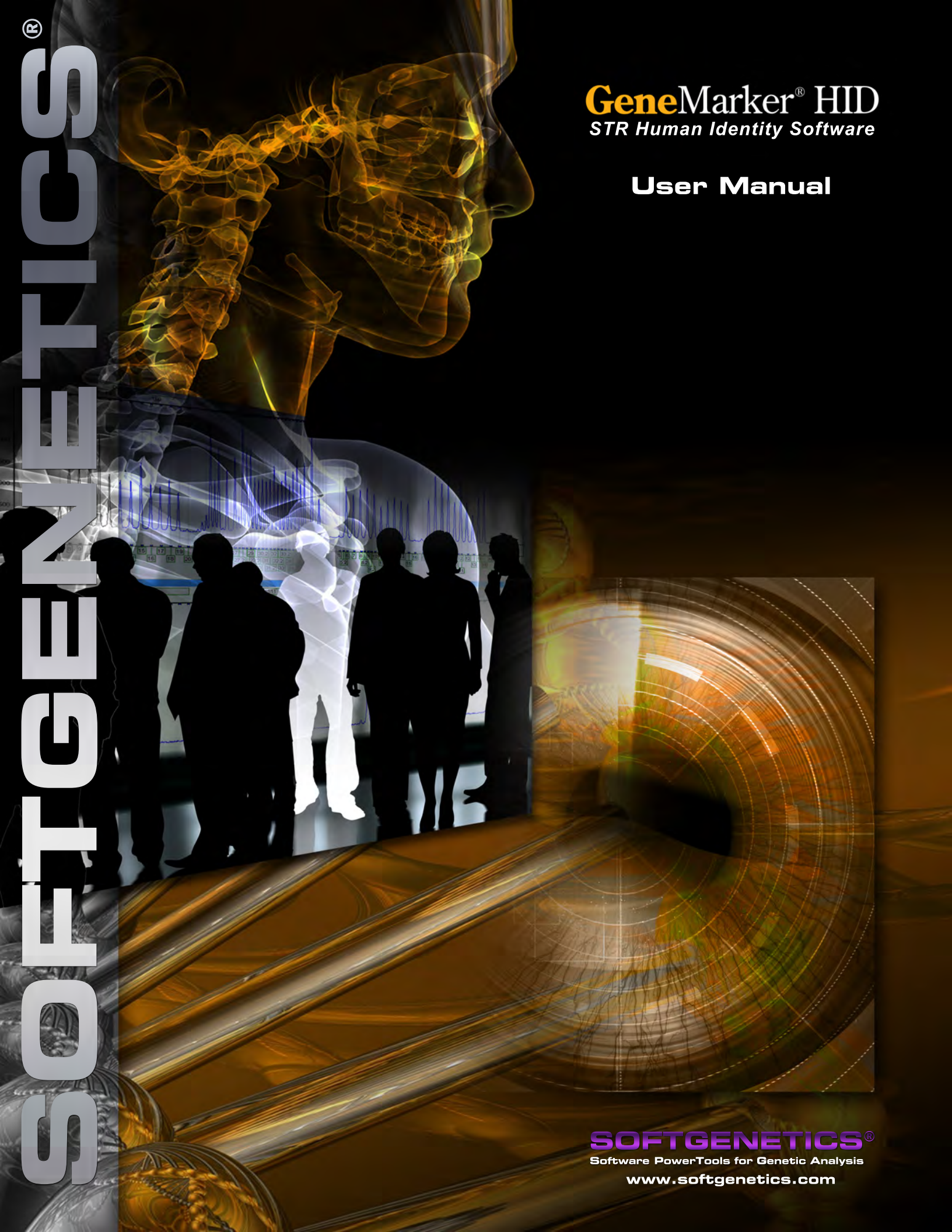

GeneMarker® HID

STR Human Identity Software

User Manual

SOFTGENETICS®

Software PowerTools for Genetic Analysis

www.softgenetics.com

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Table of Contents GeneMarker® HID v.2.6.0

Chapter 1 Installing GeneMarker HID

Computer System Requirements

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

Windows® PC
OS: Windows® XP, Vista, Windows® 7
Processor: Pentium® III, 1 GHz CPU
RAM: 512MB
Available hard disk space: 20GB

Intel® Powered Macintosh®
Parallels® desktop for Mac (Mac OS/virtual machine dependent) or Apple™ Boot Camp or VMware® Fusion (Mac OS/virtual machine dependent)
RAM: 2GB
Available hard disk space: 20GB

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

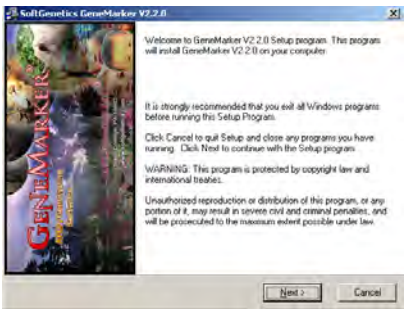
GeneMarker HID will only recognize PC file formats. To convert Macintosh file formats to PC file formats, please download the ABI PRISM® 3100 Genetic Analyzer Conversion Utilities to convert Mac files to PC files at: **http://www.appliedbiosystems.com/support/software/3100/conversion.cfm**

Validation Version

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

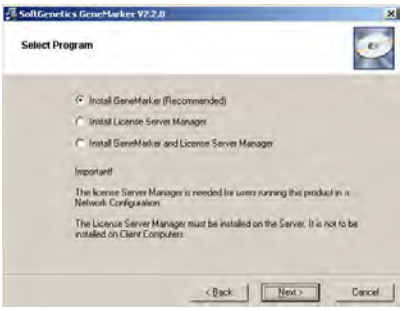
Installation

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 GeneMarker HID 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* and click the **I Agree** button in the *Read Me File* window
6. Select "Install GeneMarker HID (Recommended)" in the *Select Program* window and click Next
7. Click **Next** in the *Destination Location* window to install GeneMarker HID in the default folder. Click the **Browse** button to choose a different installation directory



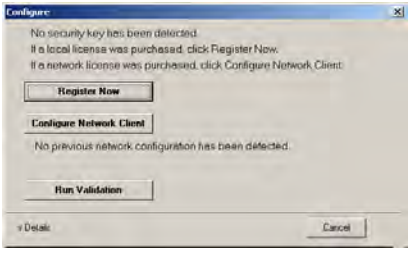
NOTE: The default *Destination Location* for the GeneMarker HID program is C:\ProgramFiles\SoftGenetics\GeneMarker HID\"version number"

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 GeneMarker HID
10. Click **Finish** in the *Installation Complete* window
11. The *Installation Wizard* will close
12. Eject the *SoftGenetics CD*
13. Launch GeneMarker HID by double-clicking the **GeneMarker HID** desktop icon *OR* open the **Start** menu and navigate to *SoftGenetics → GeneMarker HID, the version that was just installed → GeneMarker HID 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

GeneMarker HID v2.0 and above supports text-based registration for the local-licensing option—no USB device, dongle, key or hardware is required. This text-based registration ID is registered to one specific 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 will be registered to the new PC.

Installation

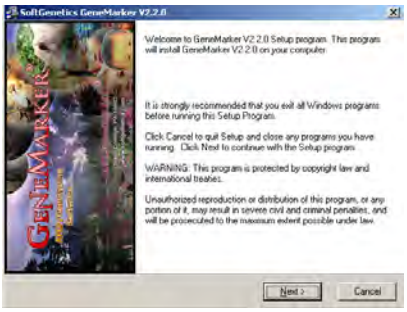
1. Insert the *SoftGenetics CD* into the optical or CD-ROM drive. If your computer is not set to automatically open a CD, navigate to the optical or CD-ROM drive on the computer and open the directory.
2. Double-click the GeneMarker HID Setup executable file (EXE)
3. The *Installation Wizard* will launch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

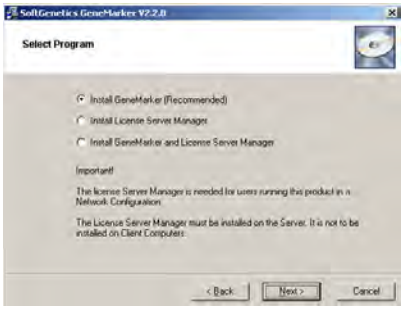
NOTE: The default *Destination Location* for the GeneMarker HID program is C:\ProgramFiles\SoftGenetics\GeneMarker HID\ver#

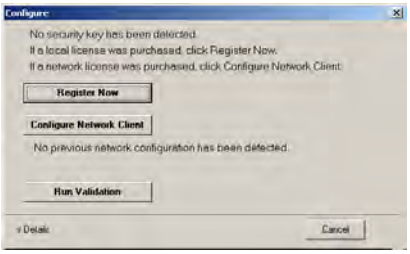
8. Click **Next** in the *Select Program Manager Group* window to accept the default *Program Manager Group*

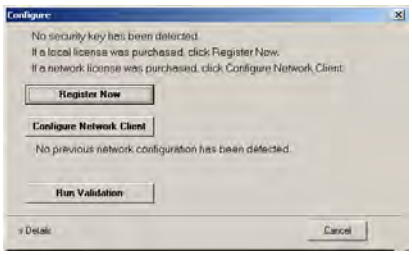
NOTE: Changing the *Program Manager Group* default may affect program operability. It is recommended to accept the default.

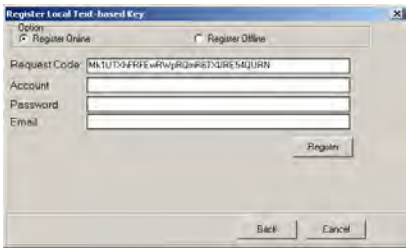
9. Click **Next** in the *Start Installation* window to install GeneMarker HID
10. Click **Finish** in the *Installation Complete* window
11. The *Installation Wizard* will close
12. Eject the *SoftGenetics CD*
13. Launch GeneMarker HID by double-clicking the **GeneMarker HID** desktop icon *OR* open the **Start** menu and navigate to *SoftGenetics → GeneMarker HID, the version that was just installed → GeneMarker HID program*
14. The *Configure/Registration* window will appear. Click **Register Now** to register the local license
15. Click **Register Local Text-based Key** from the *Choose Registration Method* dialog box











Registration

1. The *Register Local Text-based Key* window appears
2. If the computer GeneMarker HID 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

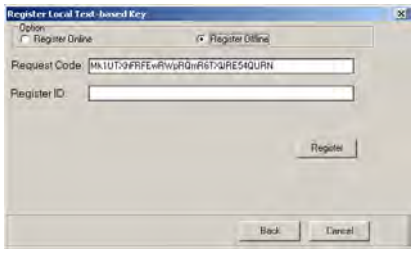
A. Locate the **Account** and **Password** on the *SoftGenetics CD*.
B. Enter your *Account*, *Password,* and *e-mail address* information in the appropriate fields
C. The *Request Code* information is automatically generated by GeneMarker HID

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 GeneMarker HID and begin analysis

Offline Registration



A. Copy and paste the entire *Request Code* string and type your *Account* and *Password* information from the *SoftGenetics CD* into the body of an e-mail
B. Send the email to tech_support@softgenetics.com
C. The *Registration ID* will be sent to you (via email) within one business day
D. Copy and paste the *Registration ID* from the e-mail into the *Registration ID* field
E. Click **Register**
F. Launch GeneMarker HID and begin analysis

Upgrade

Installing Over the Previous Version

If you choose to install the new version of GeneMarker HID over the previous version, you will need to choose the same directory for installation. Several of the old files will be replaced with newer files. Other files that are not present during installation but are created during analyses or by the user will remain in the folder and can easily be recognized by the new version of GeneMarker HID. Please make a backup copy of the target directory before installing the newer software.

Installing into a New Directory

If you choose to install the newer version in a different location, be sure to specify a unique directory name or Program Manager Group for the upgrade to prevent overwriting any previous versions of GeneMarker HID. Several files created by the users or created during analyses conducted by the previous version of GeneMarker HID will not be recognized by the new version of GeneMarker HID unless they can be found in the directory of installation. If you intend for the new version to recognize these files, then you will need to copy them from the older version's installation folder and paste them in the folder containing the new version of GeneMarker HID.

Some of the more common customized GeneMarker HID files are: GeneDB.mdb, GeneMarker HID.mdb, codis.ini, CommentsTemplate.ini, ExpTemplates.ini, Panel folder and SizeStd folder.

Upgrade Procedure – Text-based

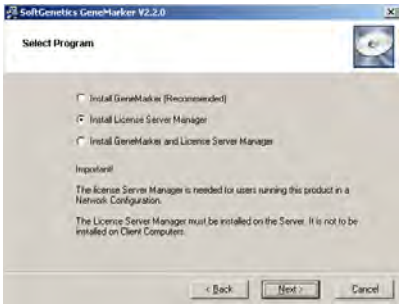
1. Before proceeding, prepare a backup copy of the previous version of GeneMarker HID (recommended)
2. Double-click the GeneMarker HID executable file (EXE) on the *SoftGenetics Upgrade CD*.
3. Proceed through the *Installation Wizard* as described in the *Installation* section above
4. Once the *Installation Wizard* is complete, launch GeneMarker HID by double-clicking the new **GeneMarker HID** desktop icon *OR* open the **Start** menu and navigate to *SoftGenetics → GeneMarker HID, the version that was just installed → GeneMarker HID* program
5. The *Configure/Registration* window appears. Click **Register Now** to register the local license
6. Click **Register Local Text-based Key** from the *Choose Registration Method* dialog box
7. Proceed through the *Registration* steps as described in the *Registration* section above
8. Launch GeneMarker HID and begin analysis

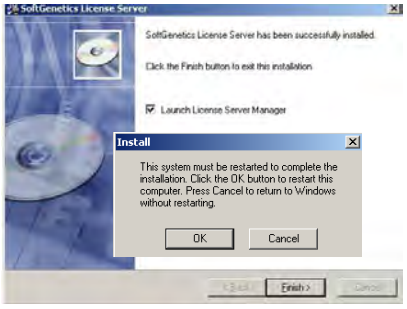
Network-licensing Option

The network-licensing version of GeneMarker HID 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 GeneMarker HID v2.00 (and above). LSM uses text-based registration—no hardware is required. Both software components 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



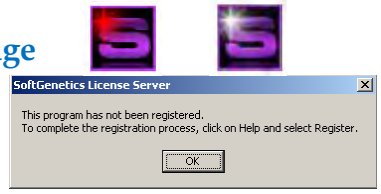


1. Insert the *SoftGenetics CD* into the optical or CD-ROM drive. If your computer is not set to automatically open a CD, navigate to the optical or CD-ROM drive on the computer and open the directory.
2. Double-click the GeneMarker HID Setup executable file (EXE)
3. The *Installation Wizard* will launch
4. Click the **Next** button in the *Welcome* window
5. Read the *SoftGenetics End User License Agreement* and click the **I Agree** button 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* and click the **I Agree** button 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

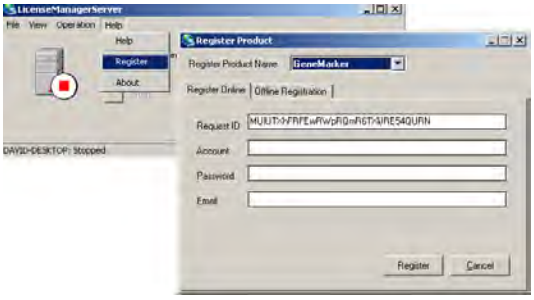
11. Click **Next** in the *Start Installation* window to install License Server Manager
12. Select the Launch License Server Manager option and click **Finish**
13. Click **OK** in the *Install* window to restart the system.
14. The *Installation Wizard* will close and the system will restart
15. Eject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

Register License Server Manager for GeneMarker HID Usage



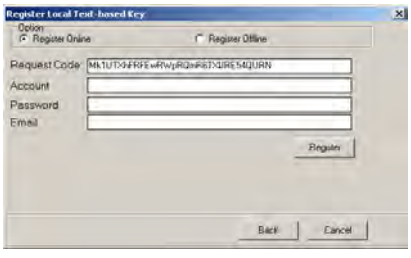
1. Open License Server from the System or Icon Tray by clicking the LSM icon

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



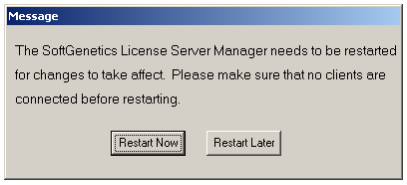
2. Click **OK** in the dialog box to proceed with registering License Server from the License Server Manager console.
3. Select Register from the Help menu to activate the Register Product window
4. Select GeneMarker HID from the Register Product Name drop-down menu.
5. SelecIf the computer License Server 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

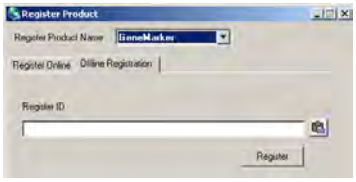
C. The *Request Code* information is automatically generated by License Server
D. Click **Register**
E. Your software will be registered automatically. A confirmation e-mail will be sent to you once registration is complete.



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.

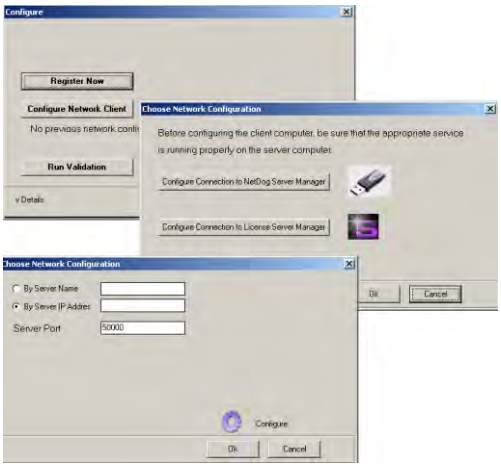
F. Restart License Server to apply the registration information.

Offline Registration



G. Copy and paste the entire *Request Code* string and type your *Account* and *Password* information from the *SoftGenetics CD* into the body of an e-mail
H. Send the email to tech_support@softgenetics.com
I. The *Register ID* will be sent to you (via email) within one business day
J. Copy and paste the *Registration ID* from the e-mail into the *Register ID* field of the Offline Registration tab
K. Click **Register**

Install GeneMarker HID Software on the Client Computer



1. Proceed with installing GeneMarker HID software on the client computer as described in the "Local-licensing Option, Installation" section above until the *Configure/Registration* window appears
2. Click **Configure Network Client** to configure the client software to contact License Server Manager
3. Click **Configure Connection to License Server Manager** from the *Choose Network Configuration* dialog box
4. Input Server Name or Server IP Address
5. Click Configure and GeneMarker HID software will automatically open if connection is properly established and a license is available.

Upgrade of License Server Manager

Activate the License Server Manager console
Proceed with step 3 of "Register License Server Manager for GeneMarker HID Usage" section above

Upgrade of GeneMarker HID Software on Client Computer

Install GeneMarker HID software on the client computer by following the procedure in the "Install GeneMarker HID software on the client computer" section above.
If the network configuration has not changed the software should activate without configuring the IP address of License Server.

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 General Procedure

Chapter 2 General Procedure
Import Data Files
Raw Data Analysis
Process Data
Adjust Analysis Parameters

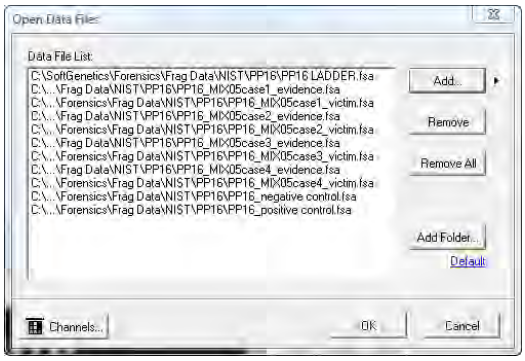
Import Data Files

After installing GeneMarker HID 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 GeneMarker HID. Note: many AB 3500 instruments have an option to export .hid files with normalized peak heights. For greatest accuracy of data analysis, GeneMarker HID reads the non-normalized peak heights of files that are compatible with the software.

ABI - .fsa, .abi, .ab1, .hid
MegaBACE - .rsd
Beckman-Coulter - .esd
Spectrumedix - .smd
Generic - .scf, .sg1

Procedure

1. Launch **GeneMarker HID**
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 GeneMarker HID



Features

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

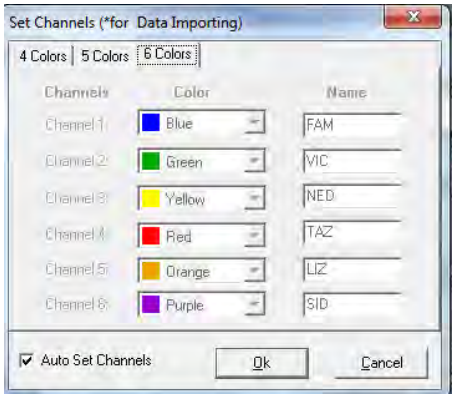
Add…
Used to locate and select raw data files for upload. Click the arrow button next to the **Add** button to see the four most recently accessed directories.

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 GeneMarker HID will always open when the **Add** or **Add Folder** buttons are clicked.

Channels
Opens the *Set Channels* dialog with 4, 5 and 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.

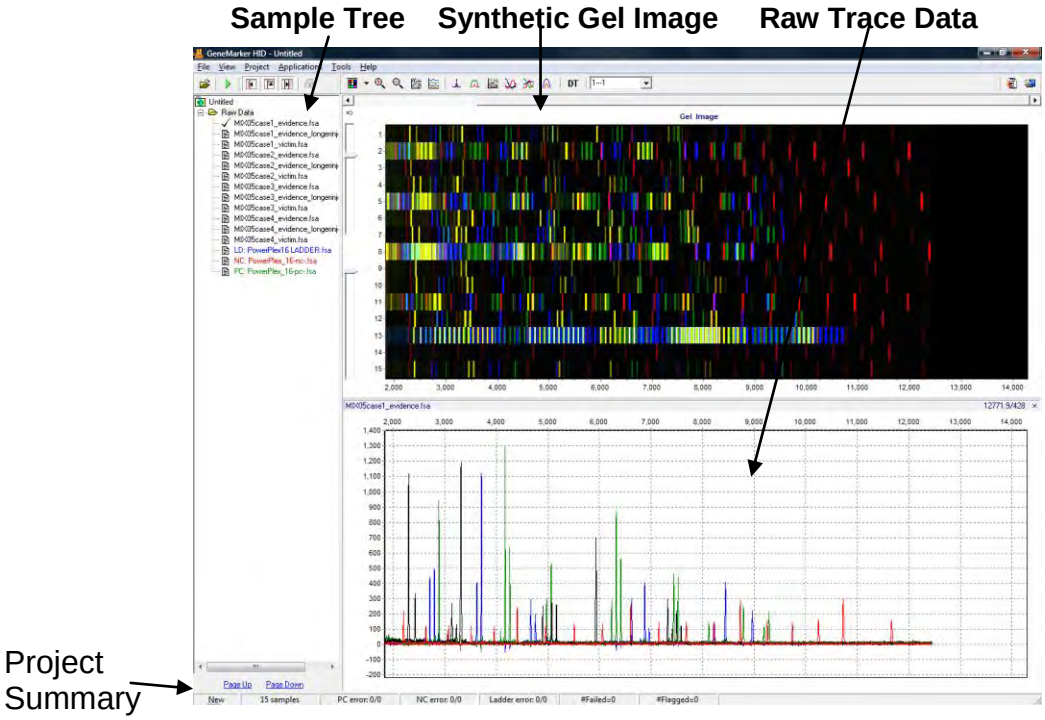


Raw Data Analysis

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.

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.

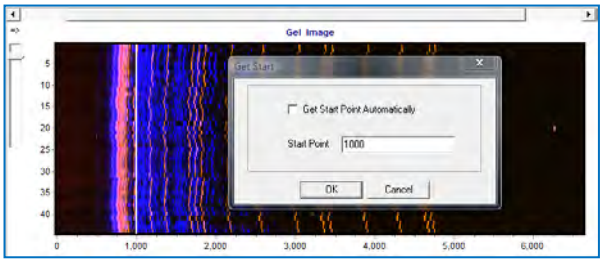
What to Expect

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

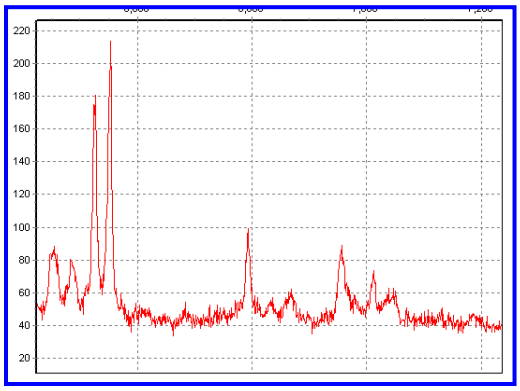
AutoRange - Analyzes from 0 to end of trace for size call
Manual Range – user-defined range

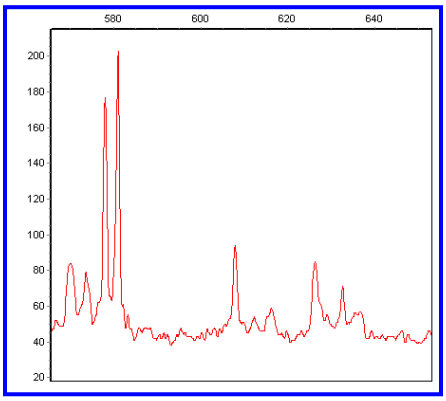
Right-click in gel image and select **Get Start Point**



Smooth

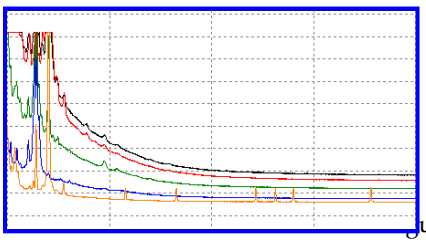
Fourier frequency transformation (FFT) to determine frequency domain
Use only top 40% of lowest frequencies
Smoothing broadens peaks and therefore you can lose resolution
Enhanced Smooth - Same as Smooth but use only top 20% of lowest frequencies

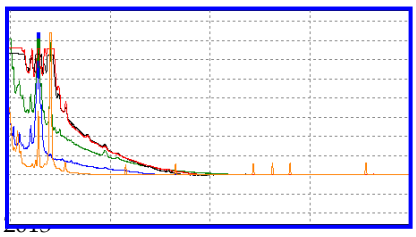




Baseline Subtraction

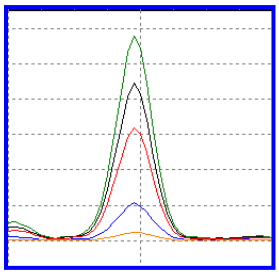
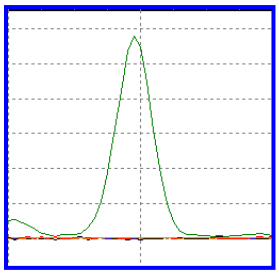
Use 20% of lowest intensities (to the right of the beginning of the range)
Looks at trace in 500-600 frame sections





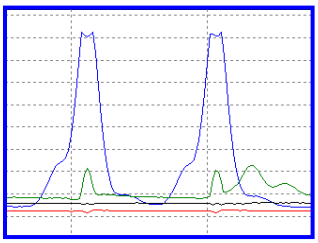
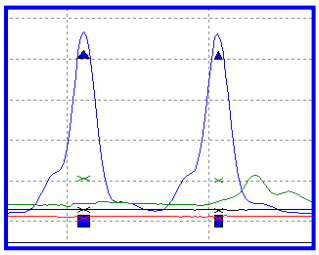
Pullup Correction

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.

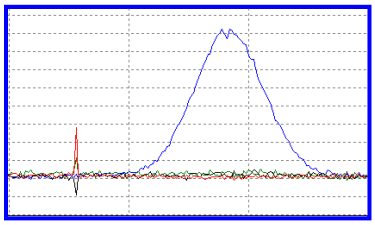
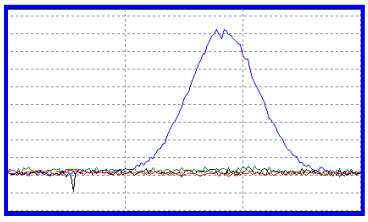
Saturated Peak Correction

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
GeneMarker HID takes the small pullup peak and adds it to the split in the saturated peak

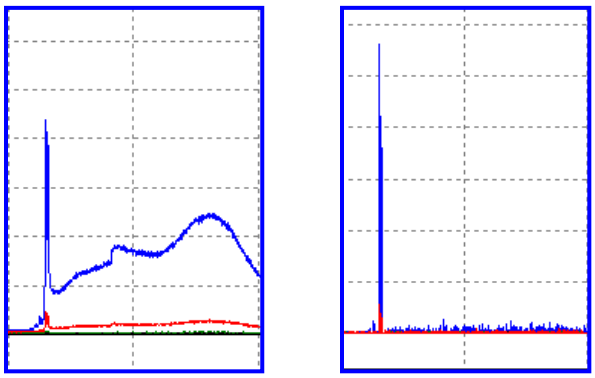



Spike Removal

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 *GeneMarker HID*, 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. *GeneMarker HID* 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 the settings for all sections of the template have been completed (Template Selection, Data Process and Additional Settings) use the Back button to return to the Template selection screen and then select Save. **Note**: only individuals with access rights to change analysis parameters may save Run Wizard Templates.

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

GeneMarker HID comes preloaded with many common kit Panels including Promega's PowerPlex kits and ABI's Identifiler kit. 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 5 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

GeneMarker HID 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 4 Fragment Sizing Standards.**

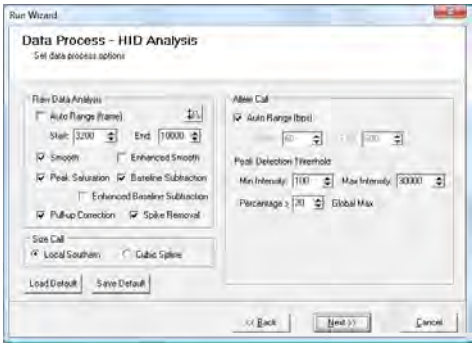
Size Template Editor: This allows the user to check sample files against a selected size standard, modify and save the size standard for future use, or create a new size standard.

Standard Color

Select the dye color which contains the internal lane standard.

Analysis Type

The *Analysis Type* option is inactivated in GeneMarker HID.



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.

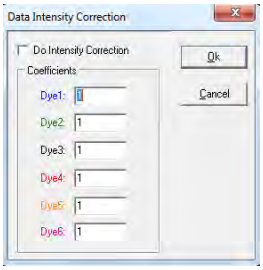
Icons and Functions

Raw Data Analysis

Auto Range (frame)

The range in camera frames will automatically find the processable 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

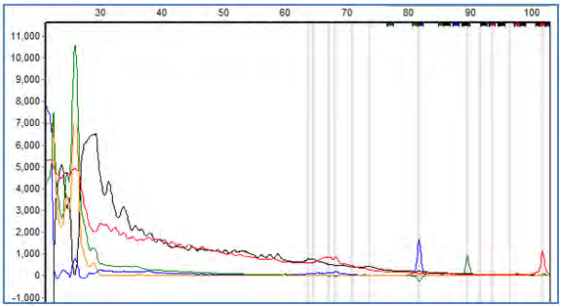
The software will analyze saturated data points by creating a synthetic estimate of the peak shape based on the curves prior to saturation. The results will be less accurate than that of non-saturated peaks.

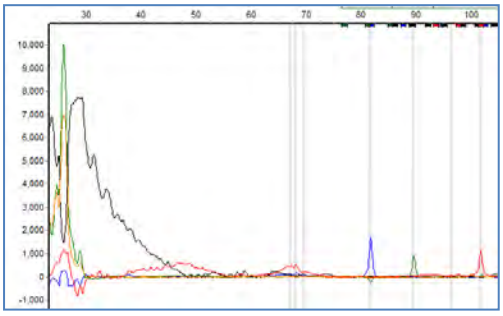
Baseline Subtraction

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

GeneMarker HID offers two sizing methods:

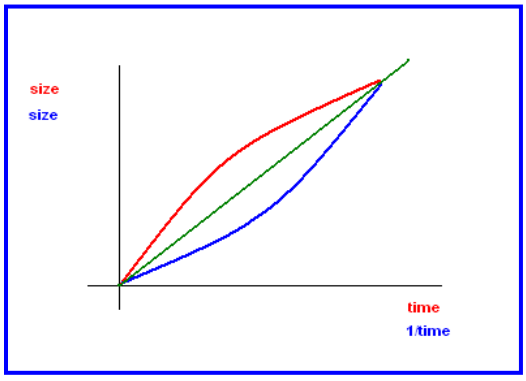
Local Southern

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).

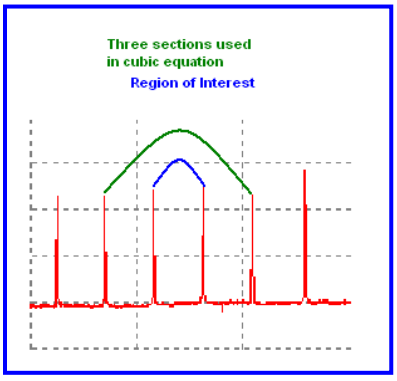
Cubic Spline Method

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 processable 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 5 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

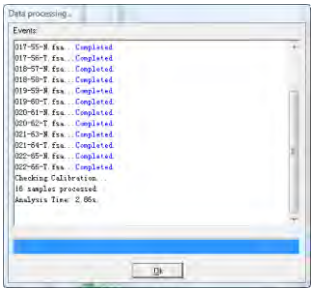
GeneMarker HID identifies ladder samples in the dataset as defined in the *View* → *Preference* → *Forensic* → *Ladder Identifier* field. Ladder samples are then compared to the chosen Panel. Each ladder that is within the range of the selected panel will pass and appears in bold font in the Sample File 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 basepair shift). Ladder samples are identified by GeneMarker HID as defined in the *View → Preference → Forensic → Ladder Identifier* field. Major alleles and variant (or virtual) alleles are specified in the Control Column in the Panel editor. See Chapter 5 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.
NOTE: Panel must be saved with signal information
For optimum Auto panel adjustment. **See Chapter 5** .

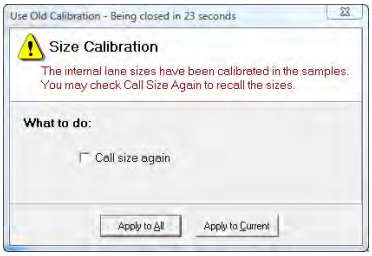
Adjust Analysis Parameters



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 3 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



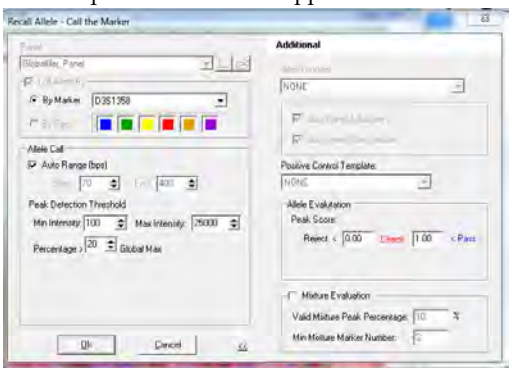
To re-analyze with the Run Wizard tool, simply click the **Run Project** icon in the main toolbar. The Run Wizard will launch and the most recently selected parameters will be displayed. Adjust parameters as necessary and click **OK** in the Run Wizard *Additional Settings* box. The *Use Old Calibration* 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.

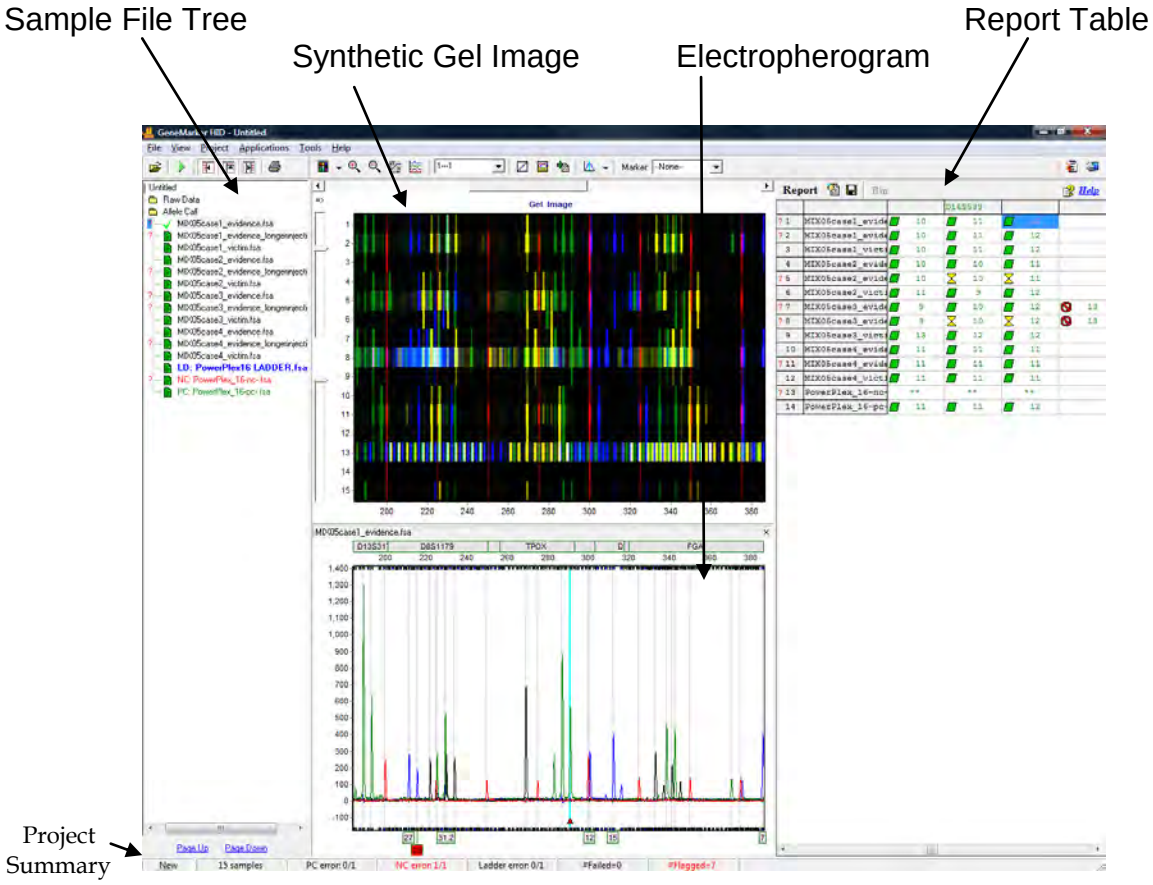
Chapter 3 Main Analysis Overview

Main Analysis Window

The main window of GeneMarker HID 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.

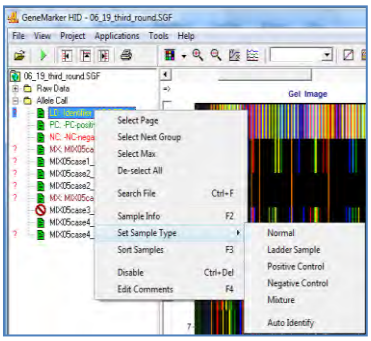
To resize the frames in the *Main Analysis* window, simply place the cursor over the partitions that separate the *Synthetic Gel Image/Electropherogram/Sample File Tree/Report Table*. The cursor will change to a two-headed arrow bisected by two vertical lines. Hold down the left mouse button and drag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

Main Analysis Window



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 2 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 4 Fragment Sizing Standards.** **A red question mark**

indicates one or more of the analysis parameters were not met for that sample (rules were fired). Detailed flagging is found in the allele label and the peak table below the electropherogram in the quality reasons column.

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

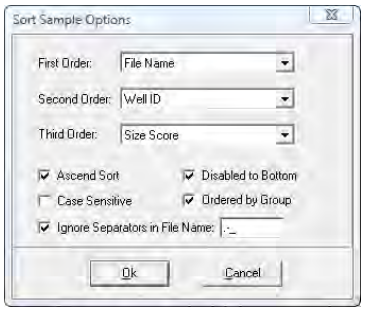
Opens electropherogram traces for the number of samples specified in the *View → Preference → Display Settings → Max # Open Charts.*

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, and Size Score. **Hot Key = F3**



Search Options

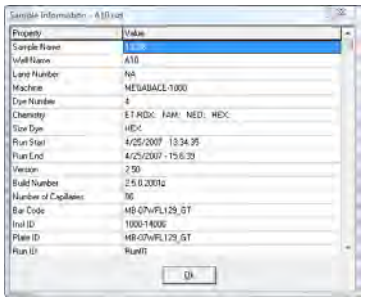
Search File

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**



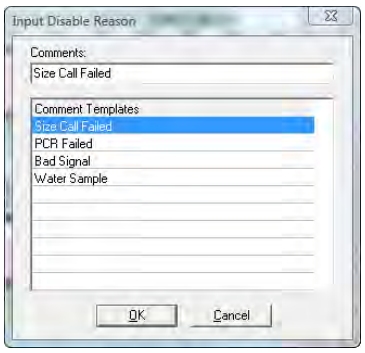
Edit Comments

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 6 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.

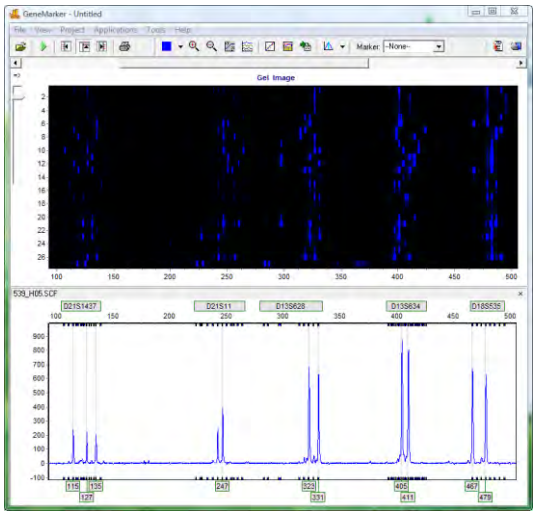
Sample Grouping

From the main toolbar, select *Project* → *Apply Sample Grouping*. The *File Name Group Editor* tool will appear (**See Chapter 8 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 GeneMarker HID 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.

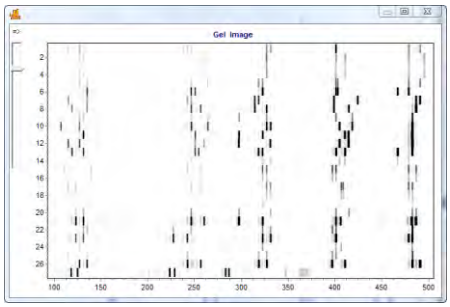
Show in Window

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 basepair sizes of the fragments. The frame units plotted along the x-axis in the original *Raw Data Analysis* window are converted to basepair 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

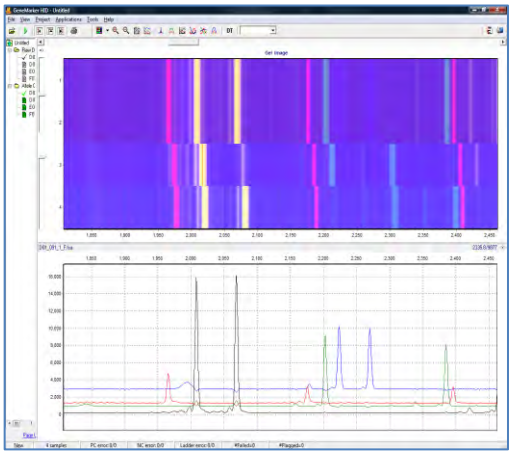
The basepair size range (x-axis) is as set in the Run Wizard *Data Process Allele Call* options box. The RFU range (y-axis) is variable and will re-adjust according to the maximum peak height in the trace. To manually set x and y-axis ranges, use the **Set Axis** icon in the main toolbar.

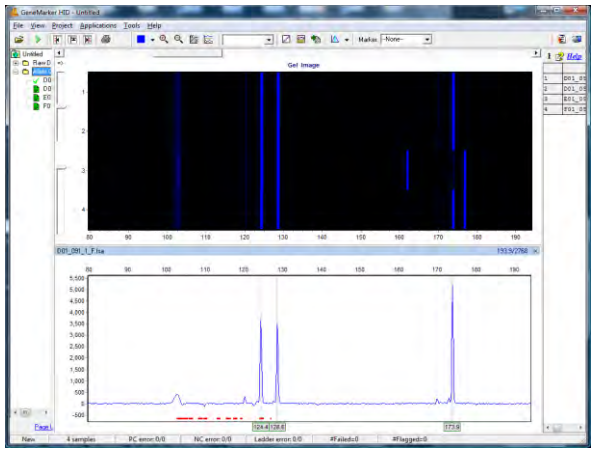
Cursor Locator

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 basepair size of the peak.

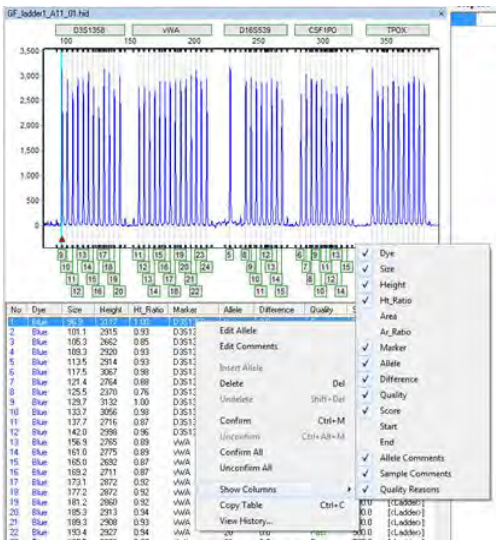




The red horizontal line (seen here in the figure on the right) is to alert analysts to trends in the data. These areas of the data have a more elevated baseline or noise-to-signal ratio (often associated with poorly resolved peaks) than the nearby regions of the trace which sometimes masks very minor peaks.

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 basepair 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 basepairs 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 2 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.

Allele Comments
Software and user edited comments appear in the *Comments* column.

Sample Comments
Added by the user with a right mouse click on the sample name in the sample list at left; select Edit Comments to add new comment or select a comment from the list

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 basepair 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. Print preview and print or save as jpeg, png or pdf for paperless record of audit trail. **See Chapter 9 User Management**.

Report Table

The *Main Analysis* window *Report Table* contains additional information about sample peaks. **See Chapter 6 Reports and Printing**.

Navigation

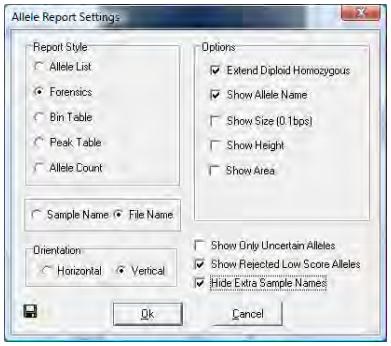
The *Report Table* is linked to the other frames in the *Main Analysis* window. Double-click on the desired allele *OR* use the **Arrow keys** to move to the cell of interest and hit **Enter** key *OR* use **Alt + Arrow keys** to move to different cells and zoom in on the peak in the *Electropherogram*.

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).

Peak Information

Hold down **CTRL** key and click the peak cell of interest. The *Allele Peak Info* box will appear containing information such as Sample, Dye, Size, Marker, Allele, Score and Comments. The information in these fields cannot be edited. This option is only available with Allele List, Marker Table, and Peak Table *Report Styles*.

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.

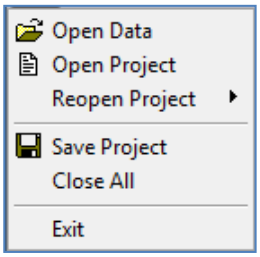
To export only selected cells in the report table, first select the cells by left-mouse drag across the cell range or hold **SHIFT** key and select cells. Right-click on the highlighted cells and select *Copy* (**Hot Key = CTRL+C**). The information is saved to the Windows clipboard and can be pasted 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 GeneMarker HID. Accepted file formats include .fsa, .ab1, .abi, .scf, .rsd, .esd, .smd, .smr. **See Chapter 2 General Procedure.**

Open Project

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

Re-Open Project

Saves the last four projects that were opened by *GeneMarker HID* and allows the user to launch any one of those four projects directly.

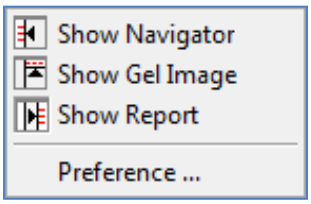
Save Project

Saves a SoftGenetics *GeneMarker HID* project (.sgf, .sfp) to a specified directory. Raw data files and analyzed data files with edits are saved within a project file. Pull-down peaks may result from changes in optical alignment or polymer of the genetic analyzer. These data are now included in the traces of saved GeneMarker HID Projects (.SGF files).

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

Exit
Closes the *GeneMarker HID* program.

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

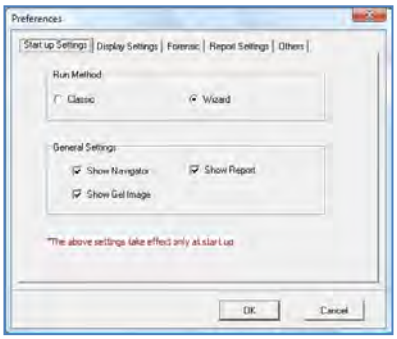


Show Navigator/ Gel Image/ Report
Toggles the *Sample File Tree, Synthetic Gel Image*, or *Report Table* frames open and closed in the *Main Analysis* window.

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 Gel Image: When selected, the *Synthetic Gel Image* 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.

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: Select this option to flag peaks detected in variant allele bins of allelic ladders.
Show All Allele Labels: Will display all allele labels when all dyes are displayed in the electropherogram (default is to display allele labels of dye 1 only when all dyes are overlaid in the electropherogram)

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.
Show Saturation Alert Line: Displays a red, horizontal line under the X axis in regions where saturation and/or elevated baseline were detected in the raw data

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.

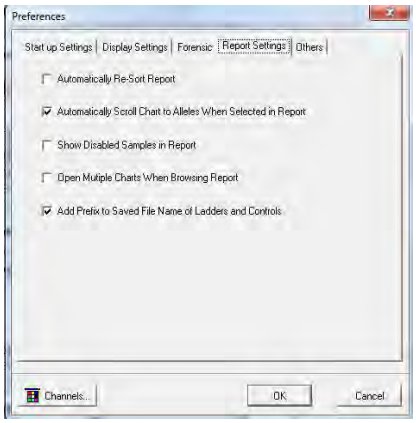
Forensic

The *Forensic* tab allows the user to determine the display of Ladders and Controls in the *Report Table* and to establish file labeling conventions for Ladders and Controls.
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, GeneMarker HID 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, GeneMarker HID 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, GeneMarker HID 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*.

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 GeneMarker HID 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.

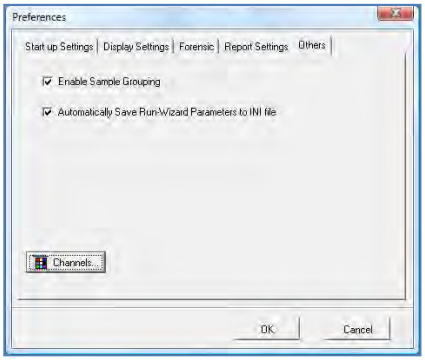
Show Disabled Samples in Report: GeneMarker HID identifies samples that failed during electrophoresis or size calling. The default setting excludes the disabled samples from the report. The option may be selected to have failed or user-disabled samples to be identified in the report.

Add Prefix to Saved File Name of Ladders and Controls: Automatically adds the prefix Ladders_ to the allelic ladder file names when saving report, and Controls_ prefix to any samples designated as PC or NC (set in the Forensic Tab of Preferences)

Others

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

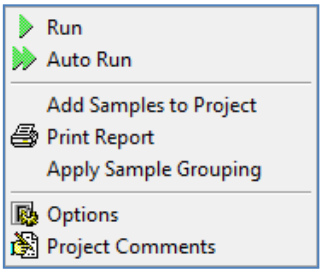
Enable Sample Grouping: When *Project* → *Apply Sample Grouping* is implemented, the *Enable Sample Grouping* option will be automatically selected. De-select *Enable Sample Grouping* to inactivate the *Apply Sample Grouping* option. The *Apply Sample Grouping* information is saved and can be recalled by selecting *Enable Sample Grouping*. **See Chapter 8 Additional Tools – Filename Group Editor.**



Automatically Save Run Wizard Parameters to INI file: 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.

Channels: Opens the *Set Channels* box and allows the user to choose from ABI, MegaBACE, and Beckman-Coulter standard dye colors. The user can also manually enter dye color and name. The default channel color setup is ABI. **See Chapter 2 General Procedure**.

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
GeneMarker HID 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.

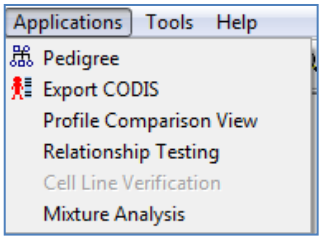
Add Samples to Project
The user can add samples to a project that has already been sized and analyzed. When selected, the *Open Data Files* box will appear. Click **Add** to select individual files to the project and click **OK**. The raw data file will be sized and processed with the same settings as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

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 2 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.



Pedigree
Display and check genotype calls using a pedigree chart. Data must be run with the correct size standard and Panel prior to using the *Pedigree* function. **See Chapter 7 Relationship Testing.**

Export CODIS
Developed for forensic scientists analyzing short tandem repeat fragment data. Exports the CMF 3.2 (.xml) and CMF 1.0 (.dat) files for upload into the FBI's CODIS database. **See Chapter 6 Reports and Printing.**

Profile Comparison View
Allows the user to graphically display any combination of samples and dye colors. This feature includes a 2-Dimensional and a 3-Dimensional view of the selected samples.

Relationship Testing
Contains tools for familial search, identities duplicate samples and potential near relatives from the relationship testing database, provides likelihood ratios for each match; kinship analysis (parent/child, sibs, half-sibs, aunt/uncle, grandparent and cousin) and automated pedigree drawing with deduced genotype of missing parent based on child(ren) and available parent where possible.

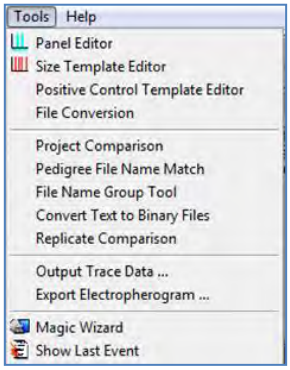
Cell Line Verification
This Application is currently under development. It will function in a similar way to the Relationship Testing and will provide immediate authentication of cell line based on published genotypes.

Mixture Analysis
GeneMarker HID identifies the presence of potential mixture samples, designates allele peaks, and calculates peak area or height ratios in the main analysis screen. The Mixture Analysis Application is activated from the Applications menu in the main analysis screen. Mixture analysis identifies the

mixture samples and any single source contributor samples in a file name tree, considers all possible allele combinations, calculates the Mixture Ratio, residual score, heterozygous imbalance for each genotype combination, and calculates the likelihood ratio for single source samples that are potential contributors to the mixture, searches database for single source file or a profile deduced from the mixture sample, matches profiles and calculates the LR.

Tools Menu

The *Tools* menu contains the *Panel* and *Size Editors* in addition to other helpful modules. Chapter 4 contains details on the Panel Editor and Chapter 5 contians details on the Size Template Editor. Please see Chapter 9 for details of all additional tools.



Panel Editor

Provides a variety of tools to adjust, edit, and create control Panels. **See Chapter 5 Panel Editor**.

Size Template Editor

Allows the comparison of sample files against a selected size standard, to modify and save the size standard for future use, or create a customized size standard. **See Chapter 4 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.

Project Comparison

Allows the user to compare the same data set (two different projects) and detect differences based on a number of parameters including peak size and height, quality score, and commented alleles. **See Chapter 8 Additional Tools.**

Pedigree File Name Match

Allows the user to automatically add additional files to a previously created pedigree tree. A .smp file is exported. **See Chapter 8 Relationship Testing.**

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 *GeneMarker HID*. **See Chapter 9 Additional Tools.**

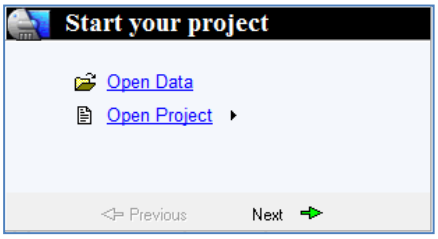
Replicate Comparison

Many labs choose to run and process multiple replicates of their samples. This ensures that a genotype is still available in cases of contamination, allele drop out, or reaction failure. Concordance between replicates can then be used to export the consensus genotype of the sample.

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.*



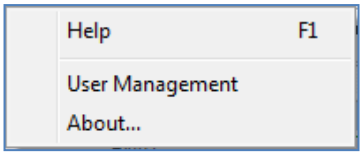
Start Your Project
Allows the user to easily access the *Open Data* or *Open Project* upload windows. The user can also re-open the four previously opened projects by selecting the black arrow next to *Open Project.*

Run
Selecting *Run* launches the Run Wizard. Selecting *AutoRun* will process the data automatically with the process options currently selected. **See Chapter 2 General Procedure.**

Report
Allows the user to *Save Project* or *Print Report*. Selecting *Print Report* will launch the *Print Report Settings* box. **See Chapter 6 Reports and Printing.**

Show Last Event
Opens the last active *Data Process* action.

Help Menu



Help
Launches a searchable version of this manual.

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 GeneMarker HID 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

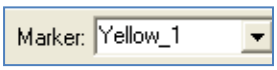
These icons are only available after the raw data has been processed and the *Sample File Tree Allele Call* folder is selected.

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

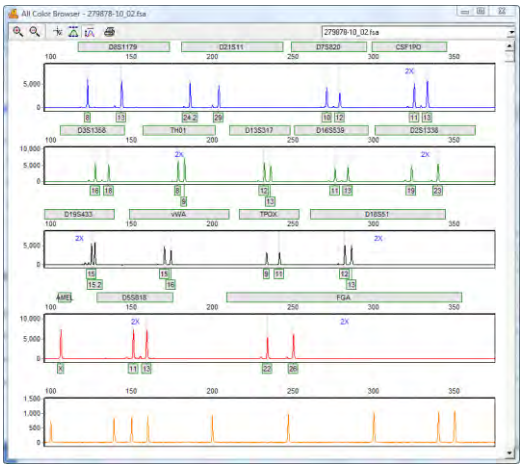
Allows the user to select which bins to include/exclude in the *Report Table*.

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 basepair 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.

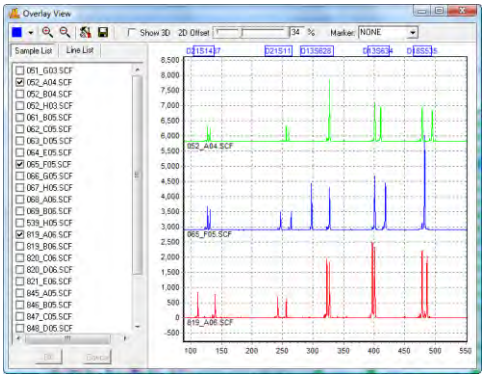
Auto Scale Markers
When selected, the RFU intensities of low peaks are adjusted to match the intensity of the highest peak in the dye color. When low peaks are increased, the intensity magnification factor is noted in the Marker (2X – 8X).

Print
Opens the *Print Report* settings box. **See Chapter 6 Reports and Printing**.

Profile Comparison View

Applications → Profile Comparison View
The *Profile Comparison View* was developed as an easy way to compare several sample traces at once.

Procedure



1. Select several samples from the *Sample List* by placing a check mark in the empty box to the left of the filename.
2. Click **OK**
3. The traces will appear in the window to the right.
4. Slide the *2D Offset* bar to the right to de-convolute the traces.
5. Select the *Line List* tab to open the list of traces present in the viewer. Select any trace to bold the trace line.
6. Select a marker from the drop-down list to view one marker at a time.
7. Select *Show 3D* to see the traces in a three dimensional view.

Icons and Functions

Dye Color
Single click to scroll through the dye colors

Zoom In/Out
Single click to zoom in or out on the center of the *Trace View* window.

Tools
All viewing options are selected by default. Unselect these options to change the *Trace View* settings.

Save
Save the *Trace View* image as a Bitmap (.bmp) file.

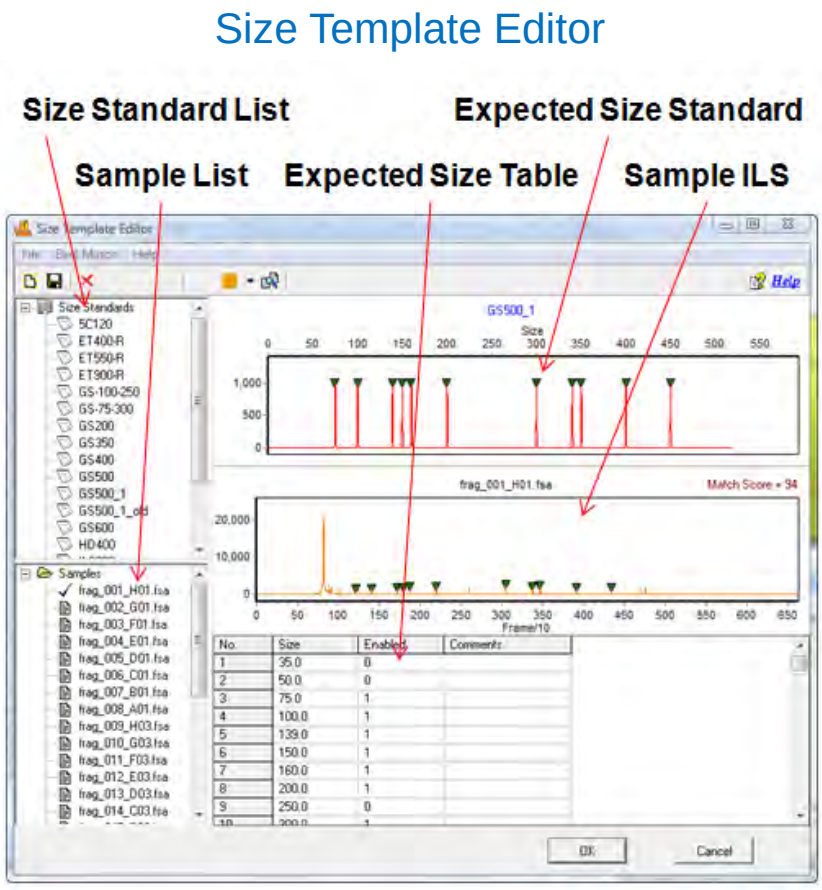
Chapter 4 Fragment Sizing Standards

Size Template Editor

The *Size Template Editor* is a tool in GeneMarker HID 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: GeneMarker HID 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 GeneMarker HID 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 GeneMarker HID 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 GeneMarker HID 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 GeneMarker HID program is closed.

Sample List

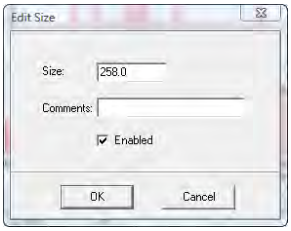
The *Sample List* contains a list of all the samples in the dataset. Double-click the filename and the sample's ILS trace will appear in the *Sample ILS* frame. Use the **Up/Down Arrow** keys to scroll through samples in the list.

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 basepair 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. The size standard files GS500_1 has disabled the 250 bp fragment as recommended by the manufacture due to differences in migration of this fragment. 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. GeneMarker HID 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 3 Main Analysis Overview.**

Procedure

As mentioned previously, Size Standards are created to assign basepair 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 basepair sizes assigned to the peaks in the ILS. GeneMarker HID'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:

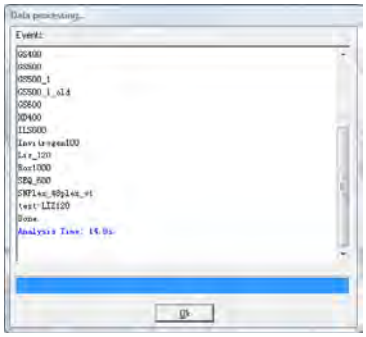
5C120
ET400-R
ET550-R
ET900-R
GS-100-250
GS-75-300
GS200
GS350
GS400
GS500
GS500_1
HD400
ILS500
ILS600
Liz120
Rox1000
SEQ_600

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. GeneMarker HID 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

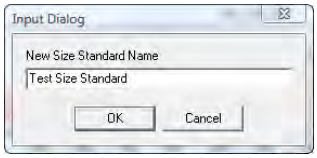


NOTE: *BestMatch* will not always choose the correct Size Standard. User inspection is required.

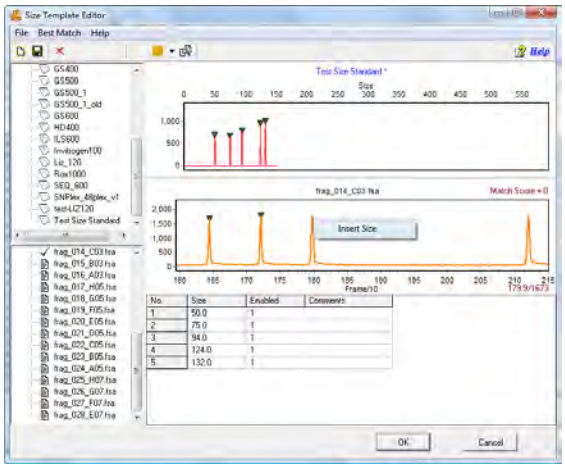
6. Once the Size Standard is chosen, click **OK** in the *Size Template Editor*
7. The selected Size Standard will then appear in the *Size Standard* field of Run Wizard *Template Selection* box and will be used to size the data.

Custom Size Standard Creation

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



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 basepair 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. GeneMarker HID 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 GeneMarker HID 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 GeneMarker HID Size Standard directory.

Import Size Standard

Opens a Windows Explorer window to the SoftGenetics GeneMarker HID 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 GeneMarker HID 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.

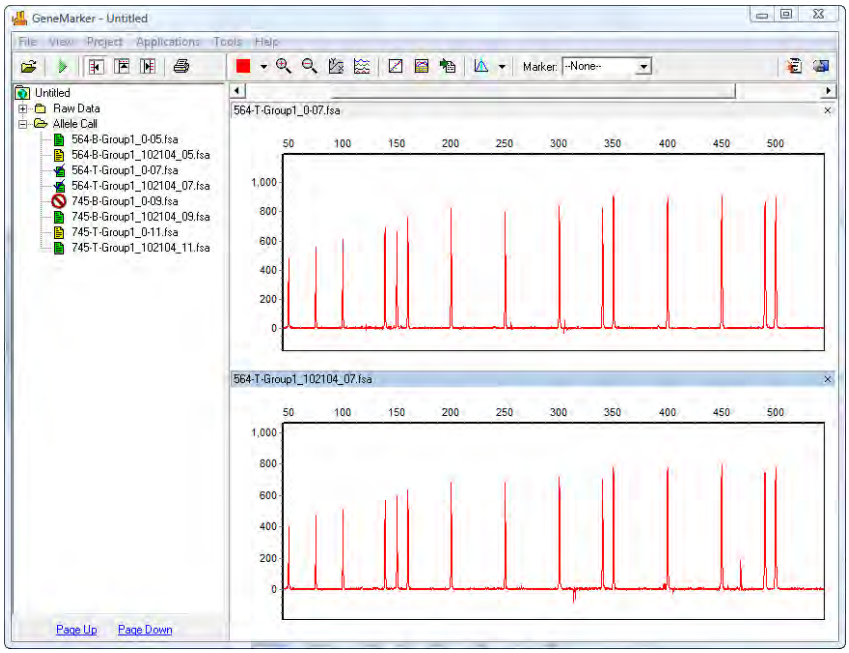
What to Expect

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 GeneMarker HID'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*.

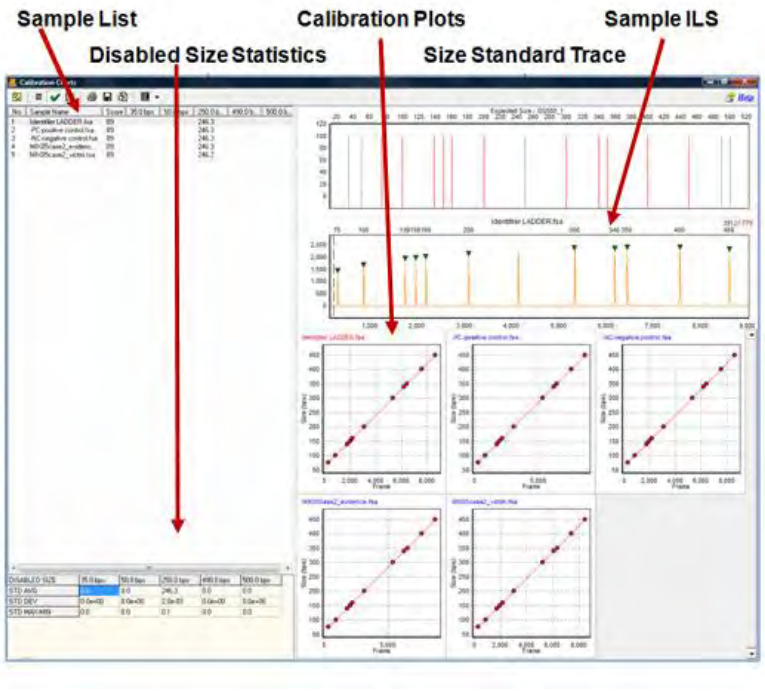
Score

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 basepair 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 basepair 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 basepair position, the standard deviation, and the difference between the maximum and minimum basepair 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 basepair 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 basepair 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 3 Main Analysis Overview**.

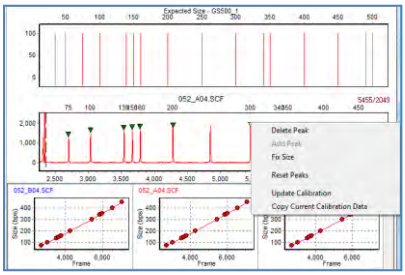
Editing Size Call

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



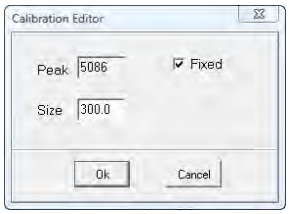
Right-click at the peak position and select *Add Peak*. A green triangle peak indicator will appear at the cursor position. To move the green triangle hold down the **CTRL** key and left-click and drag it to the desired position. The newly added peak will be included in the *Match Score* calculation. 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 basepair 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 basepair 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.

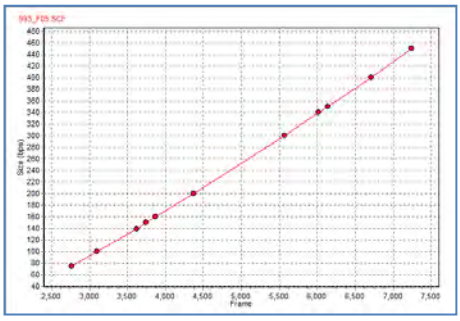
Update Calibration

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 basepair 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 basepair 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.

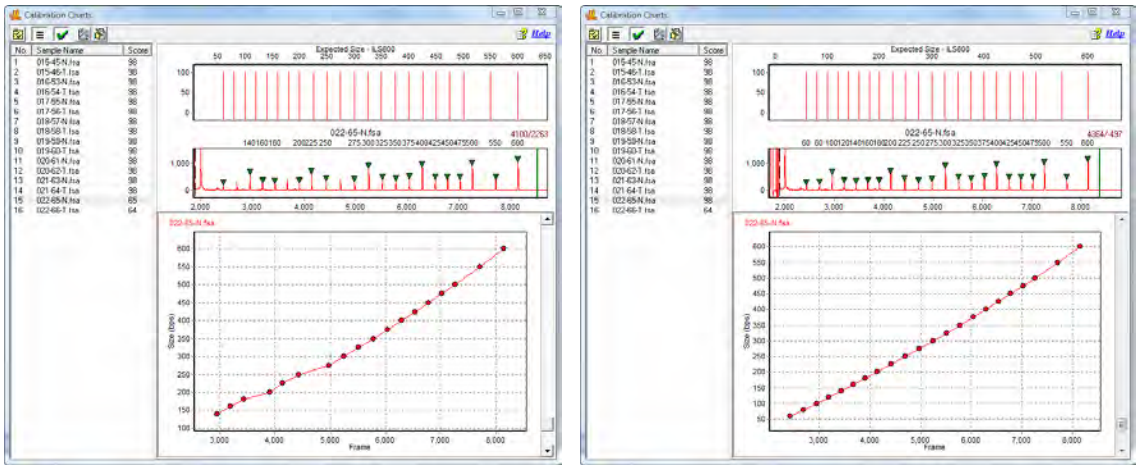
Save
Allows the user to save the size calibration pages as .png images.

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



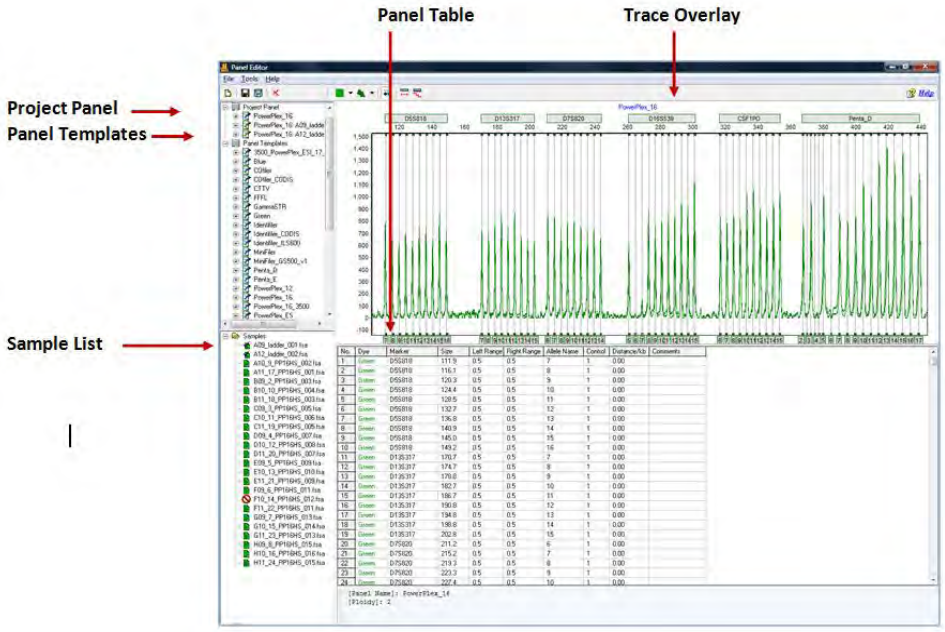
Chapter 5 Panel Editor

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 basepair position of the expected allele. In GeneMarker HID, 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 GeneMarker HID, the vertical gray bars in the electropherogram indicate the center of the detected peak.

Panel Editor

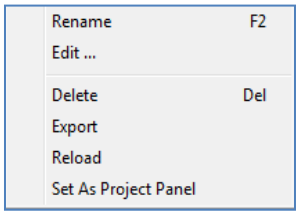


Project Panel

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. GeneMarker HID 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.

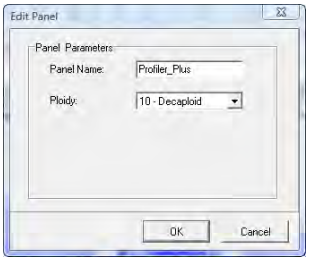
Panel Templates

The *Panel Template List* includes a list of all pre-defined and custom Panels saved to the Panels folder in the SoftGenetics GeneMarker HID 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 GeneMarker HID 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 GeneMarker HID 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 GeneMarker HID program is closed.

Sample List

The *Sample List* contains all the samples uploaded to GeneMarker HID 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.

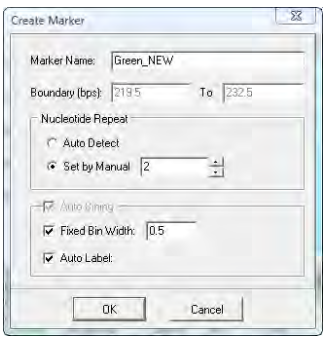
Overlay Trace

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 3 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 basepair 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

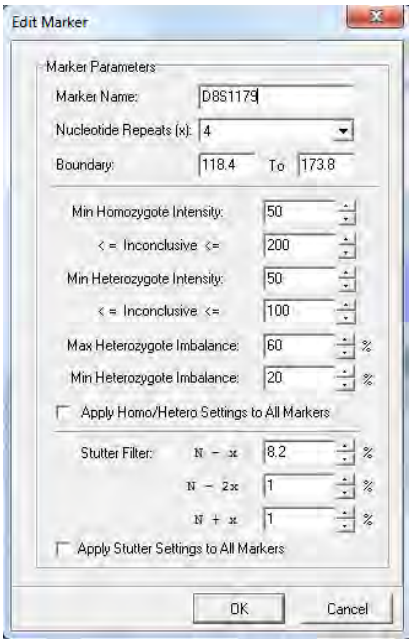
Auto Detect: Based on the peaks present in the *Overlay View*, GeneMarker HID will attempt to detect the number of nucleotides in each repeat unit of the alleles and place Bins at the appropriate interval.
Set by Manual: Select this option if the number of nucleotides in the allele repeat unit is known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

Auto Binning
Fixed Bin Width: Check this option to enter the number of basepairs 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 basepairs.
Auto Label: When deselected, the Bins are automatically labeled with the basepair size of the Bin position to the nearest tenth of a basepair. If selected, the basepair 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 basepairs 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 basepair 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 forensic 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

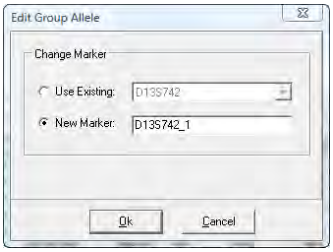
Auto Detect: Based on the peaks present in the *Overlay View*, GeneMarker HID will attempt to detect the number of nucleotides in each repeat unit of the alleles and place Bins at the appropriate interval.

Set by Manual: Select this option if the number of nucleotides in the allele repeat unit is known and GeneMarker HID will place Bins at the specified interval.

Auto Binning

Fixed Bin Width: Check this option to enter the number of basepairs 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 basepairs.

Auto Label: When deselected, the Bins will be automatically labeled with the basepair size of the Bin position to the nearest tenth of a basepair. 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 basepair position of the center of the Bin.

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.

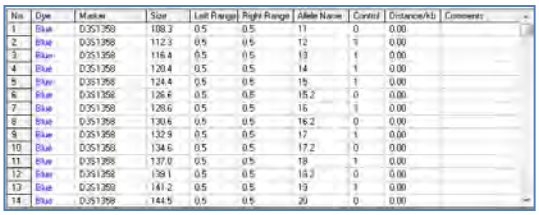
To move a bin, hold down **SHIFT** key and left-click and drag the vertical grey bar in the center of the Bin to the right or left. Let go of the **SHIFT** key and mouse button and the Bin will remain in place. To edit the range of a Bin in the *Overlay View*, click the **Trace Mode** icon to view the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

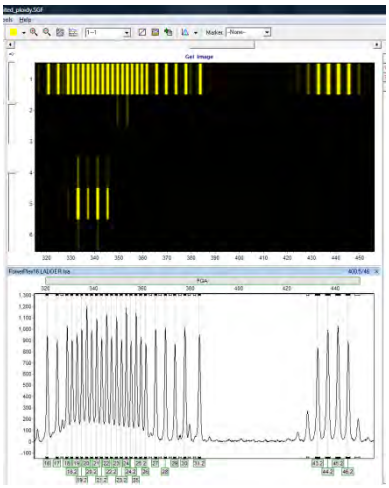
Indicates the position of the Bin center in basepairs.

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 basepairs, of expected peaks. In GeneMarker HID, 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 ABI's Identifiler and Yfiler kits. GeneMarker HID 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 GeneMarker HID's *Panel Editor* tool.

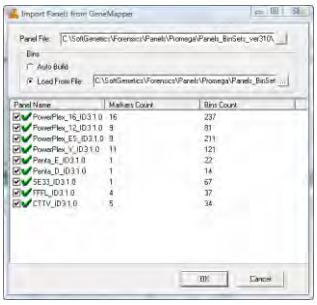
The Panels displayed by default include:

PowerPlex® 16
PowerPlex® S5
PowerPlex® Y
PowerPlex® Y23PowerPlex® ES
PowerPlex®ESX16 and 17
PowerPlex®EXI16 and 17
PowerPlex® 18
PowerPlex® 21
PowerPlex® Fusion
DIPplex®
PowerPlex® **EURO**
GenePrint® FFFL
GenePrint® CTTv
Globalfiler ®AmpFℓSTR® COfiler®
AmpFℓSTR® Identifiler®
AmpFℓSTR® MiniFiler™
AmpFℓSTR® Profiler Plus®
AmpFℓSTR® Profiler®
AmpFℓSTR® SEfiler™
AmpFℓSTR® SGM Plus®
AmpFℓSTR® Yfiler®

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**.

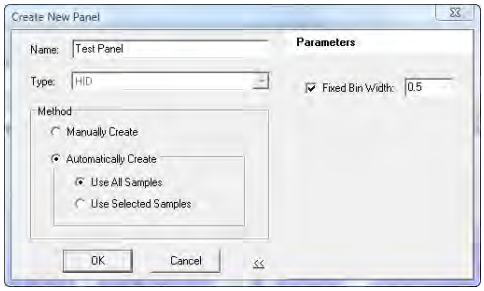
NOTE: Select *Bins Auto Build* if a Bins.txt file does not exist.

7. Click **OK** in the *Import Panels from GeneMapper* box.
8. All Panels in the Panels.txt file will be uploaded into GeneMarker HID.
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 2 General Procedure and Chapter 3 Main Analysis Overview.**

Custom Panel Creation

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

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 GeneMarker HID is HID *Analysis 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. Click the double-arrow button to expand the dialog box and see additional parameters
7. If required, check the *Fixed Bin Width* option and enter a value for the left and right Bin ranges
8. When finished, click **OK**
9. The new Panel will be created and added to the **Panel List**

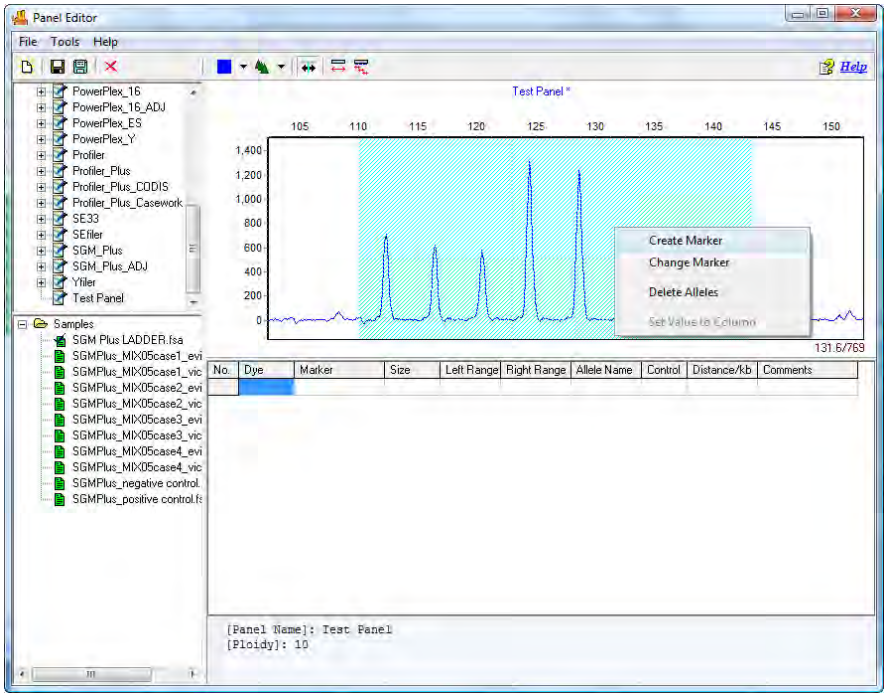
NOTE: New Panels are created based on the *Max & Average View Mode*. More intense peaks are given higher priority for Bin placement when peaks do not overlap perfectly.

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

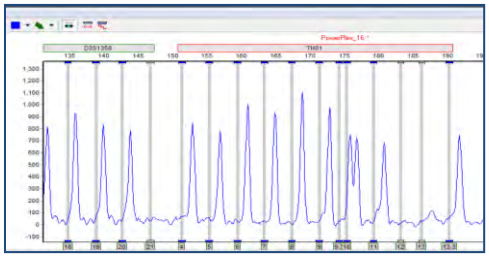
Manual Panel Creation

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 - Manual and Automated Panel Calibration

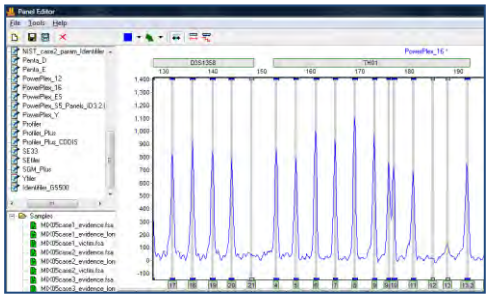


It is common for panel alignment to be shifted due to variations in genetic analyzers or run conditions (such as temperature, injection time). Markers or bins can be manually aligned to the allele ladder using the shift and mouse key or the panel can be automatically calibrated using the auto-select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

the third screen of the run wizard (as part of the analysis parameter template in Chapter 3).

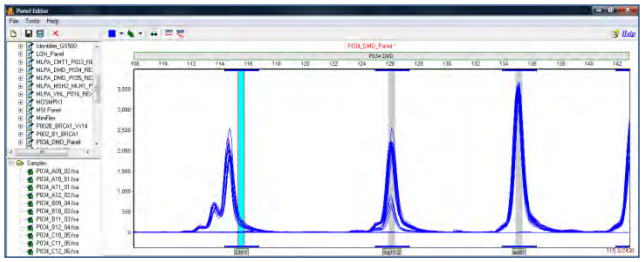
Align all of the bins within a marker

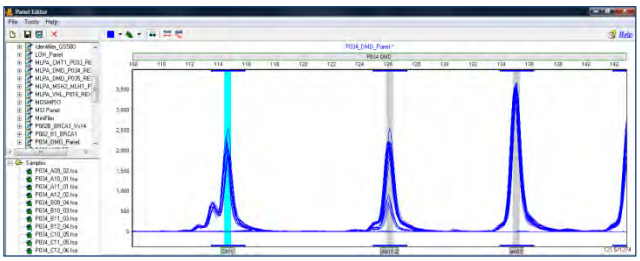
1. Hold down the shift key
2. At the same time place the mouse over the gray marker name bar at the top of the electropherogram
3. The marker rectangle will be outlined in red and the panel name will be in red font when the adjust feature is active
4. Drag the marker to align the bins with the peaks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





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 GeneMarker HID 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 GeneMarker HID 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 GeneMarker HID MLPA Panel folder. Use this option to find additional MRC Holland MLPA Panels.

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 GeneMarker HID.

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.

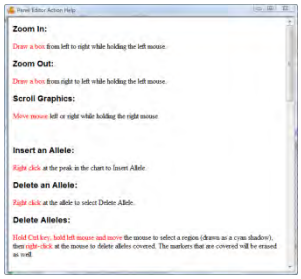
Exit
Closes the *Panel Editor* tool. Be sure to save changes to the Panel before exiting.

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*. GeneMarker HID 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 GeneMarker HID Panel directory.

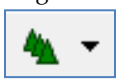
Save Changes with Signal Info
Permanently saves all Panel edits, including height information which is used with the *Major Panel Adjustment* feature.

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.

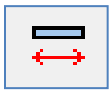


Trace Overlay displays all traces of the selected samples in the *Samples List* one dye color at a time. Single click any trace in the *Trace Overlay* frame and the trace will become bold and the associated sample will be highlighted in the *Sample List*.

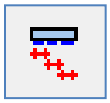
Max & Average displays two traces in the electropherogram. The darker color line corresponds to the maximum peak height at that position and the lighter color line corresponds to the average of all selected sample traces at that position.

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.

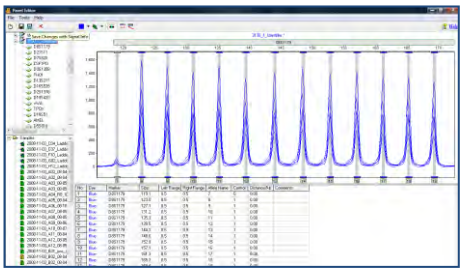


Major Adjustment of Panel Uses previously defined size and height information located in the Panel file when the panel was previously saved with signal information, to identify Marker and Bin positions. To be used when a Panel must be adjusted by 1 - 5 basepairs in order to align with the dataset peaks. **For use only with panels that do not contain variant alleles (0 in the control column)**



Minor Adjustment of Panel Aligns the center of the Bin to the center of the nearest peak (within one basepair of the Bin). . **For use only with panels that do not contain variant alleles (0 in the control column)**

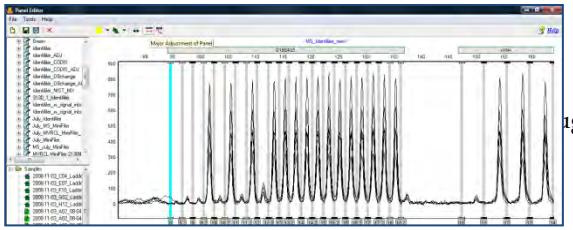
Saving a panel with signal information

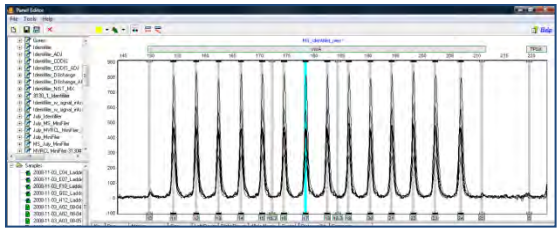


For optimal automatic panel adjustment, a panel should be adjusted manually the first time and then saved with signal information from several ladder samples from the same genetic analyzer and used for analysis of any samples from that analyzer. To manually adjust alignment hold down the shift key and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

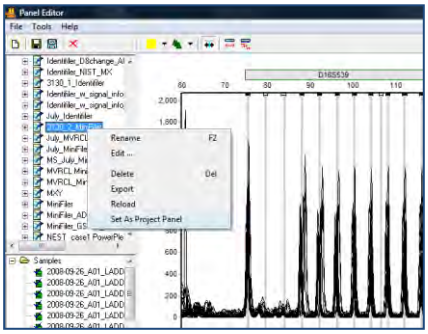
Saving the panel with signal information from the ladders provides the program with the information needed to automatically adjust the panel with subsequent data. A common occurrence is a difference due to run time variation, causing a shift in the peaks of 1-5 base pairs. The major panel adjustment 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).

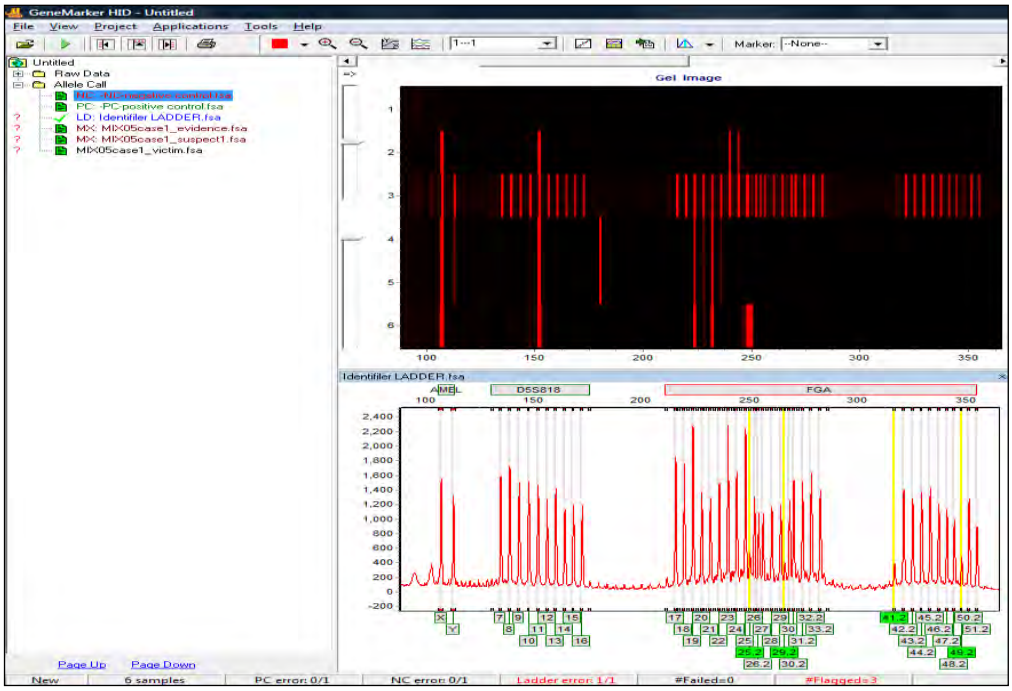


Figure 1 Yellow and green flagging alerts the analyst that although the panel is aligned with the ladder bins, some of the minor peaks in this ladder have a higher RFU than expected.

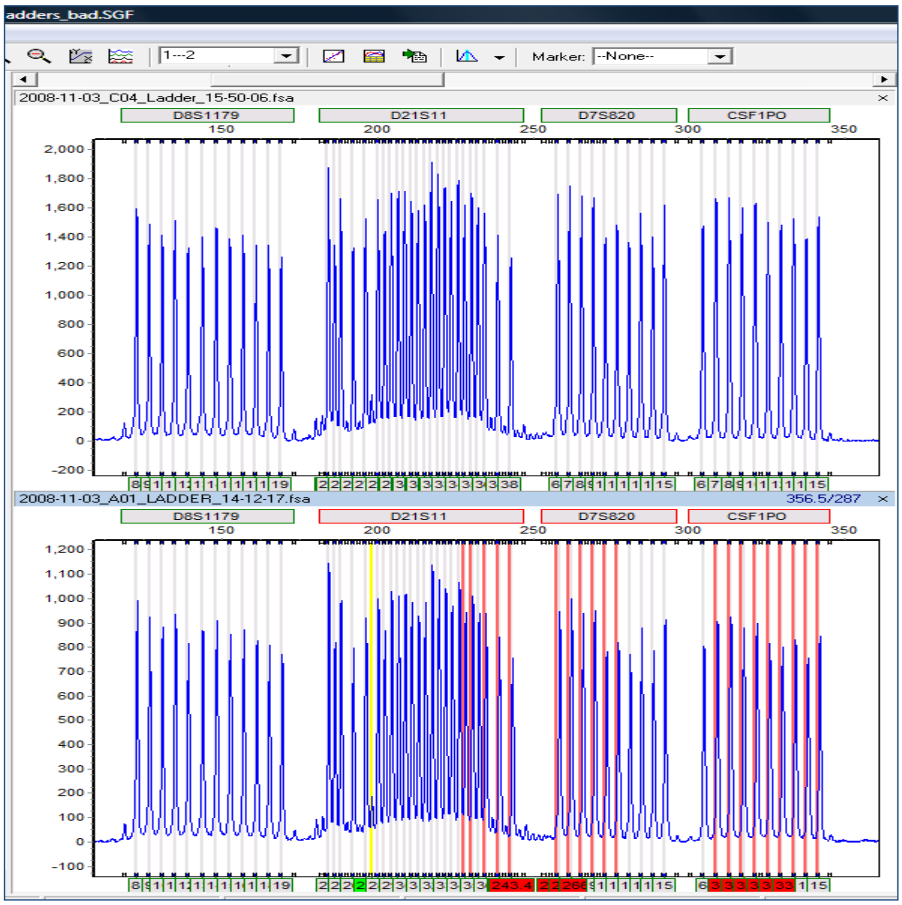
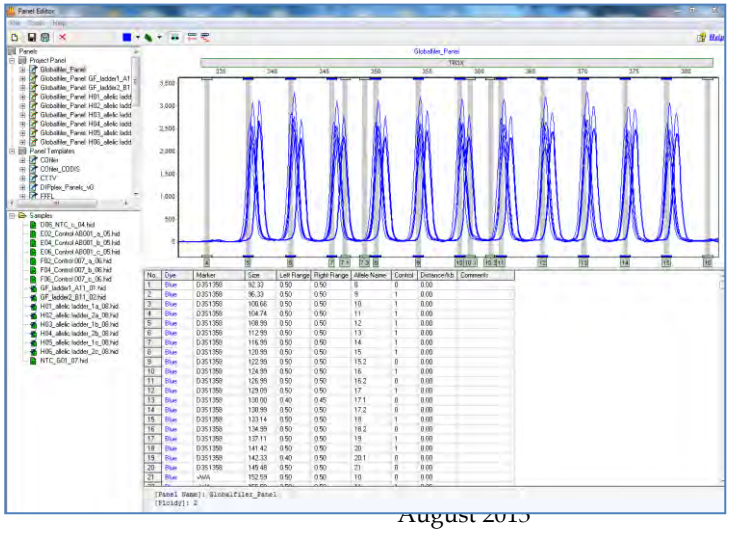
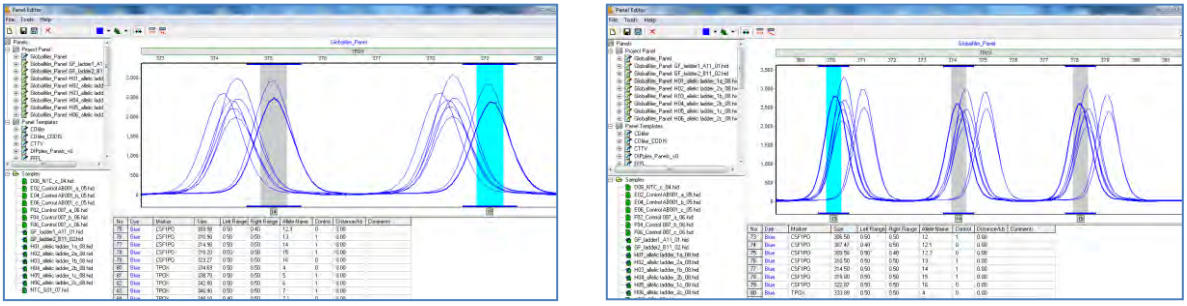


Figure 2 One of the ladder samples in this run has peaks that are out of the bins. These out of bin peaks are flagged with red allele label and red vertical bars

Often on full CE runs, there is slight migration variation from beginning to the end of the run. In the following images we see several allelic ladders that migrated more quickly than the others. The auto-panel adjust and auto select best ladder parameters provide the needed minor shift to align bins (including variant or virtual bins). A list of all passing ladders is located in the Project Panle list under the panel Name. Select the allelic ladder from this list to highlight it in the electropherogram and see the bin alignment.

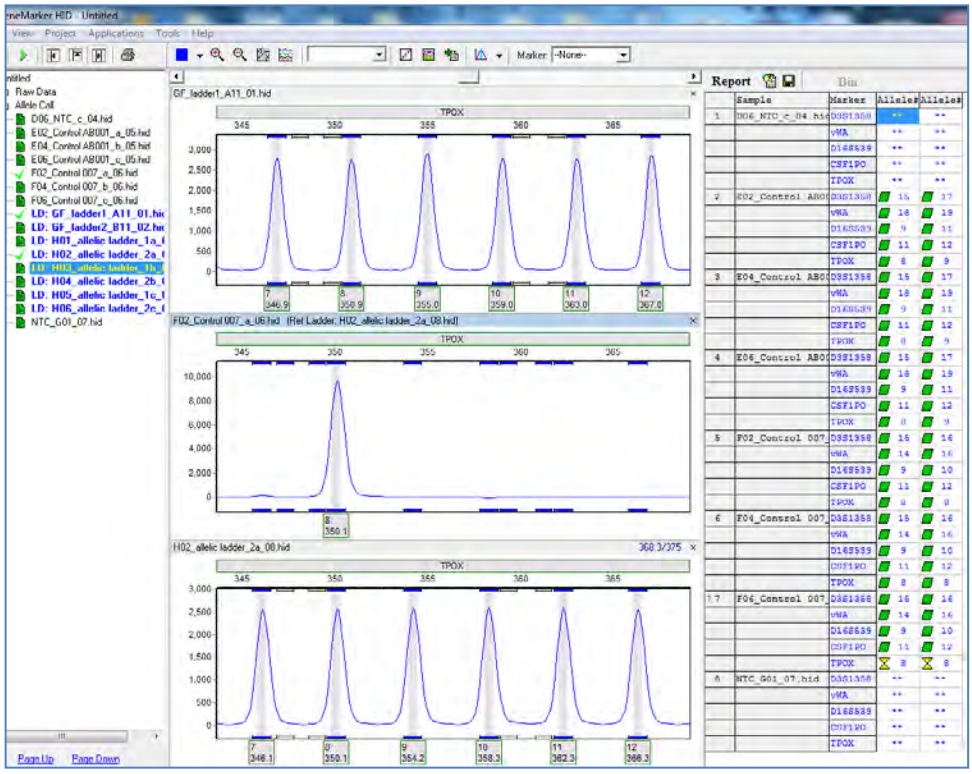


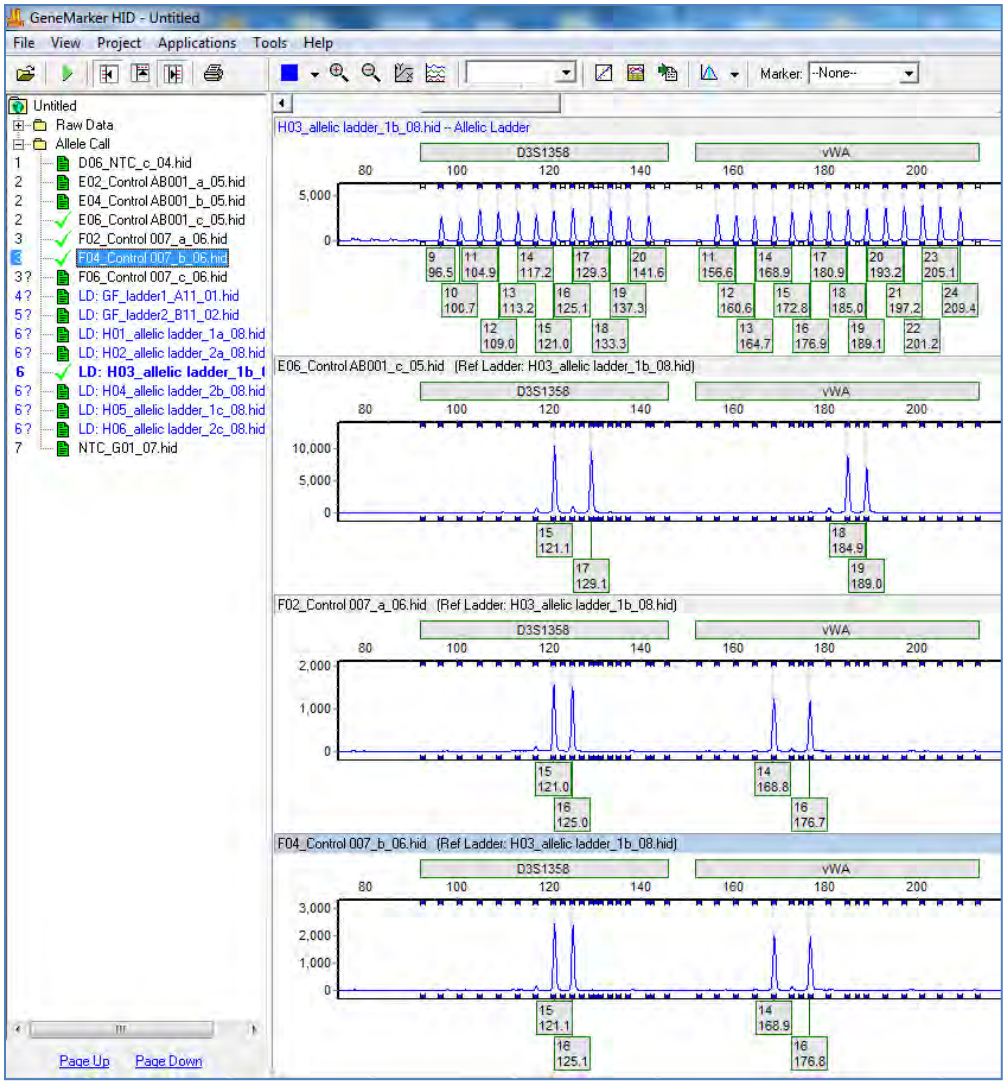


The first two allelic ladders show a migration shift Globalfiler_Panel_GF_ladder1 and ladder 2 (left). When Globalfiler_Panel_H01 is selected from the list the electropherogram displays the aligned bins for this ladder file.

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 6 Reports and Printing.





Alternative work flow – Select the desired panel from the drop-down list in the analysis settings to use one panel for all samples in a project. When this workflow is followed, the specified ladder electropherogram will be fixed at the top of the page and the page down key will open the sample electropherograms; allowing easy comparison of the allelic ladder and each sample.

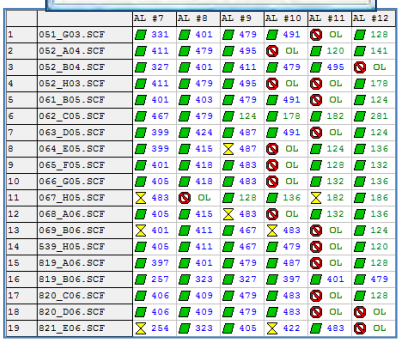
Chapter 6 Reports and Printing

Report Table

The general features of the *Report Table* were outlined in **Chapter 3 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 basepair 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	257	323	327	397	401	479
17	820_C06.SCF	406	409	479	483	OL	128
18	820_D06.SCF	406	409	479	483	OL	OL
19	821_E06.SCF	254	323	405	422	483	OL

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

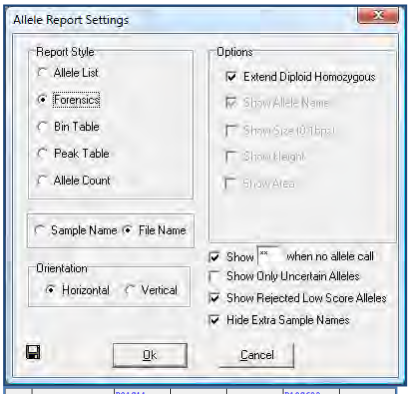
When data is displayed in *Vertical Orientation*, the sample names are repeated for each row of data that the sample is associated with. If *Hide Extra Sample Names* is selected, then the sample name will only appear once in the first of the rows it is associated with.

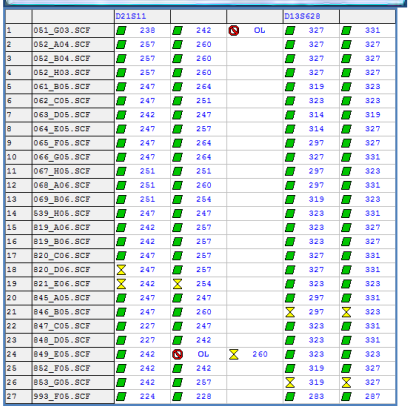
Forensics

The *Forensics 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.

Forensics is the default *Report Style* in GeneMarker HID.

NOTE: *Forensics* requires that a Panel is applied to the data. **See Chapter 5 Panel Editor.**





		D21811				D13S628	
1	051_G03.SCF	238	242	OL		327	331
2	052_A04.SCF	257	260			327	327
3	052_B04.SCF	257	260			327	327
4	052_H03.SCF	257	260			327	327
5	061_B05.SCF	247	264			319	323
6	062_C05.SCF	247	251			323	323
7	063_D05.SCF	242	247			314	319
8	064_E05.SCF	247	257			314	327
9	065_F05.SCF	247	264			297	327
10	066_G05.SCF	247	264			327	331
11	067_H05.SCF	251	251			297	323
12	068_A06.SCF	251	260			297	331
13	069_B06.SCF	251	264			319	323
14	539_H05.SCF	247	247			323	331
15	819_A06.SCF	242	257			323	327
16	819_B06.SCF	242	257			323	327
17	820_C06.SCF	247	257			327	331
18	820_D06.SCF	247	257			327	331
19	821_E06.SCF	242	254			323	323
20	845_A05.SCF	247	247			297	331
21	846_B05.SCF	247	260			297	323
22	847_C05.SCF	227	247			323	331
23	848_D05.SCF	227	242			323	331
24	849_E05.SCF	242	OL	260		323	323
25	852_F05.SCF	242	242			319	327
26	853_G05.SCF	242	257			319	327
27	993_F05.SCF	224	228			283	287

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 basepair 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.

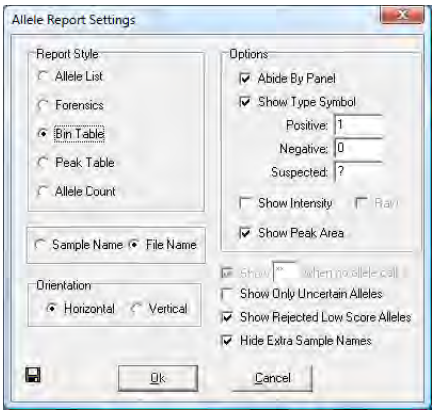
Hide Extra Sample Names: When data is displayed in *Vertical Orientation*, the sample names are repeated for each row of data that the sample is associated with. If *Hide Extra Sample Names* is selected, then the sample name will only appear once in the first of the rows it is associated with.

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



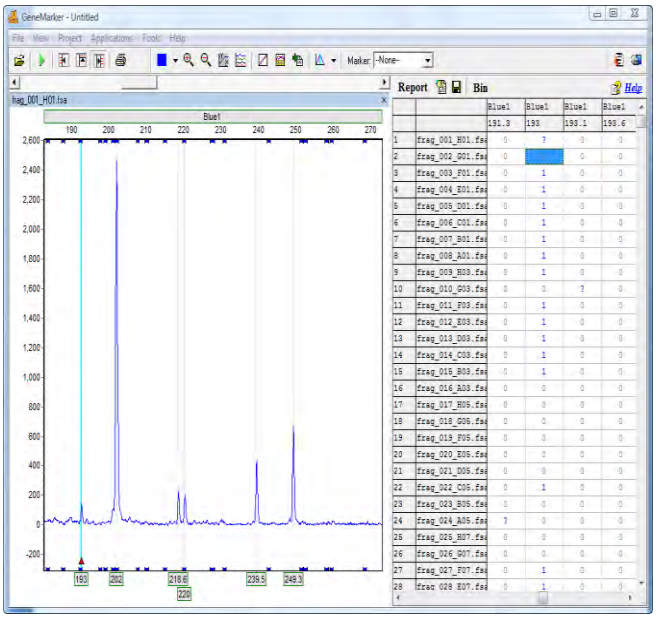
Abide By Panel: When selected, the table will show only called alleles within Panel Marker ranges. This option is only active when a Panel is applied to the data.

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 basepair 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

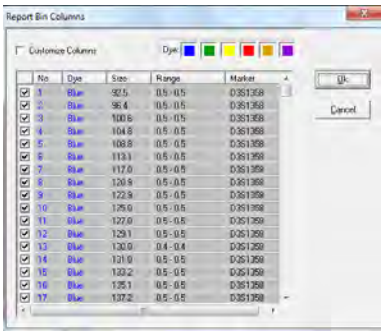
To indicate the peak present at the position is truly a peak, right-click the peak cell and select *Confirm Peaks*. Only peaks centered within a Panel *Bin* will change from Suspect Type Symbol to *Positive Type Symbol* when confirmed. Once a peak is confirmed, it cannot be unconfirmed, only deleted.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

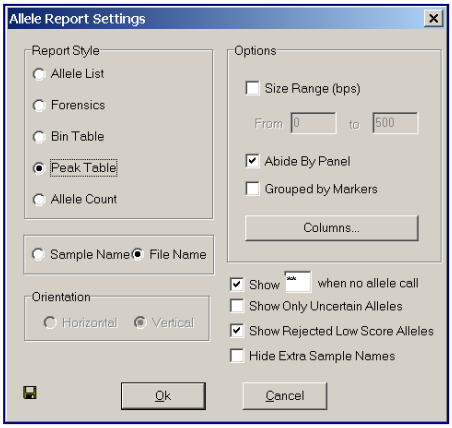
By default, all Bins will be selected with a checkmark at the beginning of the row. Individually deselect Bins for exclusion from the *Report Table* by single left-clicking the checkmark box. To deselect all, right-click any cell in the *Report Bin Columns* box and select *Uncheck All*. To deselect only 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 3 Main Analysis Overview** for column option definitions.

Features

Options

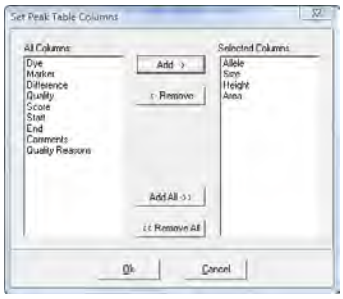
Size Range (bps): When selected, allows the user to define a specific

basepair 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).

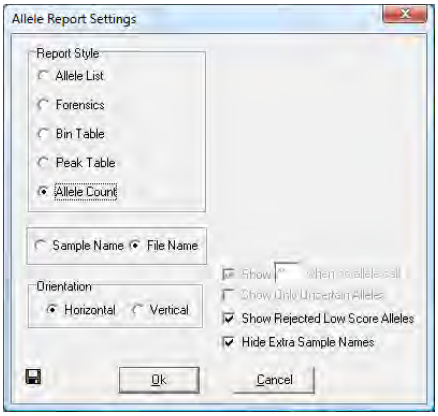
Show Rejected Low Score Alleles: When selected, the peaks with peak scores below the Run Wizard *Additional Settings Allele Evaluation Peak Score* **Reject** setting will be displayed in the table.

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#1	Area#1	Allele#2	Size#2	Height#2	Area#2
1	852_F05.SCF	D21S1435	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				
		D18S1004	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	D21S1435	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
		D18S1004	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 5 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.

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*.

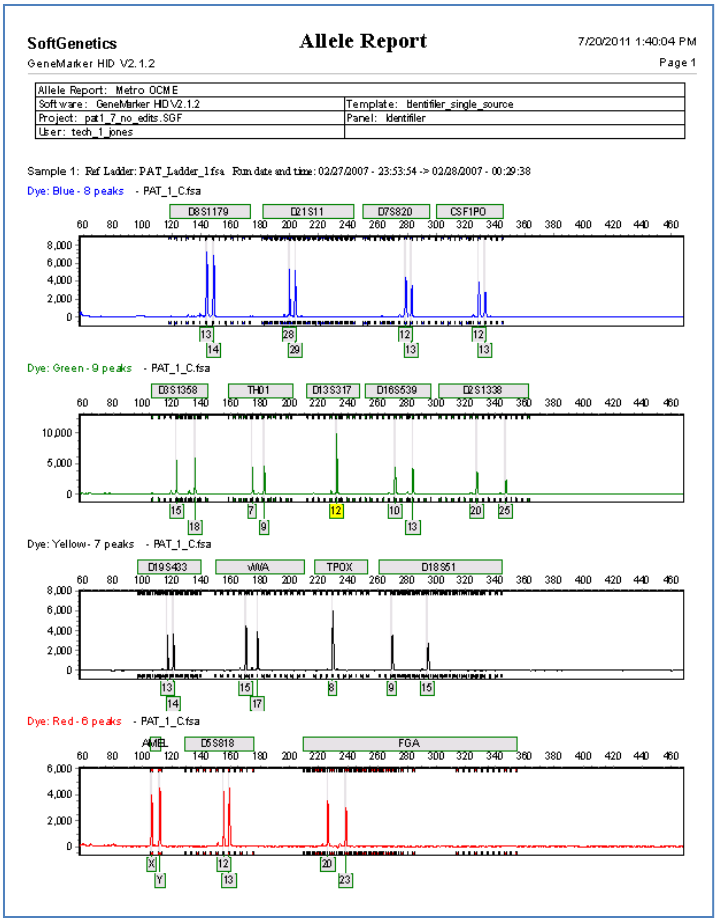
No.	Samples	D18S386	D13S305	IFNAR	D21S1411	Total Numbe
1	051_G03.SCF	2	1	2	1	22
2	052_A04.SCF	2	1	2	2	22
3	052_B04.SCF	2	1	2	2	22
4	052_H03.SCF	0	0	2	2	16
5	061_B05.SCF	2	2	2	2	23
6	062_C05.SCF	2	2	2	2	21
7	063_D05.SCF	2	2	1	1	21
8	064_E05.SCF	2	1	1	2	20
9	065_F05.SCF	1	2	2	2	22
10	066_G05.SCF	2	2	2	2	23
11	067_H05.SCF	2	2	2	2	21
12	068_A06.SCF	2	2	1	2	21
13	069_B06.SCF	2	2	1	2	23
14	539_H05.SCF	2	1	2	3	23

Print Report

The GeneMarker HID *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 and the run date and time for the sample are listed in the first line for each given sample..

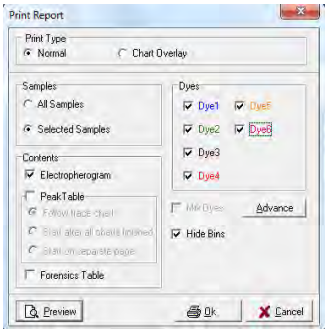
NOTE: The *View* → *Preference* → *Display Settings* options will affect how the *Print Report* is displayed.

GeneMarker HID 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.

Forensic Table: Prints the allele calls (genotype) table on the final report

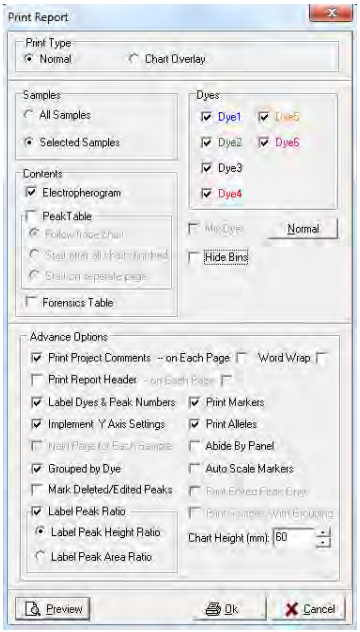
Dyes

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.

Hide Bins: uncheck this box to remove the bin markers from the X axis of electropherograms in the print report

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.

Print Report Header: Includes Institution and User ID from User Management and Template, Panel Name, from the Analysis Template

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 (not available if Grouped by Dye is selected).

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. (often used in combination with Print Samples with Grouping option).

Mark Deleted/Edited Peaks: Prints an x above a deleted peak and an E above an edited peak in the electropherogram.

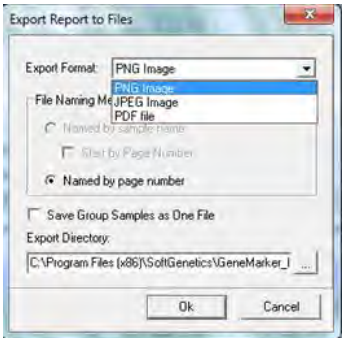
Label Peak Ratio: Select this option to print the peak ratios on the electropherogram of the print report (available when the View – Preferences – Display Settings is selected for peak top).

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

Print Samples with Grouping: print and/or save samples by group when the grouping tool is applied in the main analysis window

Icons and Functions

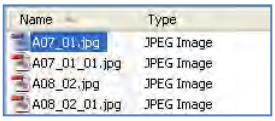
The following icons are available in the *Print Preview* window prior to printing the *Print Report*.



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

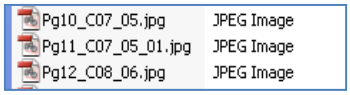
Export to File
Opens the *Export Report to Files* box. Save each page of the *Print Report* as an individual image file JPEG, PNG or PDF. Select the directory to export the files to. When saving as a group the file name will be the first file of the group. See View- Preferences – Report Settings if a prefix of Ladders and Controls is desired for allelic ladder, positive and negative control files.

Named by sample name saves each PNG or

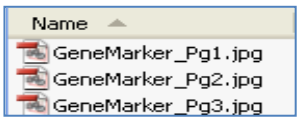


JPEG under the sample name.

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.

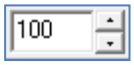


Page Setup
Opens the *Page Setup* box. Choose the paper size, margins and orientation (*Portrait* or *Landscape*).

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.

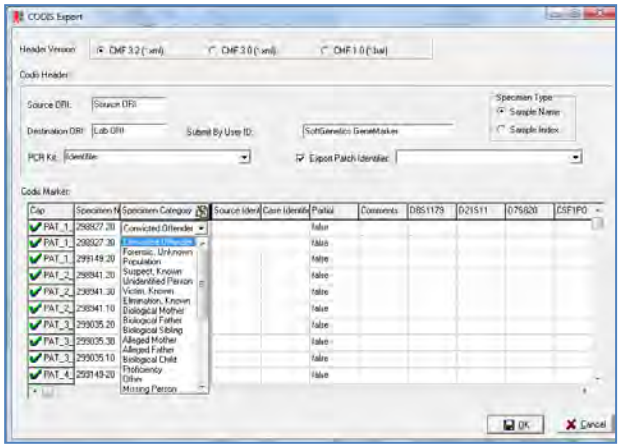
Zoom to Width
Zooms in to view the *Print Preview* page at maximum width without losing information off the screen.



Zoom Ratio
Enter percentage numbers to increase or decrease the zoom aspect of the *Print Preview* page.

CODIS Report

Tools → Export CODIS



The CODIS Report feature allows users to create an exportable report file for easy input into the CODIS database.

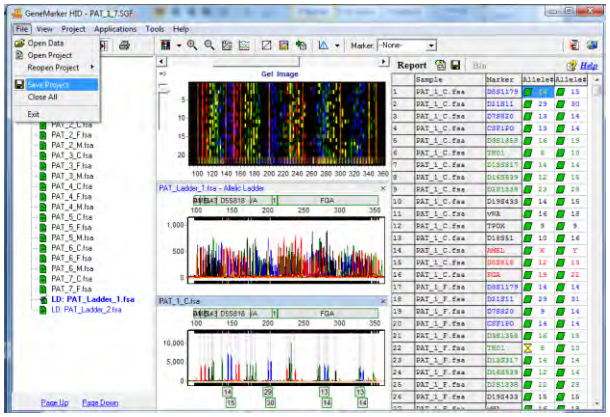
The *CODIS Export* window will appear with several options for modifying the header and classifying the samples. To change the classification information for the samples simply click the cell you wish to modify and select from the options in the drop-down menu.

Once you've updated the information click **OK** and the option to save the CODIS file will appear. Select a location and save as either a **CMF1.0** (.dat) file, **CMF3.0** (xml) or **CMF3.2** (.xml) [As per US DOJ CODIS Interface Specification (CMF 3.0) CONTRACT NO. ITOP 97-0087 Sub Task Order 26CODIS and Interface Specification (CMF 3.2) Revision 9 DOJ-FBI-2005-C-2431] file. Click **Save**.

Save Project

After a dataset is analyzed and edited, the project can be saved as a SoftGenetics GeneMarker HID 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 5 Panel Editor**. To export a custom Size Standard, see **Chapter 4 Fragment Sizing Standards**.

NOTE: Previous versions of GeneMarker HID saved projects in SFP format. This format can be opened with current GeneMarker HID 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 re-open the project, go to *File → Open Project* in the *Main Analysis* window. The last folder accessed by GeneMarker HID will appear. Navigate to the directory containing the SGF (or SFP) file and click **Open**. Additionally, the last four projects that were opened by GeneMarker HID 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 GeneMarker HID.

Chapter 7 Mixture Analysis

Overview

Mixture analysis is required for many samples that are not single source, as in the case of some crime scene and missing persons and unidentified human remains applications. The mixture analysis application was developed using recommendations of the DNA commission of the International Society of Forensic Genetics (Gill *et al*., 2006) and methods of Clayton *et al*., 1998 and Gill *et al*., 1998. GeneMarker HID mixture analysis follows the steps involved in the interpretation of STR mixture data (Clayton *et al.* 1998).

- Identify the Presence of a Mixture
- Designate Allele Peaks
- Identify the number of potential contributors
- Estimate the relative ratio of the individuals contributing to the mixture
- Consider all possible allele combinations
- Compare References Samples

GeneMarker HID identifies the presence of potential mixture samples, designates allele peaks, and calculates peak area or height ratios in the main analysis screen. The Mixture Analysis Application is activated from the Applications menu in the main analysis screen. Mixture analysis identifies the mixture samples and any single source contributor samples in a file name tree, considers all possible allele combinations, calculates the Mixture Ratio, residual score, heterozygous imbalance for each genotype combination, and calculates the likelihood ratio for single source samples that are potential contributors to the mixture. Often, as in the case of male/female cell separation, a single major contributor profile of the perpetrator can be identified in the results table. This genotype can be exported directly to the Relationship Testing database in GeneMarker to search for exact matches or close relatives, even in cases where there is no suspect reference sample.

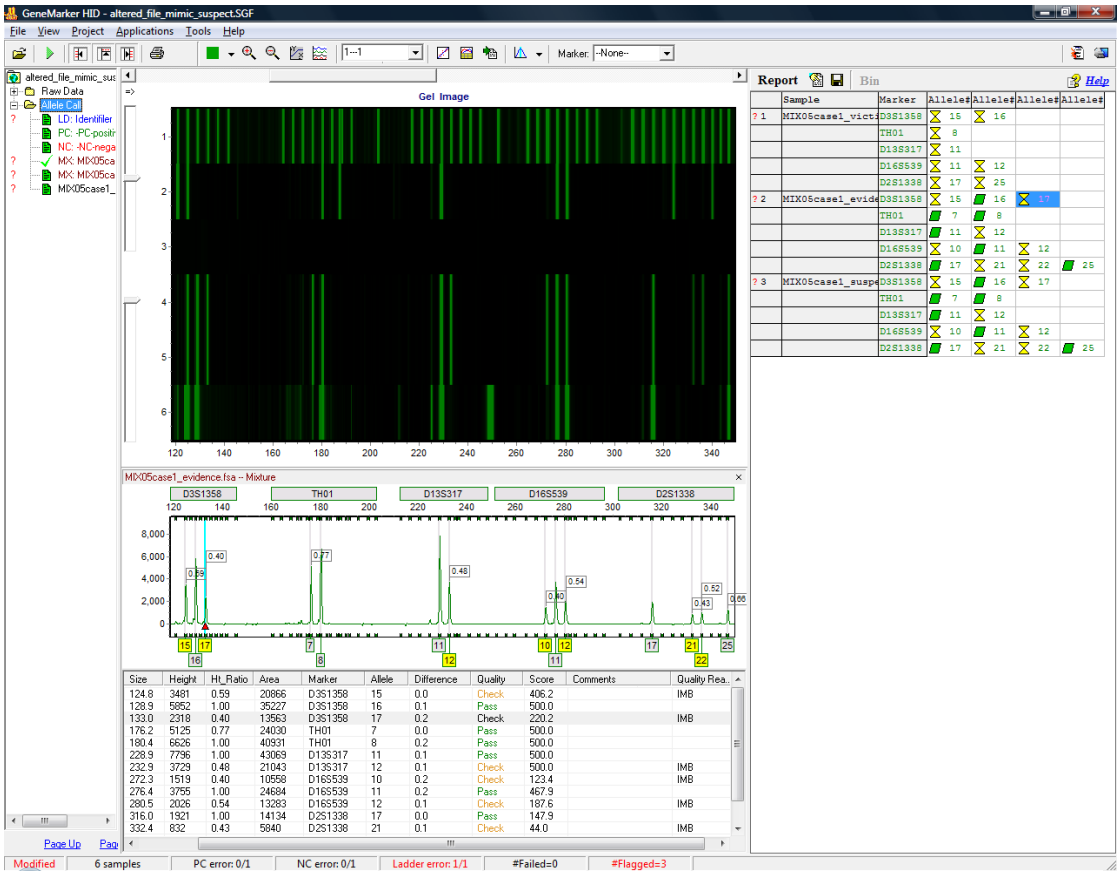
Procedure

Identify the Presence of a Mixture

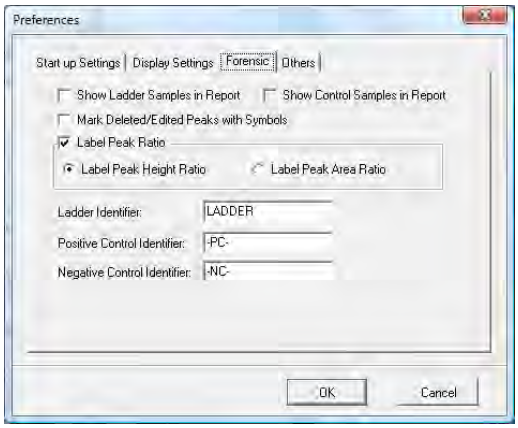
The presence of a mixture is determined by setting the needed parameters in the third plate of the Run Wizard. The criteria are used for identifying potential mixtures include: presence of more than two peaks in for a marker, severe peak height imbalance between alleles of a locus (also see Panel Editor, stutter peak filter, chapter 5), and apparent stutter product above the normally expected range.

1. From the Main Analysis Screen select File →*Open data or Open project* →*Add* →*Okay*
2. Launch Run Wizard from pop-up menu or from *Project* drop-down menu
3. Select Appropriate Template and Run Parameters (see chapter 3)
4. Select Mixture Analysis in third screen of the Run Wizard
5. Enter desired values for Valid Mixture Peak Percentage and Min Number of Peaks/Marker (3 is appropriate for a mixture from two contributors)
6. Review allele calls and summary information

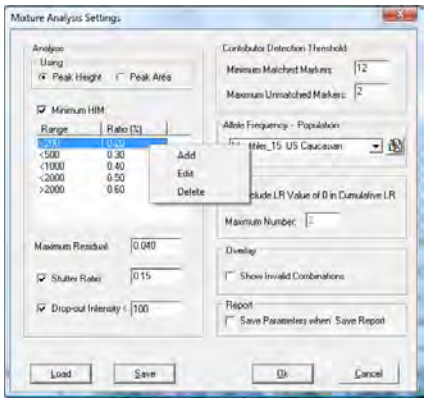
Review Results



1. Ladder and controls are automatically identified based on nomenclature specified in View →Preferences →Forensic
2. Action summary presented at the bottom of the screen indicates number of samples, automated positive and negative control concordance, ladder flagging (see chapter 5 Panel Editor), number of samples failed and flagged (caused rule firing).
3. Samples that meet the mixture criteria are identified as potential mixture - **MX:**



Mixture Analysis



1. Select Applications →*Mixture Analysis*
2. Launch Mixture Analysis Settings 

* Analysis Type – Peak Height or Area
* Minimum HIM: heterozygous imbalance minimum is on a sliding scale dependent on the Range – Right click to edit Min. HIM parameters
* Maximum Residual – "…we infer that the likelihood of the peak areas given the combination of genotypes is high if the residual is low." (P. Gill *et al*. 1998) is calculated as

$$\sum (pa\ observed - pa\ expected)^2$$

using peak areas and mean Mx to compare the observed proportions to the expected proportions.

* Minimum Major Weight parameter allows selection of potential combinations based on the Mixture Proportion (Mx)
* Set minimum stutter ratio and drop-out intensity. In cases where a valid allele combination would result with allele drop out, a Q is used to represent a possible un-amplified allele. The value set in the parameter for allele drop out is used in the HIM and Mx calculations. For example if the genotype of a mixture is 11, 12, 13 and the intensities are 1000, 1000 and 200 one of the valid combinations could be 11,12 and 13,Q. In this case the Major Mx is (1000 + 1000)/(1000 + 1000 + 200 +100) and the minor HIM is 100/200 or 0.5. But if the intensity of 13 was less than 100 the intensity of the drop-out peak would be set at the same intensity as 13.
* Contributor detection threshold allows for possible contributor when there is an incomplete profile.
* Preloaded Allele frequency values are the same as those described in Chapter 8 Relationship Testing. . The edit icon allows individuals with access right approval to easy add or remove other population allele frequency .txt tab delimited tables (see chapter 8 for file format template) **Allele Freuqency Tables**

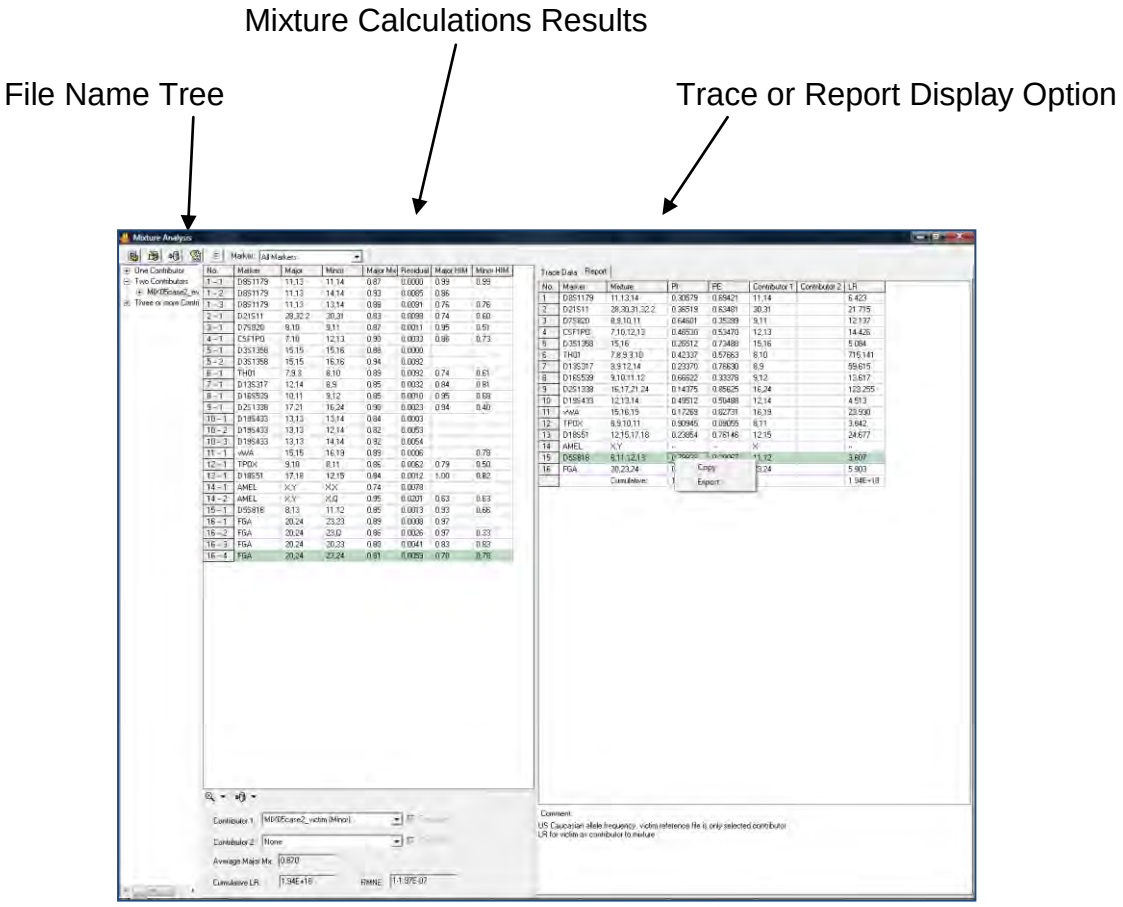
Allele frequency tables for major US populations may be selected from the drop down menus in the Select Allele Frequency Settings box. If results of all populations are preferred, select the Use all Populations box and the final report will append with the results using each of the tables sequentially. The Delete button may be used by individuals with access rights to remove any population frequency tables that are not needed by the laboratory. Use the Open folder icon to import formatted allele frequency tables for other populations.

*
* Skip Unmatched Markers in Cumulative LR calculation allows for LR calculation in the cases of incomplete profiles.
* Overlay – when Show Invalid Combinations is selected all possible genotype combinations are displayed in the results table. Red font indicates that the combination did not meet the analysis parameters. Black font indicates that the

genotype combination meets the analysis parameters. If this option is de-selected only the combinations that met the analysis parameters are displayed in the result table.

* Report – Save Parameters when Saving Report – creates an .ini file with the same name as the report; providing verification of mixture analysis parameters used to generate each report.

Mixture Analysis Results



The file name tree in mixture analysis groups files by the number of contributors. When a file under 'Two Contributors' is selected, any single source samples from the project that are potential contributors to the mixture are displayed underneath the mixture sample file name. Contributor file names are automatically listed in the dropdown menu at the bottom of the screen and peak ratio results are used to determine if the profile is a major or minor contributor.

Mixture calculation results are displayed in the results table. All possible allele combinations are displayed, along with the major Mx ratio, residual score, and major and minor heterozygous imbalance ratios. Please see Mixture Analysis Equations section at the end of this chapter for mixture analysis equations. Allele combinations that meet all of the mixture analysis parameters are in black font, other combinations are in red font.

The Report table on the right side of the mixture analysis screen lists:

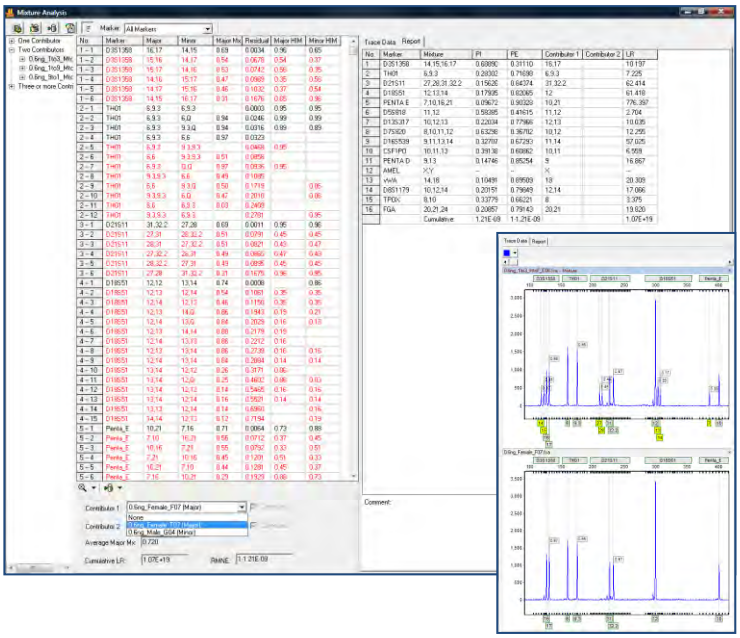
1. Marker Name (AMEL and Y markers are not included in LR calculations)
2. Alleles called in the mixture
3. Probability of Inclusion (PI) and Probability of Exclusion (PE) for any selected mixture sample (two contributors or three or more contributors)
4. Genotypes of contributors
5. Likelihood ratio for selected contributors
6. Combined Probability of Inclusion (CPI)
7. Combined Probability of Exclusion (CPE)

The analysis should record the hypothesis being tested and any other notes on the analysis in the comments section. When the result table is copy/pasted or exported as .txt files the comments are automatically saved with the report table, providing more efficient accountability and presentation of results.

What to Expect Mixture Analysis and Database Search

With Reference File(s)

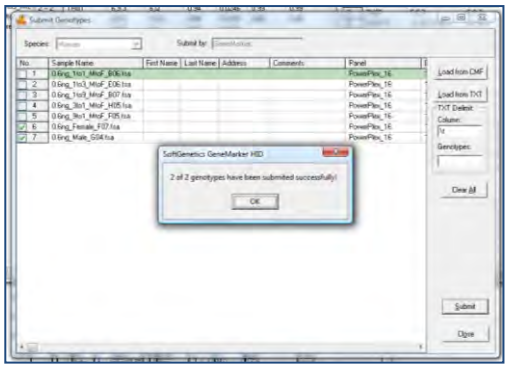
The File Name Tree indicates that two single source files are potential contributors to the evidence mixture. The analyst may select a potential contributor file from the drop-down menu at the bottom of the report. The genotypes combinations and ratio results are displayed in the result table. The Report table at the right of the screen provides the LR for each marker. The Combined LR is displayed at the bottom of the screen. The combined LR is the likelihood that this individual contributed to the mixture versus a random person from the population contributed to the mixture. Use the Trace Data to toggle from the LR report to the traces. Use the Show all combinations toggle to display either all genotypes; black font for combinations that conform to all of the mixture analysis parameters and red font the combinations that do not conform with the mixture analysis parameters OR toggle to display only the black font.



Submit a reference profile to the Relationship Testing Database

A profile from a reference sample can be submitted directly to the Relationship Testing Database from this mixture analysis screen.

1. Use the **Submit Genotypes to** 

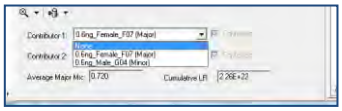


Database icon

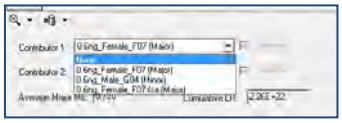
2. **Select the appropriate file(s)**
3. **Submit**
4. **OK**

Search Relationship Testing Database for exact matches

1. Use the Search Database icon to search the database for exact matches
2. Select the desired profile from the drop-down menu
3. File name(s) of matching profiles will be added to the drop-down menu
4. LR s are displayed in the Report Table



Before search database icon the drop-down list has only the single source reference files listed

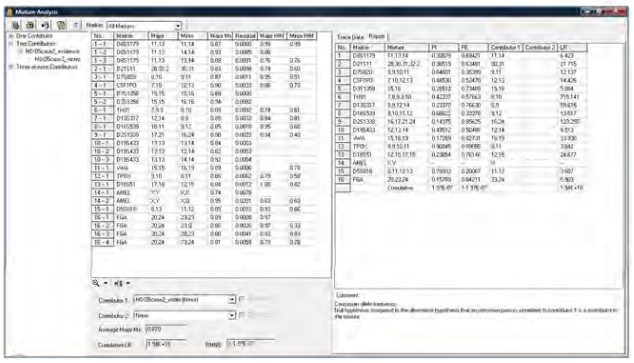


After Selecting the profile to search the database, all files that match are displayed in the drop-down menu

Select the file to display the LR results in the Report Table

What to Expect

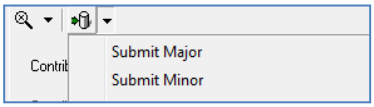
Without a Reference File



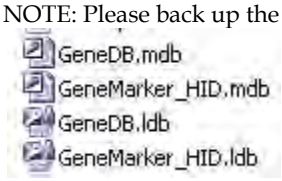
In some cases only the mixture sample and the victim reference sample are available. For example, DNA isolation from rape cases often has successful male/female cell separation that provides a mixture where the perpetrator is the major contributor and the victim is the minor contributor. Results shown in the following figure allow the analyst to identify a single profile that corresponds to the perpetrator in the Major Contributor column. This profile can be used to:

1. Search the database in GeneMarker for an exact match or match right from the mixture analysis screen
2. Search for potential relative (see Chapter 8 section Data Base Search: Finding Nearest Relatives)
3. Save and export for use with external database searches.

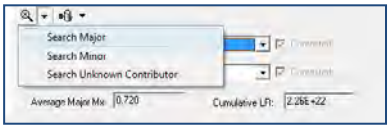
Submit a profile from deconvolution of mixture sample to the Relationship Testing Database



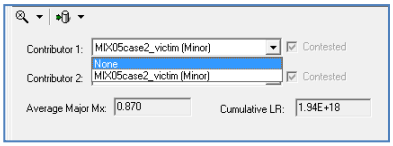
1. Toggle to display only the allele combinations that conform to the mixture analysis parameters
2. Review to confirm that a single profile has resulted for the major contributor
3. Select Submit Major, Submit and OK
   NOTE: Please back up the Database file on a regular basis.



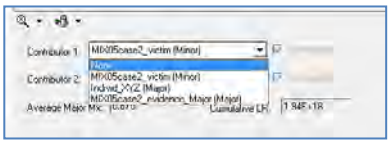
Search Relationship Testing Database for matching profiles



1. Use the Search Database icon to search the database for exact matches
2. Select the desired profile from the drop-down menu
3. File name(s) of matching profiles will be added to the drop-down menu
4. LR s are displayed in the Report Table

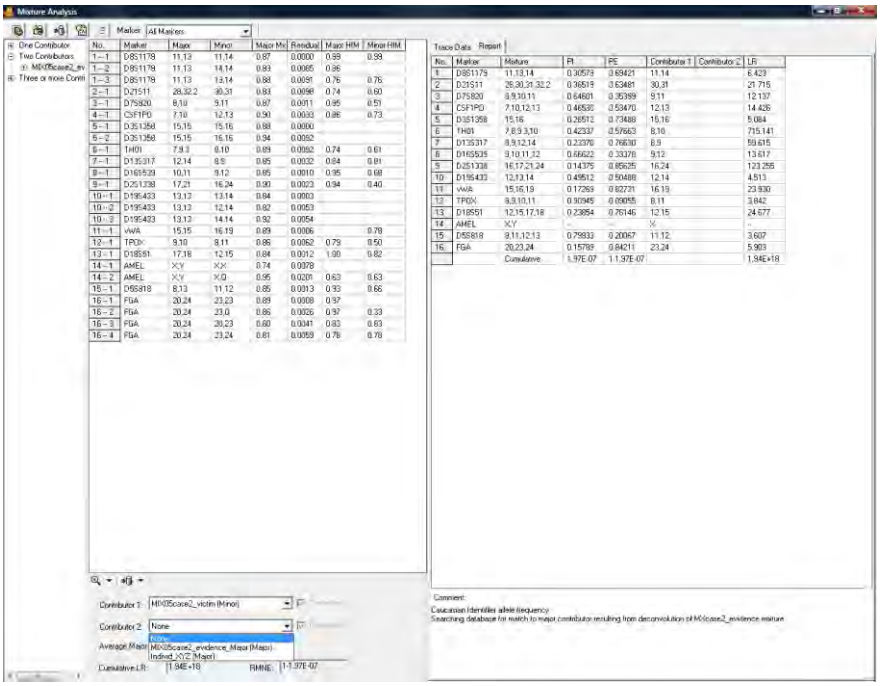


Before using the search database icon the drop-down list has only the single source reference files listed

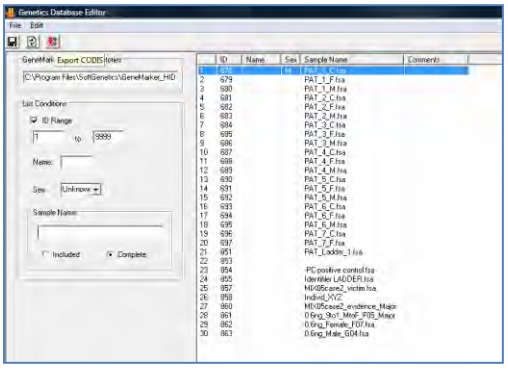


After selecting the profile to search the database, all files that match are displayed in the drop-down menu. These results indicate that there is an individual XYZ in the database that matches the major contributor in this mixture, in addition to the de-convolution profile from the mixture sample.

Select the file to display the LR results in the Report Table

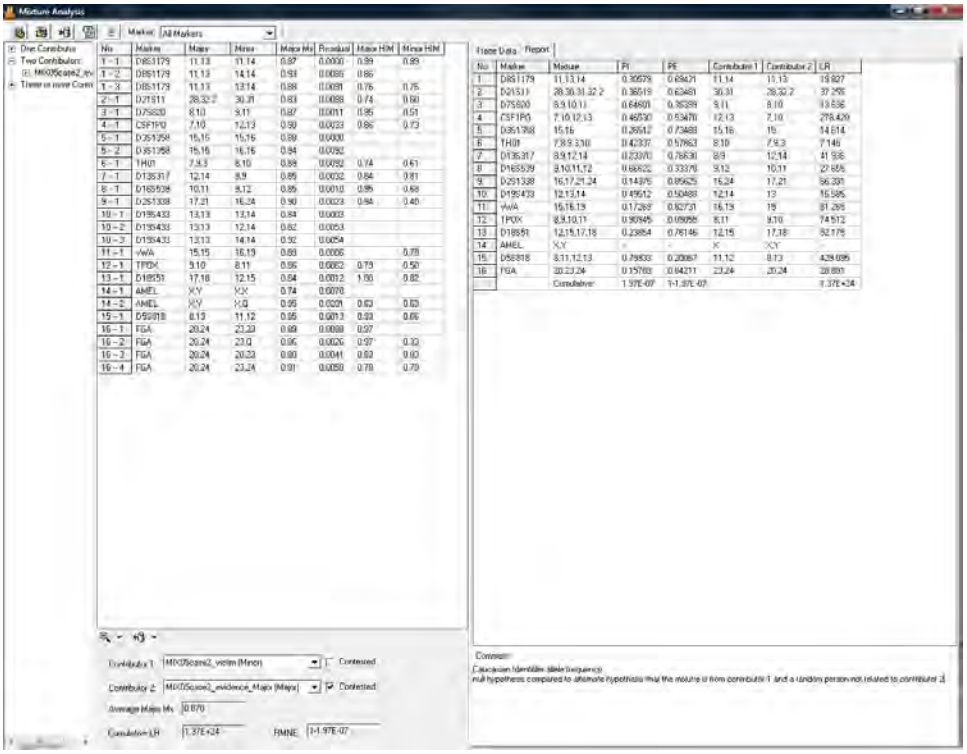


Files can be saved in CODIS format by using the Export CODIS icon from the Genetic Database Editor

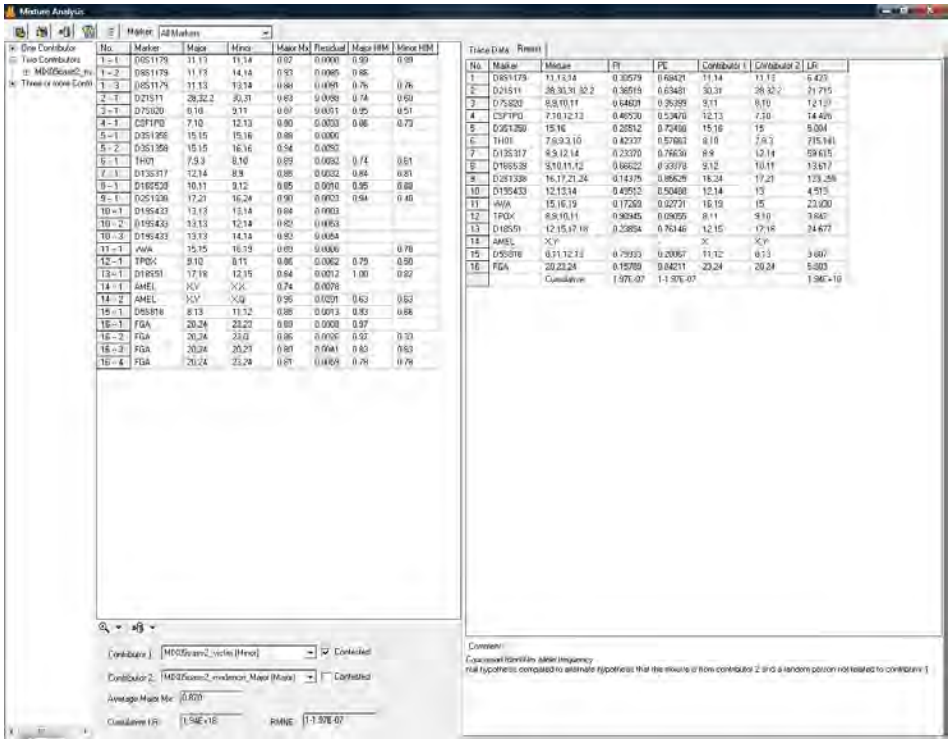


Hypothesis Testing Options

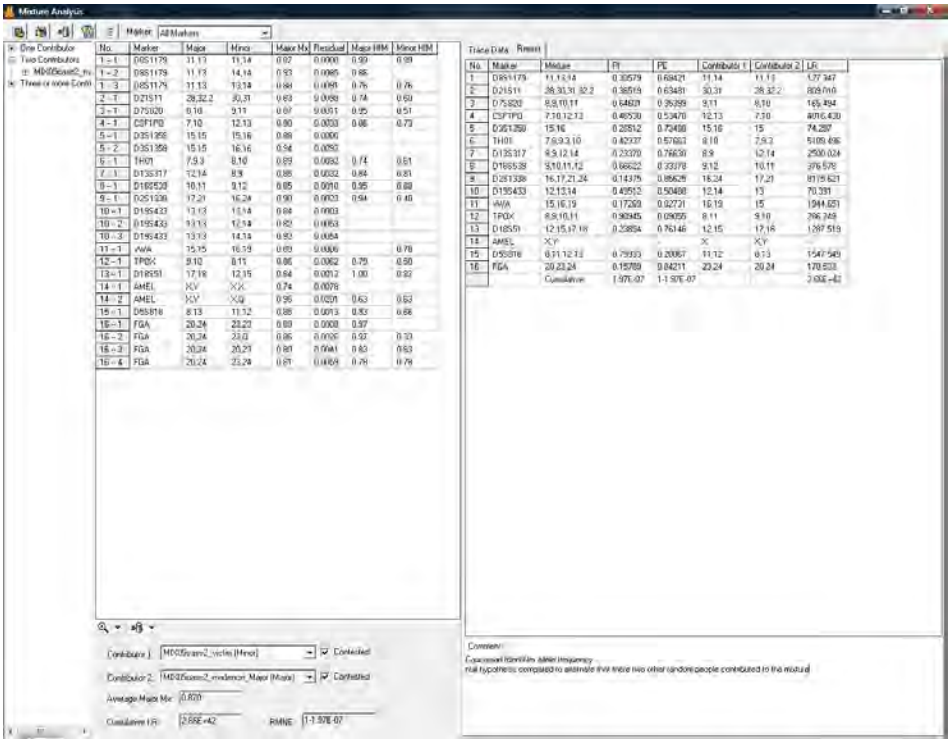
To calculate likelihood ratios from a comparison of the null hypothesis to alternate hypotheses, the analyst should select contributor 1 and contributor 2 from the drop-down menus at the bottom of the analysis screen. Check the appropriate box(es) under the Contested heading to contest contributor 1 or contest contributor 2 or contest both contributors to the mixture. Screen saves of samples and examples of comments are presented below. Please go to the Mixture Analysis – Likelihood Ratio and Hypothesis testing section for example calculations.



Contesting contributor two as a contributor to the mixture



Contesting contributor 1 as a contributor to the mixture.



Contesting both contributors to the mixture

Icons and Functions

Mixture Analysis Parameters
Launches the Mixture Analysis Settings Dialog Box

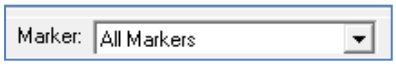
Edit Database
Navigates to the Edit Database function in Relationship Testing

Submit Genotypes to Database
Allows direct submission of reference file genotypes to the relationship testing database

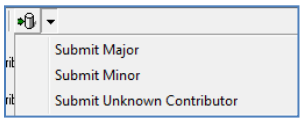
Locus Name Group Editor
Allows the user to group different format names for recognition of a single marker

Toggle
Toggle between 1) all possible allele combinations (black and red font) and 2) only the allele combinations that conform to the mixture analysis parameters (black font)

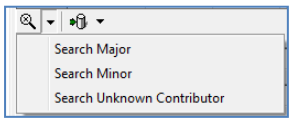
Show Color
Allows the user to select all colors to view, hide all colors, or choose a single dye layer in the trace view. Choose a single dye by single left mouse clicking on the icon.



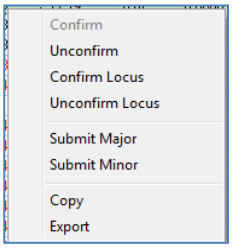
Marker
Select a Marker or Locus to view in the *Electropherogram Charts*



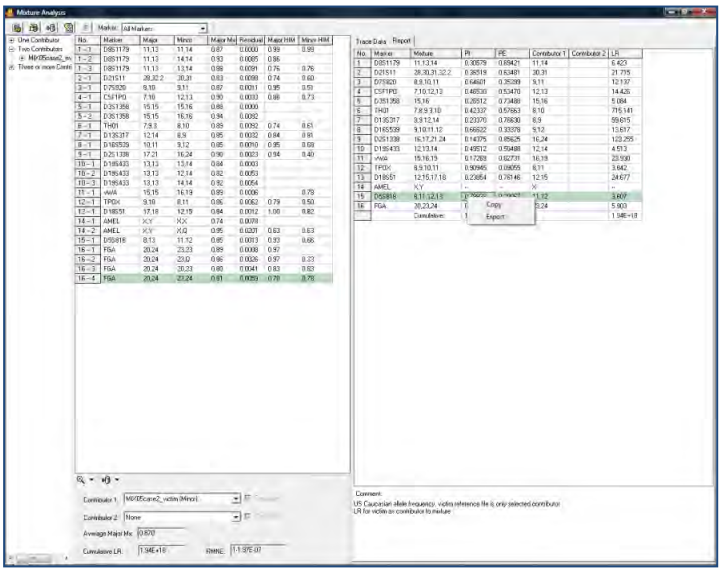
Submit to Relationship Testing Database
Submit the genotypes from deconvoluted mixture sample, Major contributor, minor contributor or unknown. The contributor label is unknown if the mixture approximately 1:1; neither file fits the definition of major or minor contributor



Search Relationship Testing Database
Search for exact matches and the likelihood ratio results without navigating to the Relationship Testing Application. Results are displayed in the mixture analysis screen.



Report Options
Right mouse click on the mixture analysis report table results to activate edit confirmation, submit, copy and save options



Save and Export Result Tables

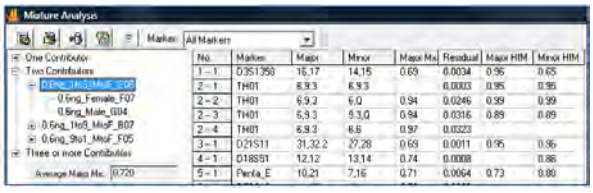
Right click on the genotype combination table or the likelihood ratio report to copy and paste the results into an existing document. Select Export to save tables as a .txt tab delimited file. Comments that are typed in by the analyst are saved with the likelihood ratio report.

Mixture Analysis Equations

Major Mx = **(**A + B) / (A + B + C + D)

User selects whether to use peak height or peak area in the analysis parameters. For these examples, peak height was selected as an analysis parameter.

Example using peak height Marker D3s1358:

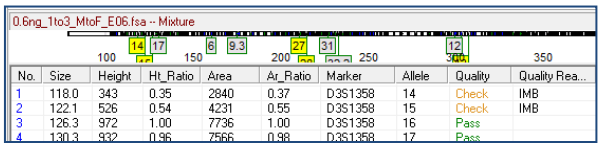


Mx =(972 +932) / (972 +932 +343 +526)

= 0.69

The major contributor accounts for 69 % of the DNA in the mixture sample.

Residual $\sum(pa\ observed - pa\ expected)^2$



Observed proportions are calculated from the data. Expected proportions are calculated using the methods of Gill *et al.* Forensic Sci. Intern. 91:41-53. (table at right)

Example for D3s1358

Observed proportions		Expected proportions
14	0.1237	(1-Mx)/2 = 0.14
15	0.1897	(1-Mx)/2 = 0.14
16	0.3505	Mx/2 = 0.36
17	0.3361	Mx/2 = 0.36

Residual = 0.0034

Heterozygous Imbalance (HIM)

Example for D3s1358

Major HIM = 932/972 = 0.96

Minor HIM = 343/526 = 0.65

The four allele model (where Mx is the proportion of sample from the first individual)

Combination	Allele			
	A	B	C	D
AB,CD	$Mx/2$	$Mx/2$	$(1-Mx)/2$	$(1-Mx)/2$
AC,BD	$Mx/2$	$(1-Mx)/2$	$Mx/2$	$(1-Mx)/2$
AD,BC	$Mx/2$	$(1-Mx)/2$	$(1-Mx)/2$	$Mx/2$
BC,AD	$(1-Mx)/2$	$Mx/2$	$Mx/2$	$(1-Mx)/2$
BD,AC	$(1-Mx)/2$	$Mx/2$	$(1-Mx)/2$	$Mx/2$
CD,AB	$(1-Mx)/2$	$(1-Mx)/2$	$Mx/2$	$Mx/2$

The three allele model

Combination	Allele		
	A	B	C
AA,BC	Mx	$(1-Mx)/2$	$(1-Mx)/2$
BB,AC	$(1-Mx)/2$	Mx	$(1-Mx)/2$
CC,AB	$(1-Mx)/2$	$(1-Mx)/2$	Mx
AB,AC	0.5	$Mx/2$	$(1-Mx)/2$
BC,AC	$Mx/2$	$Mx/2$	0.5
AB,BC	$Mx/2$	0.5	$(1-Mx)/2$
BC,AA	$1-Mx$	$Mx/2$	$Mx/2$
AC,BB	$Mx/2$	$1-Mx$	$Mx/2$
AB,CC	$Mx/2$	$Mx/2$	$1-Mx$
AC,AB	0.5	$(1-Mx)/2$	$Mx/2$
AC,BC	$Mx/2$	$(1-Mx)/2$	0.5
BC,AB	$(1-Mx)/2$	0.5	$Mx/2$

Two-allele model

Combination	Allele	
	A	B
AA,AB	$(Mx/2)+0.5$	$(1-Mx)/2$
AB,AB	0.5	0.5
AA,BB	Mx	$1-Mx$
AB,AA	$1-(Mx/2)$	$Mx/2$
BB,AA	$1-Mx$	Mx
AB,BB	$Mx/2$	$1-(Mx/2)$
BB,AB	$(1-Mx)/2$	$(Mx/2)+0.5$

Exclusion Probability for mixture profiles uses the allele frequency of a given population for each allele in a mixture sample. GeneMarker HID calculates exclusion probabilities for two person mixtures and mixtures of three or more contributors. When the CPE is multiplied by 100 (RMNE – Random man not excluded) it provides information about the percent of people in the population that are excluded from being contributors of the mixture because they have one or more alleles that are not present in the mixture.

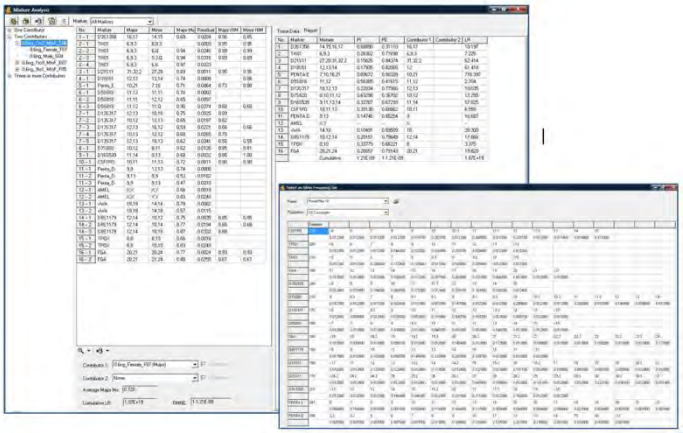
Probability of Inclusion (PI)
PI = $(p1 + p2 + \ldots. + pi)^2$

Probability of Exclusion (PE)
PE = $1 - (p1 + p2 + \ldots.pi)^2$

Combined Probability of Inclusion (CPI)
PI1 x PI2 x …..x PI*i*

Combined Probability of Exclusion (CPE)
CPE = 1 – [(1-PE1) x (1 – PE2)x….x(1 – PE*i*)]

PI, CPI, PE, CPE examples



PI for D3s1358
= $(0.1404 + 0.2463 + 0.2315 + 0.2118)^2$
= 0.6889 or 69% of population are included based on this one marker

PE for D3s1358
= $1 - (0.1404 + 0.2463 + 0.2315 + 0.2118)^2$
= 0.3111 or 31% of the population are exclude based on this one marker

CPI = 1.21E-09 or 0.000000121%
When multiplied, the PIs of each marker provide the cumulative PI (multiply by 100 to have the percent of the population that can be included as potential contributors to the mixture.

CPE or NRME = 1 – 1.21E-09 or 99.9999998% of the population can be excluded as potential contributors to this mixture.
CPE = 1 – [(1-PE1) x (1 – PE2)x….x(1 – PE*i*)]

Likelihood Ratios – Hypothesis testing and calculations

Likelihood Ratios are obtained by comparing the null hypothesis (which has a probability of 1) to an alternate hypothesis. When there are two contributors to a mixture there are several alternate hypotheses that the analyst may want to test. Several scenarios comparing Probability of the Null Hypothesis to the Probability of the Alternate Hypothesis are listed below with sample calculations.

Scenario 1
The project contains a mixture profile and one single source profile that is a potential contributor to the mixture.

Null hypothesis = this individual is a contributor to the mixture sample
Alternate hypothesis = some other random, unrelated person from the population is the contributor
Probability of Alternate hypothesis
p^2 for homozygotes
$2p_1p_2$ for heterozygotes

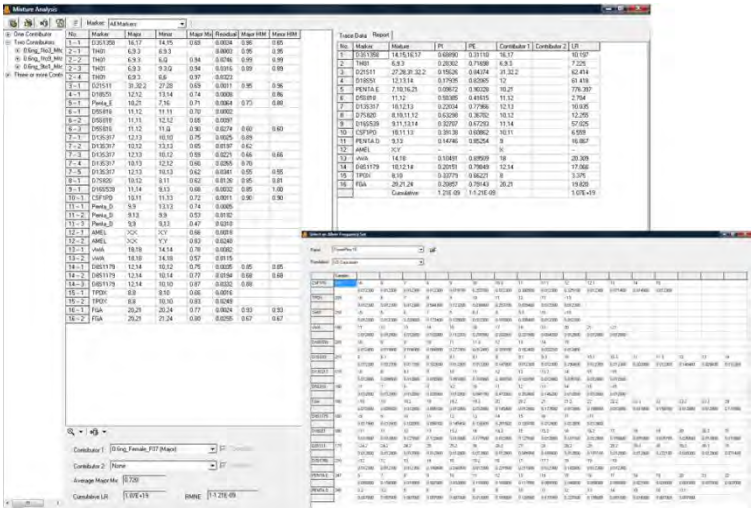
Examples

D3S1358	Alleles	Frequency
	16	0.2315
	17	0.2118

LR = 1/(2 x 0.2315 x 0.2118) = 10.197

D18S51	Allele	Frequency
	12	0.1276

LR = 1/(0.1276 x 0.1276) = 61.42



When two potential contributor profiles are available the following scenarios may be tested.

Scenario 2

<u>Null Hypothesis, Mixture is from A and B</u>
Alternate hypothesis, mixture is from person B and one unknown person unrelated to A

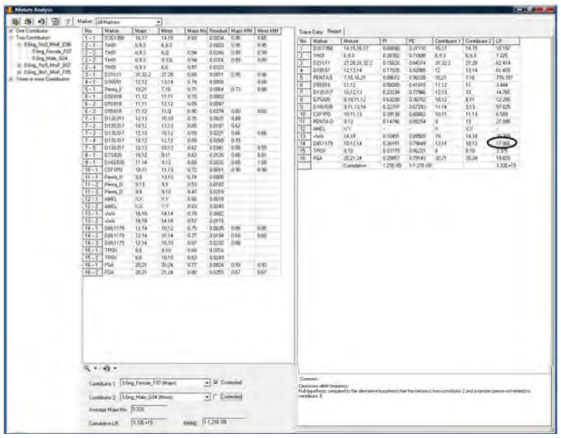
The project contains a mixture and two single source potential contributors. Both single source samples are selected and person A is contested. In the column for Major contributor allele combinations, we see there is only one viable allele combination for the major contributor (person A) – 12, 14 or (a,b)

Example for D8S1179

	Alleles
Major contributor (a,b)	12, 14
Minor contributor (a,c)	10, 12
Mixture	10, 12, 14

PowerPlex 16 US Caucasian allele frequencies

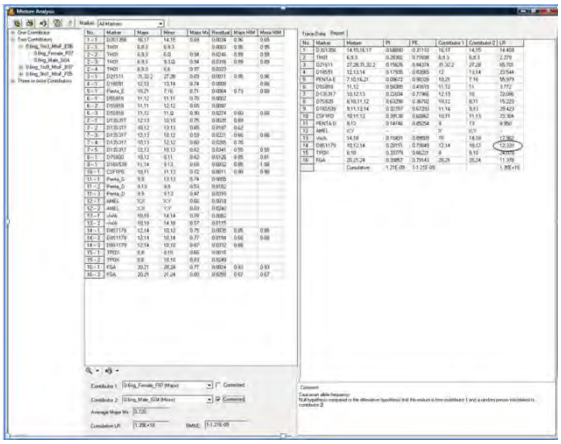
10	0.1020
12	0.1454



14 0.2015

$$LR = \frac{1}{(2 \times 0.1454 \times 0.2015)} = 17.06$$

Scenario 3



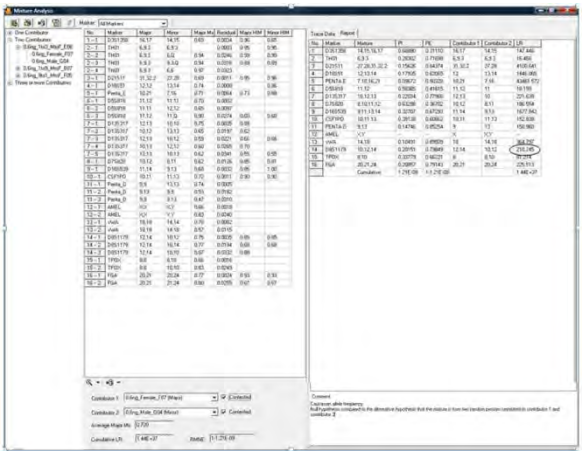
Null Hypothesis, Mixture is from A and B
Mixture is from A and an unknown person, unrelated to B

The project contains a mixture and two single source potential contributors. Both single source samples are selected and person B is contested. In the column for minor contributor allele combinations, we see there are three viable allele combinations for the minor contributor (person B) – 10,12; 10,14; 10,10 or (a,c; b,c; cc)

$$LR = \frac{1}{(0.1020 \times 0.1020) + (2 \times 0.2015 \times 0.1020) + (2 \times 0.1454 \times 0.1020)}$$

$$= \frac{1}{0.0812} = 12.32$$

Scenario 4



Null Hypothesis, Mixture is from A and B
Mixture is from two unknown people unrelated to A and B

The project contains a mixture and two single source potential contributors. Both single source samples are selected and both are contested. As in the above scenarios, there is only one viable allele combination for person A and there are three viable allele combinations for person B.

$$\text{Likelihood ratio} = \frac{1}{(2 \times a \times b) \times ((c \times c) + (2 \times b \times c) + (2 \times a \times c))}$$

LR = 1/(2x 0.1454 x 0.2015) x ((0.1020 x 0.1020) + (2 x 0.2015 x 0.1020) + (2 x 0.1454 x 0.1020))

LR = 210.245

Chapter 8 Relationship Testing

Chapter 8 Relationship Testing

Pedigree Analysis
Kinship Analysis using Identity by Descent
Data Base Search: Finding Nearest Relatives
Automated Pedigree Diagrams
Paternity Index using

Pedigree Analysis

Overview

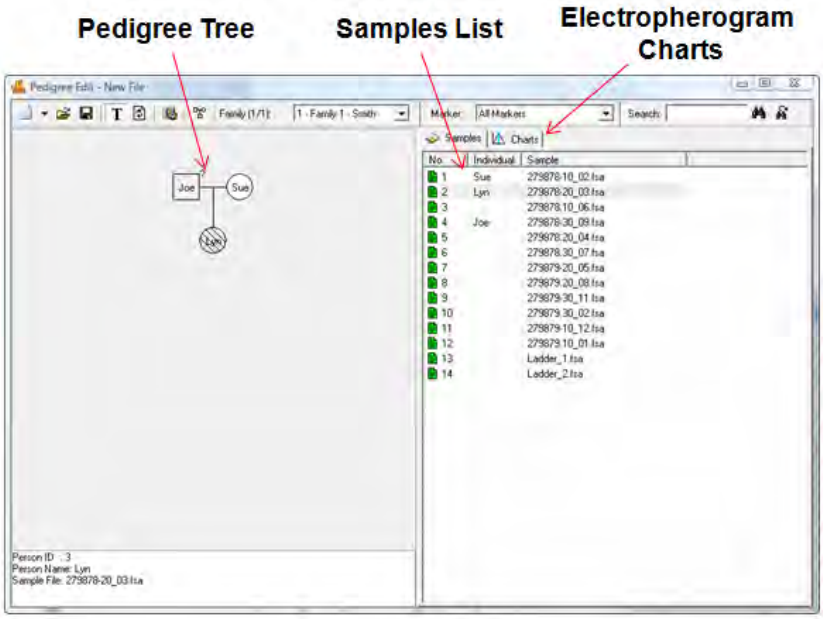
Relationship testing involves testing known genotypes in a familial relationship for simple Mendelian Inheritance laws. Since the core loci selected for forensic genotyping demonstrate the Mendelian characteristics of segregation and independent assortment, the **Pedigree** tool in GeneMarker HID is an excellent choice to determine genetic relationships between family members.

The *Pedigree* module is designed to aid identification of inheritance patterns and abnormalities for medical forensics. All individuals in the *Pedigree Tree* are directly linked to their corresponding *Electropherograms*. To display the *Electropherogram* for a sample, simply double-click on the sample's node in the *Pedigree Tree* and the *Electropherogram* will appear in the *Charts* tab. The link between the *Pedigree Tree* and *Electropherogram Charts* makes relationship analysis quick and efficient.

To activate the *Pedigree* function, select **Applications → Pedigree** from the *Main Analysis* window menu bar.

NOTE: The dataset must be sized and a Panel applied prior to using the Pedigree function.

Pedigree Application



Pedigree Tree

The *Pedigree Tree* displays, in a standard format, the relationship between different nodes or individuals in the sample dataset. Older generations appear at the top of the *Pedigree Tree*, younger generations near the bottom. Males are represented as square nodes, females as circle nodes. Horizontal lines indicate mates, vertical lines indicate offspring.

Once a node is added to the *Pedigree Tree*, right-click the node to see additional options.

Select/Deselect Individual Node

To view the electropherogram for an individual, right-click their node and select *Select Node* OR double-click the node and the electropherogram trace will appear in the *Charts* tab on the right. To hide the sample's electropherogram, right-click the node and select *Deselect Node*. When a node is selected in the *Pedigree Tree*, the corresponding sample in the *Sample List* becomes highlighted.

Add/Edit/Delete Individual

If an individual is the first to be added to the *Pedigree Tree*, a family must be designated for the person. If the person is already added to the *Pedigree Tree*, right-click and select *Edit Node* to change that person's characteristics. To delete an individual from the *Pedigree Tree*, right-click the node and select *Delete Node*.

Family Name

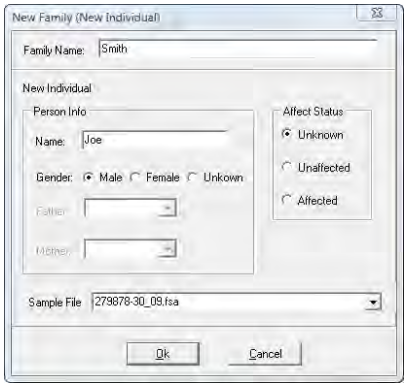
Enter a name for the family in the free form text box. The *Family Name* field will not appear after the first individual is added to the *Pedigree Tree*. All subsequent individuals added will be considered members of the family.

Person Info

Name: Free form text box to enter a name for the individual. Display the individual's name in the *Pedigree Tree* by clicking the **Show Individual ID** icon in the toolbar.

Gender: Select either Male, Female, or Unknown gender for the individual. Male nodes are squares, female nodes are circles, and Unknown gender nodes are displayed as a circle within a square.

Father/Mother: When more than one mate is displayed for a Mother or Father, a drop-down menu allows the user to choose which possible parent to associate with the child.



NOTE: The *Gender* and *Father/Mother* options are not available when adding a mate.

Affected Status

Affected Status options are available to mark individual nodes for genetic linkage calculations. Before marking individual nodes with *Affected Status*, click the **Pedigree Parameters** icon in the toolbar and adjust settings accordingly.

Unknown: The individual's phenotype is unknown. The node is displayed as an empty square or circle.

Unaffected: The individual does not show signs of the expected phenotype. The node is filled in white.

Affected: The individual expresses the phenotype. The node is filled in with diagonal hashed lines.

Sample File

Select the individual's sample file from the drop-down list. Only samples in the current dataset will be available. If no sample file is chosen, the node will be grayed out. Additionally, drag and drop a sample from the *Sample List* onto a node to associate the sample with the individual node.

Add Family Members

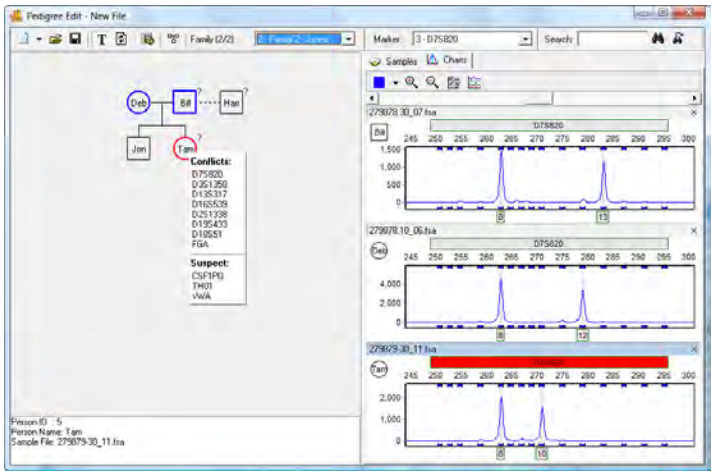
To add family members to the *Pedigree Tree*, right-click a node and select *Add Mate* or *Add Child*. The *Add Mate* or *Add Child* box will appear. Enter the individual's information and click **OK**.

Display Conflicts

Once a family is created in the *Pedigree Tree*, GeneMarker HID will automatically identify any Mendelian inheritance conflicts between Parents and Children or between Siblings. Click the **Show Conflict with Parents/Siblings** icon in the toolbar to alternate between the two modes. Nodes with conflicts will appear red. Mouse over the red node and a list of Markers or loci with conflicts will appear. Single left-click the Marker name and the family members' electropherograms will appear in the *Charts* tab on the right. The conflicting Marker will appear red.

Additionally, suspect Markers are indicated in the Pedigree Tree by a question mark symbol "?" next to the individual's node. Suspect Markers include those which contain additional peaks or low quality peaks. Mouse over the node with suspect Markers and a list of Markers or loci with suspect peaks will appear. Single left-click the Marker name and the individual's electropherogram will appear in the *Charts* tab on the right. The suspect Marker will appear red.

Show Inheritance Conflicts



Samples List

The *Samples List* displays the filename and individual ID for each sample in the current dataset. Drag and drop samples from the *Sample List* to nodes in the *Pedigree Tree* to associate individuals with the family.

Electropherogram Charts

The electropherogram traces for the samples in the *Sample List* appear in the *Charts* tab. Double-click a node or single-click the Marker name in the Conflict or Suspect list of the *Pedigree Tree* and the electropherogram trace for the Marker will appear. If a Child node is selected, the Mother and Father traces will also appear in the *Charts* tab.

Navigation and allele editing in the *Electropherogram Charts* is similar to navigation options in the *Main Analysis* window. **See Chapter 3 Main Analysis Overview**.

NOTE: After alleles are edited in the *Electropherogram Charts*, click the **Update Sample Data** icon in the toolbar. Conflict and Suspect Marker identification will be adjusted accordingly. Additionally, allele changes made in the *Pedigree* tool will be applied in the *Main Analysis* window.

Edit Suspect Alleles

Procedure

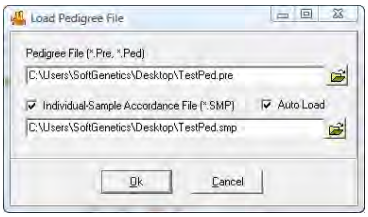
Although allele calls can be edited in the *Pedigree* tool, it is easier to begin a relationship test analysis with good, clean traces. In order to begin with the best sample traces, complete size calling, Panel alignment, and allele editing in the *Main Analysis* window prior to launching the *Pedigree* tool. To launch the *Pedigree* tool, select *Applications* → *Pedigree* from the menu bar of the *Main Analysis* window.

File types accepted or generated by GeneMarker HID *Pedigree* module:

Pedigree File (PRE, PED) – Contains information about the *Pedigree Tree* display.
Individual Sample Accordance File (SMP) – Contains Family and Individual ID information associated with specific sample file names.
Loci Description File (DAT) – Contains gene frequency information generated in the *Pedigree Parameters* box.

There are two ways to begin a relationship test with the Pedigree tool: upload a previously created pedigree file, OR create an entirely new pedigree file. The procedures are described below.

Upload Previously Created Pedigree

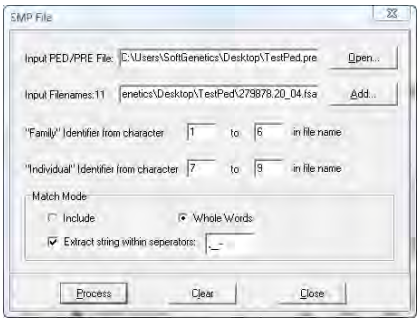


1. In *Pedigree* tool, click the **Open Pedigree File** icon
2. The *Load Pedigree File* box appears
3. Click the **Open Files** icon next to the *Pedigree Files* field
4. Use the Windows Explorer window to locate the Pedigree File (PED, PRE)
5. Select the file and click **Open**

NOTE: The *Pedigree File* selected must be associated with the samples currently uploaded to GeneMarker HID. If the sample filenames are not the same, the *Pedigree Tree* will appear grey and will not link with the *Sample List* or *Electropherogram Chart*.

6. The directory path will appear in the *Pedigree File* field
7. The same directory path and filename will appear in the *Individual Sample Accordance File* field if the SMP file is located in the same folder
8. If the SMP file is located in a different directory, click the **Open Files** icon next to the *Individual Sample Accordance File* field, select the correct directory, and click **Open**
9. Click **OK** in the *Load Pedigree File* box
10. The information in the Pedigree and SMP files will recreate the *Pedigree Tree* and associate the nodes with the samples in the dataset.

Create an SMP File from a PED/PRE File



If an SMP file does not exist for a *Pedigree File* or has been lost, a new SMP file can be generated with the *Pedigree File Name Match* tool.

1. In the *Main Analysis* window menu bar, select *Tools* → *Pedigree File Name Match*
2. The *SMP File* box appears
3. Click the **Open** button
4. Select a previously created Pedigree File (PED or PRE)
5. Click the **Add** button
6. Select all the samples included in the Pedigree File
7. Click the **Process** button
8. The *SMP Table* box will appear with filenames aligned in family groups
9. Click the **Save As** button
10. Save the SMP file to the same directory that contains the Pedigree File
11. Proceed through steps **1-10** above.

Create New Pedigree

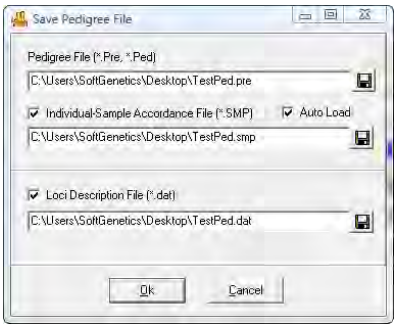
1. In *Pedigree* tool, click the **New Pedigree File** icon
2. The *New Family (New Individual)* box appears
3. Enter a Family Name, the Individual's Name, Gender, and the Sample File to associate with the Pedigree Tree node
4. Click **OK**
5. The individual's node will appear in the *Pedigree Tree* and the individual ID information will appear next to the sample name in the *Sample List*.
6. Continue to add family members by right-clicking at the node and selecting *Add Mate/Child*
7. Mouse over red highlighted nodes in the *Pedigree Tree* to view Conflicts and Suspect Markers – see section above – *Pedigree Tree*
8. Edit alleles in the *Electropherogram Charts* as required – see section above – *Electropherogram Charts*

Save Pedigree File

After the Pedigree has been created and modified, there are two options for saving the information: save the Pedigree File and/or export the *Pedigree Tree* as an image.

Save Pedigree File

1. In *Pedigree* tool, click the **Save Pedigree File** icon
2. The *Save Pedigree File* box appears
3. Click the **Save** icon next to the *Pedigree File* field
4. The Windows Explorer directory window appears
5. Enter a filename and select a directory
6. Click **Save**
7. The directory path and filename will appear in all three fields of the *Save Pedigree File* box
8. Click **OK**
9. Three files will be saved to the specified directory (PRE, SMP, and DAT).
10. To reopen the files in *Pedigree* tool, follow steps **1-10** of the *Upload Previously Created Pedigree* section above

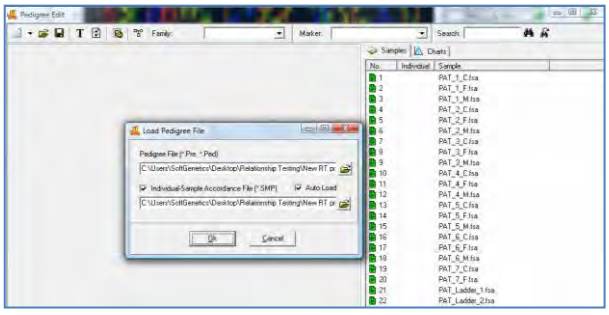


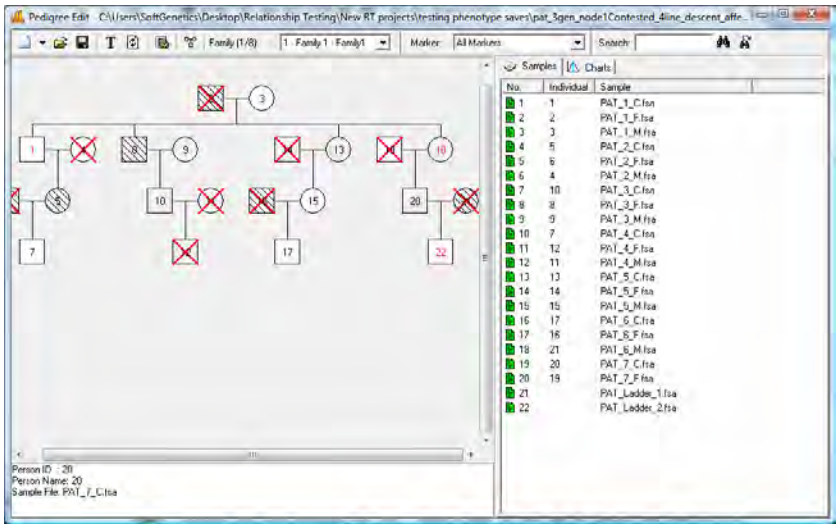
Save Pedigree Tree

1. Right-click anywhere in the *Pedigree Tree*
2. Select **Export Bitmap**
3. The *Save As* window appears
4. Enter a filename, choose a directory, and click **Save**
5. The *Pedigree Tree* will be saved as an image file (BMP)

Extended Pedigree Files

All personal information is saved and retrieved with the pedigree, smp and dat files, including affected status and deceased. Extended family trees can be built saved and added to, as additional information becomes available.





Icons and Functions

New Pedigree File
Select *New Pedigree File* to create a new pedigree with multiple families *OR* select *New Family* to add a family to the pedigree file. Enter the first family member's information into the *New Family (New Individual)* box and click **OK** to create a new *Pedigree Tree*.

Open Pedigree File
Launches the Load Pedigree File box. Select a PED or PRE file to upload (the SMP file will automatically upload) and click OK.

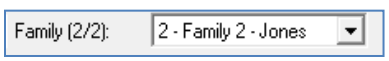
Save Pedigree File
Launches the *Save Pedigree File* box. Enter filename and change directory to save the Pedigree Files (PRE, SMP, DAT).

Show Individual Name
When selected, the individual ID will be displayed in the nodes of the *Pedigree Tree*.

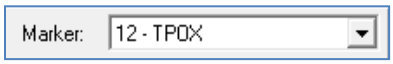
Update Sample Data
Select to refresh the Mendelian inheritance calculation after a node or allele is edited.

Pedigree Parameters
Launches the *Loci Description* box. Enter the Affection Locus Description, Gene Frequencies, Select Markers, Number of Liability Classes, Penetrances, Recombination Values, and view the Allele Label and Frequencies.

Show Conflict
Toggle between *Show Conflict with Parents* and *Show Conflict with Sibling*. Conflicting and suspected Markers based on Mendelian inheritance are highlighted.



Family
Select a family from the currently uploaded pedigree file to view and edit.



Marker
Select a Marker or Locus to view in the *Electropherogram Charts*.

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.

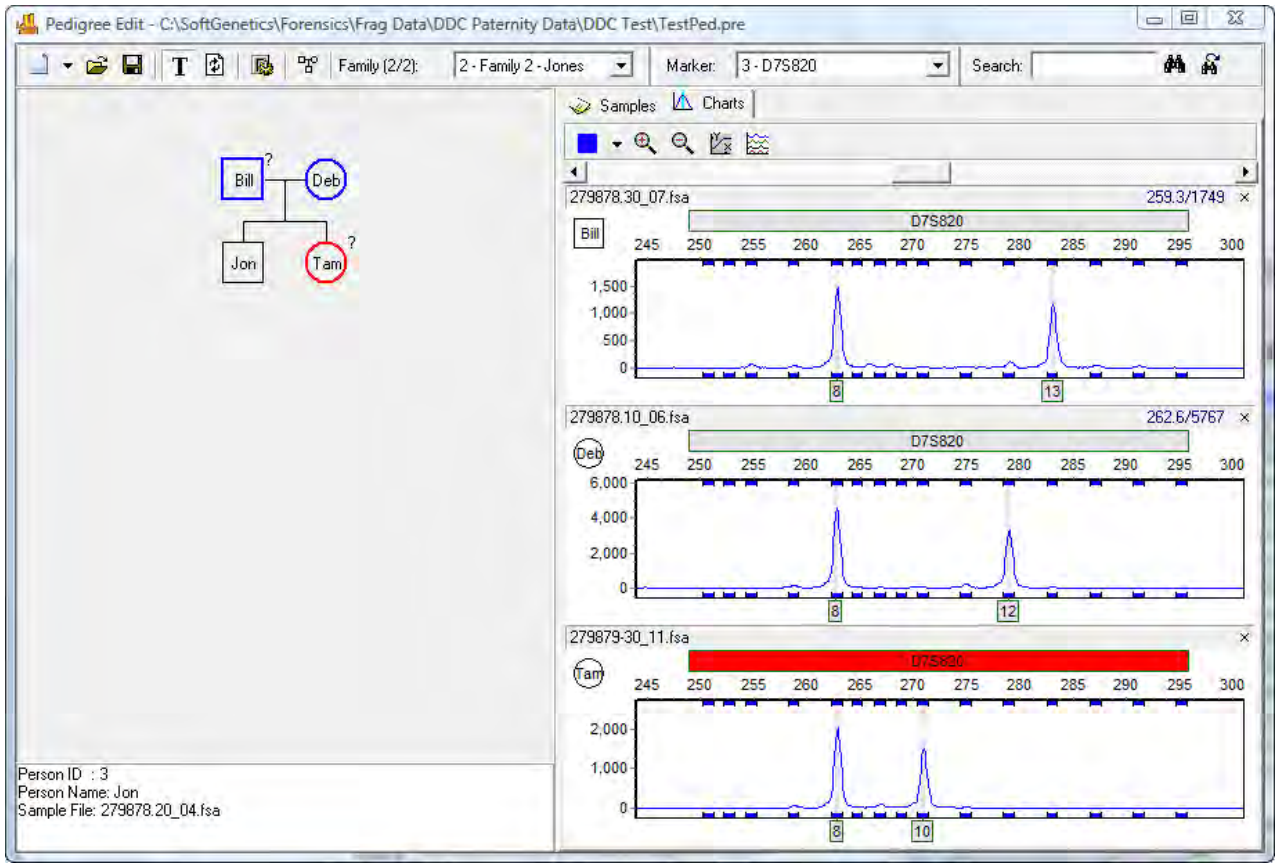
What to Expect

Mendelian inheritance is guided by Mendel's two basic laws: Segregation and Independent Assortment. The law of segregation describes how phenotypes are an expression of two alleles inherited independently, one from each parent. Independent assortment is the idea that the inheritance of one gene or allele does not affect the likelihood of inheritance of a different gene. Today we have found that this is not true of all genes, some genes are invariably linked and inherited together. Linked genes do not follow Mendelian inheritance patterns.

With GeneMarker HID's Pedigree module, Mendelian inheritance patterns can be analyzed. That is, alleles are inherited independently, one from each parent, and alleles are not influence by the inheritance of other alleles. Forensic STR loci were selected because they follow the laws of Mendelian genetics.

In the example below, the child's Marker D7S820 is highlighted red. The child's Allele 8 matches with the mother and father; however, the child's Allele 10 does not match with either the father or the mother. Because Allele 10 does not match an allele from the father or mother, it can be assumed that one of the parents is not related to the child. Because all three individuals share Allele 8, it cannot be determined which parent is not related to the child. Review additional loci to determine which parent is unrelated.

Mendelian Inheritance of Alleles



Kinship Analysis using Identity by Descent

Overview

STR profiles of two individuals can be compared to determine the likelihood that they have a specific relationship versus the likelihood that they are unrelated. Kinship analysis compares STR profiles from individuals to determine likelihood of a family relationship versus the likelihood that two individuals with these STR profiles are unrelated. The formulas used to calculate the level of kinship depend on:

1. Probabilities that 2, 1 or 0 alleles will be shared (IBD identity by descent) given a specific relationship

2. The probability of a specific genotype X given genotype Y at all loci, under the conditions that X and Y have 2, 1, or 0 alleles IBD

GeneMarker HID uses established, rigorous statistical analysis (Kinship formulas from Brenner 2004; Eisenberg and Planz 2007) to calculate probabilities and likelihood ratios for different relationship levels including: Parent/child, Siblings, Half-siblings, Uncle/Nephew, Cousins and Grandparents.

$$[P_{2(xy)}\,\Phi_2] + [P_{1(xy)}\,\Phi_1] + [P_{0(xy)}\,\Phi_0]$$

Where:
$P_{2(xy)}$ = Probability of 2 alleles IBD (I) given the genotypes of sample x and sample y
$P_{1(xy)}$ = Probability of 1 alleles IBD (T) given the genotypes of sample x and sample y
$P_{0(xy)}$ = Probability of 0 alleles IBD (O) given the genotypes of sample x and sample

Identity by Descent (IBD Φ_2, Φ_1, Φ_0) for each relationship category:

	Φ_2	Φ_1	Φ_0
Parent – Child	0	1	0
Siblings	0.25	0.5	0.25
Half-siblings	0	0.5	0.5
Cousins	0	o.25	0.75
Uncle/nephew	0	0.5	0.5
Grandparent/ Grandchild	0	0.5	0.5

Kinship Formula Transition Matrices

$$I = \begin{array}{c|ccc} & A_1A_1 & A_1A_2 & A_2A_2 \\ A_1A_1 & 1 & 0 & 1 \\ A_1A_2 & 0 & 1 & 0 \\ A_2A_2 & 0 & 0 & 1 \end{array}$$

$$T = \begin{array}{c|ccc} & A_1A_1 & A_1A_2 & A_2A_2 \\ A_1A_1 & p_1 & p_2 & 0 \\ A_1A_2 & 0.5\,p_1 & 0.5(p_1+p_2) & 0.5p_2 \\ A_2A_2 & 0 & p_1 & p_2 \end{array}$$

$$O = \begin{array}{c|ccc} & A_1A_1 & A_1A_2 & A_2A_2 \\ A_1A_1 & p_1^2 & 2\,p_1p_2 & p_2^2 \\ A_1A_2 & p_1^2 & 2\,p_1p_2 & p_2^2 \\ A_2A_2 & p_1^2 & 2\,p_1p_2 & p_2^2 \end{array}$$

From C C Li and Sachs, 1954

Relationship Testing
Kinship Formula:
$[P_2(xy)\ x\ \Phi_2] + [P_1(xy)\ x\ \Phi_1] + [P_0\ (xy)\ x\ \Phi_0]$
Where:
Φ_2, Φ_1, Φ_0 = Identity by descent coefficients for sharing 2, 1 or 0 alleles
$P_2(xy)$ = The probability of genotype y given genotype x with 2 of their alleles IBD
$P_1(xy)$ = The probability of genotype y given genotype x with 1 of their alleles IBD
$P_0\ (xy)$ = The probability of genotype y given genotype x with 0 of their alleles IBD
Formulas for each possible combination of alleles, derived from stochastic matrices of Li and Sachs.[5]
$p_A\ p_B\ p_C\ p_D$ = Probability of that allele for a given population

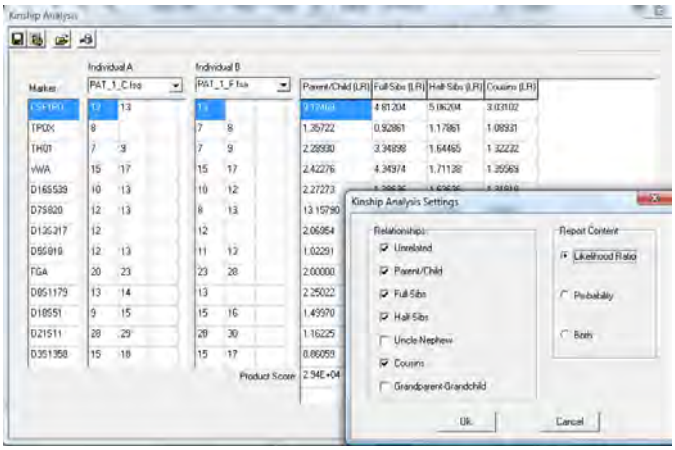
Genotype combination	Frequency
AB AB	$\Phi_2 + 0.5\,\Phi_1(p_A + p_B) + 2\,\Phi_0 p_A\,p_B$
AA AA	$\Phi_2 + \Phi_1 p_A + \Phi_0 p_A^2$
AA AB	$\Phi_1 p_B + 2\Phi_0\,p_A\,p_B$
AB AC	$0.5\,\Phi_1\,p_C + 2\Phi_0\,p_A\,p_C$
AB CD	$2\Phi_0 p_C p_D$
AA BB	$\Phi_0 p_B^2$
AA BC	$2\,\Phi_0 p_B p_C$

Procedure

Although allele calls can be edited in the *Relationship Testing* tool, it is easier to begin a relationship test analysis with good, clean traces. In order to begin with the best sample traces, complete size calling, Panel alignment, and allele editing in the *Main Analysis* (see chapter 2 General Procedure) window prior to launching the *Relationship Testing* tool.
File types accepted or generated by GeneMarker HID *Pedigree* module:
Pre/Ped files
SGF
TXT
BMP

The *Save to DataBase* function allows easy updates of the relationship testing database. The default database includes 13 CODIS markers and should be used with complete profile samples. Additional markers can be added to the database.

To launch the *Kinship Analysis* function, select *Applications* → *Relationship Testing* from the menu bar of the *Main Analysis* window.

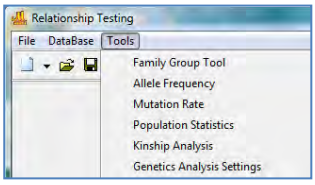


1. Open data file or previously saved project
2. Run Wizard to call alleles
3. Select Relationship Testing from the Applications drop down menu
4. Select Allele Frequency of the appropriate population from the Tools drop down menu
5. Select Kinship Analysis Tool – use drop down menus to select individuals for comparison
6. Select the desired relationship level and likelihood ratio, probability or both at the kinship analysis settings

   Probabilities for the occurrence of the genotypes within the population having a specific relationship or being unrelated for each locus and all loci combined are displayed in table form.
7. Likelihood ratios for each locus and combined likelihood ratio of a related (parent/child, sibling, half sibling) are presented in table form.

Icons and Functions

Relationship Testing Main Drop-down menus
Select from File, DataBase or Tool options



Relationship Testing Tools include:
Family Group Tool – for automated pedigree trio drawing
Allele Frequency for major US populations (Budwole 2001 *et al.*)
Mutation Rate specified by AABB
Population Statistics for the family groups under analysis
(2pq = heterozygotes and $p^2 + p(1-p)\theta$ where $\theta=0.01$) = homozygotes
Genetic Analysis Settings – allows setting the above options at the same time

New Pedigree File
Select *New Pedigree File* to create a new pedigree with multiple families *OR* select *New Family* to add a family to the pedigree file. Enter the first family member's information into the *New Family (New Individual)* box and click **OK** to create a new *Pedigree Tree*.

Open Pedigree File
Launches the Load Pedigree File box. Select a PED or PRE file to upload (the SMP file will automatically upload) and click OK.

Save Pedigree File Launches the *Save Pedigree File* box. Enter filename and change directory to save the Pedigree Files (PRE, SMP, DAT).

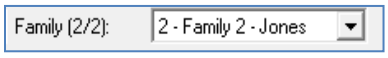
Show Individual Name
When selected, the individual ID will be displayed in the nodes of the *Pedigree Tree*.

Update Sample Data
Select to refresh the Mendelian inheritance calculation after a node or allele is edited and after selection a different family when the *'show genotype'* display is used

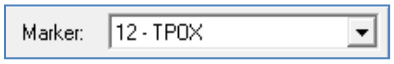
Relationship Testing Parameters
Launches the *Relationship Testing Settings* box. Options for selected samples or all samples, selecting the appropriate allele frequency for the population, mutation rate, and prior probability, gender known and limits based on LR or minimum number of retrieved samples.

Show Conflict
Toggle between *Show Conflict with Parents* and *Show Conflict with Sibling*. Conflicting and suspected Markers based on Mendelian inheritance are highlighted.

Show Genotype
Toggle between *Displaying and Not Displaying the Genotypes* of the selected node



Family
Select a family from the currently uploaded pedigree file to view and edit.



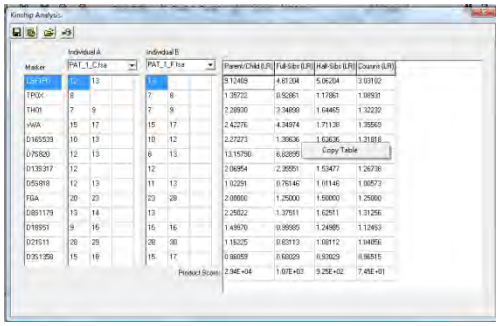
Marker
Select a Marker or Locus to view in the *Electropherogram Charts*.

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.

Save Kinship Report as .txt file or right click to copy/paste

Database Search: Locate Duplicate Samples and Nearest Relatives

Overview

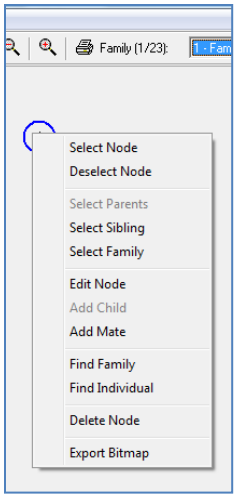
Missing person identification may be necessary in many situations: mass natural disasters (earthquakes, tsunamis), human attacks (Sept 11 World Trade Center), war, or in the cases of thousands of missing children and adults reported every year. The database function of GM HID is capable of closed system searches for same sample matches – in the case where a DNA sample of the missing individual is available from personal use items. In the case where there is no personal sample available the kinship formulas (IBD) are used to find highest likelihood ratio scores for each relationship level. The database includes all CODIS markers and should be used with complete profile samples. The *Save to DataBase* function allows easy updates of the relationship testing database. It is strongly recommended to back up the database files on a regular basis.

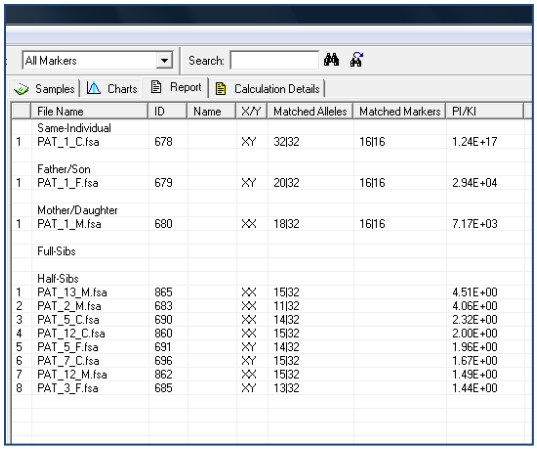
Procedure

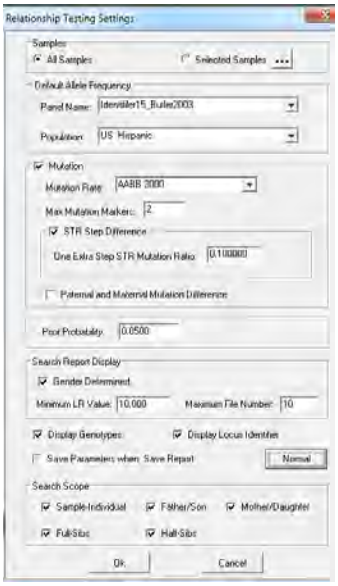
1. Open data file or previously saved project
2. Run Wizard to call alleles
3. Select Relationship Testing from the Applications drop down menu
4. Select Tools - Allele Frequency of the appropriate population from the Tools drop down menu
5. Select Tools – Genetic Analysis Settings if settings other than the defaults are desired

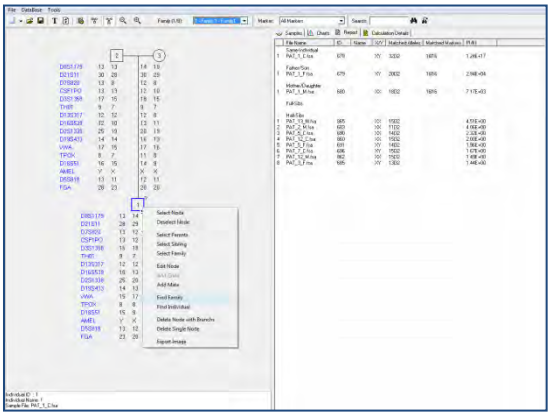
Individual sample

1. Tools > *family group tool* > *OK* to allow selection of each individual in the file as a separate node
2. Use the Family dropdown menu to select the individual file
3. Right click on the node and select *find family* from the drop-down menu
4. Left click on the Report icon in the tool bar to display the file name and any duplicates of that file found in the data base. The samples with the highest LR for each relationship type are displayed in descending order, in addition to the sex, number of matched alleles and matched markers









Sample in Pedigree Tree

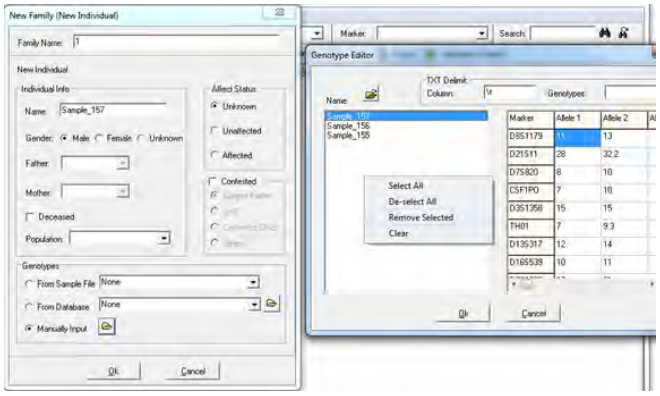
1. Import or draw Pedigree Tree **See Chapter 7 Pedigree Analysis and Automated Pedigree Tree.**
2. Right click on the node and select *find family* from the drop-down menu
3. Left click on the Report icon in the tool bar to display the file name and any duplicates of that file found in the data base. The samples with the highest LR for each relationship type are displayed in descending order, in addition to the sex, number of matched alleles and matched markers

Search Database for .txt file genotype

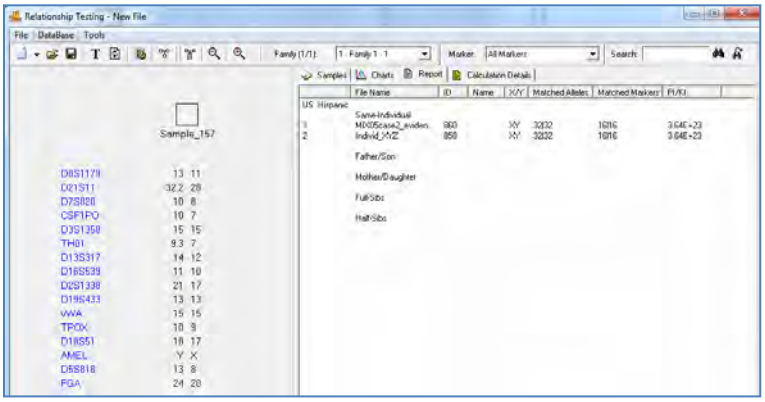
Use this function if the genotype is only available from an archived file --the .fsa or .hid data file is not available. Format the .txt tab delimited file as in the example below – the exact format is required for the program to recognize the genotype. The file must be saved as .txt tab delimited.

AA3 | fx | 18

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
1	AID	D8S1179	D8S1179	D21S11	D21S11	D7S820	D7S820	CSF1PO	CSF1PO	D3S1358	D3S1358	TH01	TH01	D13S317	D13S317	D16S539	D16S539
2	Sample_157	11	13	28	32.2	8	10	7	10	15	15	7	9.3	12	14	10	11
3	Sample_156	12	13	27	28	7	8	8	11	15	14	7	7	12	12	10	11
4	Sample_155	11	11	32.2	32.2	8	8	10	10	15	15	9.3	9.3	14	14	10	11



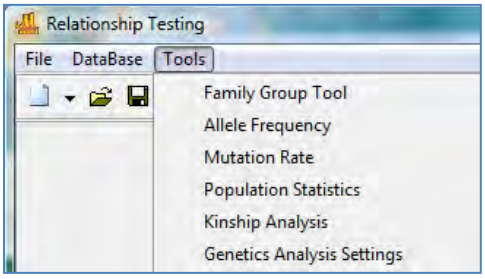
1. Open a saved project in the main analysis screen
2. Select Applications – Relationship Testing
3. Select Tools – Allele frequency and select the appropriate table
4. Select File --- New Pedigree
5. Fill in the sample identifier or name and click on Manual Input and the open folder Icon
6. Use the open folder icon in the Genotype Editor to add genotypes from a .txt file
7. Select the desired genotype for the search (right click on the open area at the left to Remove Selected Files or to Clear all file in order to import additional genotypes).
8. Select Ok in both the Genotype Editor and New Individual Dialogs to display the node of the selected individual – then right click and select find family as in the above searches to search the database



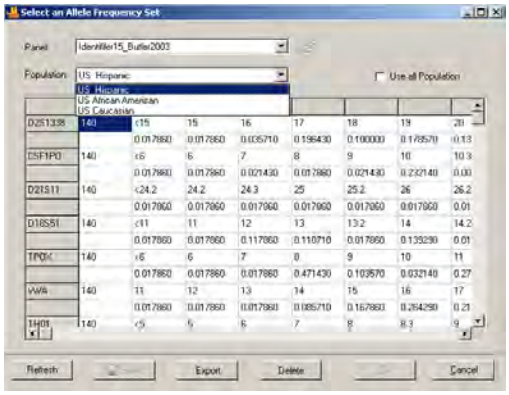
Icons and Functions

Relationship Testing Main Drop-down menus
Select from File, DataBase or Tool options

Relationship Testing Tools include:



Family Group Tool – for automated pedigree trio drawing
Allele Frequency for major US populations
Mutation Rate specified by AABB
Population Statistics for the file under analysis
Genetic Analysis Settings – allows setting the above options at the same time, additional settings include limiting the number of files retrieved in the database search by a minimum number, minimum likelihood ratio, gender known or gender not known, and Advanced settings -- to limit the search to specified relationship levels (the default is to search and display potential same individual, father/son, mother/daughter, sibling and half sibling).



Allele Frequency Tables
Allele frequency tables for major US populations may be selected from the drop down menus in the Select Allele Frequency Settings box. If results of all populations are preferred, select the Use all Populations box and the final report will append with the results using each of the tables sequentially. The Delete button may be used by individuals with access rights to remove any population frequency tables that are not needed by the laboratory. Use the Open folder icon to import formatted allele frequency tables for other populations.

Allele Frequency Tables and Sources:

PowerPlex 16_2001_Levedakou_et al.

Caucasian American, African American, Hispanic American and Asian American Levedakou, E.N. *et al.* (2001) Allele frequencies for fourteen STR loci of the PowerPlex™ 1.1. and 2.1 multiplex systems and Penta D locus in caucasians, african-americans, hispanics, and other populations of the United States of America and Brazil. [published erratum appears in *J. Forensic Sci.* (2001) Nov.;46(6)] *J. Forensic Sci.* **46**,736–61. http://www.promega.com/applications/hmnid/referenceinformation/popstat/custstat_Allelefreq.htm

CODIS 13_2001_Budowle et al.

Budowle B, Shea B, Niezgoda S, Chakraborty R.
CODIS STR loci data from 41 sample populations. J Forensic Sci 2001:46;(3):453–489. http://projects.nfstc.org/workshops/resources/literature/CODIS%20STR%20Loci%20Data%20from%2041%20Sample.pdf
African American, US Caucasian and Hispanic from the FBI column in Budowle 2001 were previously published in: Budowle B, Moretti TR, Baumstark AL, Defenbaugh DA, Keys KM.
Population data on the thirteen CODIS core short tandem repeat loci in
African Americans, U.S. Caucasians, Hispanics, Bahamians, Jamaicans,
and Trinidadians. J Forensic Sci 1999;44:1277–86.

ESX_ESI 2010_Hill et al.
Carolyn R. Hill, David L. Duewer, Margaret C. Kline, Cynthia J. Sprecher, Robert S. McLaren, Dawn R. Rabbach, Benjamin E. Krenke, Martin G. Ensenberger, Patricia M. Fulmer, Douglas R. Storts, John M. Butler Concordance and population studies along with stutter and peak height ratio analysis for the PowerPlex® ESX 17 and ESI 17 Systems
FSI:Genetics April 2010
http://www.cstl.nist.gov/strbase/NISTpop.htm
http://www.fsigenetics.com/article/S1872-4973(10)00071-2/abstract

GlobalFiler allele frequency table is populated from the marker information of the Identifiler and ESX publications

Fusion Butler, J.M., Hill, C.R., Coble, M.D. (2012) Variability of new STR loci and kits in U.S. population groups. *Profiles in DNA*.

Identifiler_15_2003_Butler et al.
John M. Butler, Ph.D.; Richard Schoske, M.A.; Peter M. Vallone, Ph.D.; Janette W. Redman; and Margaret C. Kline, M.S. Allele Frequencies for 15 Autosomal STR Loci on U.S. Caucasian, African American, and Hispanic Populations *J Forensic Sci*, July 2003, Vol. 48, No. 4 Paper ID JFS2003045_484
http://www.cstl.nist.gov/biotech/strbase/pub_pres/Butler2003a.pdf

New Pedigree File
Select *New Pedigree File* to create a new pedigree with multiple families *OR* select *New Family* to add a family to the pedigree file. Enter the first family member's information into the *New Family (New Individual)* box and click **OK** to create a new *Pedigree Tree*.

Open Pedigree File
Launches the Load Pedigree File box. Select a PED or PRE file to upload (the SMP file will automatically upload) and click OK.

Save Pedigree File
Launches the *Save Pedigree File* box. Enter filename and change directory to save the Pedigree Files (PRE, SMP, DAT).

Show Individual Name
When selected, the individual ID will be displayed in the nodes of the *Pedigree Tree*.

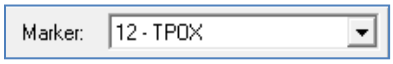
Update Sample Data
Select to refresh the Mendelian inheritance calculation after a node or allele is edited and after selection a different family when the *'show genotype'* display is used

Relationship Testing Parameters
Launches the *Relationship Testing Settings* box. Options for selected samples or all samples, selecting the appropriate allele frequency for the population, mutation rate, and prior probabilitiy.

Show Conflict
Toggle between *Show Conflict with Parents* and *Show Conflict with Sibling*. Conflicting and suspected Markers based on Mendelian inheritance are highlighted.

Show Genotype
Toggle between *Displaying and Not Displaying the Genotypes* of the selected node

Family
Select a family from the currently uploaded pedigree file to view and edit.



Marker
Select a Marker or Locus to view in the *Electropherogram Charts*.

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.

Importing Population Specific Allele Frequency and Mutation Rate Information

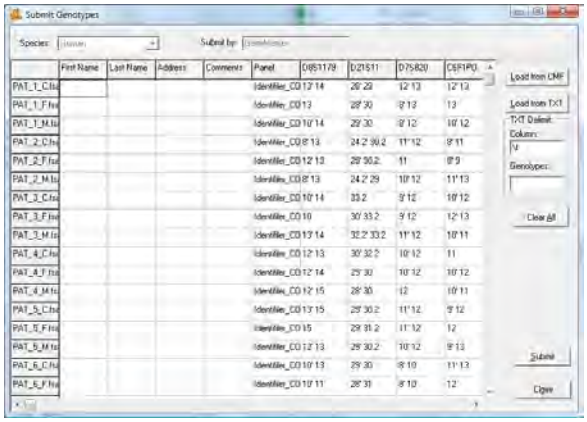
The Relationship Testing application in GeneMarker HID has the allele frequencies for major USA populations and the mutation rates specified by AABB pre-loaded. Customization with population specific allele frequency and mutation rates is easily accomplished using the open folder icon of the allele frequency and mutation rate tools. Population specific allele frequency and mutation rate tables must follow the format of preloaded files and be saved as a .txt tab delimited file.

Tools →Allele Frequency  → → Save

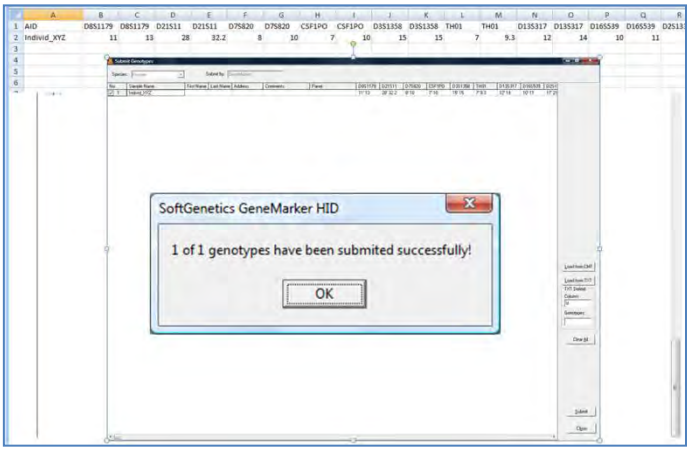
Building the Database

DataBase > Save to DataBase and select from three different routes.

1. Directly from the current project – Submit
2. From a CMF file – select Load from CMF and navigate to the saved file(s)
3. From a TXT file – select Load from TXT and navigate to the saved file(s)



Use the format of the spread sheet shown here for importing genotypes as .txt tab delimited files. The AID column has the file name. Each marker heading is in duplicate and the extended homozygous format is used for the allele calls. If there is a marker with allele drop out or null allele double asterisk ** should be used in that cell. Please see Report Table in Chapter 3.



Genotypes saved to the database from a .txt file must have the format at follows:
AID column = sample identification
Row 1 B – The marker names (case sensitive) repeated as below

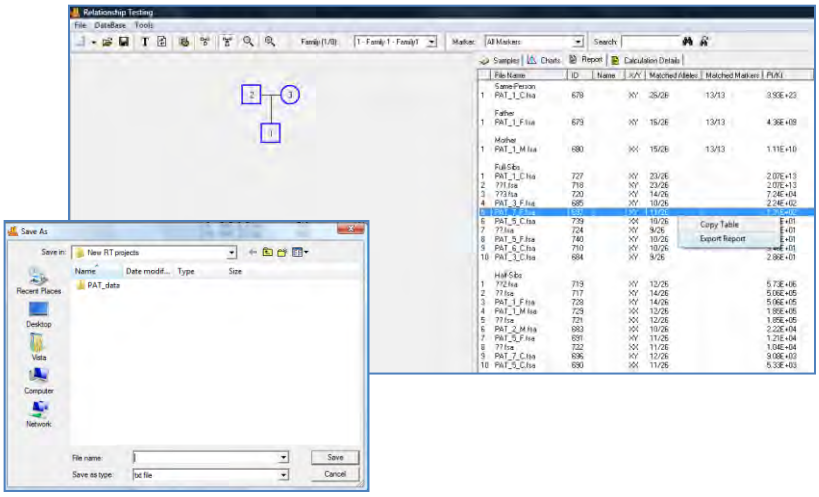
AA3 | 18

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
1	AID	D8S1179	D8S1179	D21S11	D21S11	D7S820	D7S820	CSF1PO	CSF1PO	D3S1358	D3S1358	TH01	TH01	D13S317	D13S317	D16S539	D16S539
2	Sample_157	11	13	28	32.2	8	10	7	10	15	15	7	9.3	12	14	10	11
3	Sample_156	12	13	27	28	7	8	8	11	15	14	7	7	12	12	10	11
4	Sample_155	11	11	32.2	32.2	8	8	10	10	15	15	9.3	9.3	14	14	10	11

Save and Print Report

'Find Family' Report – right click on the report to copy/paste directly into an existing document or report

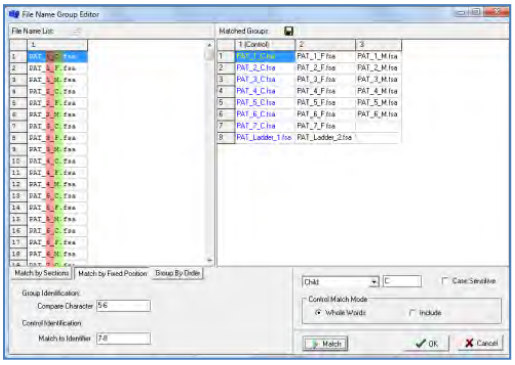
Or export as a .txt file



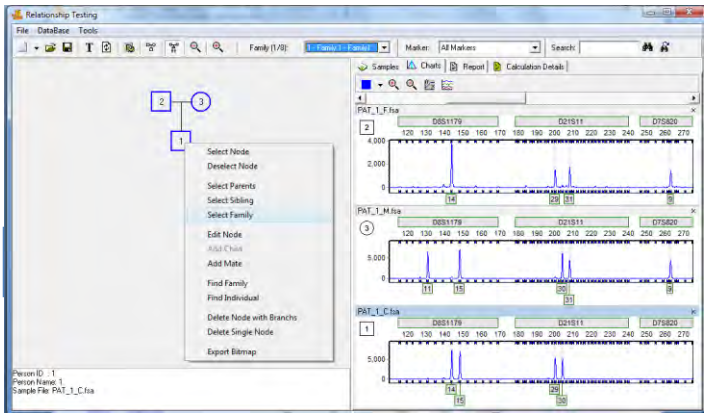
Automated Pedigree Trio Diagrams and Analysis using Family Group Tool

When a naming convention is followed the Family Group Tool enables matching of files into family groups.

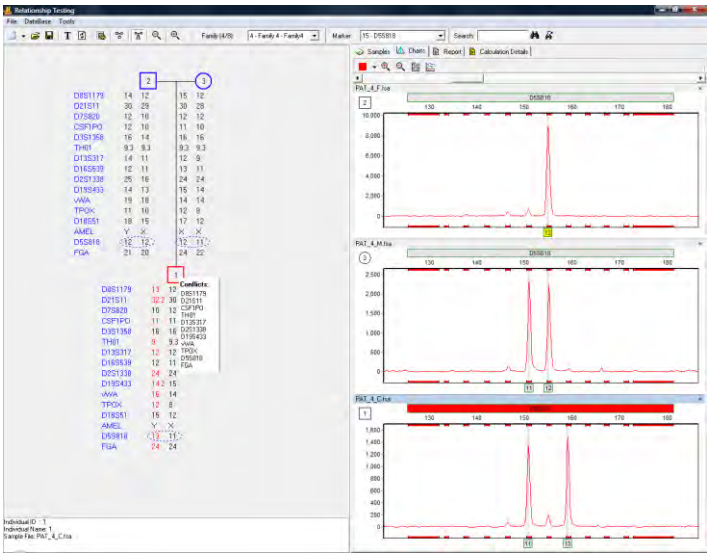
Procedure:



1. Select *Applications* → *Relationship Testing* from the menu bar of the *Main Analysis* window
2. Match by Sections, Positions or Group Order and then Match Whole Words → OK
3. The Pedigree for the families is drawn and displayed at the left of the Sample List.



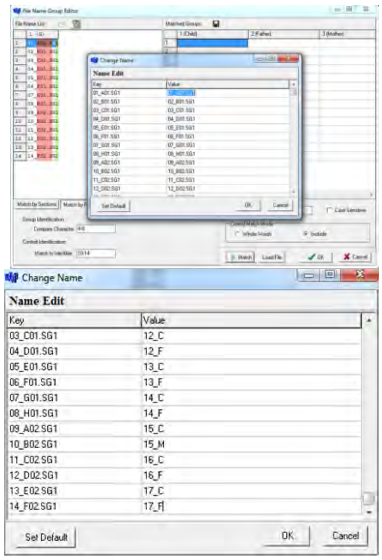
4. Right click on a node for edit or analysis options.
5. *Select Family* displays all electropherograms for the pedigree tree at the right.
6. *Select Node* or parents, siblings displays electropherograms
7. *Edit Node* allows editing of file or electropherogram information – Be sure to use the refresh key after any changes
8. *Add Mate or child* to expand the pedigree



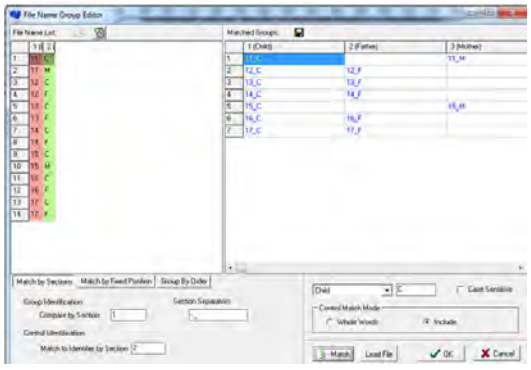
Allele conflicts are listed and the node is highlighted in red. Clicking on the marker in the list links to the section of the electropherogram where the conflict can be visualized.

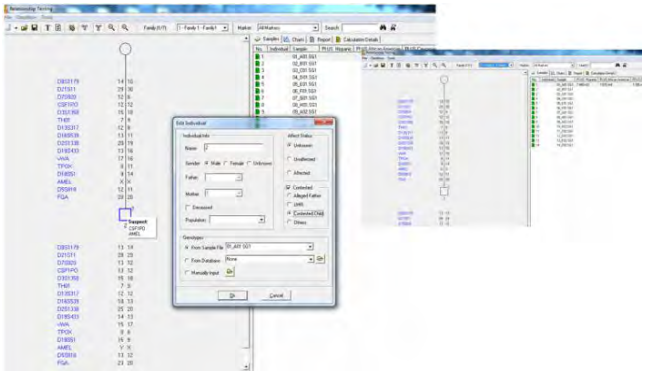
Renaming Tool:
If the file names are not adequate for use in the family group editor, the renaming tool provides transient naming conventions and allows the user to use the automated pedigree drawing instead of manually diagramming the pedigree.

Procedure:



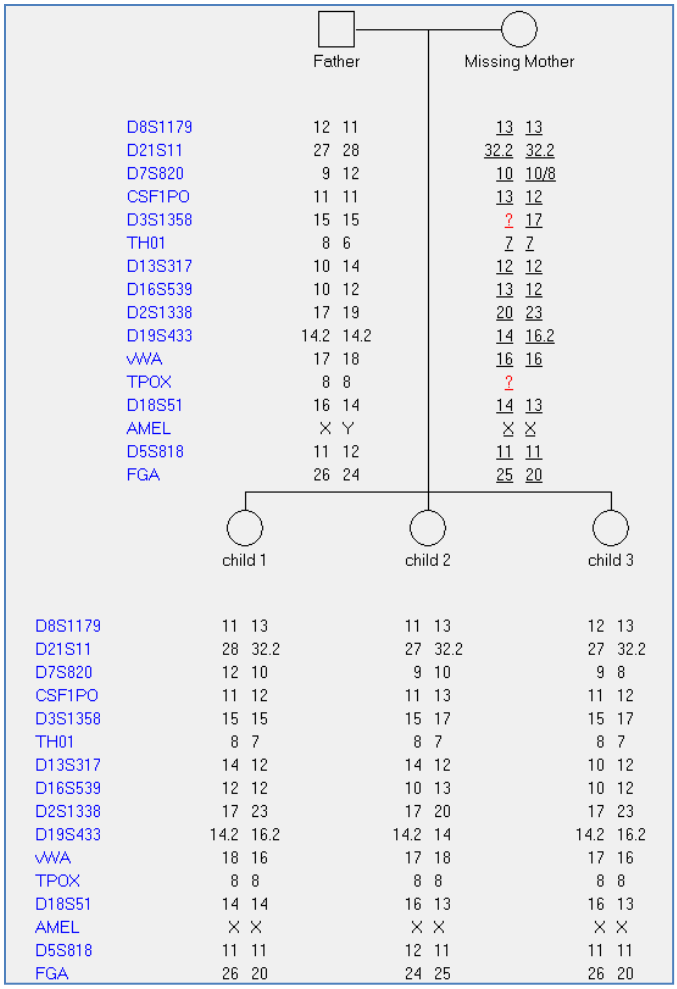
1. Click on the  icon to activate the renaming tool
2. Enter values that have a family identifier file_relationship field
3. C = Child, F=Father, M=Mother – for several children in a family group, number them C1, C2, C3…
4. Enter Group Identification and Control Identification values as previously described for the family Grouping Tool
5. Click Match and then OK to draw the pedigrees
6. Right mouse click on the parent node – select Edit Node
7. Click the 'contested' box to obtain the PI calculation results using the trio or motherless case PI equations from AABB Recommendations for Relationship Testing





Deducing Missing Parent Genotype

The partial genotype of a missing parent is deduced based on the allele calls of the available parent and the child(ren). In the example below the father and three children genotypes were available. The Missing Mother genotype is deduced based on the genotypes of children and father. Deduced allele calls are indicated with underlines. Question marks indicate allele calls that cannot be made with the available data.



Editing Personal Information

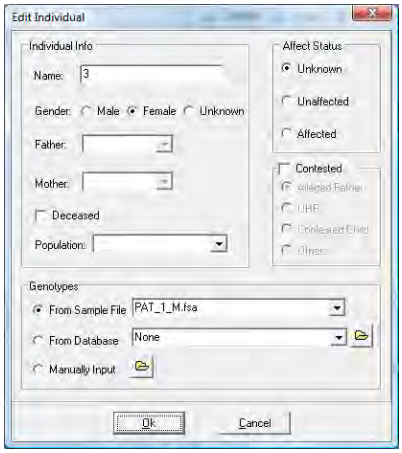
Add/Edit/Delete Individual

If an individual is the first to be added to the *Pedigree Tree*, a family must be designated for the person. If the person is already added to the *Pedigree Tree*, right-click and select *Edit Node* to change that person's characteristics. To delete an individual from the *Pedigree Tree*, right-click the node and select *Delete Node*.

Family Name

Enter a name for the family in the free form text box. The *Family Name* field will not appear after the first individual is added to the *Pedigree Tree*. All subsequent individuals added will be considered members of the family.

Person Info



Name: Free form text box to enter a name for the individual. Display the individual's name in the *Pedigree Tree* by clicking the **Show Individual ID** icon in the toolbar.

Gender: Select either Male, Female, or Unknown gender for the individual. Male nodes are squares, female nodes are circles, and Unknown gender nodes are displayed as a circle within a square.

Father/Mother: When more than one mate is displayed for a Mother or Father, a drop-down menu allows the user to choose which possible parent to associate with the child.

NOTE: The *Gender* and *Father/Mother* options are not available when adding a mate.

Affected Status
Affected Status options are available to mark individual nodes for genetic linkage calculations. Before marking individual nodes with *Affected Status*, click the **Pedigree Parameters** icon in the toolbar and adjust settings accordingly.
Unknown: The individual's phenotype is unknown. The node is displayed as an empty square or circle.
Unaffected: The individual does not show signs of the expected phenotype. The node is filled in white.
Affected: The individual expresses the phenotype. The node is filled in with diagonal hashed lines.

Contested
Select Contested to activate the ability to identify a file as an alleged father, unidentified human remains (UHR) or a contested child

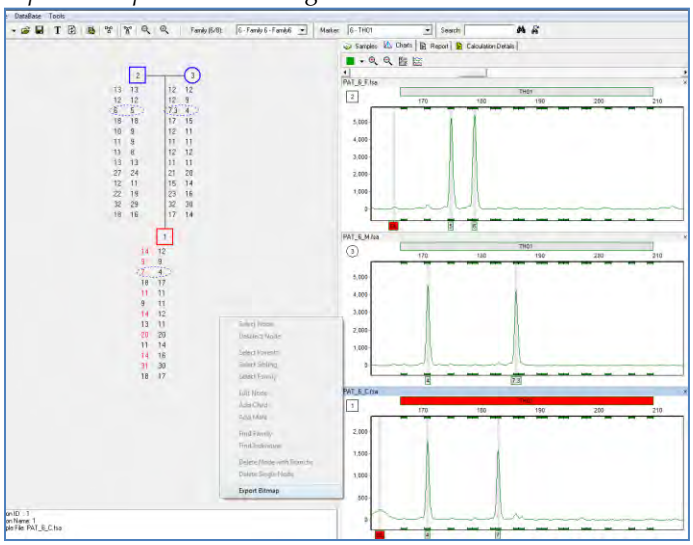
Sample File
Select the individual's sample file from the drop-down list. Only samples in the current dataset will be available. If no sample file is chosen, the node will be grayed out. Additionally, drag and drop a sample from the *Sample List* onto a node to associate the sample with the individual node.

From Database
Select the individual's sample file from the drop-down list. Any sample that was previously saved to the database will be available.

Add Family Members
To add family members to the *Pedigree Tree*, right-click a node and select *Add Mate* or *Add Child*. The *Add Mate* or *Add Child* box will appear. Enter the individual's information and click **OK**.

Save Report

Export Bitmap to save the diagram



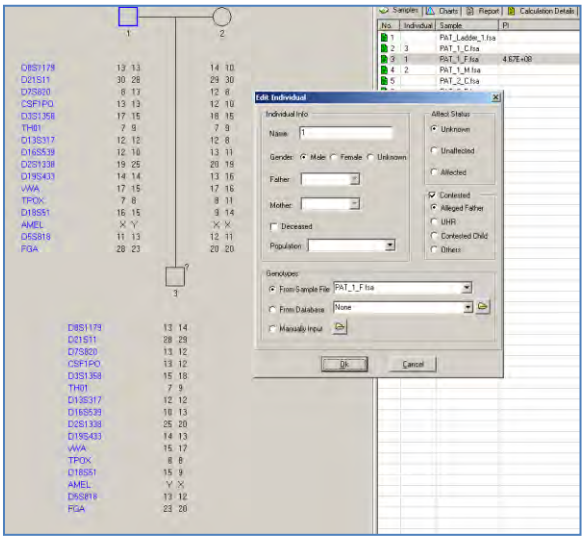
Paternity Index Calculations

Overview

Paternity index (PI) is calculated using the Recommendations of AABB Standards For Relationship Testing Laboratories, Appendix 8). The combined PI is displayed to the right of the file of the alleged father and the Calculation Details tab contains the results for each marker. As with the Identity by Descent calculations, the user may opt to allow for mutation (allele conflicts) using the analysis parameters and the AABB mutation rates.

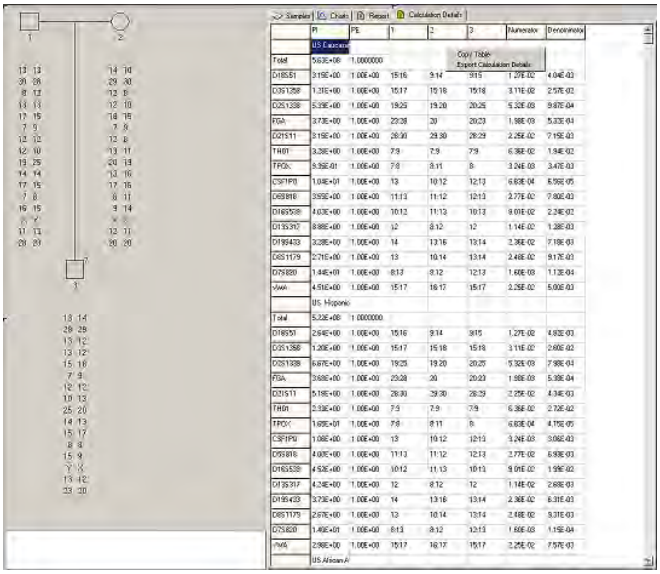
Procedure

1. Open a saved project file or import raw data and complete analysis as in chapter 3.
2. Tools – Select the desired Allele Frequncy Table (or use all populations)
3. Tools – Relationship Testing – Tools – Family Grouping Tool
4. Group families as in the previous section.
5. Right mouse click on the alleged father node and edit node.
6. Select Alleged Father in the Contested section.
7. The PI is displayed in the table.
8. Select Calculation Details for the results at each locus
9. Right mouse click on the table to copy/paste or save as .txt tab delimited file



Save and Print Reports

Activate the Save or Copy/Paste for Print reports by using a right mouse click anywhere in the results table. Tables are saved as .txt tab delimited files or may be copy/pasted into an existing report.

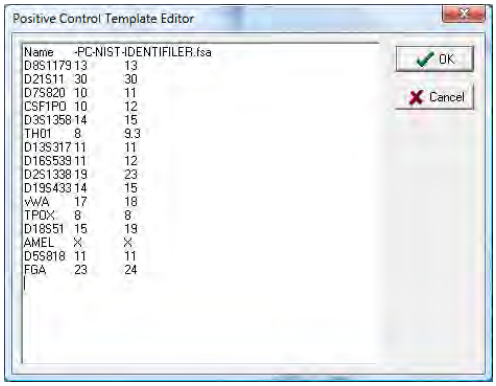


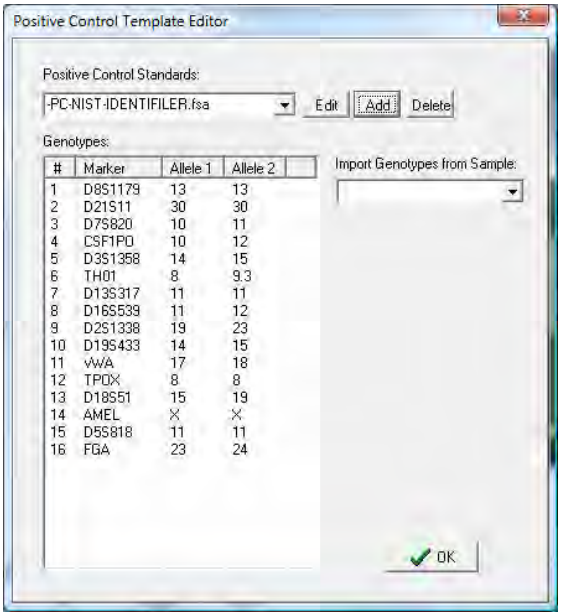
Chapter 9 Additional Tools

Automated Control Concordance

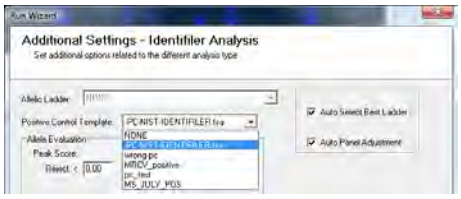
Positive Control Template Editor

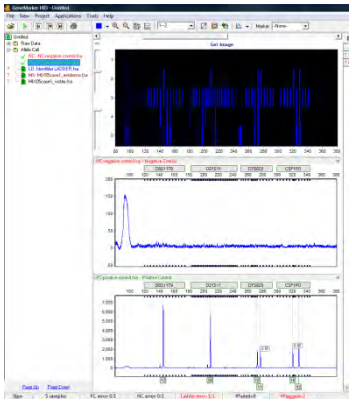
1. Tools →Positive Control Template Editor to launch dialog box
2. Import Genotypes from samples using dropdown menu
3. Select from functions to Add new positive control samples, Edit or Delete files





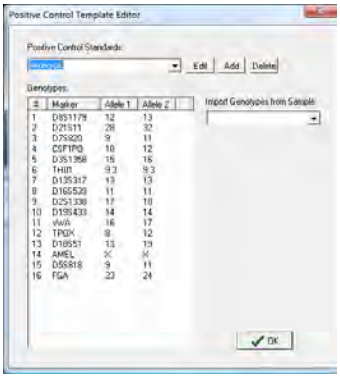
4. Select the appropriate positive control file from the dropdown menu in the run wizard. Summary message is located at the bottom of the main analysis window. For example, if a project has one positive control file that is in agreement with the positive control template, the message is PC error 0/1.

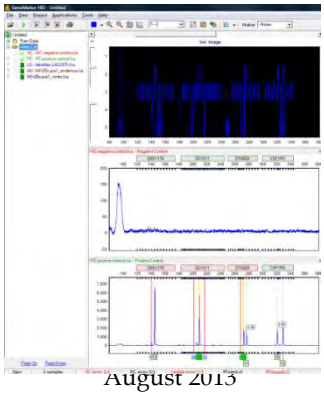




Example of Positive and Negative samples in concordance with controls

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.**

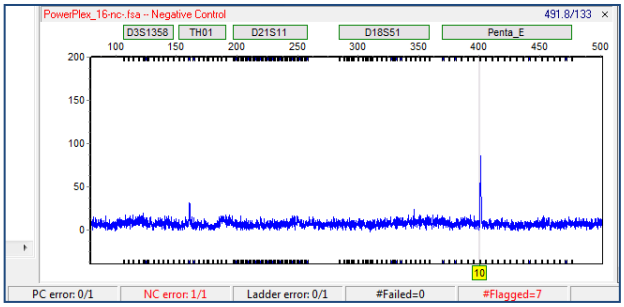




Example of Positive sample not in concordance with positive control template

Negative Control Concordance

1. Use View →Preferences →Forensic 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

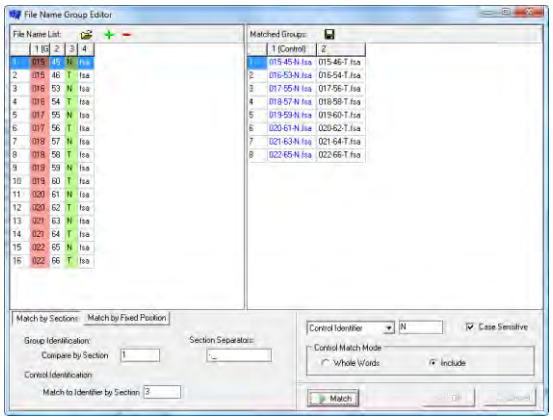


Filename Group Editor

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

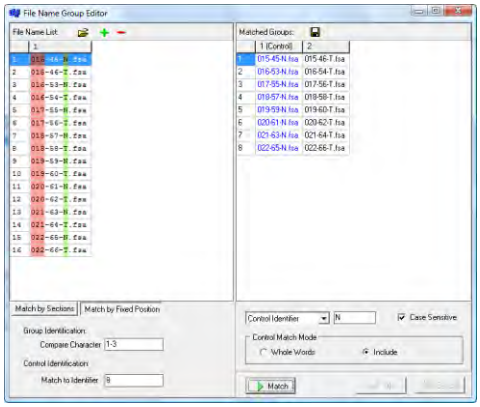
Automatically separates the sample filenames into groups based on the specified *Section Separators*.

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.

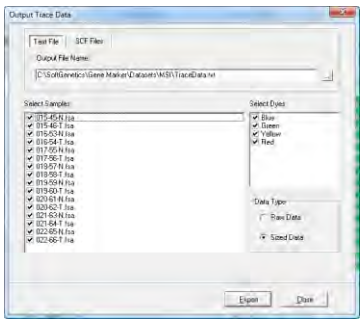
Control Match Mode

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.

Project Comparison

Tools → Project Comparison

The *Project Comparison* tool can serve three functions. First, it can be used to compare two independent analysts' analyses. Second, it can be used as a validation tool to determine differences in allele calls based on analysis parameters or instrument runs. Third, it can be used to compare the projects if the sample names are not identical, as would be the case if the samples were analyzed on two different genetic analyzers.

Procedure for comparison of projects with same file names

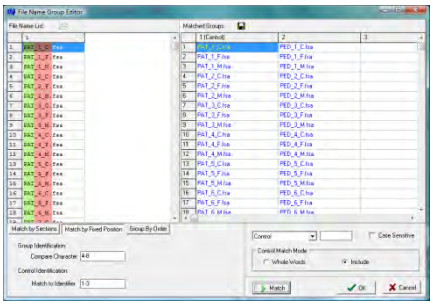
1. After initial dataset analysis, select *Tools → Project Comparison*
2. The *Project Comparison* window appears
3. Click the **Open Project to Compare** icon
4. Use the file directory window to locate and select a previously saved SoftGenetics project file (.sgf, .sfp)

NOTE: Projects with similar datasets and analysis types should be chosen.

5. Click **Open** and the second project will be uploaded to the *Project Comparison* tool
6. The first project originally loaded into GeneMarker HID will be marked as the ***Reference (R=>)*** and the second project uploaded to the *Project Comparison* tool is marked as the ***Sample (S=>)***
7. Click the **Project Comparison Settings** icon to choose parameters to compare between the projects
8. Differences will be indicated in the report table on the right. When a difference is selected, each project's electropherogram and peak table will be displayed on the left.

Procedure for comparison of projects where the same samples have different names

1. Follow steps 1-4 above
2. Click Open and both projects will be listed at the right of the screen
3. Click on the 'Edit File Groups' icon to group files of same sample together
4. Click Match and Ok
5. Same as 6-8 above



Project Comparison Tool

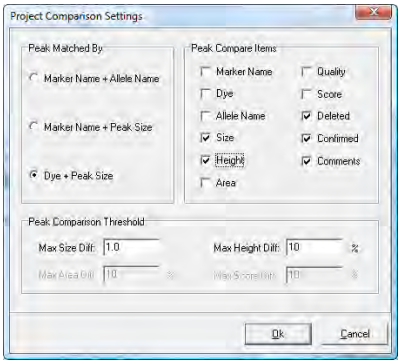
Icons and Functions

Open Project to Compare

Opens a directory window for the user to identify a similar project to compare to the project already running in GeneMarker HID. The first project in GeneMarker HID will be considered the Reference project and the project uploaded to the *Project Comparison* tool will be considered the Sample project.

Project Comparison Settings

Launches the *Project Comparison Settings* box with several options for running the comparison.



Peak Matched By: Allows the user to choose the principal parameters for comparison.

Peak Compare Items: Options for which parameters should be compared and marked as different.

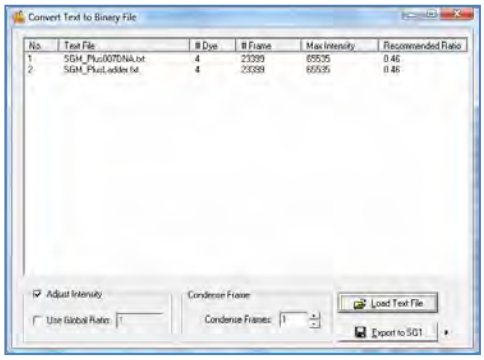
Peak Comparison Threshold: Allows the user to qualify the ranges for detecting differences in peak attributes.

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 GeneMarker HID and translated into chromatograms. This tool is useful for institutions developing their own fragment analysis instruments.

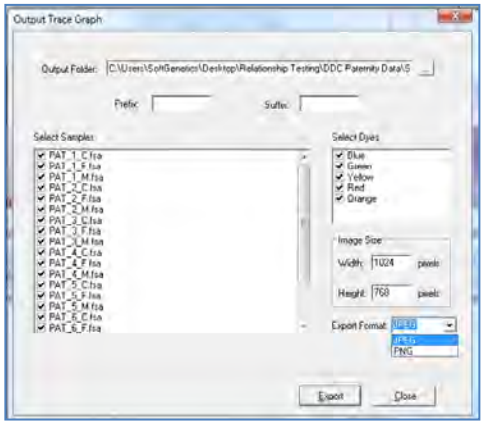
Procedure



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.

Replicate Comparison Tool

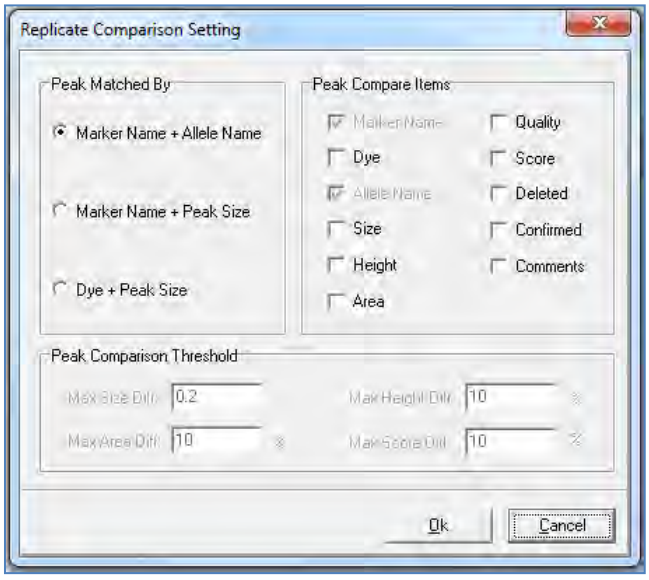
Tools → Replicate Comparison Tool

Many labs choose to run and process multiple replicates of their samples. This ensures that a genotype is still available in cases of contamination, allele drop out, or reaction failure. Concordance between replicates can then be used to deduce the genotype of the sample.

The *Replicate Comparison Tool* is designed for projects in which multiple replicates of each sample have been uploaded. This tool compares replicates from the same sample to each other, with the goal of determining a "Consensus Genotype". The results of this comparison are displayed in the tool's Report Table. If a comparison resulted in a conflict (e.g. one replicate had the genotype 11, 12 and its counterpart had 11, 13) the marker is flagged. The user has the ability to address these flags by entering the deduced genotype through a dropdown menu. Replicates must be grouped prior to utilizing the tool. This can be done in the in the tool itself (Edit file groups icon) or via the "apply sample groupings" option in the project menu of the main analysis window.

Procedure

11. Import raw data files.
12. Process data using the *Run Wizard*
13. Use the Main Analysis Window and Report Table to review flagged size and allele calls, and to make any necessary edits.
14. Group replicates using the *File Name Group Editor* (project → apply sample grouping).
15. Select Tools → **Replicate Comparison Tool**
16. Choose comparison settings or use defaults.
17. View results in the Report Table.
18. If desired export results.



Icons and Functions

Replicate ComparisonSettings
Opens the replicate comparison settings window, where the user may determine which qualities of each replicate are compared to one another.

Peak Compare Items – select which quantities to include in the comparison. Marker Name and Allele Name are initially selected.

Peak Match By – For convenience, some peak comparison items are grouped into three categories. Select one of the three categories to automatically select the relevant comparison parameters.

Peak Comparison Threshold – As described below, any conflicts arising from the comparison of two or more replicates are flagged. Here, the user may restrict flagging to only cases in which the difference surpasses a pre-set threshold.

As an example, suppose the height of a peak was 1000 RFU, and the height of the corresponding peak in a replicate was 1050 RFU. If the *Max Height Difference* was set to 10%, this conflict would not be flagged, as it is a difference of only 5%.

NOTE: Peak comparison thresholds will be grayed-out unless the corresponding comparison item is selected in the *Peak Comparison Items* section.

Edit File Groups
Opens the *File Name Group Editor*, allowing the user to group or re-group samples within the Replicate Comparison Tool.

Viewing Options
Use these icons to scroll through dye colors, zoom in, zoom out, and set the axis ranges, respectively. These icons are synonymous with their counterparts in the Main Analysis Screen.

Browse by All Color
Opens the All Color Browser, which allows the user to see the alignment of each separate dye trace for a given sample. Use the sample dropdown menu in the upper right corner to change samples.

Show Chart/Table
Displays a chart below each electropherogram which includes information such as peak size, heights, and user comments. Right click on the table to modify its contents.

Save Report
Use this icon to save the Report Table as a tab-delimited text file. Click the inverted triangle to switch between export formats. All exports contain a header with project and analysis information.

Whole Report – With this option selected, the Report Table will be exported in its entirety.

Final Report – Exports a header, the status column, and the final genotype column only.

Final Report With Only Valid Alleles – Identical to the Final Report option, with the exception that markers with **none** selected as the final genotype (see below) will be excluded from the export.

Regardless of which option is selected, the user will receive a warning if they attempt to export the report table if Discordant (D) calls are present. Use the pop up dialog box to bypass this message.

Sort Report
Use this icon to sort replicate groups in the Report Table. There are two sorting options.

Sort by Group Sequence – With this option selected, replicate groups will be sorted according to their order in the Main Analysis Window file tree.

Sort by Status – With this option selected, replicate pairs with Discordant calls (D) or Null calls (N) will be sorted to the top of the table. Accordingly, Concordant (C) replicate pairs will be sorted to the bottom of the Report Table.

Select the preferred sorting option from the dropdown, and then click the refresh icon (white paper with arrows) to have the sorting take effect.

The Report Table

In the replicate comparison tool, synonymous replicates are compared according to the parameters set in the *Replicate Comparison Settings* window. The results of this comparison are organized and displayed in the Report Table.

The replicate Group Number is in the first column of the table, and the name of the first sample of each group is listed in the second column of the table, under the Sample Name header. Allele calls for each replicate are enumerated in the next columns and rows.

The Status Column – The status column is the focal point of the Report Table, as it displays the concordance of related replicates. The status column is automatically filled with a **C**, for **Concordant**, if the replicates are identical, or if differences are less than predetermined thresholds. If the replicates are different, or differences are greater than predetermined thresholds, the status column is filled with a **D**, for **Discordant**. Finally, if only one replicate produced any allele calls at a given marker, the status column is filled with an **N**, for **Null**.

Final Genotypes Column – The final genotypes column displays the consensus genotype for each marker of each replicate group.

Concordant Case - If the status column is filled with a C, the final genotype column is automatically filled with the genotype of both replicates.

Null Case - If the status column is filled with an N, the final genotype column is automatically filled with the genotype of the replicate which has allele calls.

Discordant Case – If the status column is filled with a D, the Final Genotypes column is left blank.

In this case, it is up to the user to manually determine the final genotype. This can be done using a dropdown menu in the corresponding cell of the Final Genotypes column.

From the dropdown menu, select the genotype for the entire locus. The dropdown menu can be accessed from any of the final genotype cells in the same row as the discordant marker. After selecting a genotype, the status will change to **E,** for **Edit**, to reflect that the locus has been manually changed.

Report

No	Sample Name						Status	Final Geno	
1	1120708-10_A08_032		Rep1		Rep2				
		Marker	Allele1	Allele2	Allele1	Allele2		Allele1	Allele2
		D3S1358	17		17		C	17	
		TH01			9	9.3	N	9	9.3
		D21S11	30		30		C	30	
		D18S51	18		12	18	D		
		Penta E	5	20	5	20	C	5	20
		D5S818	9	11	9	11	C	9	11
		D13S317	12	13	12	13	C	12	13
		D7S820	9	10	9	10	C	9	10
		D16S539	11	13	11	13	C	11	13
		CSF1PO	11		11		C	11	
		Penta D	12		12		C	12	
		AMEL	X		X		C	X	
		vWA	15	18	15	18	C	15	18
		D8S1179	14	16	14	16	C	14	16
		TPOX	8	11	8	11	C	8	11
		FGA	21	25	21	25	C	21	25
		D19S433	13	14	13	14	C	13	14
		D2S1338	17		17		C	17	
2	1120708-20_B08_031		Rep1		Rep2				
		Marker	Allele1	Allele2	Allele1	Allele2		Allele1	Allele2
		D3S1358	17		17		C	17	
		TH01	9	9.3	9	9.3	C	9	9.3

The user also has the option of selecting "none" instead of a consensus genotype. Markers for which none is selected are not exported in the "Final Report with Valid Alleles Only" export option.

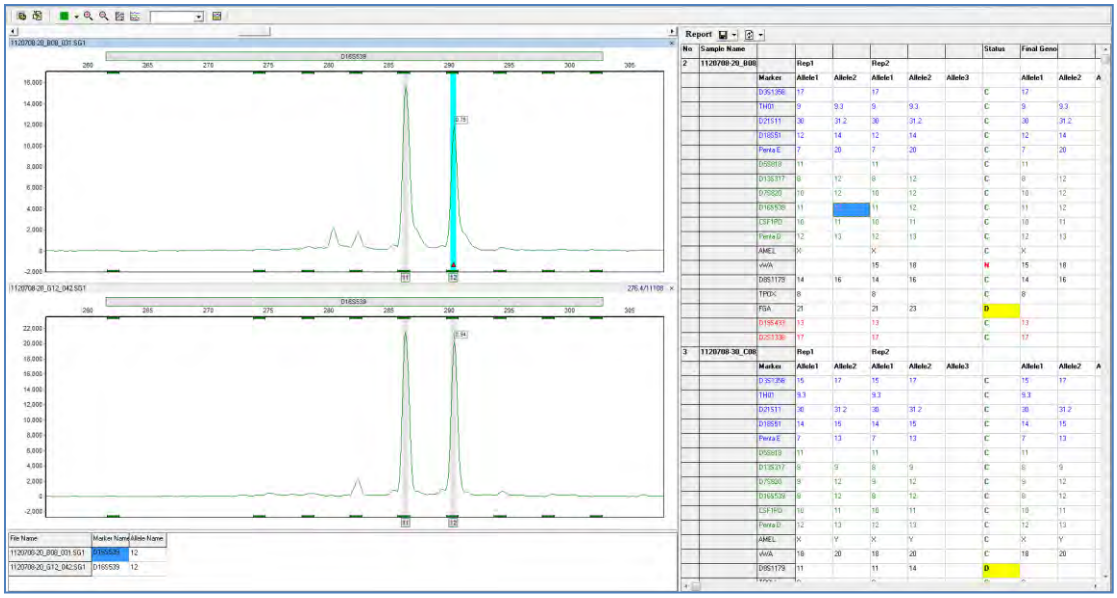
Selecting the consensus genotype:

Sample Name						Status	Final Geno	
1120708-10_A08_032		Rep1		Rep2				
	Marker	Allele1	Allele2	Allele1	Allele2		Allele1	Allele2
	D3S1358	17		17		C	17	
	TH01			9	9.3	N	9	9.3
	D21S11	30		30		C	30	
	D18S51	18		12	18	D		
	Penta E	5	20	5	20	C	18 12, 18 None	18
	D5S818	9	11	9	11	C		11
	D13S317	12	13	12	13	C	12	13

The Electropherogram

An electropherogram is displayed for each sample in a replicate group. The electropherograms are positioned directly to the left of the report table. It displays the alignment of samples within replicate groups. The electropherogram field behaves exactly like its counterpart in the main analysis window: draw a box from left-to-right to zoom in, and draw a box from right-to-left to zoom out. However, allele calls cannot be edited while in the Replicate Comparison Tool.

Clicking on an allele call in the report table will take you directly to the marker containing that call in the electropherogram trace. Double-clicking on the C, D, or N in the status column will also display the corresponding locus in the Electropherogram.

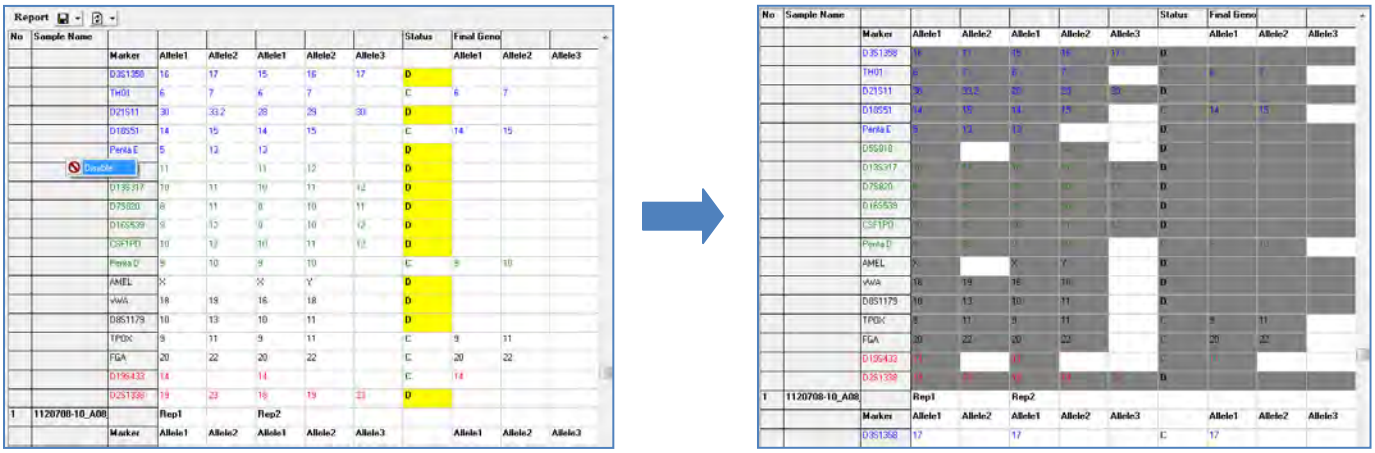


Other Features and Considerations

Disable Entire Replicates

The replicate comparison tool allows the user to remove entire replicate groups from the comparison. To do this, simply right-click on the Sample Name column of a replicate group and select **Disable**. Allele and status calls of disabled replicates will be colored dark grey. Simply right click on the Sample Name column and click **Enable** to undo these effects.

Disabled replicates are never exported, regardless of which reporting option is selected.



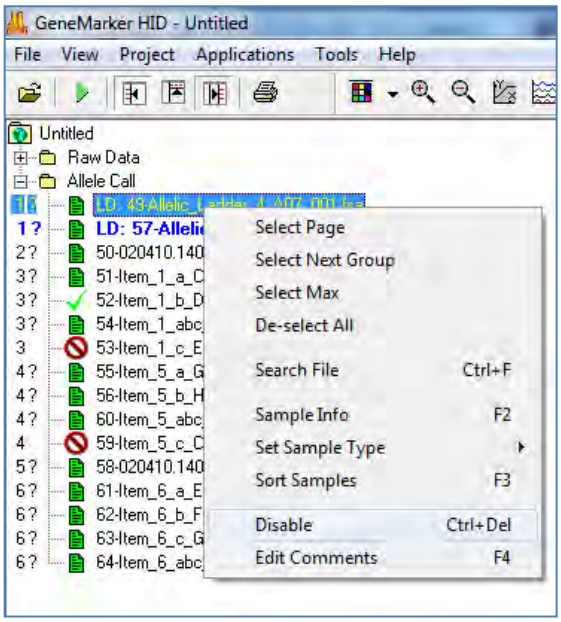
Disabling Allelic Ladders

Because the report table expands to include every allele call for a given replicate group, it is recommended that Allelic ladders be disabled prior to entering the tool. Otherwise the report table will expand to include every ladder peak, making analysis of relevant peaks more difficult.

To disable ladder samples, in the Main Analysis Window simply right click on a ladder sample and select Disable.

NOTE: You may be prompted to re-process your samples. If the disabled ladder was used in your analysis procedure, select **No**.

File grouping must be done **after** allelic ladder samples have been disabled. Otherwise, the ladders will be included in the replicate comparison window.

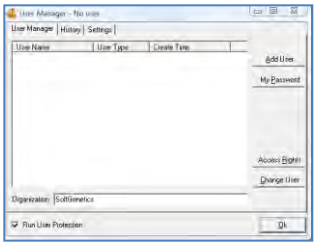


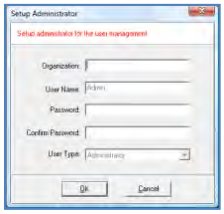
Chapter 10 User Management

Overview

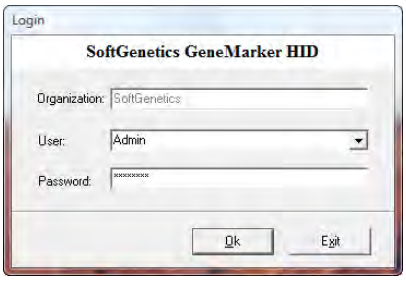
User management may be implemented after installation of GeneMarker HID. The administrator activates User Management from the Help drop-down menu. User management provides control of user access rights and automatically generates an audit trail of all edits.

Procedure



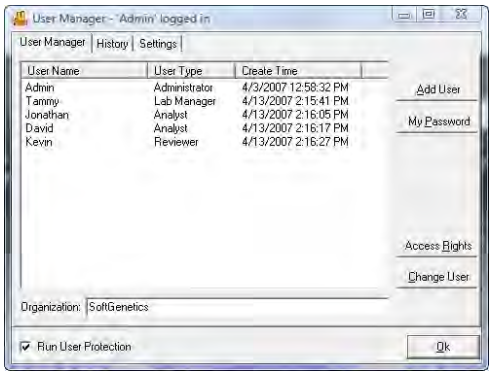


1. Select *Help* → *User Management*
2. The *Login* box appears
3. Click Run User Protection to activate the setup Administrator
4. Enter Organization Name, an Administrator username and password
5. Click **OK**
6. You are now logged in as the *Administrator*
7. Click the **Add User** button to add additional 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 GeneMarker HID after the User Manager is activated.



User Manager

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

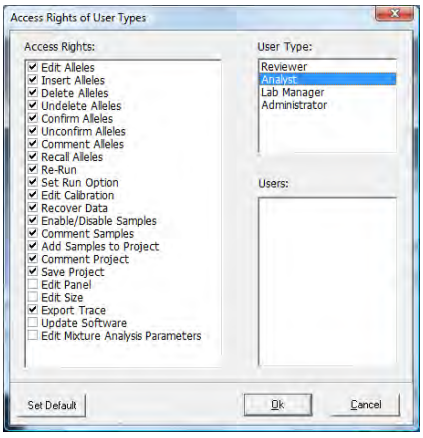


User Window
Displays all users by name, type and creation date

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 GeneMarker HID 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.



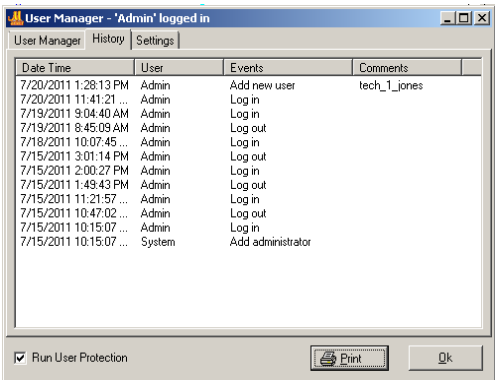
My Password
Launches the *Change Password* box where the user that is logged in can enter a new password. The new password must be entered twice to ensure accuracy.

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.



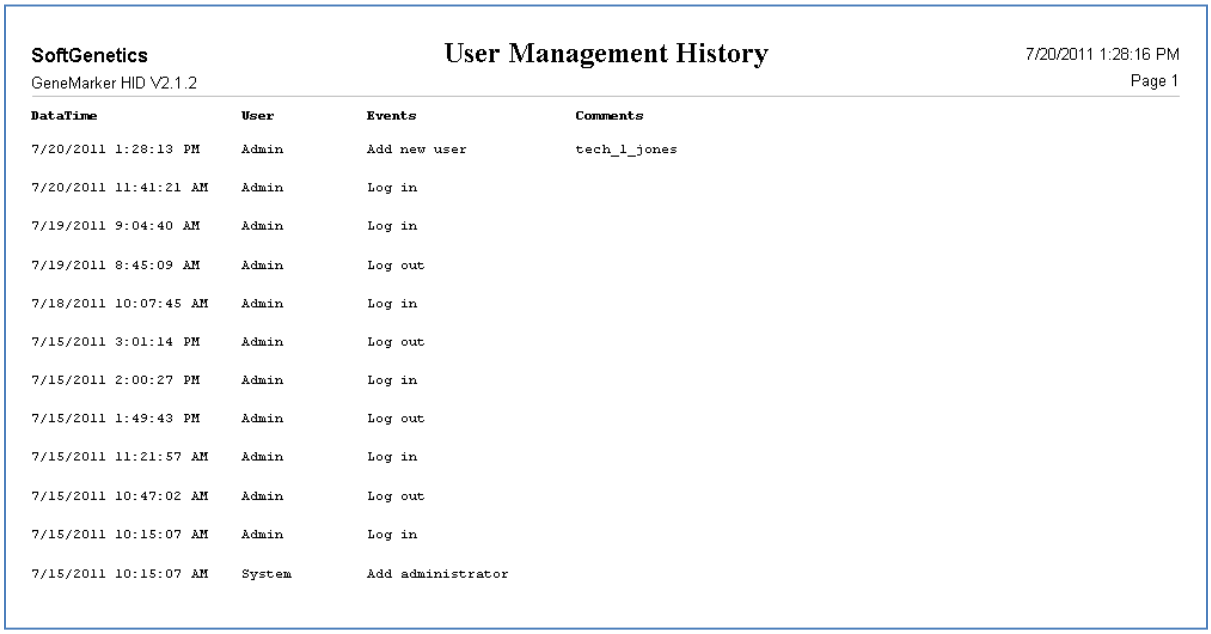
Date/Time
Records the computer's date and time for the activity.

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*.

Print
Use the Print button to preview the User Management History, print the history or save as a .png, pdf or .jpeg file.



SoftGenetics 7/20/2011 1:28:16 PM
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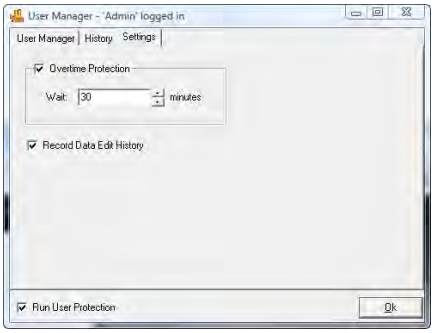
User Management History

DataTime	User	Events	Comments
7/20/2011 1:28:13 PM	Admin	Add new user	tech_1_jones
7/20/2011 11:41:21 AM	Admin	Log in	
7/19/2011 9:04:40 AM	Admin	Log in	
7/19/2011 8:45:09 AM	Admin	Log out	
7/18/2011 10:07:45 AM	Admin	Log in	
7/15/2011 3:01:14 PM	Admin	Log out	
7/15/2011 2:00:27 PM	Admin	Log in	
7/15/2011 1:49:43 PM	Admin	Log out	
7/15/2011 11:21:57 AM	Admin	Log in	
7/15/2011 10:47:02 AM	Admin	Log out	
7/15/2011 10:15:07 AM	Admin	Log in	
7/15/2011 10:15:07 AM	System	Add administrator	

Settings

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



Overtime Protection
When selected, GeneMarker HID 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).

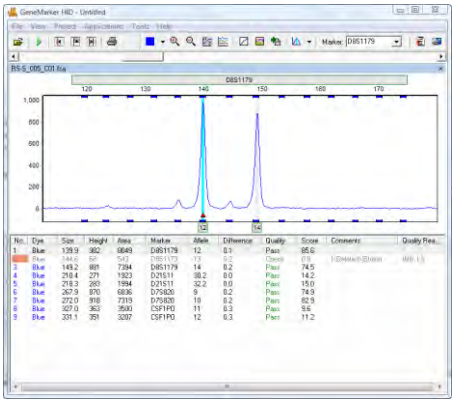
Record Data Edit History

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

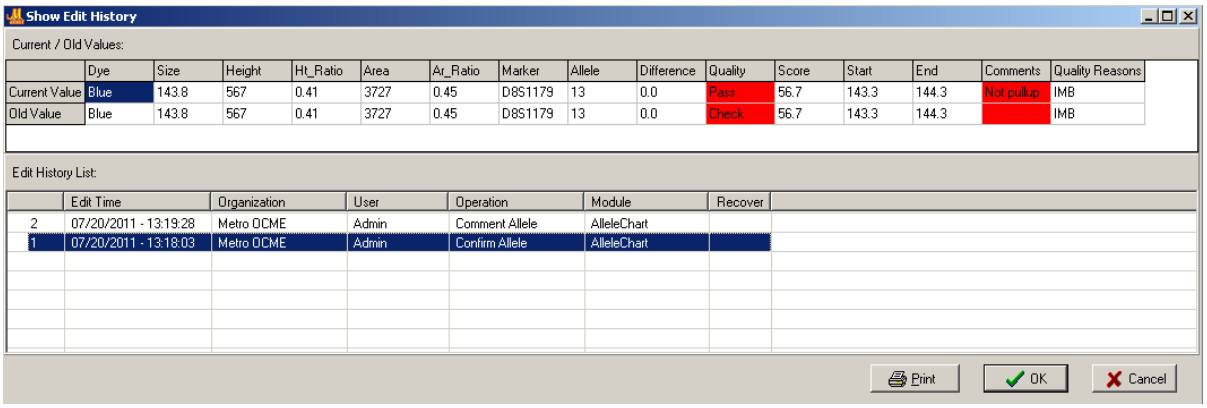
When **Record Data Edit History** is selected in the *User Manager Settings* box (see *User Management Settings* section above), any change to allele calls in the analysis will be recorded. Changes can also be recovered in the *Edit History* feature.

Procedure

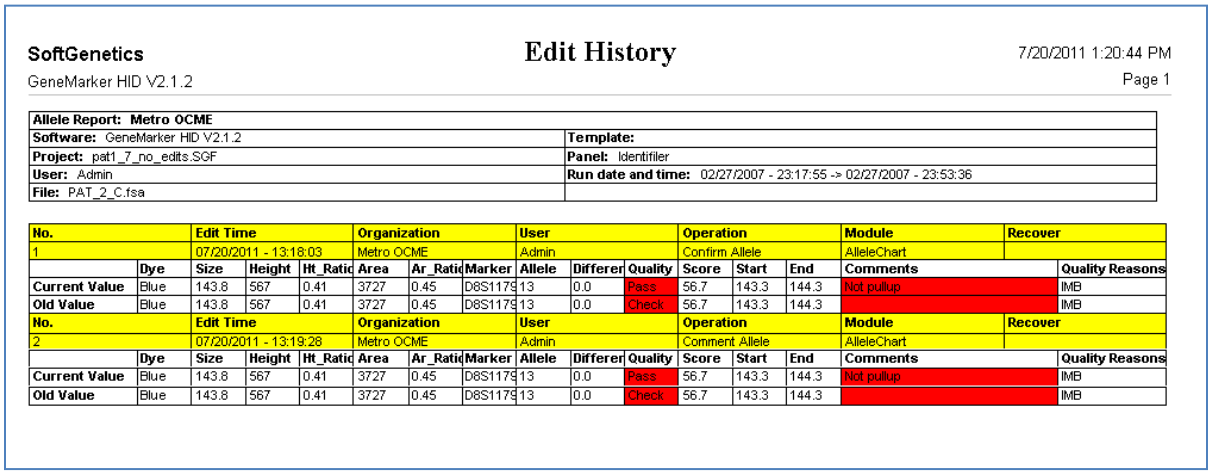


1. Click the **Show Chart/Table** icon in the *Main Analysis* window.
2. The *Peak Table* will appear below the sample electropherogram.
3. Make changes to allele calls by right-clicking any cell in that allele's row in the *Peak Table* or right-click the grey vertical bar at the center of the peak in the electropherogram.
4. Choose to *Edit Allele, Edit Comments, Add/Delete Allele*, and *Confirm*. **See Chapter 3 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



Use the Print Preview button in the lower right to Print the Edit History Window or save it as a .png or .jpeg file.



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Allele Report: Metro OCME	
Software: GeneMarker HID V2.1.2	**Template:**
Project: pat1_7_no_edits.SGF	**Panel:** Identifiler
User: Admin	**Run date and time:** 02/27/2007 - 23:17:55 -> 02/27/2007 - 23:53:36
File: PAT_2_C.fsa	

No.	**Edit Time**	**Organization**	**User**	**Operation**	**Module**	**Recover**
1	07/20/2011 - 13:18:03	Metro OCME	Admin	Confirm Allele	AlleleChart	

	Dye	**Size**	**Height**	**Ht_Ratio**	**Area**	**Ar_Ratio**	**Marker**	**Allele**	**Differer**	**Quality**	**Score**	**Start**	**End**	**Comments**	**Quality Reasons**
Current Value	Blue	143.8	567	0.41	3727	0.45	D8S1179	13	0.0	Pass	56.7	143.3	144.3	Not pullup	IMB
Old Value	Blue	143.8	567	0.41	3727	0.45	D8S1179	13	0.0	Check	56.7	143.3	144.3		IMB

No.	**Edit Time**	**Organization**	**User**	**Operation**	**Module**	**Recover**
2	07/20/2011 - 13:19:28	Metro OCME	Admin	Comment Allele	AlleleChart	

	Dye	**Size**	**Height**	**Ht_Ratio**	**Area**	**Ar_Ratio**	**Marker**	**Allele**	**Differer**	**Quality**	**Score**	**Start**	**End**	**Comments**	**Quality Reasons**
Current Value	Blue	143.8	567	0.41	3727	0.45	D8S1179	13	0.0	Pass	56.7	143.3	144.3	Not pullup	IMB
Old Value	Blue	143.8	567	0.41	3727	0.45	D8S1179	13	0.0	Check	56.7	143.3	144.3		IMB

The Print / Saved Edit History Report contains the project header with institution, User, run date time for the project and parameters. The table provides a record of each edit, time, organization, user, and operation.

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