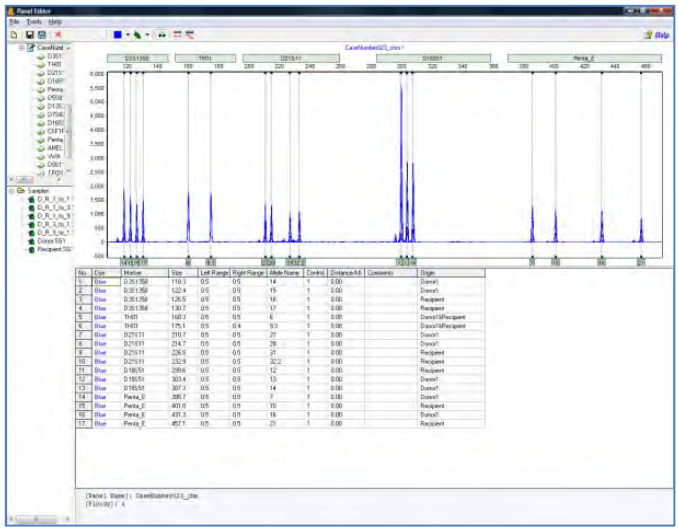
Genotyping and Chimertyping Panels

ChimerMarker includes two 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 will use 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 has been applied to the project.

File →Create Panel
Select User Donor/Recipient Select OK to automatically create a case specific chimertyping panel. If a naming convention was 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.



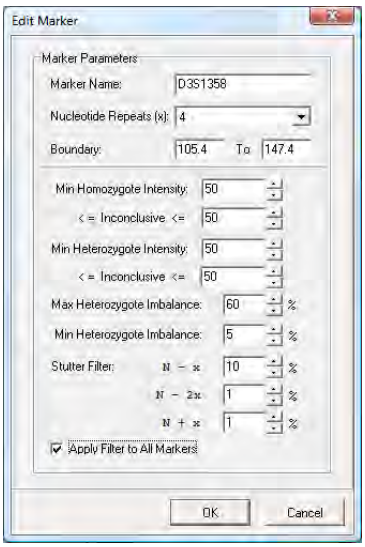
This automatically creates a chimertyping panel with the alleles found in all of the participants of the study (donors and recipients). When used in the chimerism analysis (please see Chapter 7, Chimerism Analysis), the panel calls only those peaks that are consistent with the donor(s) and recipient 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 (please see Chapter 7 Chimerism Analysis).



Editing a Chimertyping Panel

It is very likely that some of the peaks will be 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:

1. Right mouse click on one of the Marker (Loci) label above the electropherogram and select Edit Marker
2. Enter the appropriate value into the Min. Heterozygous Imbalance box
3. Select the Apply to all Markers 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 will indicate 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 will be called. Peaks that are **less than 5**% of the taller peak height **will not** be called. To ensure that all peaks outside the stutter position are called, set the minimum heterozygous imbalance to 1.

Options, Functions and Icons

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

Menu Options

The *Panel Editor* contains three menu options – *File, Tools*, and *Help*. 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 box 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* box 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 window 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* box. 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. Be sure to save changes to the Panel before exiting.

Tools Menu

Match Ladder
Opens the *Select Ladder* box. Choose an allelic ladder sample from the drop-down menu. Click **OK** and the Panel will adjust 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
Select a Panel from the *Panel List* and click *Virtual Panel* in the *Tools* menu. The *Create Virtual Panel* process box will appear. Click **OK** and a new Panel will be 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. Use *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* box appears.

Toolbar Icons

Panel Editor
Found in the Run Wizard *Template Selection* box *OR* the *Tools* menu.

Create New Panel
Opens the *Create New Panel* box. 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 RunWizard 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

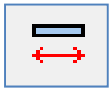
Single left-click to cycle through the options or use the drop-down menu.

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 will become bold and the associated sample will be highlighted in the *Sample List*.

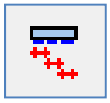
Max & Average displays two traces in the electropherogram. The darker color line corresponds to the maximum peak height at that position and the lighter color line corresponds to the average of all selected sample traces at that position.

Gel Image displays selected samples as a synthetic gel image. Bin ranges in the *Gel Image* mode appear as white vertical lines and can be manipulated by holding down **SHIFT** and dragging the white lines left or right.

Check Range in Edit When activated, the software will warn 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.



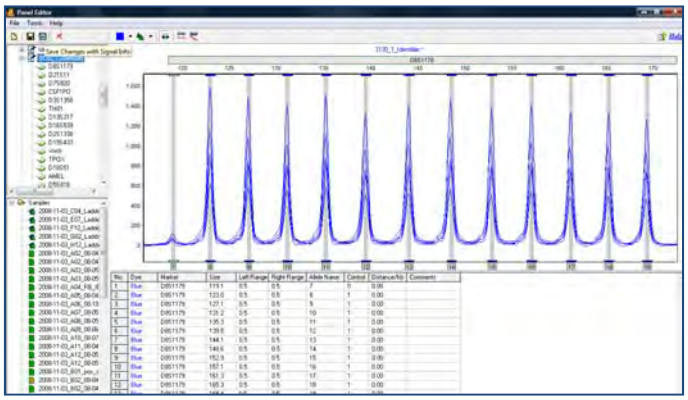
Major Adjustment of Panel Uses previously defined size and height information located in the Panel file when the panel was previously saved with signal information, to identify Marker and Bin positions. To be used when a Panel must be adjusted by 1 - 5 base pairs in order to align with the dataset peaks. 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.

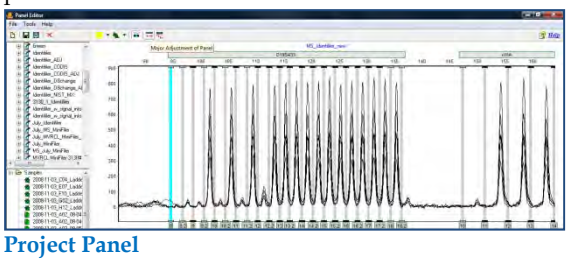
Saving a panel with signal information

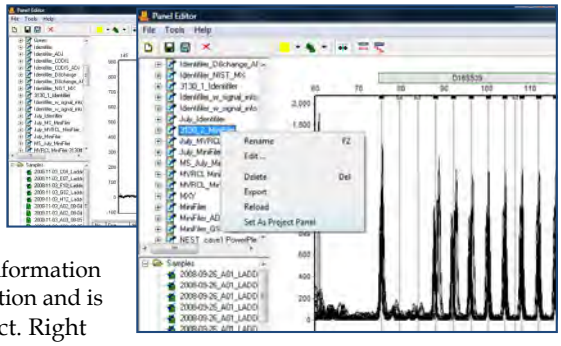
For optimal automatic panel adjustment, a panel should be adjusted manually the first time and then saved with signal information from several ladder samples from the same genetic analyzer and used for analysis of any samples from that analyzer. To manually adjust alignment hold down the shift key and 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.



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.

Saving the panel with signal information from the ladders provides the program with the information needed to automatically adjust the panel with subsequent data. 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 can be used to automatically align the panel to the current ladders.





Project Panel

Once a panel has been aligned and saved with ladder signal information it can be set as the project panel. This panel has signal information and is used to automatically adjust to multiple ladder files in a project. Right

click on the panel name in the list of panels and select Set As Project Panel. The panel name will be displayed at the top of the panel list under the heading Project Panel. Exit from the panel editor and the project panel will be applied to the project.

What to Expect

Once a Panel has been created, aligned and saved with signal information, it can be applied to the dataset. Save the edited Template Panel with signal information in *Panel Editor* then exit the *Panel Editor*. If the *Panel Editor* was accessed via the Run Wizard *Template Selection* box icon, then the selected Panel will appear in the *Panel* field. If the *Panel Editor* was accessed via the *Tools* menu, then click the **Run Process** icon in the *Main Analysis* toolbar. The Run Wizard will appear. Select the Panel from the *Panel* drop-down menu in the Run Wizard *Template Selection* box. Proceed through the other Run Wizard boxes and click **OK** when the *Data Process* window is complete. The Panel will be 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 1). If any peaks are not in the expected bin they are flagged with red allele labels and red vertical bars (figure 2). The analyst can return to the panel editor to determine if there were problems with the capillary for that particular ladder (figure 3).

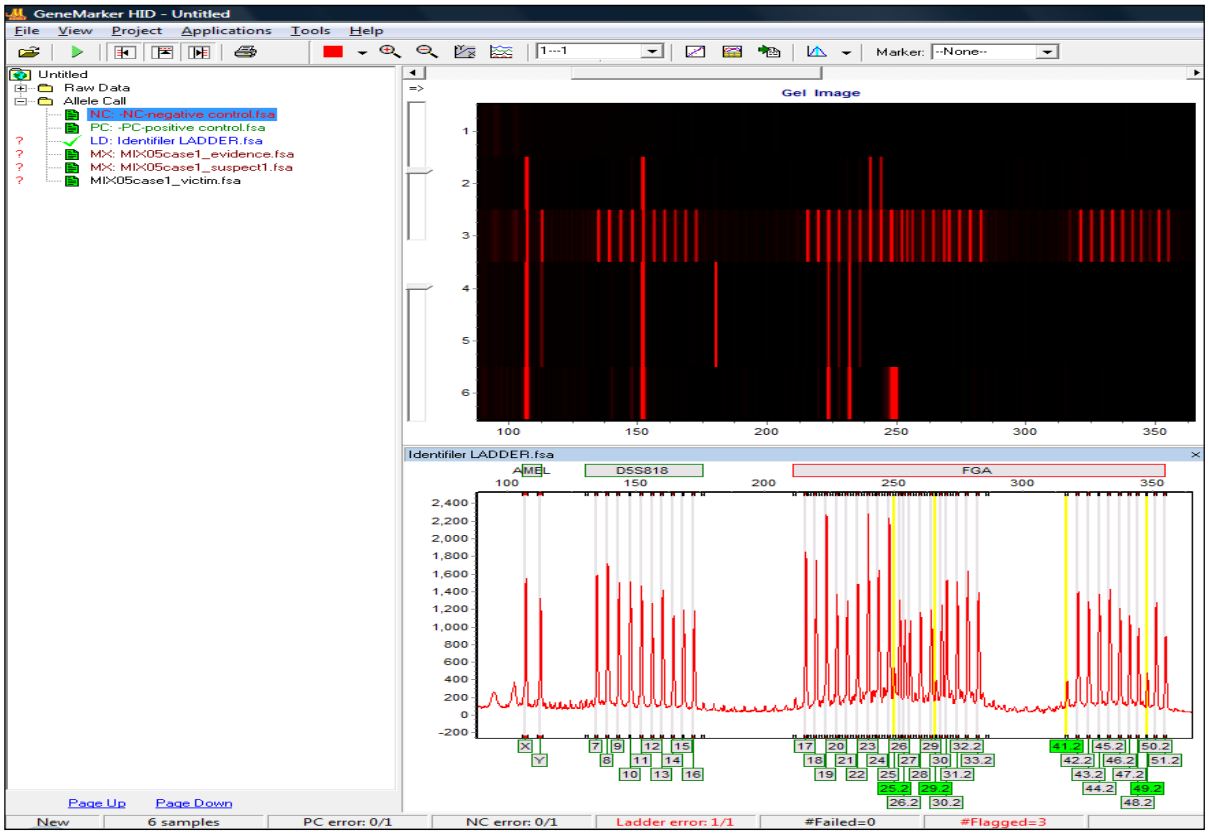


Figure 1 Yellow and green flagging alerts the analyst that although the panel is aligned with the ladder bins, some of the minor peaks in this ladder have a higher RFU than expected.

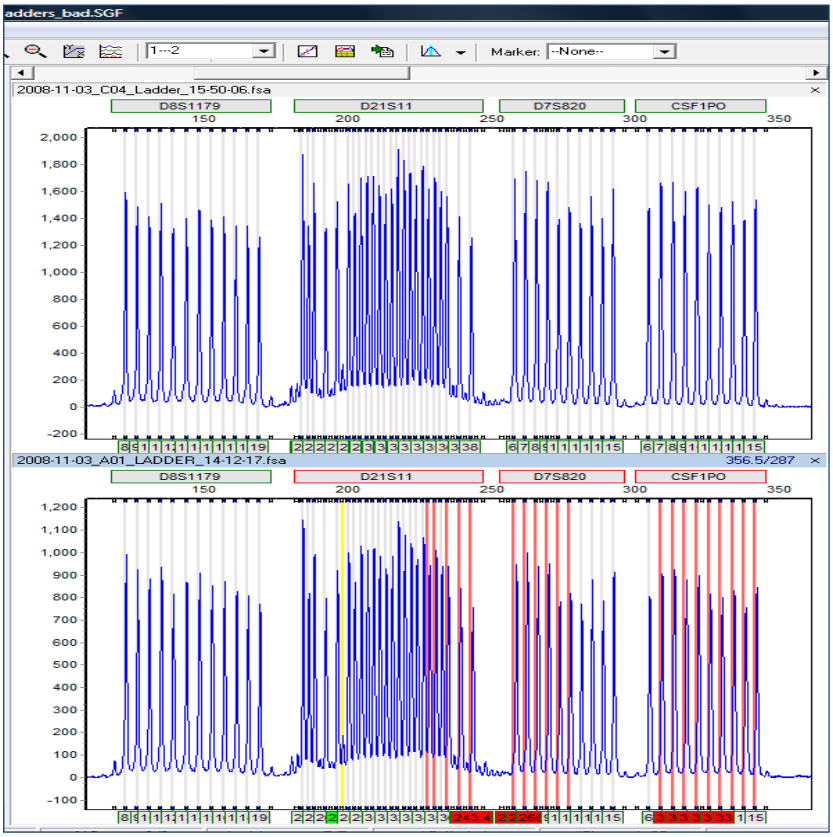


Figure 2 One of the ladder samples in this run has peaks that are out of the bins. These out of bin peaks are flagged with red allele label and red vertical bars

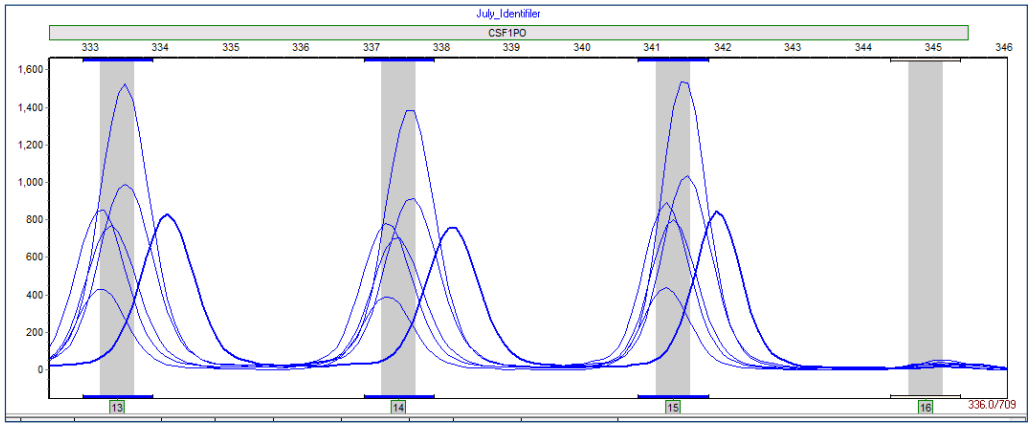
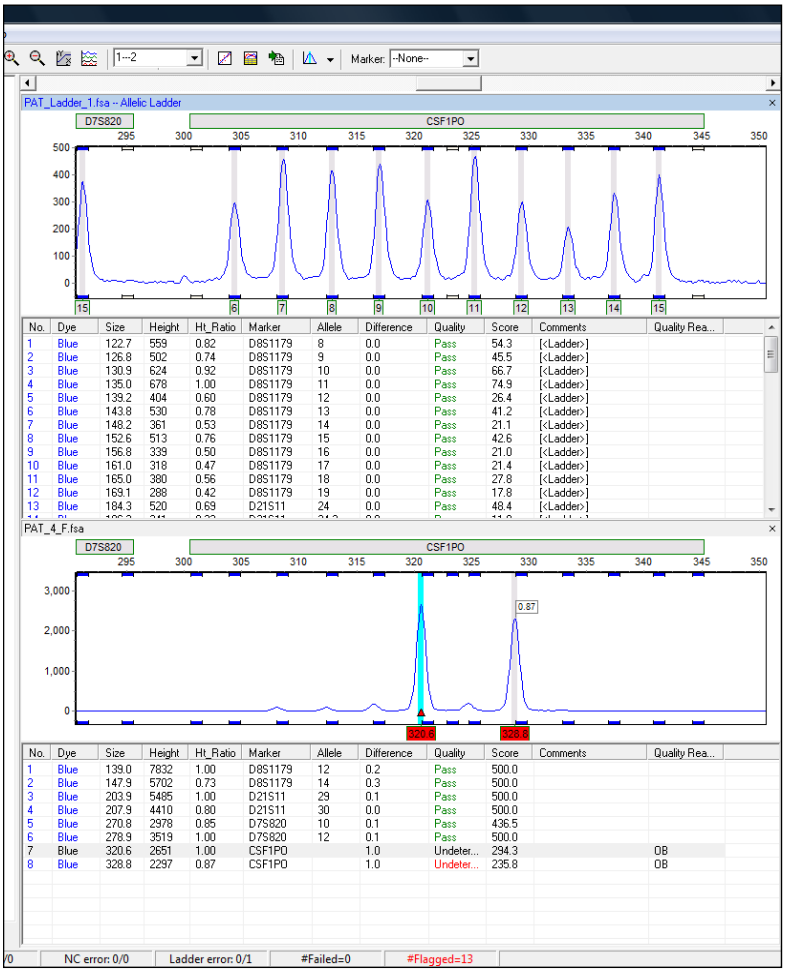


Figure 3 The trace overlay view in the panel editor shows the automatic adjustment of the panels to the ladder samples, illustrating that the ladder file in error is an outlier. The analyst may elect to disable this ladder sample in the main analysis window.

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. The file name of the ladder used for a sample is displayed in the allele report. See Chapter 7 Reports and Printing.



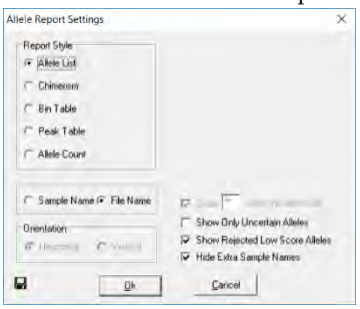
Chapter 7 Reports and Printing

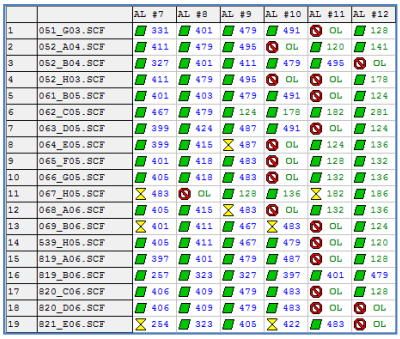
Report Table

The general features of the *Report Table* were outlined in **Chapter 4 Main Analysis Overview**. Here we will discuss and give examples of each *Report Style* available in the *Report Table*.

Allele List

The *Allele List Report Style* displays the base pair size (or Allele Label, if a Panel is applied) of the called peaks. The sample names are listed in rows in the far left column and peaks are numbered in columns at the top of the table.





		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	267	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

Features

Report sample by Sample Name or File Name

Show Only Uncertain Alleles

When selected, displays only the peaks with Quality ranks of Check (yellow) and Undetermined (red).

Show Rejected Low Score Alleles

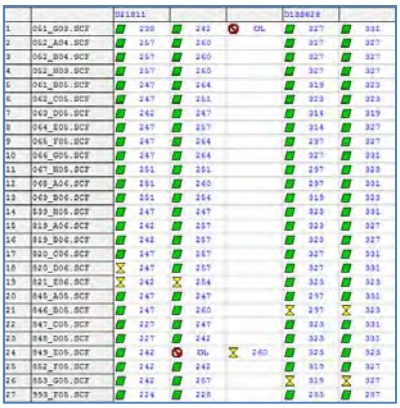
When selected, the peaks with peak scores below the Run Wizard *Additional Settings Allele Evaluation Peak Score* **Reject** setting will be displayed in the table.

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 will only appear once in the first of the rows it is associated with.

Chimerism

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.



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.

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 will be 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 Allele Evaluation Peak Score* **Reject** setting will be displayed in the table.

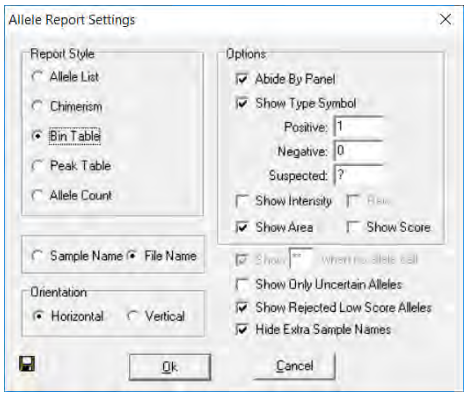
Hide Extra Sample Names: When data is displayed in *Vertical Orientation*, the sample names are repeated for each row of data that the sample is associated with. If *Hide Extra Sample Names* is selected, then the sample name will only appear once in the first of the rows it is associated with.

Bin Table

If a peak is detected in at least one sample, the *Bin Table Report Style* will report the presence or absence of a peak at that position for the rest of the samples in the dataset.

Features

Options



Abide By Panel: When selected, the table will show only called alleles within Panel Marker ranges. This option is only active when a Panel is applied to the data.

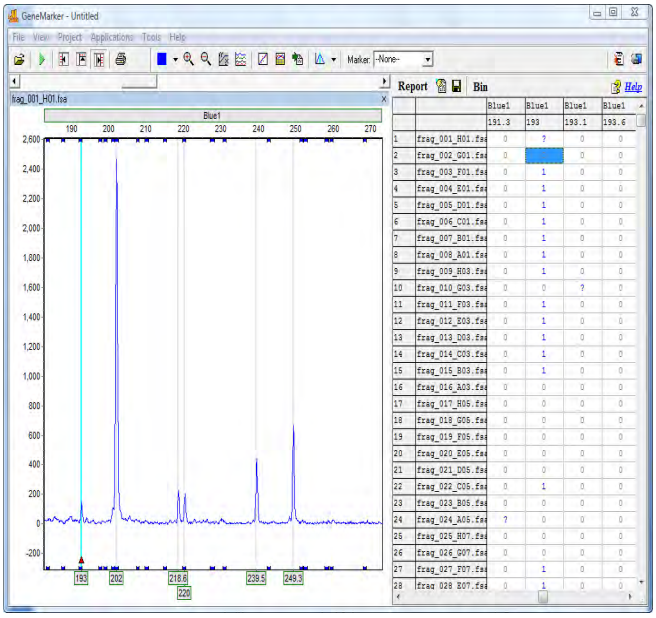
Show Type Symbol: Enter values to indicate the presence of a peak at the position (*Positive*), the absence of a peak at the position (*Negative*), and a Check or Undetermined Quality rank at the position (*Suspected*).

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* will show 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.

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 will be 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 will only appear 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 has been labeled with a *Negative Type Symbol*, right-click the cell and select *Insert a Peak at this Bin Site*. The *Negative Type Symbol* will change 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 has been labeled with a *Positive* or *Suspect Type Symbol*, right-click the peak cell and select *Delete*. The *Type Symbol* will change to *Negative*. (**Hot Key = DEL**)

Confirm

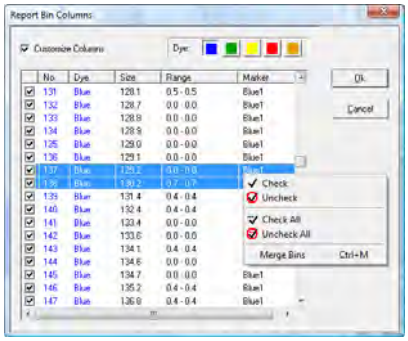
To indicate the peak present at the position is truly a peak, right-click the peak cell and select *Confirm Peaks*. Only peaks centered within a Panel *Bin* will change from Suspect Type Symbol to *Positive Type Symbol* when confirmed. Once a peak is confirmed, it cannot be unconfirmed, only deleted. (**Hot Key = CTRL+M**)

Delete Bin Columns

To completely 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 will be deleted (not the columns which 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* box will appear.



Display Bins

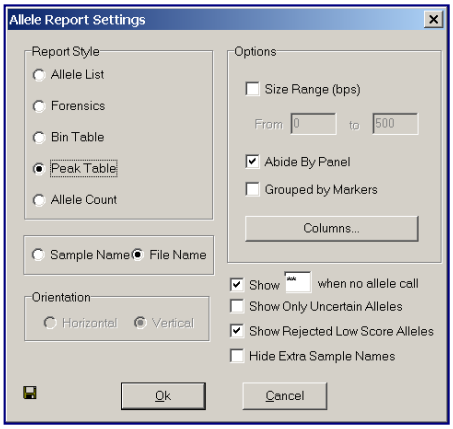
By default, all Bins will be selected with a checkmark at the beginning of the row. Individually deselect Bins for exclusion from the *Report Table* by single left-clicking the checkmark box. To deselect all, right-click any cell in the *Report Bin Columns* box and select *Uncheck All*. To deselect only a few Bins, left-click a cell to highlight the row then hold **CTRL** or **SHIFT** key and select additional rows. Next, right-click and select *Check* or *Uncheck* to include or exclude the Bins, respectively. Click **OK** in the *Report Bin Columns* box when finished and only the Bins with checkmarks will be displayed in the *Report Table*.

Merge Bins

To make two or more Bins become one Bin, single left-click a row to highlight it. Next, hold down **SHIFT** key to select additional rows. Right-click the highlighted rows and select *Merge Bins*. (**Hot Key = CTRL+M**) Click **OK** in the *Report Bin Columns* box when finished, and the selected Bins will be 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 will be reported.

Peak Table

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*. **See Chapter 4 Main Analysis Overview** for column option definitions.

Features

Options

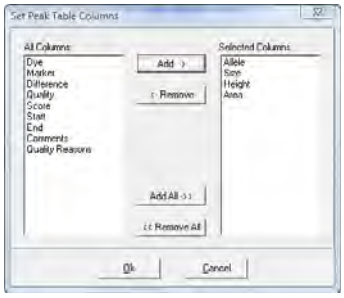
Size Range (bps): When selected, allows the user to define a specific base pair range. Only the peaks within the range will be displayed within the *Report Table*.

Abide By Panel: When selected, the table will show 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 will be listed one after the other in the columns at the top of the table. When de-selected, each allele will be 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.

Columns
Click the **Columns** button to open the *Set Peak Table Columns* box. All column options are listed in the *All Columns* field on the left. The columns currently being displayed in the *Report Table* are listed in the *Selected Columns* field on the right.

Selecting Columns
Single left-click options in the *All Columns* field and click the **Add** button to add the column option to the *Selected Columns* field. 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
Single left-click options in the *Selected Columns* field and click **Remove** to move the column option to the *All Columns* field. 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.



Click **OK** in the *Set Peak Table Columns* box and the *Allele Report Settings* box when finished. The options in the *Selected Column* field will be displayed along the top of the table in columns.

Show Only Uncertain Alleles: When selected, displays only the peaks with Quality ranks of *Check* (yellow) and *Undetermined* (red).

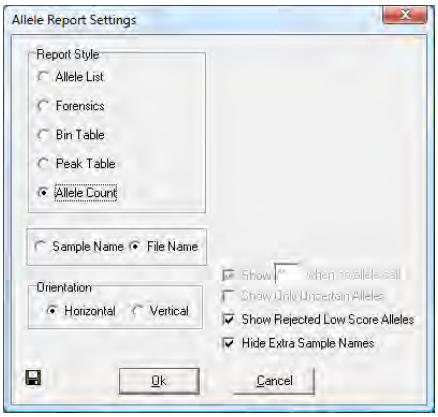
Show Rejected Low Score Alleles: When selected, the peaks with peak scores below the Run Wizard *Additional Settings Allele Evaluation Peak Score* **Reject** setting will be displayed in the table.

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 will only appear once in the first of the rows it is associated with.

	Sample	Marker	Allele#1	Size#1	Height#	Area#1	Allele#2	Size#2	Height#	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				
		D18S1003	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
		D18S1003	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

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.
NOTE: *Allele Count* requires that a Panel is applied to the data. **See Chapter 6 Panel Editor.**



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.

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	S39_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 will be displayed in the table.

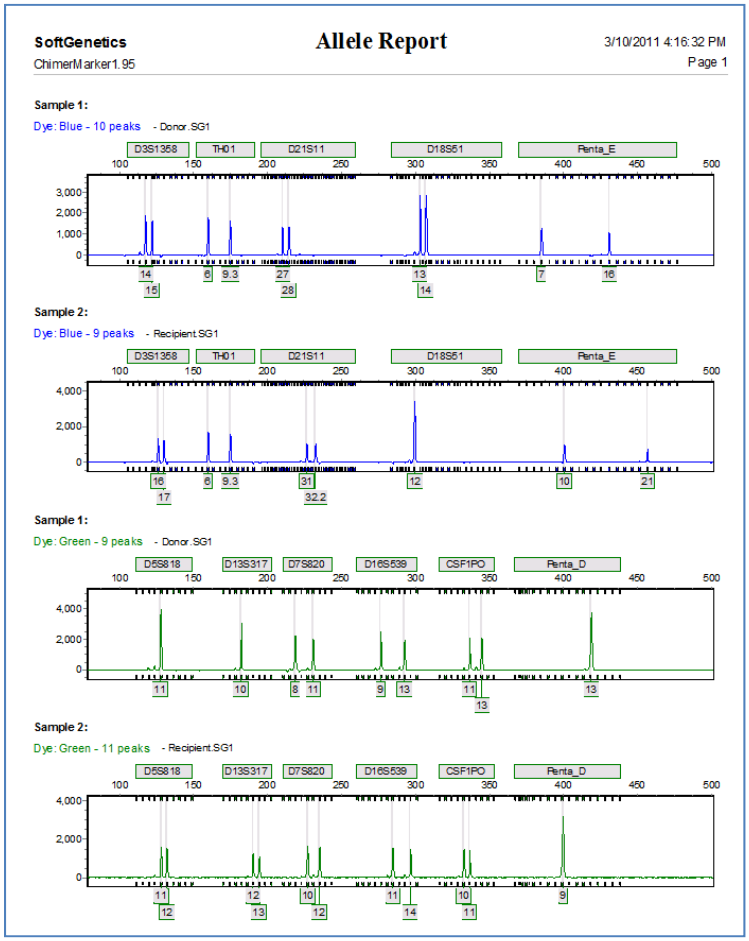
Hide Extra Sample Names: This feature is not active for *Allele Count Report Style*.

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 click the **Print Report** icon in the *Main Analysis* window. The *Print Report* options box will appear. 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.

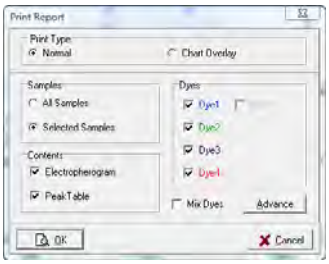
NOTE: The *View* → *Preference* → *Display Settings* options will affect how the *Print Report* is displayed.

ChimerMarker Print Report



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 will be automatically labeled with its respective sample file name in the printed report. The *Advanced* button provides more options.



Print Type

Normal: All *Print Report* options are available when *Normal Print Type* is selected.

Chart Overlay: Prints only the *Electropherogram* with the report.

Samples

All Samples: Prints all the samples in the project.

Selected Samples: Prints only those sample files that have been selected in the *Main Analysis* window *Sample File Tree*.

Contents

Electropherogram: Prints the peak trace for each dye color and sample selected.

NOTE: The zoom setting of the *Electropherogram* in the *Main Analysis* window will be 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* will contain a list of each dye color selected for each sample selected and the allele count within each dye color.

Dyes

Dye 1-6: Click the checkbox to include the dye color in the *Print Report*.

Mix Dyes: Prints all selected dye colors on one electropherogram.

Advanced Options

Print Project Comments: Includes the *Project Comments* at the top of the *Print Report*. Select *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* window *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 on 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 i.e. 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.

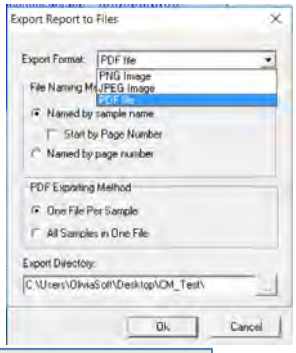
Chart Height: Use this feature to customize the size of the electropherogram in the print report.

Icons and Functions

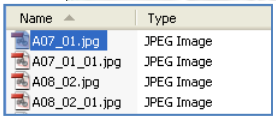
The following icons are available in the *Print Preview* window prior to printing the *Print Report*.

Print
Opens the *Print* options box. Select a printer, the print range and the number of copies.

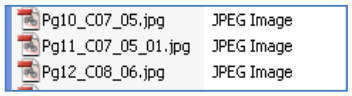
Export to File
Opens the *Export Report to Files* box. Save each page of the *Print Report* in JPEG, PNG, or PDF format. Select the directory to export the files to.



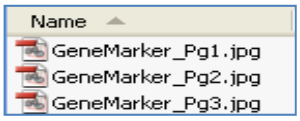
Named by sample name saves each PNG or JPEG under the sample name.



Start by Page Number combines the page number and the sample name for the saved file name.

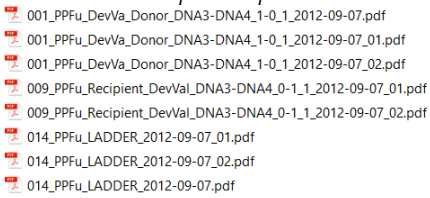


Named by page number saves each file by the page number within the report.



When *PDF file* is selected as the *Export Format*, the PDF can be exported in two ways.

1. *One File per Sample:*



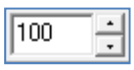
2. *All Samples in One File:* File is named using the Project name

Page Setup
Opens the *Page Setup* box. Choose the paper size, margins and orientation (*Portrait* or *Landscape*).

Content Options
Opens the *Print Report* options box. See the section above - *Report Content Options*.

Zoom to Fit
Zooms out to view the entire *Print Preview* page.

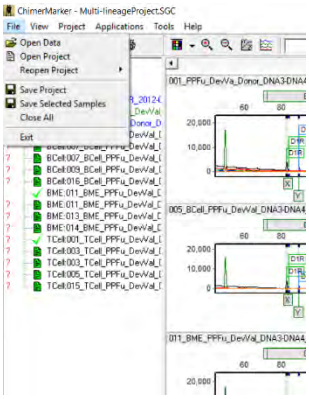
Zoom to Width
Zooms in to view the *Print Preview* page at maximum width without losing information off the screen.



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 can be 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 Standards**.



NOTE: Previous versions of ChimerMarker saved projects in SFP format. This format can be opened with current ChimerMarker versions.

To save a project, go to *File* → *Save Project* in the *Main Analysis* window. The *Save Project* box will open. Select a directory and enter a project name. Click **Save**.

To save a sub-set project, containing the files selected in the *Main Analysis* window, go to *File* → *Save Selected Samples*. The *Save Project* box will open. Select a directory and enter a project name. Click **Save**.

To re-open the project, go to *File* → *Open Project* in the *Main Analysis* window. The last folder accessed by ChimerMarker will appear. Navigate to the directory containing the SGF (or SFP) file and click **Open**. Additionally, the last four projects that were opened by ChimerMarker appear when the *File* → *Reopen Project* fly-out menu is selected. Click a project from the fly-out menu and it will be uploaded to ChimerMarker.

Chapter 8 Maternal Cell Contamination Analysis

Maternal Cell Contamination (MCC) Analysis

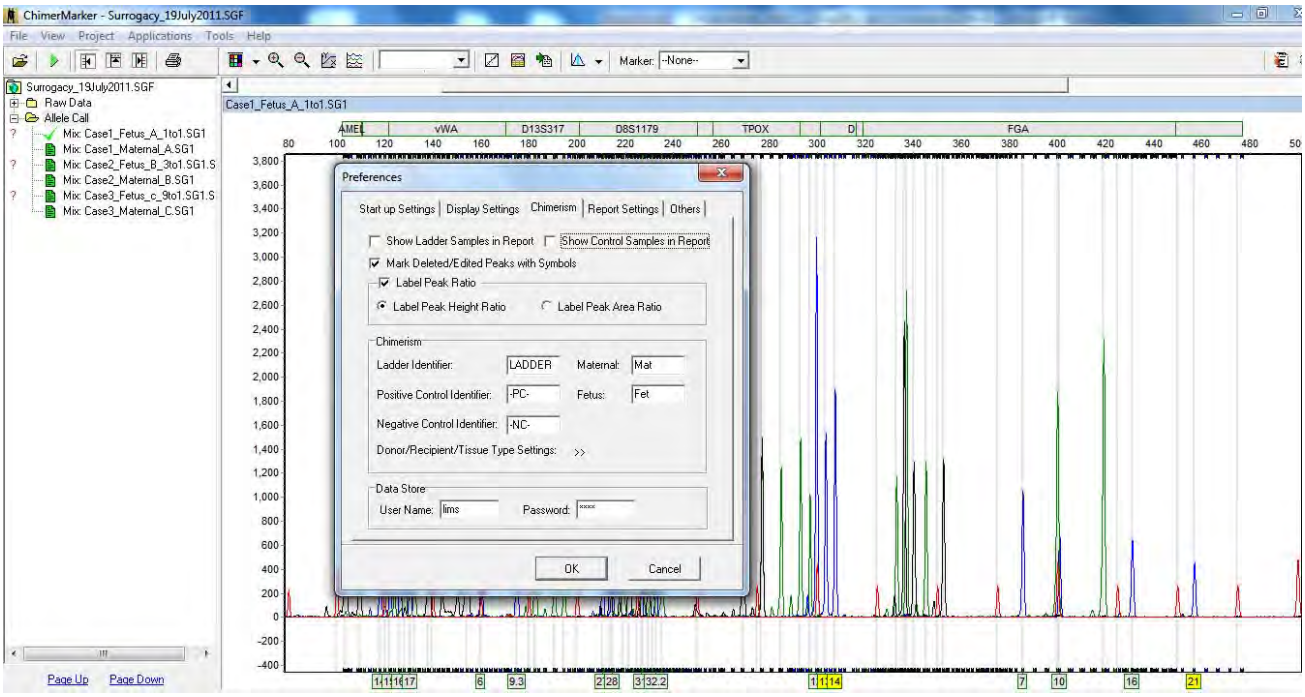
Overview

Please follow steps from Chapter 3 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 can be accessed under *Application* tab in the main menu toolbar.

Procedure

Genotyping of all sample data:

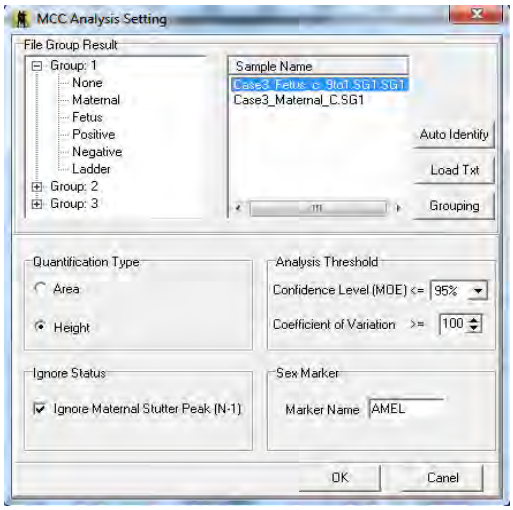
Follow the steps for importing and processing data from Chapter 2, General Procedure. Review the results in the main analysis window.



After reviewing the results and making any desired edits (Chapter 3, Main Analysis), go to View-> Preference-> Chimerism tab to specify identifiers for maternal and fetus sample.

Maternal Cell Contamination Analysis:

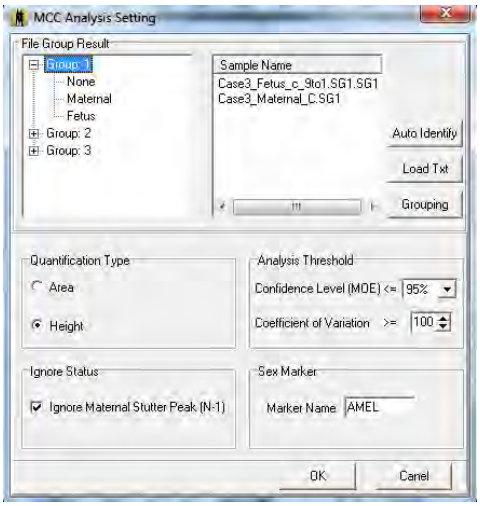
After genotyping and specifying the identifiers for the maternal and fetus samples, go to Application-> MCC analysis.



Auto Identify button will automatically group fetus sample and maternal sample using identifiers specified in
View ->Preference->Chimerism Tab.

Load Txt button can group samples by using a text file created from Excel (tab delimited txt file) or other word editing programs. Refer to page 123 for more information.

Grouping button brings up a dialog window that is used to group maternal and fetus samples into different groups or patients.



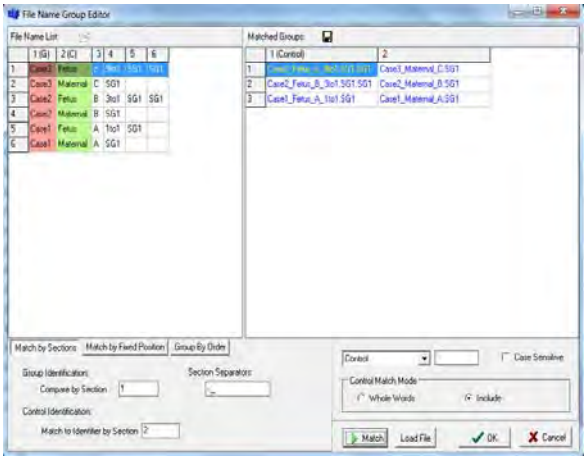
Quantification Type:
1) Area: uses area of allele for %MCC calculations
2) Height: uses height of allele for %MCC calculations

Analysis Threshold:
Set desired threshold for analysis.

Ignore Status:
If selected, any maternal allele detected in the stutter position of fetus sample will be ignored.

Sex marker:
Specify marker name that is used for sex identification.

Grouping Options: Match by Section, Fixed Position or Order



Match by Section:

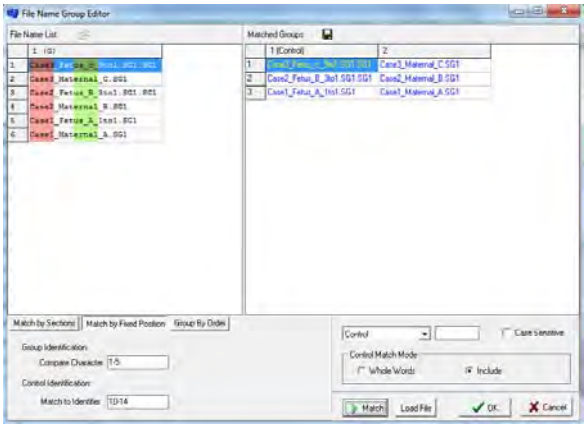
Group Identification: specify which column to group each set of samples/individual patients.

Control identification: specify which column the identifiers specified in the View->Preference->Chimerism tab can be found.

Control: specify identifiers to set as control (usually maternal sample).

Match: group samples and control according to selected method.

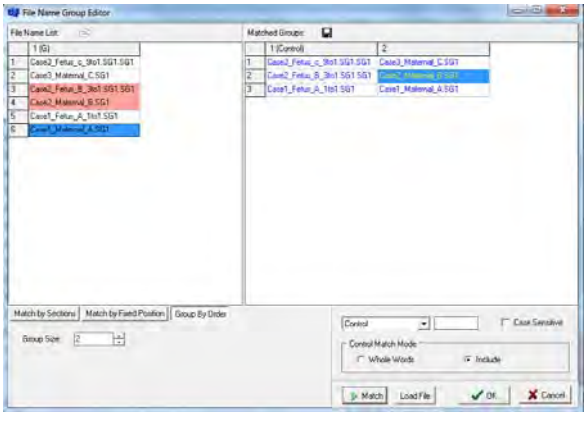
Load File: Load grouping file from text.



Match by Fixed Position:

Group Identification: specify characters location to group each set of samples/individual patients.

Control Identification: specify characters location that the identifiers specified in the View->Preference->Chimerism tab can be found.



Match by Order:

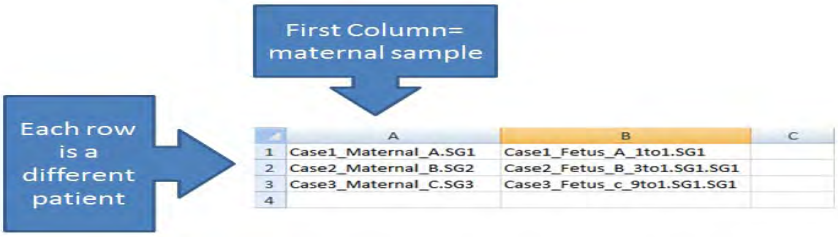
Group Size: select the number of samples to group by order.

Grouping by Text File Rules:

1) Sample name in text file must be identical to sample name loaded in project.
2) Each Row correspond to an individual group/patient
3) First column is recognized as maternal sample
4) Each sample name within a row is separated by a "tab"

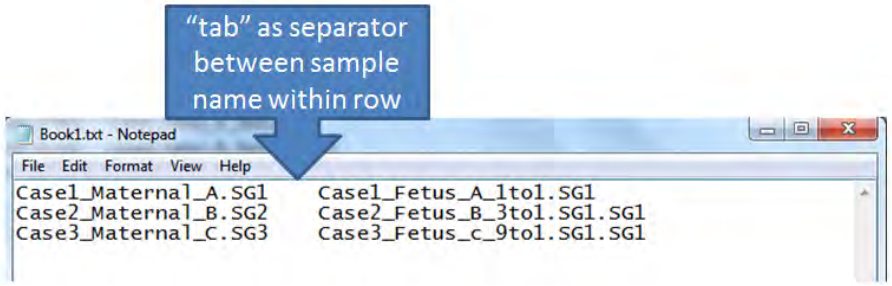
    * If using Excel to create group information, the file must be save as a tab delimited text file. The following illustration shows an example of grouping information created in Excel and Notepad.

Excel Example:



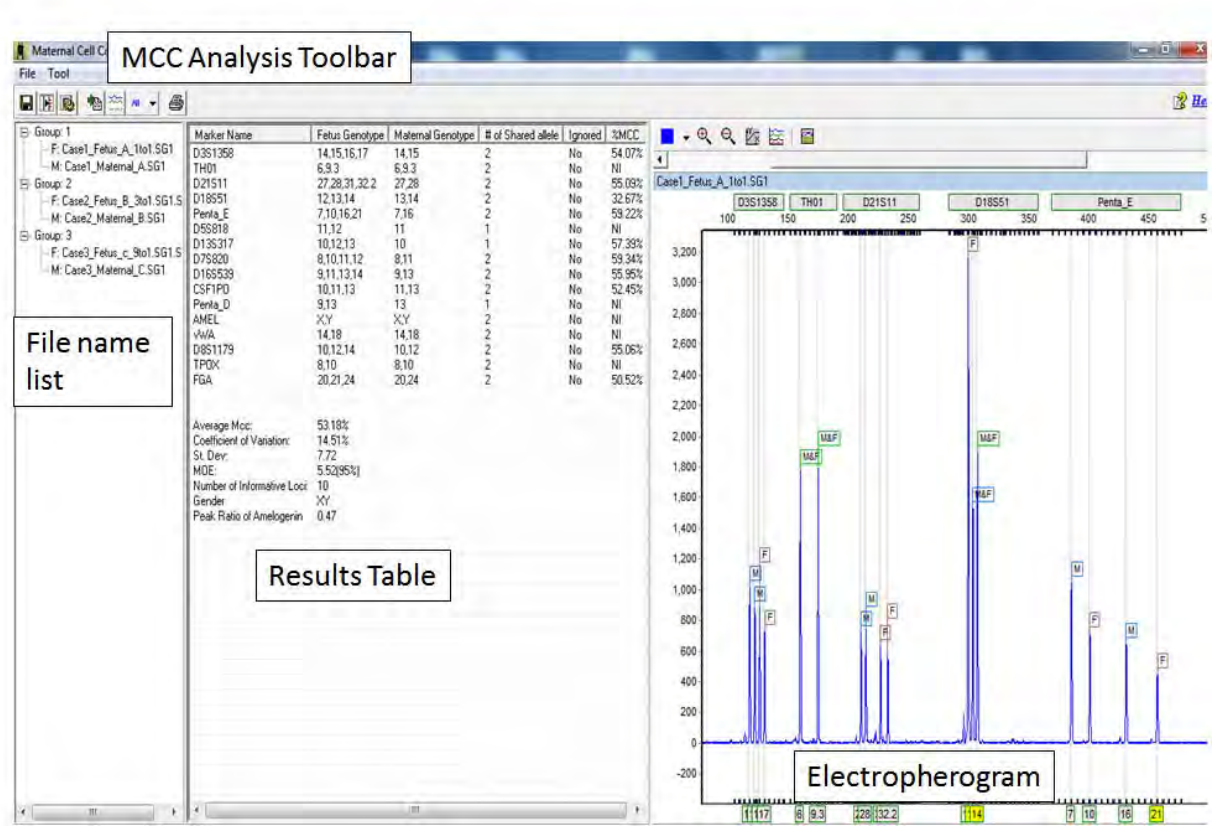
After creating the file, go to File-> select "Save As"->" Save as type"-> select Text (Tab delimited) (.txt)

Text File Example:

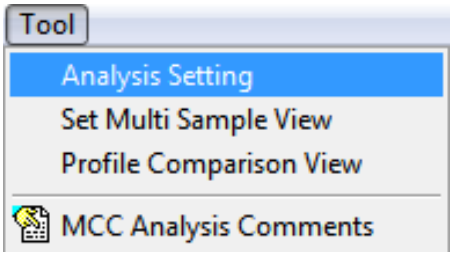


Results

The automated MCC calculations are displayed in the center table. 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.



Tools and Icons



Analysis Setting-Reopens MCC Analysis settings dialog box.

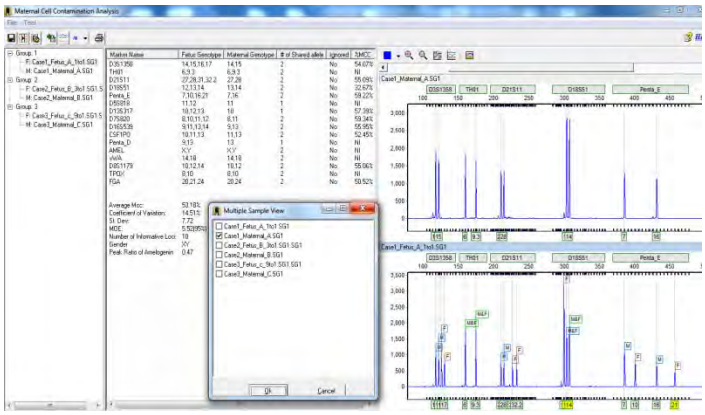
Set Multi Sample View- Select multiple samples to view electropherograms.

Profile Comparison View- to view selected traces and level of 2D or 3D offset

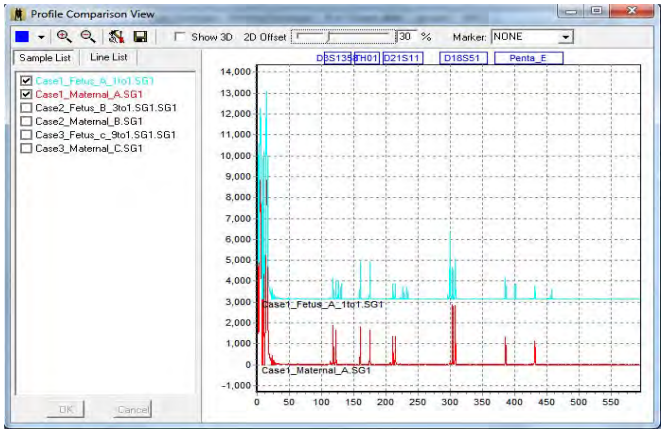
MCC Analysis Comments- to record any case notes to be saved and printed with the final report (saves up to 6400 characters).

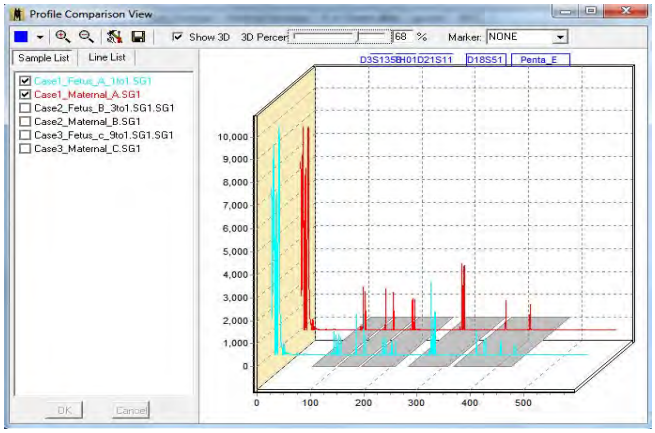
Examples Screens of Tool Selections

Multi Sample View to display electropherograms of several samples

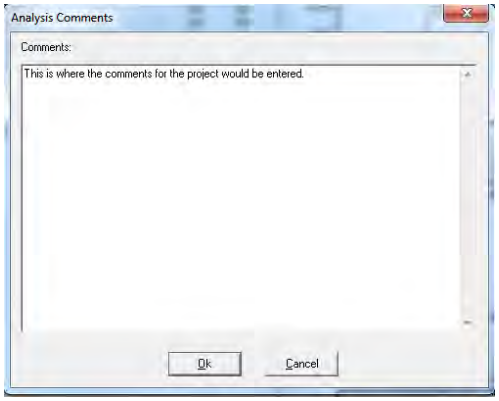


Profile Comparison View with 2D and 3D Options





MCC Analysis Comments saved with project and displayed in comprehensive report



Save the report table in .xls or .txt file format

Show or Hide – toggle this icon to show or hide the electropherograms

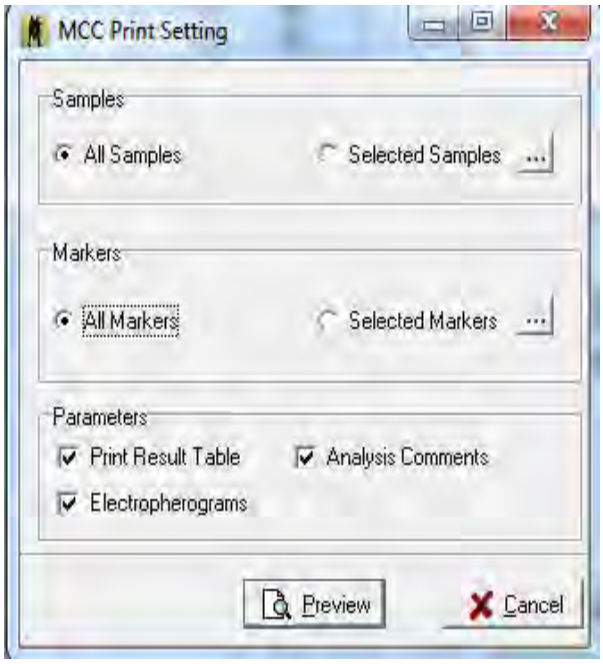
Analysis Parameter icon to relaunch the Chimerism Analysis Settings Box

Multiple Sample view – alternative path to display electropherograms of multiple samples

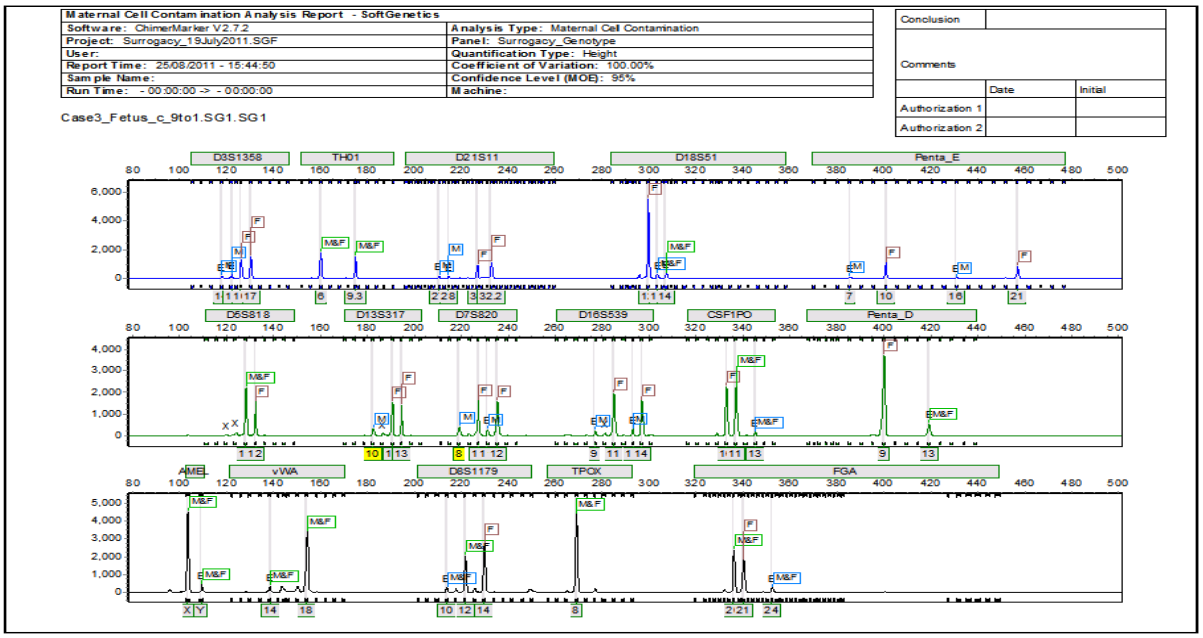
Profile Comparison – alternative path to display profile comparison view

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



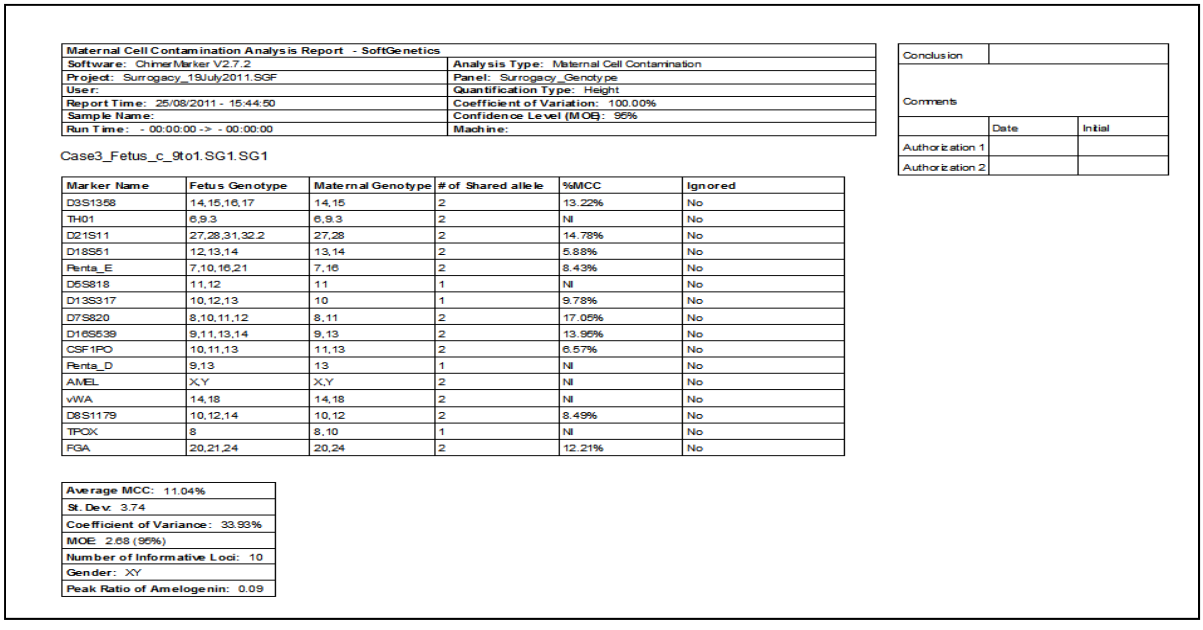
Comprehensive Reports



First page of report includes header, electropherograms (traces) and report table.

The report header uses information from user management, signature box, 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 box, and the results table that was displayed in the MCC analysis results window.



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:

Case3_Fetus_c_9to1.SG1.SG1

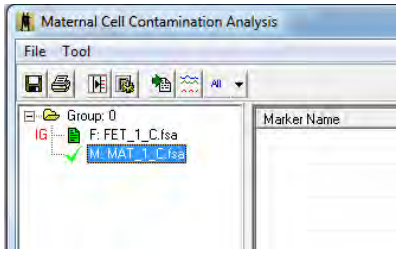
Conclusion		
Comments		
	Date	Initial
Authorization 1		
Authorization 2		

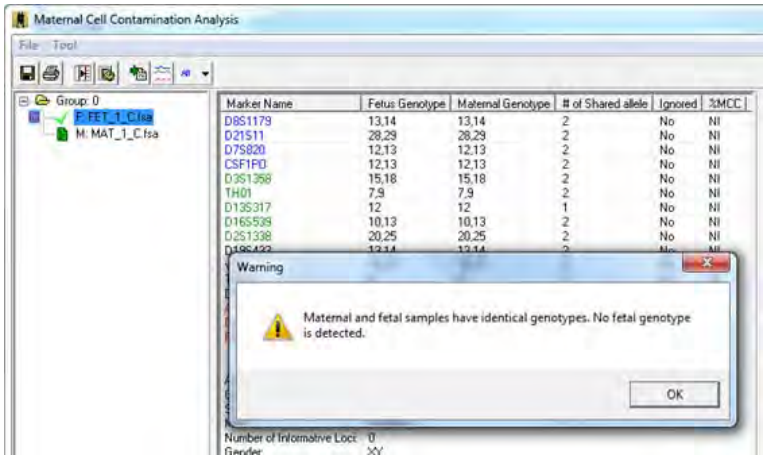
Marker Name	Fetus Genotype	Maternal Genotype	# of Shared allele	%MCC	Ignored
D3S1358	14,15,16,17	14,15	2	13.22%	No
TH01	6,9.3	6,9.3	2	NI	No
D21S11	27,28,31,32.2	27,28	2	14.78%	No
D18S51	12,13,14	13,14	2	5.88%	No
Penta_E	7,10,16,21	7,16	2	8.43%	No
D5S818	11,12	11	1	NI	No
D13S317	10,12,13	10	1	9.78%	No
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 will alert the user when the Fetal sample is selected. "IG" will be displayed in front of the Fetal sample and a red font message will be included in the report.



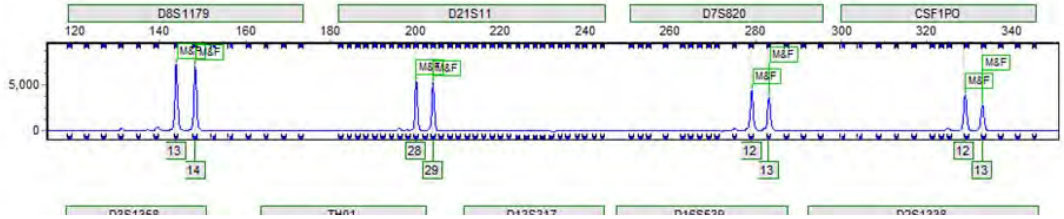


Maternal Cell Contamination Analysis Report - SoftGenetics	
Software: ChimerMarker V3.1.5	**Analysis Type:** Maternal Cell Contamination
Project:	**Panel:** Identifiler
User: Admin	**Quantification Type:** Height
Report Time: 11/04/2016 - 14:53:24	**Coefficient of Variation:** 10.00%
Sample Name: 298927.20	**Confidence Level (MOE):** 95%
Run Time: 02/27/2007 - 23:53:54 -> 02/28/2007 - 00:29:38	**Machine:** Clyde

Sample Comments:

FET_1_C.fsa (Maternal and fetal samples have identical genotypes. No fetal genotype is detected.)

Conclusion		
Comments		
	Date	Initial
Authorization 1		
Authorization 2		

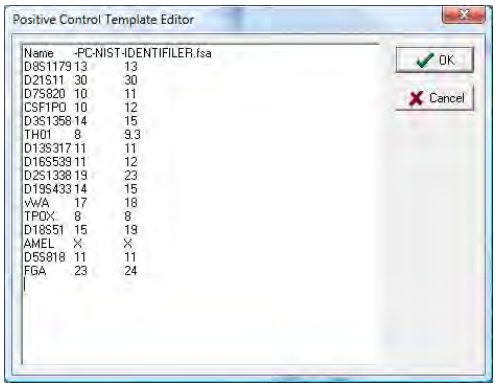


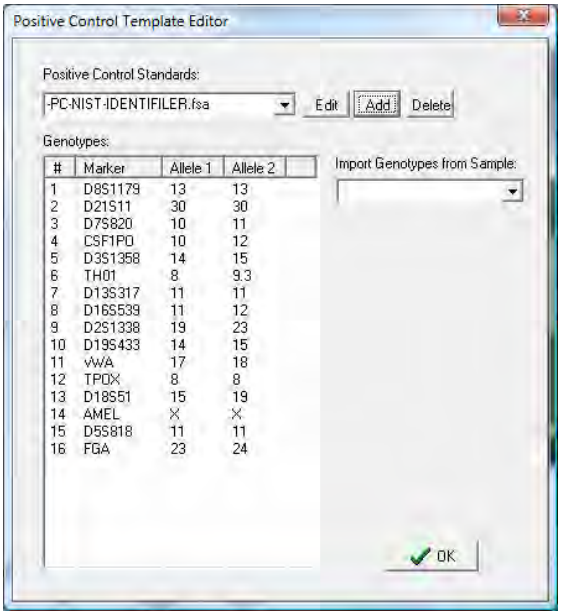
Chapter 9 Additional Tools

Automated Control Concordance

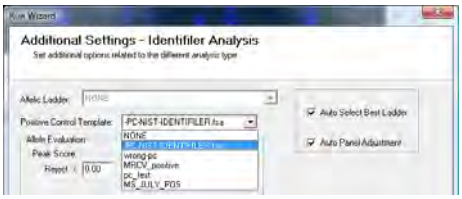
Positive Control Template Editor

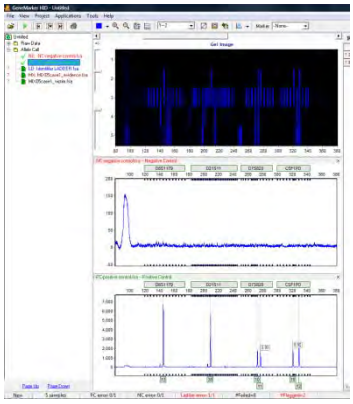
1. Tools →Positive Control Template Editor to launch dialog box
2. Import Genotypes from samples using dropdown menu
3. Select from functions to Add new positive control samples, Edit or Delete files





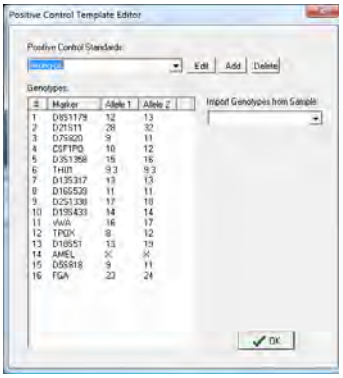
4. Select the appropriate positive control file from the dropdown menu in the run wizard. Summary message is located at the bottom of the main analysis window. For example, if a project has one positive control file that is in agreement with the positive control template, the message is PC error 0/1.

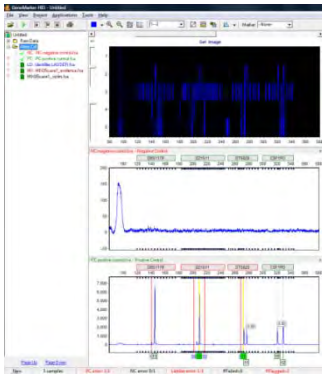




Example of Positive and Negative samples in concordance with controls

5. **If the positive control samples are not in agreement with the template the message will be PC error 1/1 and red lines will indicate the peaks in error.**

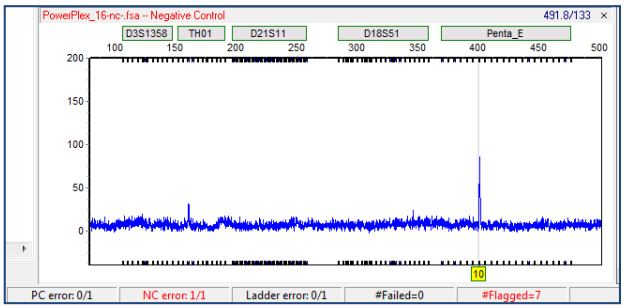




Example of Positive sample not in concordance with positive control template

Negative Control Concordance

1. Use View →Preferences →Chimerism to select the file name recognition
2. GeneMarker automatically changes font of negative control file name to red in Filename Tree
3. Any negative control sample that has peak(s) will result in a negative control (NC error) in the Project Summary Bar

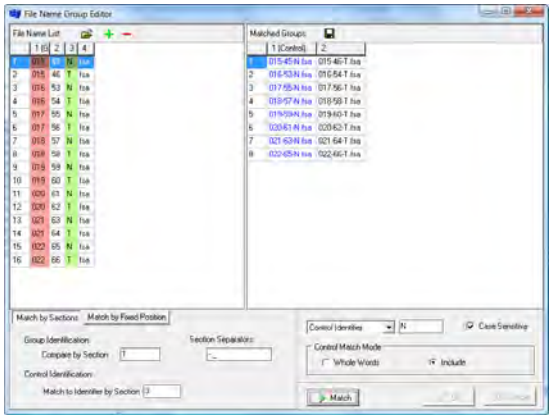


Filename Group Editor

Project → Apply Sample Grouping *OR* **Tools → File Name Group Tool**

The *Filename Group Editor* can be used to group family members or other related samples in the dataset based on their filenames for simplified analysis.

Procedure



1. Select *Project → Apply Sample Grouping*
2. The *File Name Group Editor* window appears
3. 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
4. Choose *Match by Sections* or *Match by Fixed Position*
5. Enter values for the *Group Identification* and *Control Identification* fields
6. Enter a *Control Identifier* value and click **Match**
7. The samples from the *File Name List* will be paired into groups in the *Matched Groups* window
8. 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
9. The *File Name Group Editor* window will close and the grouping information will appear next to the sample filenames in the *Sample File Tree* in the *Main Analysis* window (if *Project → Apply Sample Grouping* was chosen).
10. To navigate by group in the *Sample File Tree*, hold down the **CTRL** key and hit the **PageUp/Down** keys. Sample groups will be opened consecutively.

Icons and Functions

Load Files
Opens a directory window where raw data files can be located and uploaded to the *Filename Group Editor*

Add Files
Opens a directory window where additional raw data files can be 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* will be in the first column of the *Matched Groups* tab-delimited Text file.

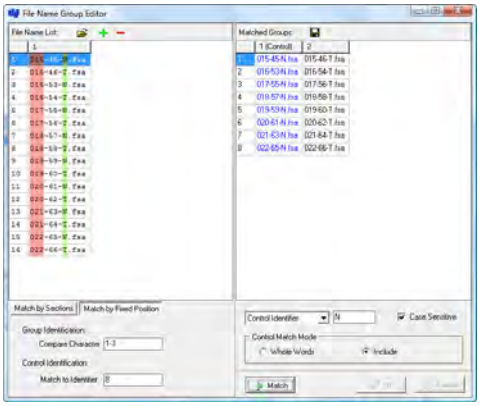
Match by Sections

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 into the *Compare by Section* field. The section of the filename specified will be 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 will be highlighted green in the *File Name List*.

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 will be 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 will be highlighted green in the *File Name List*.

Control Identifier

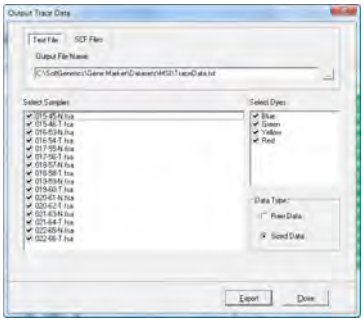
Enter the character from the *Control Identification* section (highlighted green) that describes the control or reference sample. Example: N = normal or R = reference. Select **Case Sensitive** if the *Control Identifier* needs to be identified by upper or lower case letters.

Control Match Mode

Choose either **Whole Words** or **Include**.
Whole Words should be used if the characters entered into the *Control Identifier* field need to match exactly.
Include should be selected if the characters in the *Control Identifier* field only need to be identified in the filename, i.e. not an exact match.

Output Trace Data

Tools → Output Trace Data



The *Output Trace Data* tool exports raw or sized data of uploaded sample files as Text (.txt) or SCF (.scf) files.

Procedure

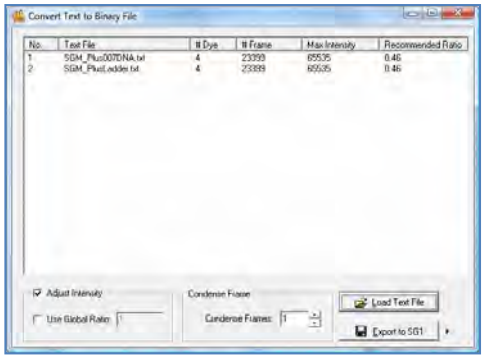
1. Select whether to export the data as a *Text* or *SCF* file
2. Choose the directory and folder to save the exported data to in the *Output File Name* field.
3. Select the samples to include in the output file from the *Select Samples* field.
4. Select which dye color data to export from the *Select 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

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.

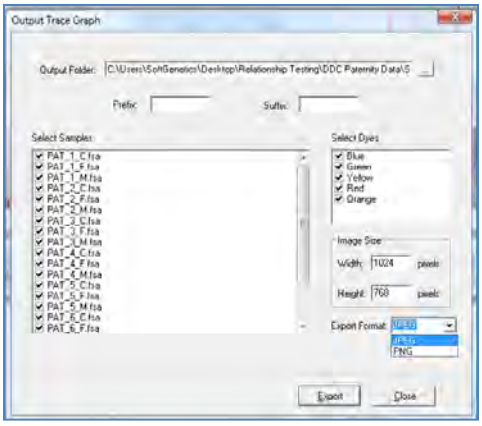
Procedure



1. Click the **Load Text File** button and select Text (.txt) files to convert
2. Once files are uploaded, they will appear in the *Text File* field
3. The software will automatically calculate a *Recommended Ratio* for the user to condense the number of frames in a single trace
4. Enter a condense frames by XX number in the *Condense Frames* field
5. Click **Export to SG1** if exporting a five-color trace, click **Export to SCF** if exporting a four-color trace.

Export Electropherogram

Tools →Export Electropherogram



The *Export Electropherogram* tool allows the user to export the trace images to a specified folder.

Procedure

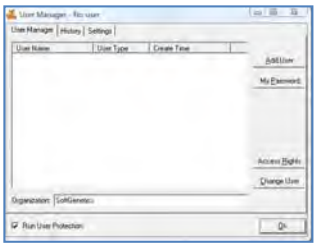
1. Use a dropdown menu to specify the output folder.
2. Specify the prefix and suffix for the exported file name. The full file name will be Prefix+Sample name+"_"+Dye name+Suffix+Extension name.
3. Select samples, Dyes and Image Size
4. Use a dropdown menu to specify the export format. JPEG and PNG are both available. PNG is recommended.

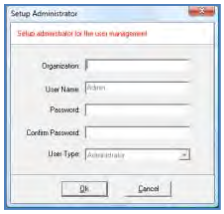
Chapter 10 User Management

Overview

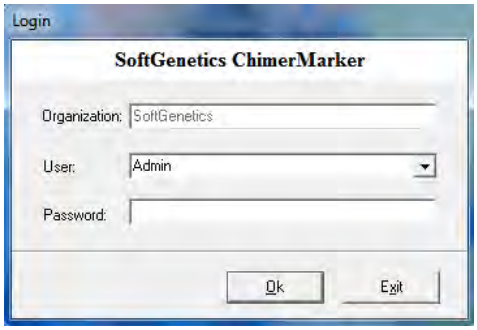
User management may be implemented after installation of ChimerMarker. The administrator activates User Management from the Help drop-down menu. User management provides control of user access rights and automatically generates an audit trail of all edits.

Procedure



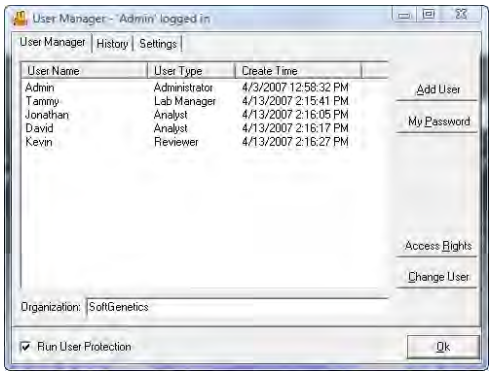


1. Select *Help* → *User Management*
2. The *Login* box appears
3. Click Run User Protection to activate the setup Administrator
4. Enter Organization Name, an Administrator username and password
5. Click **OK**
6. You are now logged in as the *Administrator*
7. Click the **Add User** button to add additional users
8. Click the **Access Rights** button to set up user type access permissions
9. Be sure to select *Run User Protection* and click **OK** to exit
10. Login is required to open ChimerMarker after the User Manager is activated.



User Manager

The *User Manager* tab displays user information and contains options for creating and deleting users.

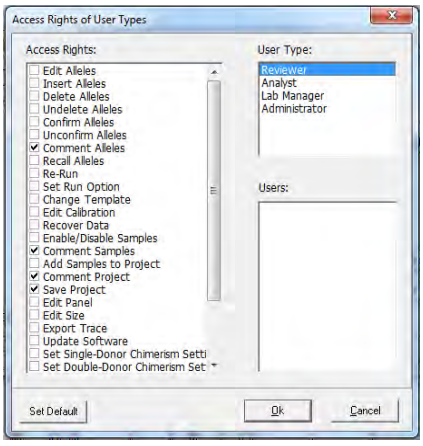


User Window
Displays all users by name, type and creation date

Organization
Enter your organization name

Run User Protection
When selected, users will be prompted to log on with a user name and password. When deselected, any person can launch ChimerMarker without a username and password.

Add User
Launches the *Add User* box where a new username and password can be input. This is also where the user type can be chosen. A user can be deleted by right-clicking the username and selecting *Delete User*.
NOTE: Only the *Administrator* can add and delete users.



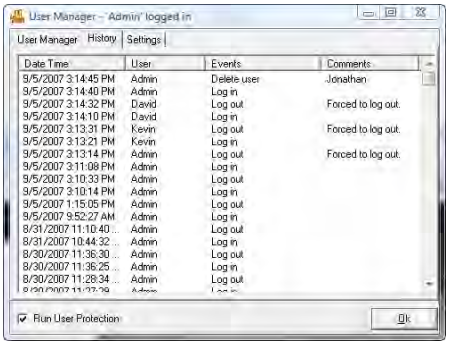
My Password
Launches the *Change Password* box where the user that is logged in can enter a new password. The new password must be entered twice to ensure accuracy.

Access Rights
Launches the *Access Rights of User Types* box where the different access rights available to each user type can be identified. Clicking the **Set Default** button will return the *Access Rights* for the *User Type* selected back to factory defaults.
NOTE: Only the *Administrator* can change *Access Rights* for a *User Type*.

Change User
Prompts for a confirmation of action then launches the *Login* box.

History

The *User Manager History* tab monitors user activity associated with the user manager function.



Date/Time
Records the computer's date and time for the activity.

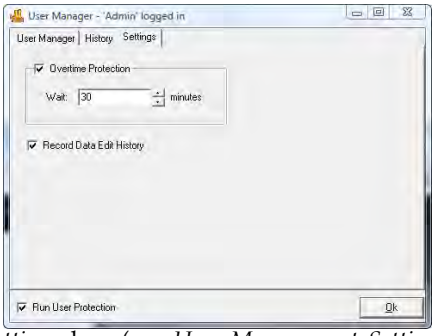
User
Identifies the username of the person that performed the activity.

Events
Records the user manager activity that was performed.

Comments
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*.

Settings

The *User Manager Settings* tab contains additional options for the *User Management* function.



Overtime Protection
When selected, ChimerMarker will logout 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).

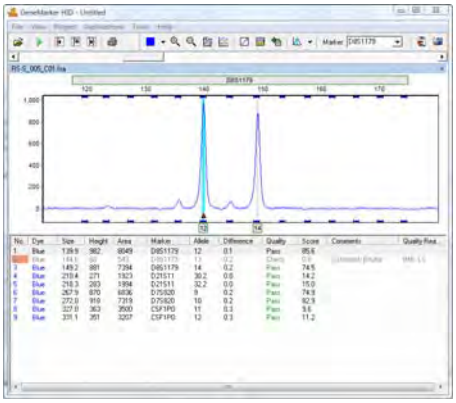
Record Data Edit History
When selected, any changes made to the allele calls of the project will be saved in the *Edit History* log. Please see *Edit History* section below for more information.

Edit History/Audit Trail

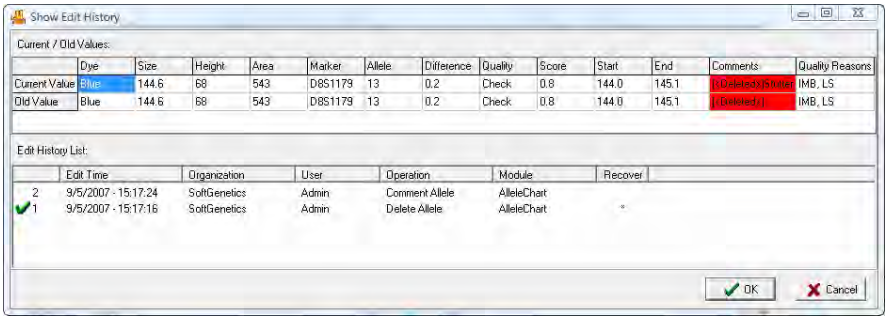
When **Record Data Edit History** is selected in the *User Manager Settings* box (see *User Management Settings* section above), any change to allele calls in the analysis will be recorded. Changes can also be recovered in the *Edit History* feature.

Procedure

1. Click the **Show Chart/Table** icon in the *Main Analysis* window.
2. The *Peak Table* will appear below the sample electropherogram.
3. 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.
4. Choose to *Edit Allele, Edit Comments, Add/Delete Allele*, and *Confirm*. **See Chapter 4 Main Analysis Overview**.
5. 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 has been made to that allele.
6. Right-click any changed allele and select *View History*.
7. The *Show Edit History* window appears.
8. Select a change from the *Edit History List* to view changes in the *Current/Old Values* table. Changes will be highlighted in red.
9. To recover a change, right-click the row in the *Edit History List* and select **Recover Old Value**. A star will appear in the *Recover* column.
10. Click **OK** and click **Yes** when the warning prompts you to confirm.



Edits History Window



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