

GeneMarker® HID



TOOLS

SOFTGENETICS®  
Software PowerTools for Genetic Analysis

## **Copyright, Licenses and Trademarks**

©2001-2019 SoftGenetics LLC. All rights reserved. No part of this publication may be reproduced, transmitted, transcribed, or translated into any language in any form by any means without the written permission of SoftGenetics LLC. The software is copyrighted and cannot be altered or given to a third party without the written authorization from SoftGenetics LLC. The software may be licensed from SoftGenetics, LLC. Mutation Explorer, Mutation Surveyor, JelMarker, ChimerMarker, NextGENe, GeneMarker and MaSTR are trademarks of SoftGenetics LLC. All other product names and/or logos are trademarks of their respective owners.

## **Limited Liability of Using the Software**

In no event shall SoftGenetics LLC. be liable for direct, indirect, incidental, special, exemplary, or consequential damages (including, but not limited to, procurement of substitute goods or services; loss of use, data, or profits; or business interruption), however caused and any theory of liability, whether in contract, strict liability, or tort (including negligence or otherwise) arising in any way out of use of this software, even if advised of the possibility of such damage.

# **SoftGenetics End User License Agreement**

## **GeneMarker® HID & GeneMarker® HID auto & GeneMarker® HID auto lite**

**NOTICE TO USER: PLEASE READ THIS CONTRACT CAREFULLY. BY DOWNLOADING, INSTALLING, OR USING ALL OR ANY PORTION OF THE SOFTWARE YOU ACCEPT ALL THE TERMS AND CONDITIONS OF THIS AGREEMENT.**

**CLICKING ON THE "ACCEPT," OR "I AGREE," BUTTON IS THE SAME AS SIGNING THIS AGREEMENT. IF YOU ARE INSTALLING THIS SOFTWARE FOR ANOTHER USER, YOU ACKNOWLEDGE THAT THE ULTIMATE END USER HAS READ AND AGREED TO THE TERMS OF THIS AGREEMENT. IF YOU ARE INSTALLING THIS SOFTWARE FOR AN ORGANIZATION, YOU ACKNOWLEDGE AND AGREE THAT YOU ARE AUTHORIZED TO BIND THE ORGANIZATION TO THE TERMS OF THIS AGREEMENT. IF AN ORGANIZATION USES THIS SOFTWARE, THE ORGANIZATION AGREES TO ALL THE TERMS AND CONDITIONS OF THIS AGREEMENT.**

**IF YOU DO NOT AGREE TO ANY OF THE TERMS CONTAINED IN THIS AGREEMENT, DO NOT DOWNLOAD, INSTALL, OR USE THIS SOFTWARE.**

1. Definitions. "Software" means (a) all of the contents of the files (delivered electronically or on physical media), disk(s), CD-ROM(s) or other media with which this Agreement is provided, including but not limited to (i) SoftGenetics' or third party computer information, data, or software; (ii) related explanatory written materials or files ("Documentation"); and (iii) fonts and other output mechanisms; and (b) upgrades, modified versions, updates, additions, and future copies of the Software, if any, licensed to you by SoftGenetics (collectively, "Updates").

2. "Use" or "Using" means to access, install, download, copy or otherwise benefit from using the functionality of the Software.

3. "Permitted Number" means one (1) unless otherwise indicated under a valid license (e.g. volume license) granted by SoftGenetics.

4. "Computer" means a virtual machine or a physical electronic device that accepts information in digital or similar form and manipulates it for a specific result based on a sequence of instructions.

5. "SoftGenetics" means SoftGenetics, LLC State College, PA 16803

6. Software License. As long as you comply with the terms of this End User License Agreement (the "Agreement") and pay all license fees for the Software, SoftGenetics grants to you a non-exclusive license to Use the Software for the purposes described in the Documentation. Some third party materials included in the Software may be subject to other terms and conditions, which are typically found in a "Read Me" file located near such materials.

6.1. General Use. You may install and Use a copy of the Software on your compatible computer. You may also use the Software on a computer file server provided concurrent use does not exceed the Permitted Number. No other network use is permitted, including but not limited to, using the Software either directly or through commands, data or instructions from or to a computer not part of your internal network, for internet or web hosting services or by any user not licensed to use a copy of the Software through a valid license from SoftGenetics.

6.2. Distribution. This license does not give you the right to distribute or sublicense the Software to others. A separate agreement with SoftGenetics is required for any distribution or sublicensing of the Software.

6.3. Backup Copy. You may make one backup copy of the Software, provided your backup copy is not installed or used on any Computer other than for archival purposes. You may not transfer the rights to a backup copy unless you transfer all rights in the Software to subsequent authorized user.

6.4. Home Use. You, as the primary user of the computer on which the Software is installed, may also install the Software on one of your home computers. However, the Software may not be used on your home computer at the same time the Software is being used on any other computer.

6.5. Research Use Only. User acknowledges and agrees that the Software is licensed by SoftGenetics for research use only. Any violation of this restriction on use shall constitute a breach of this Agreement. User assumes all risk for use of the Software. User further acknowledges that User is responsible for validating the Software for use in User's intended applications. Due to the nature of computers, software, operating systems and installation procedures, SoftGenetics cannot accept any liability or responsibility for validation of the Software in any of User's applications.

7. Intellectual Property Rights. The Software and any copies that you are authorized by SoftGenetics to make are the intellectual property of and are owned by SoftGenetics and its suppliers. The structure, organization and code of the Software are the valuable trade secrets and confidential information of SoftGenetics and its suppliers. The Software is protected by copyright, including without limitation by United States Copyright Law, international treaty provisions and applicable laws in the country in which it is being used. You may not copy the Software, except as set forth in this Agreement ("Software License"). Any copies that you are permitted to make pursuant to this Agreement must contain the same copyright and other proprietary notices that appear on or in the Software. You also agree not to (a) modify, adapt, translate, or create derivative works based on the Software, or (b) reverse engineer, decompile, disassemble or otherwise attempt to discover the source code of the Software except to the extent that (i) you may be expressly permitted to decompile under applicable law, (ii) it is essential to do so in order to achieve operability of the Software with another software program, and (iii) you have first requested SoftGenetics to provide the information necessary to achieve such operability and SoftGenetics has not made such information available. SoftGenetics may impose reasonable conditions and request a reasonable fee before providing such information. Any information supplied by SoftGenetics or obtained by you, as permitted hereunder, may only be used by you for the purpose described herein and may not be disclosed to any third party or used to create any software which is substantially similar to or performs the same function(s) as the Software. Requests for information should be directed to SoftGenetics.

8. Trademarks shall be used in accordance with accepted trademark practice, including identification of trademarks owners' names. Trademarks can only be used to identify printed output produced by the Software and such use of any trademark does not give you any rights of ownership in that trademark. SoftGenetics and other owners of any trademarks contained in the Software reserve all rights to limit and/or condition use of any of their trademarks.

9. Except as expressly stated above, this Agreement does not grant you any intellectual property rights in the Software.

Indemnity. You agree to hold harmless, indemnify and defend SoftGenetics, its officers, directors, employees, agents, and affiliates from and against any claims, demands, losses, damages, fines and expenses (including attorney's fees and costs) arising out of your use of the Software, any instrument on which the Software is operating, and related Documentation. Without limiting the foregoing, you also agree to hold harmless, indemnify and defend SoftGenetics, its officers, directors, employees, agents, and affiliates from and against any matters relating to any claims that you have (i) viewed, downloaded, encoded, compressed, copied or transmitted any materials (other than materials provided by SoftGenetics) in connection with use of the Software or any instrument on which the Software is operating in violation of another party's rights or in violation of any

law.

10. **Transfer.** You may not rent, lease, sublicense or transfer your rights in the Software, or authorize all or any portion of the Software to be copied onto another user's Computer except as may be expressly permitted herein. You may, however, transfer all your rights to Use the Software to another person or legal entity provided that: (a) you also transfer this Agreement, any instrument on which the Software operates, the Software and all other software or hardware bundled or pre-installed with the Software, including all copies, Updates and prior versions, and all copies of font or other output software converted into other formats, to such person or entity; (b) you retain no copies, including backups and copies stored on a Computer; and (c) the receiving party accepts the terms and conditions of this Agreement and any other terms and conditions upon which you legally purchased a valid license to the Software. Notwithstanding the foregoing, you may not transfer education, pre-release, or not-for-resale copies of the Software.

11. **Multiple Environment Software / Multiple Language Software / Dual Media Software / Multiple Copies/ Bundles / Updates.** If the Software supports multiple platforms or languages, if you receive the Software on multiple media, if you otherwise receive multiple copies of the Software, or if you received the Software bundled with other software or with an instrument, the total number of your Computers on which all versions of the Software are installed may not exceed the Permitted Number. You may not rent, lease, sublicense, lend or transfer any versions or copies of such Software you do not Use. If the Software is an Update to a previous version of the Software, you must possess a valid license to such previous version in order to Use the Update. You may continue to use the previous version of the Software on your Computer after you receive the Update to assist you in the transition to the Update, provided that: the Update and the previous version are installed on the same Computer; the previous version or copies thereof are not transferred to another party or Computer unless all copies of the Update are also transferred to such party or Computer; and you acknowledge that any obligation SoftGenetics may have to support the previous version of the Software may be ended upon availability of the Update.

12. If the Software is used to connect with or download information from any other software program, system, or database, you agree that SoftGenetics does not control the features, operations, or information practices of any third party websites, computer systems, or databases.

13. **LIMITED WARRANTY.** SoftGenetics warrants to the person or entity that purchases a license for the Software for use pursuant to the terms of this license that the Software will perform substantially in accordance with the Documentation for a period of thirty (30) days following receipt of the Software when used on the recommended hardware configuration. Any non-substantial variation of performance from the Documentation does not establish a warranty right. THIS LIMITED WARRANTY DOES NOT APPLY TO UPDATES, SOFTWARE CONVERTED INTO OTHER FORMATS, PRE-RELEASE (BETA), TRYOUT, PRODUCT SAMPLER, OR NOT-FOR-RESALE (NFR) COPIES OF THE SOFTWARE. To make a warranty claim, you must return the Software to the location where you obtained it along with proof of purchase within such ninety (90) day period. If the Software does not perform substantially in accordance with the Documentation, the entire liability of SoftGenetics and your exclusive remedy shall be limited to either, at SoftGenetics option: (1) the replacement of the Software, or (2) the refund of the license fee you paid for the Software. THE LIMITED WARRANTY SET FORTH IN THIS SECTION GIVES YOU SPECIFIC LEGAL RIGHTS. YOU MAY HAVE ADDITIONAL RIGHTS WHICH VARY FROM JURISDICTION TO JURISDICTION.

14. **DISCLAIMER.** THE FOREGOING LIMITED WARRANTY STATES THE SOLE AND EXCLUSIVE REMEDIES FOR SOFTGENETICS'S OR ITS SUPPLIER'S BREACH OF WARRANTY. SOFTGENETICS AND ITS SUPPLIERS DO NOT AND CANNOT WARRANT THE PERFORMANCE, MERCHANTABILITY OR RESULTS YOU MAY OBTAIN BY USING THE SOFTWARE. EXCEPT FOR THE FOREGOING LIMITED WARRANTY, YOU ACKNOWLEDGE AND AGREE THAT SOFTGENETICS HAS MADE NO OTHER EXPRESS WARRANTIES TO YOU REGARDING THE SOFTWARE AND THAT THE SOFTWARE IS BEING PROVIDED TO YOU "AS IS" WITHOUT WARRANTY OF ANY OTHER KIND. SOFTGENETICS SPECIFICALLY DISCLAIMS ALL OTHER WARRANTIES WITH REGARD TO THE SOFTWARE, EXPRESS OR IMPLIED, INCLUDING, WITHOUT LIMITATION ANY IMPLIED WARRANTIES OF FITNESS FOR A PARTICULAR PURPOSE, MERCHANTABILITY, MERCHANTABILITY QUALITY, NONINFRINGEMENT OF THIRD PARTY RIGHTS, INTEGRATION, OR SATISFACTORY QUALITY. SOFTGENETICS AND ITS SUPPLIERS MAKE NO OTHER WARRANTIES, CONDITIONS, REPRESENTATIONS OR TERMS, EXPRESS OR IMPLIED, WHETHER BY STATUTE, COMMON LAW, CUSTOM, USAGE OR OTHERWISE AS TO ANY OTHER MATTERS. The provisions of this disclaimer shall survive the termination of this Agreement, howsoever caused, but this shall not imply or create any continued right to Use the Software after termination of this Agreement.

15. **LIMITATION OF LIABILITY.** IN NO EVENT WILL SOFTGENETICS OR ITS SUPPLIERS BE LIABLE TO YOU FOR ANY LOSS OF USE, INTERRUPTION OF BUSINESS, DAMAGES, CLAIMS OR COSTS WHATSOEVER OR ANY CONSEQUENTIAL, INDIRECT, INCIDENTAL DAMAGES, OR ANY LOST PROFITS OR LOST SAVINGS, EVEN IF A SOFTGENETICS REPRESENTATIVE HAS BEEN ADVISED OF THE POSSIBILITY OF SUCH LOSS, DAMAGES, CLAIMS OR COSTS, OR FOR ANY CLAIM BY ANY THIRD PARTY. THE FOREGOING LIMITATIONS AND EXCLUSIONS APPLY TO THE EXTENT PERMITTED BY APPLICABLE LAW IN YOUR JURISDICTION. SOFTGENETICS'S AGGREGATE LIABILITY AND THAT OF ITS SUPPLIERS UNDER OR IN CONNECTION WITH THIS AGREEMENT SHALL BE LIMITED TO THE AMOUNT PAID FOR THIS LICENSE, IF ANY. SoftGenetics is acting on behalf of its suppliers for the purpose of disclaiming, excluding and/or limiting obligations, warranties and liability as provided in this Agreement, but in no other respects and for no other purpose.

16. **Export Rules.** You agree that the Software will not be shipped, transferred or exported into any country or used in any manner prohibited by the United States Export Administration Act or any other export laws, restrictions or regulations (collectively the "Export Laws"). In addition, if the Software is identified as export controlled items under the Export Laws, you represent and warrant that you are not a citizen, or otherwise located within, an embargoed nation (including without limitation Iran Iraq, Syria, Sudan, Libya, Cuba, North Korea, Serbia and Crimea) and that you are not otherwise prohibited under the Export Laws from receiving the Software. All rights to Use the Software are granted on condition that such rights are forfeited if you fail to comply with the terms of this Agreement.

17. **Governing Law.** This Agreement will be governed by and construed in accordance with the substantive laws in force in the State of Pennsylvania, United States of America.

18. **General Provisions.** This is the entire agreement between you and SoftGenetics regarding the license of the Software, and it supersedes any prior representations, discussions, undertakings, communications, or advertising related to the Software. This Agreement may only be modified by a writing executed by an authorized officer of SoftGenetics, except that Updates may be licensed to you by SoftGenetics with additional or different terms. SoftGenetics may assign this Agreement to an affiliate or any other third party at SoftGenetics's sole discretion.

# Table of Contents GeneMarker®HID v.3.0

<b>CHAPTER 1 INSTALLING GENEMARKERHID</b>	<b>5</b>
COMPUTER SYSTEM REQUIREMENTS	6
LOCAL-LICENSING OPTION	6
<i>Installation</i>	6
<i>Local Registration</i>	7
<i>Local Upgrade</i>	7
NETWORK-LICENSING OPTION	7
<i>Install License Server Manager</i>	8
<i>Register License Server Manager for GeneMarkerHID Usage</i>	8
<i>Install GeneMarkerHID Software on the Client Computer</i>	9
<i>Upgrade of License Server Manager</i>	10
<i>Upgrade of GeneMarkerHID Software on Client Computer</i>	10
QUESTIONS	10
<b>CHAPTER 2 GENERAL PROCEDURE</b>	<b>11</b>
IMPORT DATA FILES	12
<i>Procedure</i>	12
<i>Features</i>	12
RAW DATA ANALYSIS	13
.....	14
<i>Main Toolbar Icons</i>	14
<i>What to Expect</i>	15
PROCESS DATA	17
<i>Run Wizard Template Selection</i>	17
<i>Run Wizard Data Process</i>	18
<i>Run Wizard Additional Settings</i>	21
ADJUST ANALYSIS PARAMETERS	22
<i>Re-analyze with Run Wizard</i>	22
<b>CHAPTER 3 MAIN ANALYSIS OVERVIEW</b>	<b>25</b>
MAIN ANALYSIS WINDOW	26
<i>Sample File Tree</i>	26
<i>Synthetic Gel Image and Electropherogram with Peak Table</i>	28
<i>Report Table</i>	33
MENU OPTIONS	34
<i>File Menu</i>	34
<i>View Menu</i>	35
<i>Project Menu</i>	39
<i>Applications Menu</i>	39
<i>Tools Menu</i>	40
<i>Help Menu</i>	41
MAIN TOOLBAR ICONS	42
ADDITIONAL ANALYSIS OPTIONS	43
<i>Browse By All Colors</i>	43
<i>Profile Comparison View</i>	44
<b>CHAPTER 4 FRAGMENT SIZING STANDARDS</b>	<b>45</b>
SIZE TEMPLATE EDITOR	46
<i>Procedure</i>	48
<i>Icons and Functions</i>	49

Table of Contents

<i>What to Expect</i> .....	51
SIZE CALIBRATION CHARTS.....	51
<i>Procedure</i> .....	54
<i>Icons and Functions</i> .....	54
<i>What to Expect</i> .....	55
<i>SQ Flagging</i> .....	55
<b>CHAPTER 5 PANEL EDITOR .....</b>	<b>57</b>
OVERVIEW.....	58
<i>Project Panel</i> .....	58
<i>Panel Templates</i> .....	58
<i>Sample List</i> .....	59
<i>Overlay Trace</i> .....	59
<i>Minimum Intensity(RFU) Filter</i> .....	60
<i>Min Heterozygote Intensity:</i> .....	60
<i>Inconclusive Range (Stochastic Range Flagging):</i> .....	61
<i>Max Heterozygote Imbalance flagging:</i> .....	61
<i>Min Heterozygote Imbalance filter:</i> .....	61
<i>Stutter Filter by marker:</i> .....	61
<i>Allele Specific Stutter Filter:</i> .....	61
<i>Panel Table</i> .....	63
PROCEDURE.....	64
<i>Pre-Defined Panels</i> .....	64
<i>Custom Panel Creation</i> .....	65
<i>Adjusting Panels - Manual and Automated Panel Calibration</i> .....	66
OPTIONS, FUNCTIONS AND ICONS.....	67
<i>Menu Options</i> .....	67
<i>Toolbar Icons</i> .....	68
<i>Project Panel</i> .....	69
WHAT TO EXPECT.....	69
<b>CHAPTER 6 REPORTS AND PRINTING.....</b>	<b>73</b>
REPORT TABLE.....	74
<i>Allele List</i> .....	74
<i>Forensics</i> .....	74
<i>MaSTR™ and STRMix™ compatible format:</i> .....	74
<i>Bin Table</i> .....	75
<i>Peak Table</i> .....	77
<i>Allele Count</i> .....	78
LIMS REPORT.....	78
PRINT REPORT.....	79
<i>Report Content Options</i> .....	80
<i>Include Customizable Logo in the Print Report</i> .....	83
<i>Icons and Functions</i> .....	84
CODIS REPORT.....	85
SAVE PROJECT.....	86
SAVE SELECTED SAMPLES.....	86
<b>CHAPTER 7 MIXTURE ANALYSIS.....</b>	<b>87</b>
OVERVIEW.....	88
PROCEDURE.....	88
<i>Identify the Presence of a Mixture</i> .....	88
<i>Mixture Analysis</i> .....	90

Table of Contents

MIXTURE ANALYSIS RESULTS.....	91
WHAT TO EXPECT MIXTURE ANALYSIS AND DATABASE SEARCH .....	92
<i>With Reference File(s)</i> .....	92
<i>Without a Reference File</i> .....	93
<i>Hypothesis Testing Options</i> .....	94
ICONS AND FUNCTIONS .....	96
SAVE AND EXPORT RESULT TABLES .....	97
MIXTURE ANALYSIS EQUATIONS .....	98
<b>CHAPTER 8 RELATIONSHIP TESTING .....</b>	<b>103</b>
KINSHIP ANALYSIS USING IDENTITY BY DESCENT .....	104
<i>Overview</i> .....	104
<i>Procedure</i> .....	105
<i>Icons and Functions</i> .....	106
<i>Multi-Pair Kinship Analysis</i> .....	107
DATABASE SEARCH: LOCATE DUPLICATE SAMPLES AND NEAREST RELATIVES.....	108
<i>Overview</i> .....	108
<i>Procedure</i> .....	108
<i>Icons and Functions</i> .....	110
<i>Importing Population Specific Allele Frequency and Mutation Rate Information</i> .....	112
<i>Building the Database</i> .....	113
<i>Save and Print Report</i> .....	113
AUTOMATED PEDIGREE TRIO DIAGRAMS AND ANALYSIS USING FAMILY GROUP TOOL .....	114
<i>Procedure:</i> .....	114
<i>Deducing Missing Parent Genotype</i> .....	116
<i>Editing Personal Information</i> .....	116
<i>Save Report</i> .....	117
PATERNITY INDEX CALCULATIONS .....	118
<i>Overview</i> .....	118
<i>Procedure</i> .....	118
<i>Reverse Parentage</i> .....	118
<i>Save and Print Reports</i> .....	119
.....	119
<b>CHAPTER 9 CELL LINE AUTHENTICATION – PERCENT SAME GENOTYPE SEARCH .....</b>	<b>121</b>
OVERVIEW.....	122
BUILDING YOUR DATABASE.....	122
SEARCHING THE DATABASE .....	124
<b>CHAPTER 10 ADDITIONAL TOOLS .....</b>	<b>127</b>
AUTOMATED CONTROL CONCORDANCE .....	128
NEGATIVE CONTROL CONCORDANCE .....	129
CONTAMINATION CHECK AND ELIMINATION DATABASE .....	129
FILENAME GROUP EDITOR .....	134
OUTPUT TRACE DATA .....	136
PROJECT COMPARISON .....	136
CONVERT TXT TO BINARY.....	137
EXPORT ELECTROPHEROGRAM.....	138
REPLICATE COMPARISON TOOL.....	138
QUALITY SENSOR EVALUATION (QS) .....	143
<b>CHAPTER 11 USER MANAGEMENT .....</b>	<b>145</b>
OVERVIEW.....	146



Table of Contents

PROCEDURE.....	146
USER MANAGER.....	146
HISTORY.....	147
SETTINGS .....	147
EDIT HISTORY/AUDIT TRAIL .....	148
<i>By Sample</i> .....	148
<i>By Project</i> .....	148
INDEX.....	<b>149</b>



# Chapter 1 Installing GeneMarkerHID

## **Chapter 1 Installing GeneMarkerHID**

Computer System Requirements

Local-Licensing Option

Network-Licensing Option

Questions

## Computer System Requirements

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

### Windows® PC

OS: Windows® 7 - 10

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 GeneMarkerHID is not supported on Linux or UNIX-based operating systems.

GeneMarkerHID 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>

## Local-licensing Option

The local-licensing option is the “traditional” setup. Each license is installed on and registered to a specific PC.

### Installation

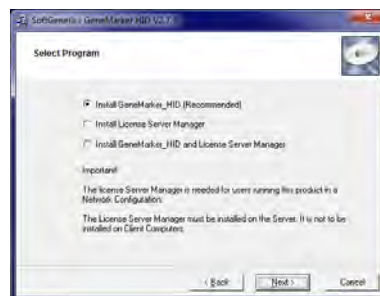
1. Insert the *SoftGenetics* CD into the optical or CD-ROM drive. If your computer is not set to automatically open a CD, navigate to the optical or CD-ROM drive on the computer and open the directory.
2. Double-click the GeneMarkerHID Setup executable file (EXE)
3. The *Installation Wizard* will launch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

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

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

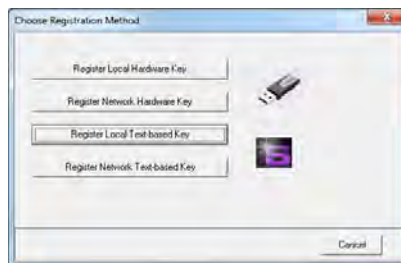
**NOTE:** Changing the *Program Manager Group* default may affect program operability. It is recommended to accept the default.

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



## Local Registration

1. The *Register Local Text-based Key* window appears
2. If the computer GeneMarkerHID 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 GeneMarkerHID
- D. Click **Register**
- E. Your software will be registered automatically. A confirmation e-mail will be sent to you once registration is complete.

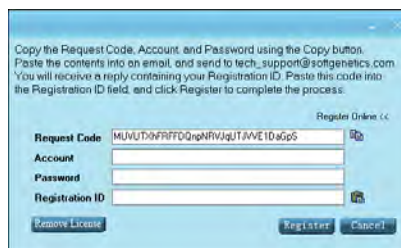
**NOTE:** Some characters can commonly be misread. If you get an error trying to register, check for number “1” and lower case letter “L” or number “0” and upper case letter “O” confusion.

- F. Launch GeneMarkerHID 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](mailto: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 GeneMarkerHID and begin analysis



## Local Upgrade

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

## Upgrade Procedure – Text-based

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

## Network-licensing Option

In the network licensing option, GeneMarkerHID programs share their licensing information over a local network. GeneMarkerHID can be installed on any number of PCs, but the maximum number of simultaneous users is equal

to the number of purchased network licenses. The 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.

## Install License Server Manager

**NOTE:** The LSM controls which computers are able to access GeneMarkerHID. If the computer running the LSM is turned off, *no* computer will be able to access GeneMarkerHID, as the LSM will be nonfunctional. For this reason it is recommended that the LSM be installed on a computer that is always on, or always on during working hours.

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 GeneMarkerHID 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

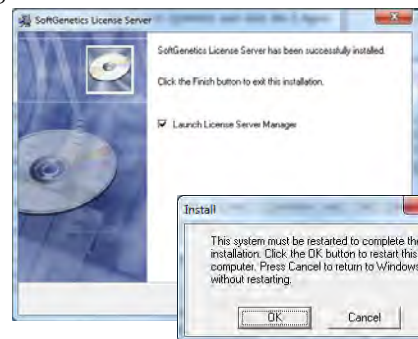
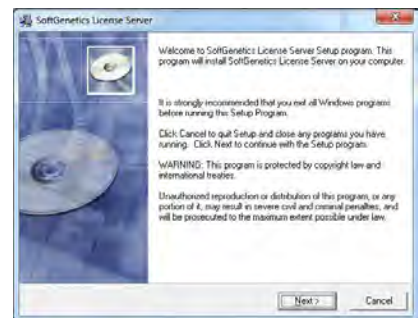
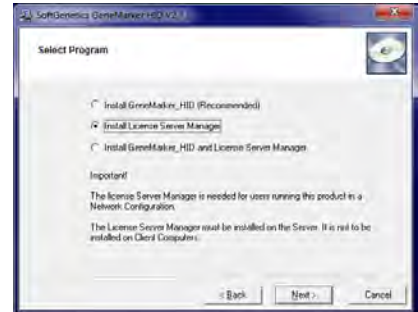
## Register License Server Manager for GeneMarkerHID Usage

1. Open License Server from the System or Icon Tray by clicking the LSM icon, which is a purple ‘S’.

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

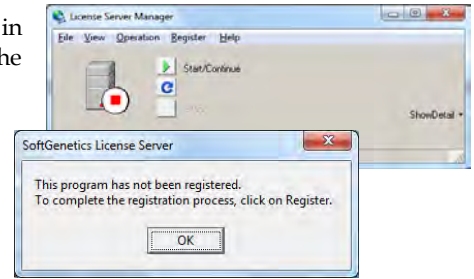
**NOTE:** If the purple ‘S’ icon is not present in your icon tray, you may need to first run the LSM. To do this, open the main Start menu and navigate to:

```
All Programs
  SoftwareGenetics
    License Server
      License Server (EXE)
```



After clicking the License Server EXE, the Purple S icon should be available in your Icon Tray.

2. You may receive a message that the LSM is not registered. Click **OK** in the dialog box to proceed with registering License Server from the License Server Manager console.
3. Click the Register button to activate the Register Product window
4. Select GeneMarkerHID from the Register Product Name drop-down menu.
5. If the computer License Server is being installed on has an internet connection, select the **Online Registration** tab (default). If the computer does not have an internet connection select the **Offline Registration** tab.

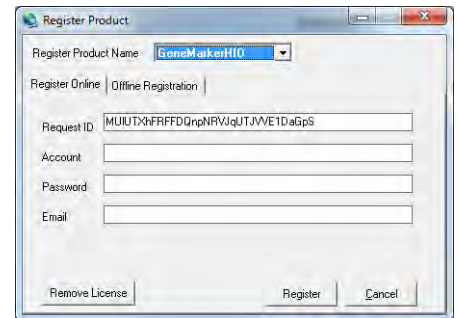


### Online Registration

- 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. For this reason copy/pasting is strongly recommended.

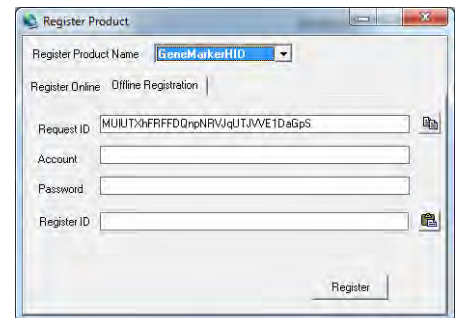
- F. Restart License Server to apply the registration information.



### 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](mailto: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**

This should complete the registration process.



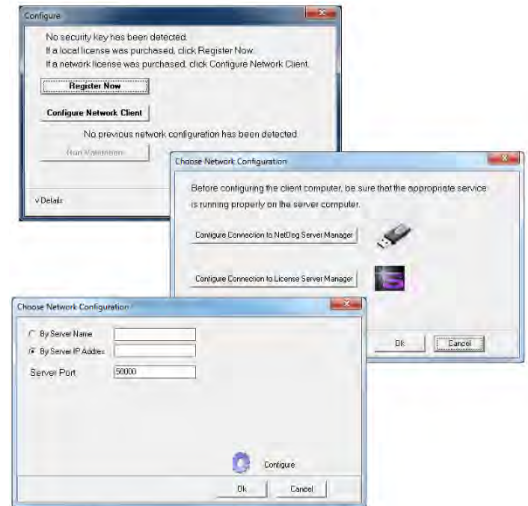
## Install GeneMarkerHID Software on the Client Computer



Now that the LSM has been registered, GeneMarkerHID must be installed on client computers, and then *configured* to the License Server Manager.

1. Proceed with installing GeneMarkerHID 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 GeneMarkerHID software will automatically open if connection is properly established and a license is available.

**NOTE:** It may not be obvious, but **Configure** is a button in the third window to the right. Click this button to test the configuration of the specified Server Name or IP Address.



### Upgrade of License Server Manager

Activate the License Server Manager console

Repeat “Register License Server Manager for GeneMarkerHID Usage” section above

### Upgrade of GeneMarkerHID Software on Client Computer

Install GeneMarkerHID software on the client computer by following the procedure in the “Install GeneMarkerHID 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](mailto:tech_support@softgenetics.com)

## Chapter 2 General Procedure

### **Chapter 2 General Procedure**

Import Data Files

Raw Data Analysis

Process Data

Adjust Analysis Parameters

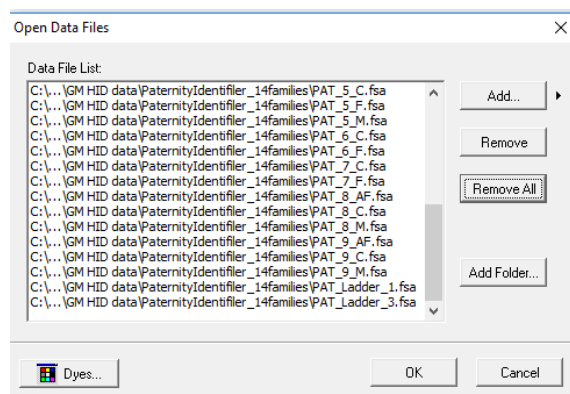


## Import Data Files

After installing GeneMarkerHID 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 GeneMarkerHID. Note: many AB 3500 instruments have an option to export .hid files with normalized peak heights. For greatest accuracy of data analysis, GeneMarkerHID reads the non-normalized peak heights of files that are compatible with the software, including data files from *ThermoFisher* .fsa,.hid; *IntegenX*, .fsa; *ANDE*® .fsa and *generic* .scf or .sg1 files

### Procedure

1. Launch **GeneMarkerHID**
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 GeneMarkerHID



### 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. Use the View - Preferences - Startup settings tab to set default locations.

#### 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.

**Dyes** Reporting, especially print reports from non-color printers, may require that the dye name is included. Dye names of Applied Biosystem, AB (ThermoFisher), Promega and Qiagen available for the imported data files. Initial default dye names are set to AB dye names. To change the dye name setting for the data channels:

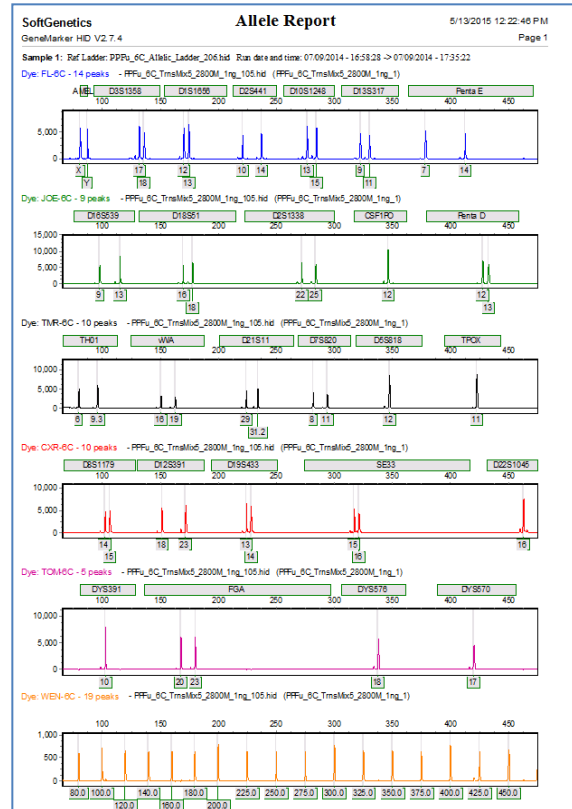
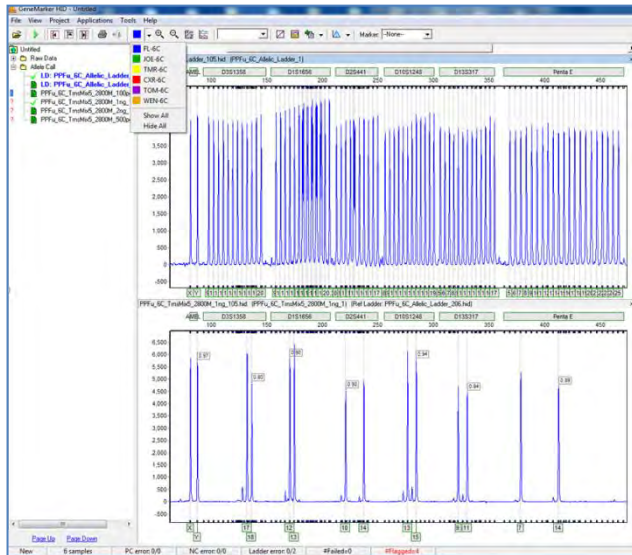
1. Select the Dyes button
2. Select the tab for the number of colors (4, 5, or 6 dye chemistry)
3. Select Change Chemistry
4. Select the appropriate manufacturer
5. Click Save to set this as the default for the dye names
6. To create an option for a new chemistry, select the page icon and enter the appropriate manufacturer and dye names



## Chapter 2 General Procedure

For simplicity in reporting or presentation of results from different chemistries, some labs choose to enter the color, rather than the dye name. The dye names will be displayed in the Main analysis screen and in any print report where the dye display is selected in the print report settings (see chapter 6 for Report and Printing). Data amplified with PowerPlex Fusion®-6C is used in the figures below to illustrate the display and reporting that includes dye names.

Dye icon in the main tool bar displays the dye names for each channel. Allele reports displaying the dye name for each trace; especially beneficial for allele reports printed in black.

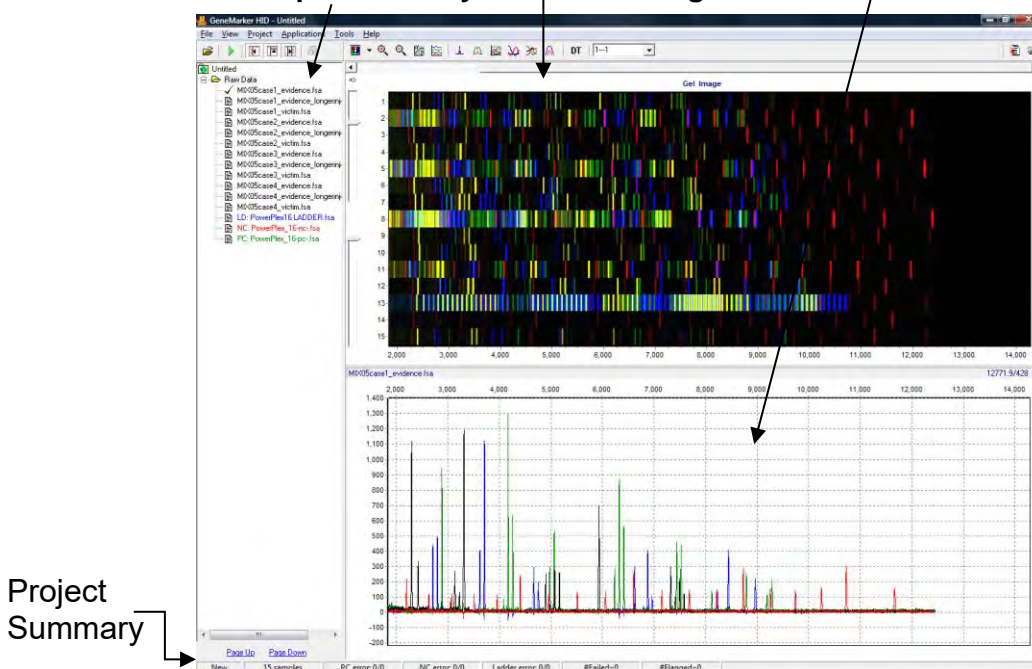


## Raw Data Analysis

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*. If selected from the tool bar toggle, 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

Sample Tree Synthetic Gel Image Raw Trace Data



### 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 smooths 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 recommended to de-select *Pull-up Correction* in the Run Wizard when using this function.



**2<sup>nd</sup> 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.

**Note:** Raw data icons are applied to the raw data. These icons are recommended for **research applications**; user management can exclude access rights for analysts.

## What to Expect

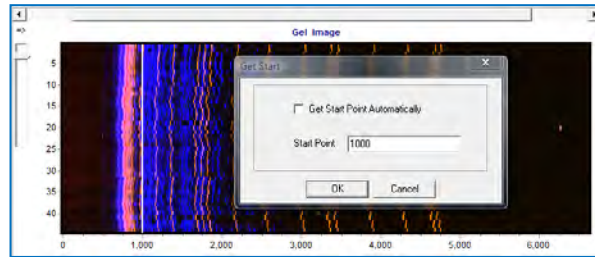
The raw data correction icons can be selected individually in the *Raw Data Analysis* window. The images below demonstrate how the data will look before (left image) and after (right image) the parameter is applied.

### 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**



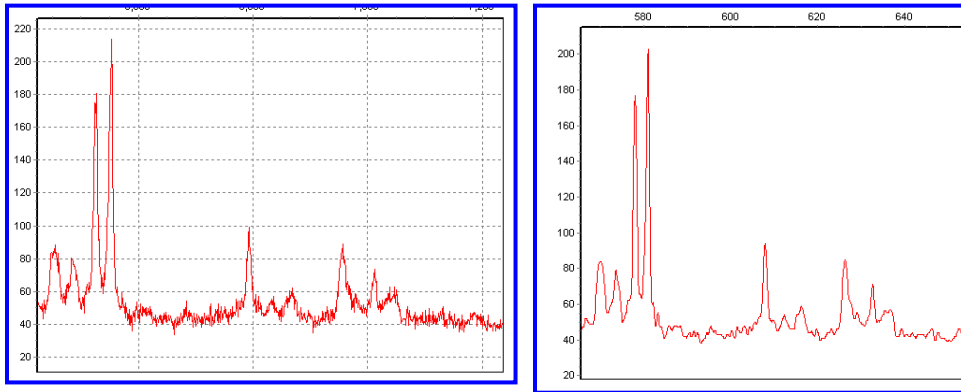
### Smooth

Fourier frequency transformation (FFT) to determine frequency domain

Use only top 40% of lowest frequencies

Smoothing broadens peaks and therefore you can lose resolution

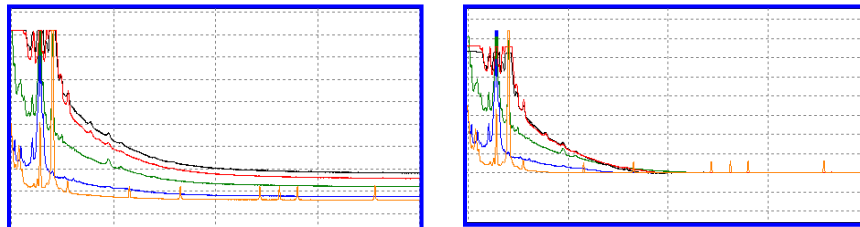
**Enhanced Smooth** - Same as Smooth but use only top 20% of lowest frequencies



### 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

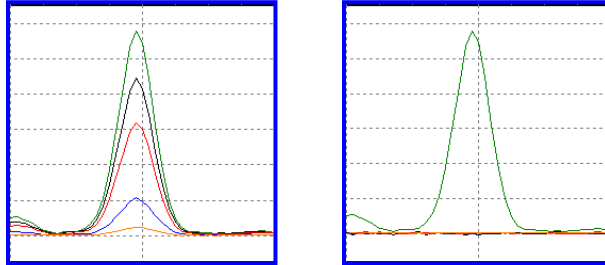
$Ax=B$

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.



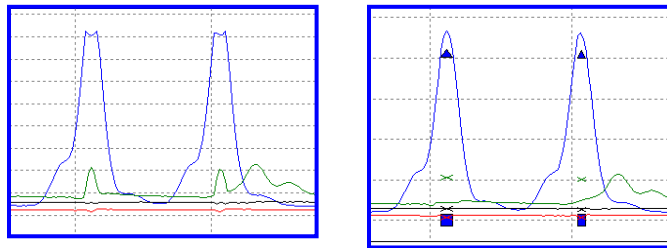
### Saturated Peak Correction

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

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



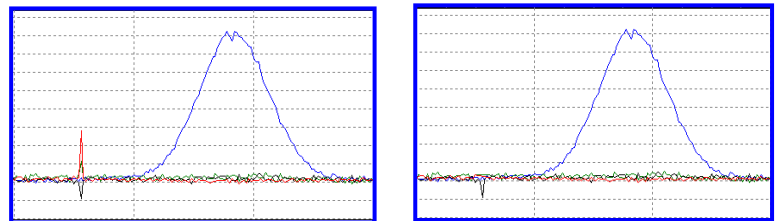
### Spike Removal

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 1<sup>st</sup> DT outliers (3-5 sigma)

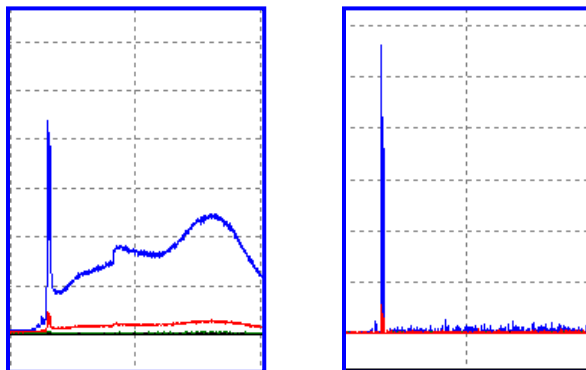


### Second Derivative Trace

$$(A1-A2)-(A2-A3) = A1+A3-2(A2)$$

Use when you have a fat base to your peaks (ex. Dye blob under peak, etc)

**NOTE:** Do not use 2<sup>nd</sup> DT with *Spike Removal* because real peaks look like spikes.



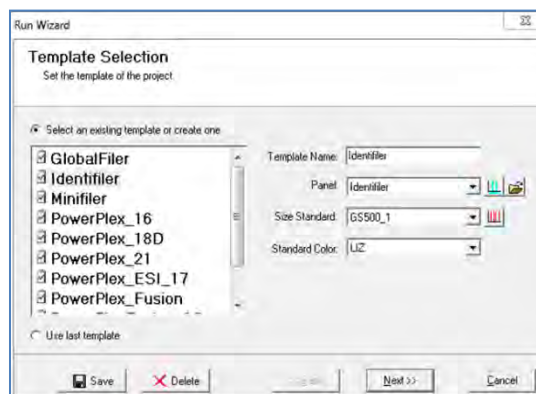
## Process Data

After the raw data files have been uploaded to *GeneMarkerHID*, 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. *GeneMarkerHID* 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

GeneMarkerHID comes preloaded with many common kit Panels including Promega's PowerPlex kits and ABI's Identifiler and Globalfiler kits, Qiagen's Investigator kits. 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

GeneMarkerHID comes preloaded with many common size standards including GeneScan 500, GS600, ILS 500, ILS600. A custom Size Standard can be created by selecting the *Size Template Editor* icon next to the *Size Standard* field. GS500\_1 is a commonly used size standard .xml file; the two smallest fragment, the 250 bp fragment and the two largest fragments are disabled - 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.

## 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 in the primer front, de-select *Auto Range* and manually input the required data range.

##### Smooth

Smooths the baseline by eliminating smaller noise peaks.

##### Enhanced Smooth

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

##### Pull Up Correction

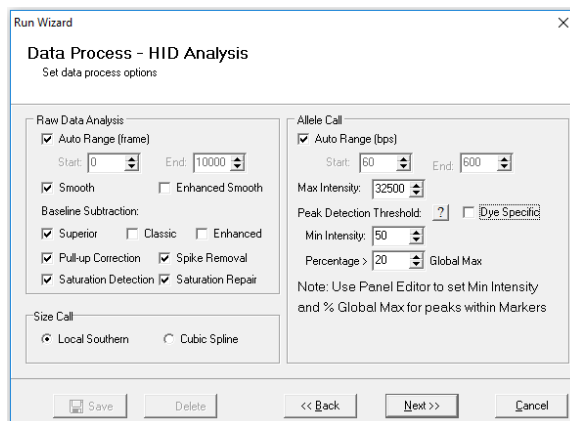
Makes minor matrix corrections to pull up peaks (up to 20%) directly underneath the major peak. This function removes peaks caused by wavelength bleed-through to other wavelengths. The function should be disabled if a *Manual Pull-up Correction* was used in the *Raw Data Analysis* window.

##### 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 *2<sup>nd</sup> Derivative Trace* has been applied.

##### Saturation Detection

Uses a conservative algorithm to evaluate peaks for saturation detection. Flags the allele in yellow - quality reason SD (saturation detection) displayed in peak table





### Saturation Repair

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. Flags the allele in yellow – quality reason SR (saturation repair)

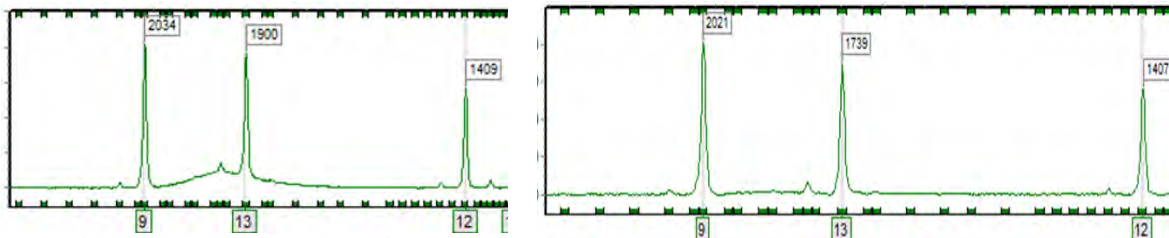
### Classic Baseline Subtraction

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. This is the same as the Baseline Subtraction setting in GeneMarkerHID v.2.7.6 and earlier versions.

### Superior Baseline Subtraction

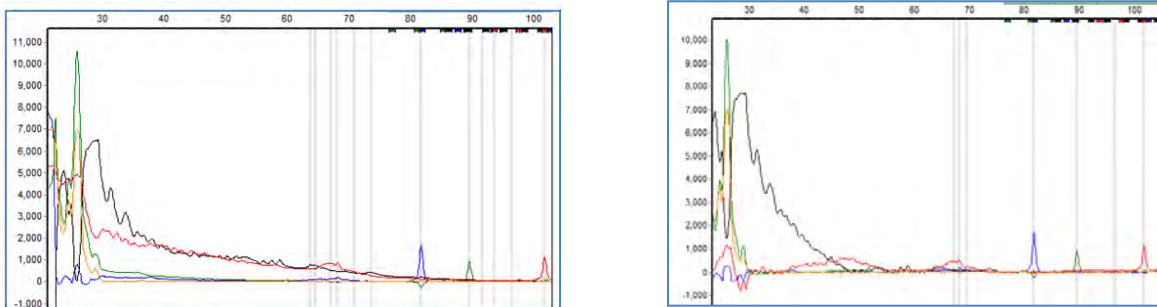
Is a similar algorithm to classic baseline subtraction but it looks at the trace in 100 frame sections. This baseline subtraction method is excellent for samples that have a raised bubble that is occasionally present in the low molecular weight; and for samples with closely spaced peaks.

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



### 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.

## Size Call

*GeneMarkerHID* offers two sizing methods:

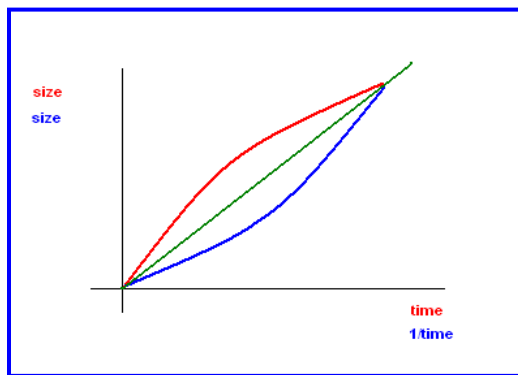
### Local Southern

Used in most genotyping software applications and is recommended for most analyses – local Southern and modified local Southern. 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).

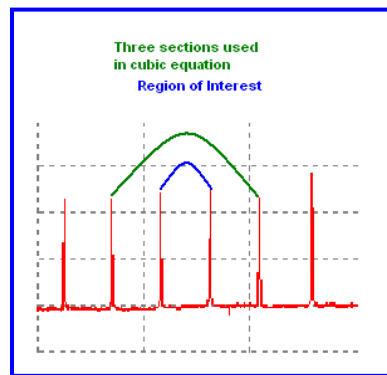
### Cubic Spline Method

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.

### 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.

## 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](#).

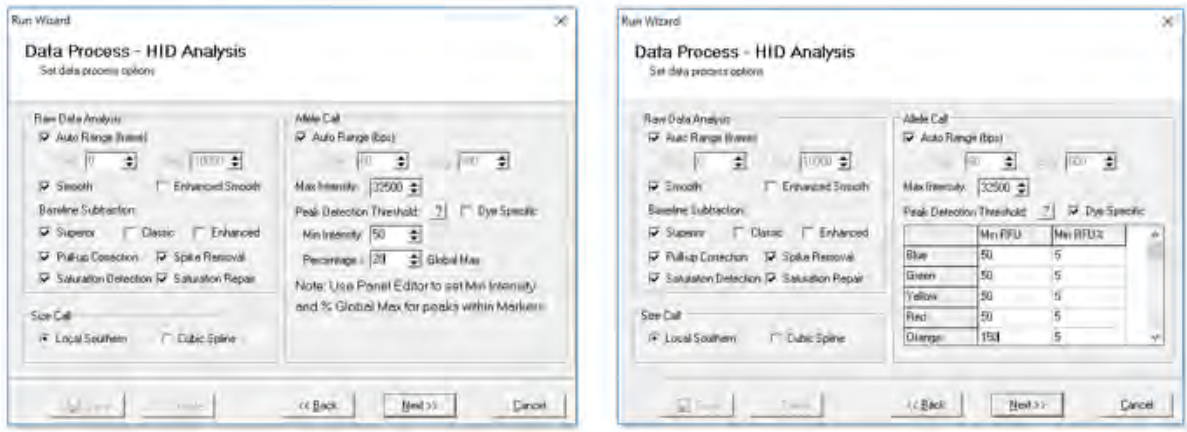
The peak detection thresholds setting has the option to apply the same minimum intensity and percent global max to all dyes (figure left) or to assign values per dye (figure right)

### Min Intensity

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

### Percentage Global Max

Relative minimum intensity of allele peaks to the 5<sup>th</sup> highest peak in the dye color used for peak detection. Peaks below this value will not be called.



**Note:** Versions 1.6-2.85 had an automatic peak score filter (0.02). Version 1.9.0 had the option to turn off the score filter in the Data Process screen of the analysis template. Feedback from forensic laboratories indicated that peak height and height ratio were the preferred method for filtering peaks, therefore, in Version 2.9.5 and above the score filter has been removed from the software.

Save Icon will save any changes to the analysis template (Run Wizard Settings)

## Run Wizard Additional Settings Procedure

7. The Run Wizard *Additional Settings* box appears
8. Select an Allelic Ladder and adjust the Peak Score parameters or check Auto Select Best ladder and Auto Panel Adjust.
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.

### Positive Controls

During validation, the laboratory can use the positive control template tool to include positive control genotypes to automate positive control concordance. See [Chapter 10, Additional Tools](#), for details on positive control concordance settings.

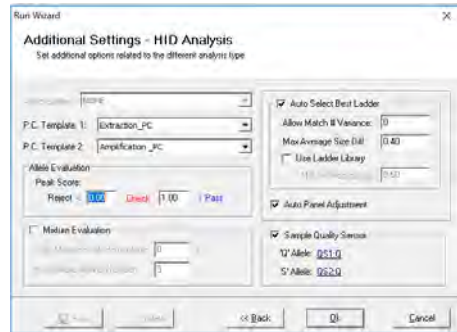
### Allele Evaluation

#### 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 peaks have a red allele flag, peaks that need to be checked have a yellow allele flag, and samples that have passed appear in green.

#### Mixture Evaluation

Select this option to identify mixture samples. This option must be selected to provide access to the Mixture Application for deconvolution and LR results of two person mixtures (See [Chapter 7 Mixture Analysis](#)).



### Auto Select Best Ladder

GeneMarkerHID 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 specified 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. The file name used for each sample is displayed above the electropherogram after the sample file name.

**Additional options under Auto Select Best Ladder are available in versions 2.9.5 and above.** The setting in the image above are consistent with previous versions of GeneMarkerHID. New settings: Allow Match Number Variance, Min Average Size Difference and Use Ladder Library enable the laboratory to place additional criteria on the best ladder selection pool for projects where a collection of ladders (ladder library) are used.

### Auto Panel Adjustment

Performs calibration of the panel.xml to the allelic ladder files, 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 GeneMarkerHID 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.

### 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.

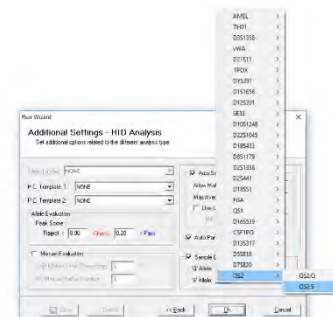
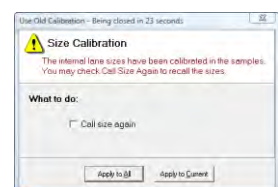
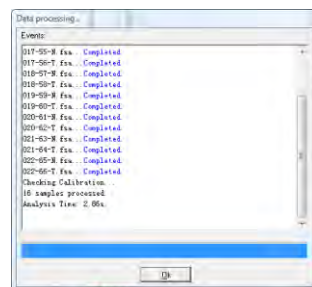
**NOTE: Manual edits will be lost when data is re-analyzed.**


### 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.

### Quality Sensor Evaluation

If the chemistry has quality sensor peaks, such as the Investigator®24plex kit, this setting can be used to flag samples that break the rules set in the *View* - *Preferences* - *Sample Quality* tab. Select the appropriate Q and S alleles. Then click **OK**. Any peaks that do not meet the criteria set in the *Sample Quality* tab will be flagged qS. A mouseover pop-up message will indicate potential issues with the sample.



Researchers may have the need to Re-analyze Individual Samples - select the tool bar icon 

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**

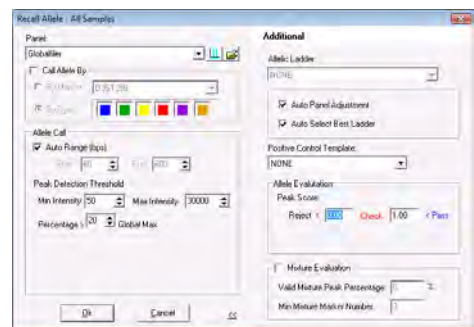
Applies the new parameter settings only to the sample highlighted in the *Sample File Tree*.

**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

### **Chapter 3 Main Analysis Overview**

Main Analysis Window

Menu Options

Main Toolbar Icons

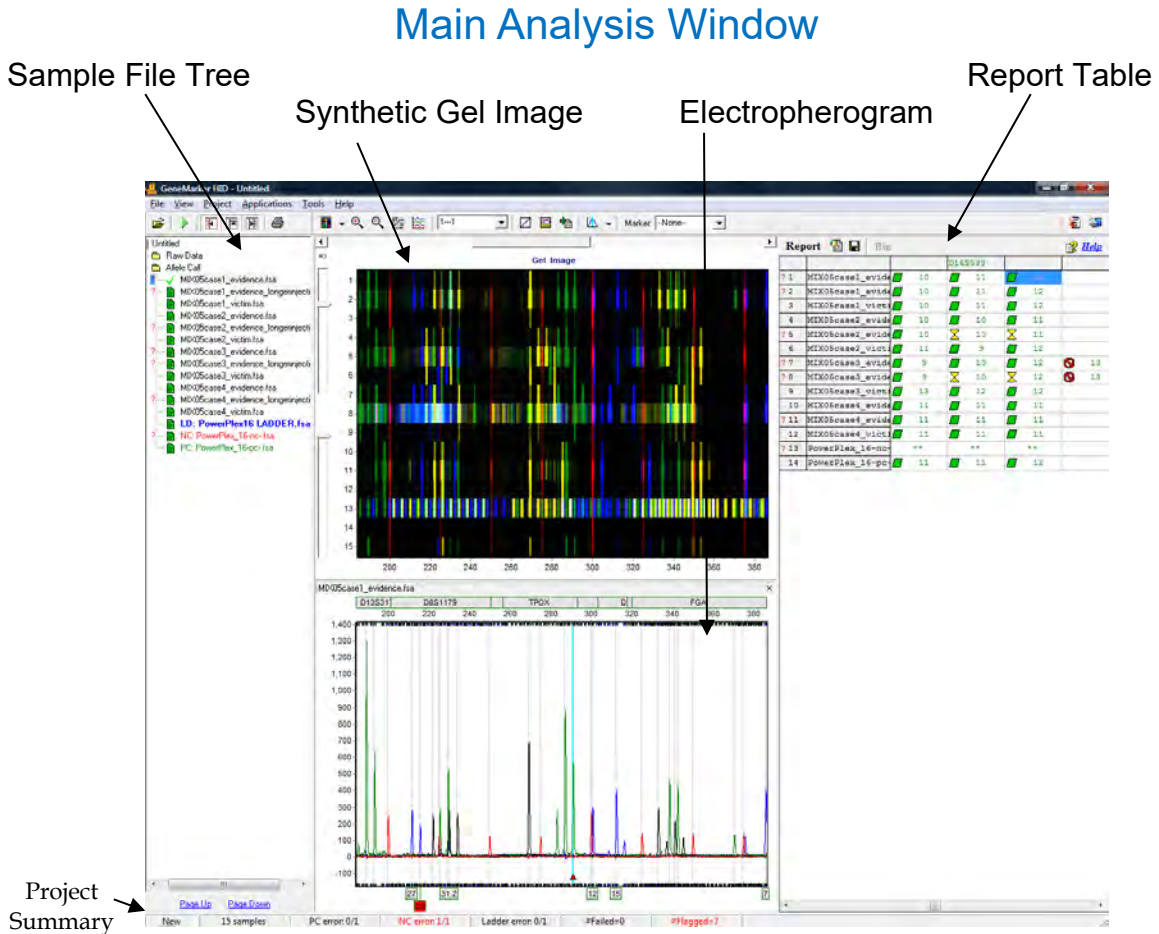
Additional Analysis Options



## Main Analysis Window

The main window of GeneMarkerHID 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.

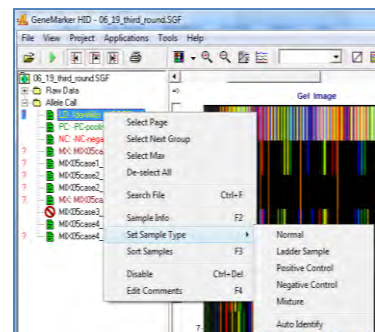
To resize the frames in the *Main Analysis* window, simply place the cursor over the partitions that separate the *Electropherogram/Sample File Tree/Report Table*. The cursor will change to a two-headed arrow bisected by two vertical lines. Hold down the left mouse button and drag the gray vertical line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



## Sample File Tree

The *Sample File Tree* of the main analysis window contains two folders. The first is the **Raw Data** folder which, when expanded, displays a list of all the dataset samples. When a sample is double-clicked its preprocessed electropherogram trace will appear in the *Raw Data Analysis* window. See [Chapter 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. Red question mark flagging is also assigned to samples with null marker(s) and samples with AMEL and Y-STR marker conflict(s) when amplified with a multiplex that contains AMEL and Y-STRs in addition to autosomal markers. If the analyst resolves all of the quality reason flag(s) for a sample, the red question mark is replaced by an E. This provides a very quick way for any reviewer to select only the samples that had flagging completely resolved by the analyst; as is the scenario for use of the software as an expert system where technical review is needed for any sample that required analyst intervention to pass the criteria for CODIS export.

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

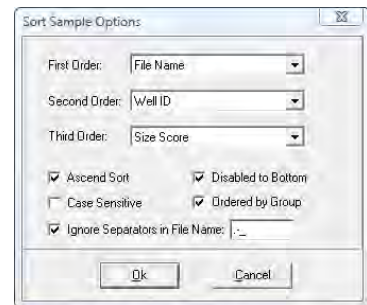
Opens electropherogram traces for the number of samples specified in the *View* → *Preference* → *Display Settings* → *Max # Open Charts*.

### 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**

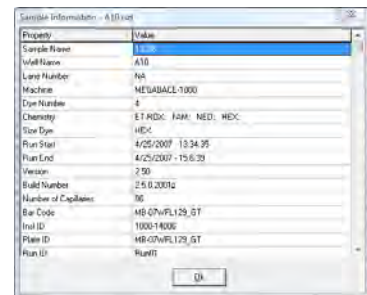
## Sample Information

### 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**

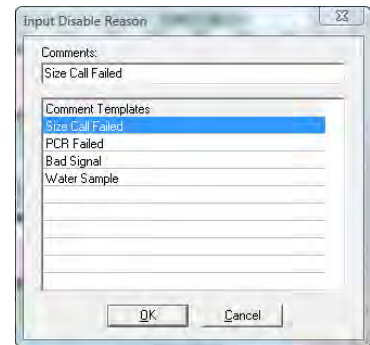


## Disable Samples

### 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 *View* → *Preferences* → *Others* → *Enable Sample Grouping*. Then return to the main toolbar and 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 GeneMarkerHID 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

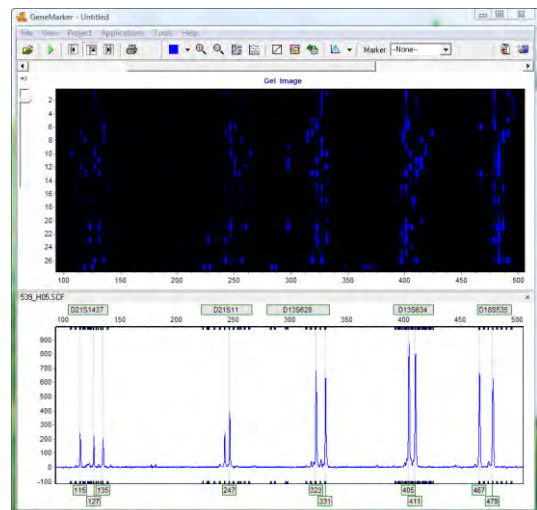
In the *Synthetic Gel Image* or the *Electropherogram*, hold down the left mouse button and drag a box from upper left to lower right around the area you would like to zoom in on. To zoom back out, hold down the left mouse button and drag a box in the opposite direction from lower right to upper left. 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.

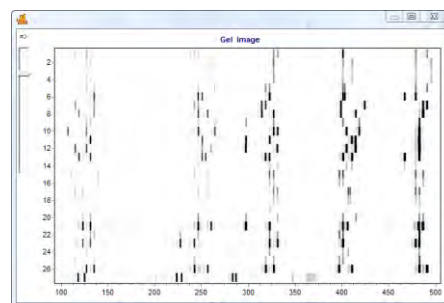
### Image Display

#### 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.

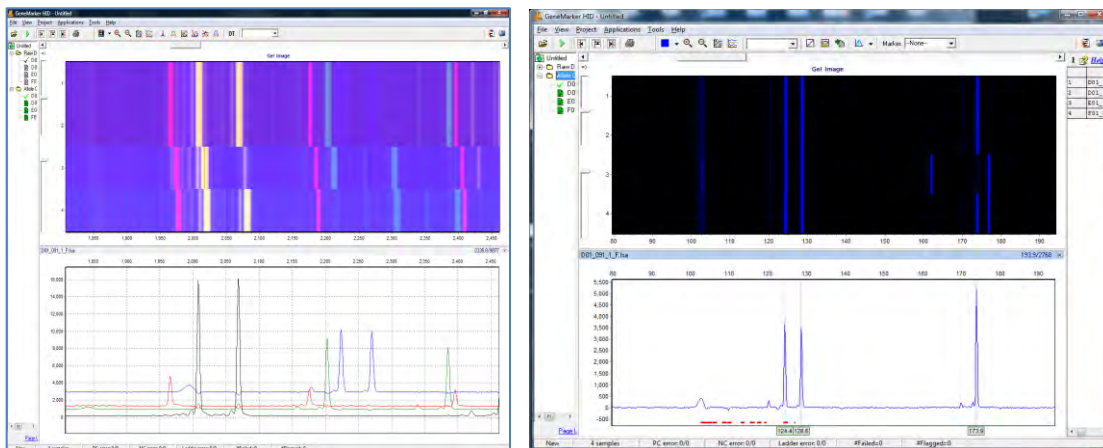
#### Cursor Locator

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.

#### File Name

Reports the full file name of the .hid or .fsa file

#### Sample Name

Reports the sample name from the Property sample name in the .fsa or .hid file

#### 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. **Note:** During data review it is sometimes helpful to select a different peak for comparison. To change the reference peak used for the height ratio, right mouse click on the peak in the electropherogram or in the report table and select Set Reference peak height to refresh the calculated peak height ratios.

#### FWHM Full Width at Half Maximum

The peak width at half height is displayed in this column.



**Area**

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

**Area Ratio**

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

**Marker** (*Panel Only*)

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

**Allele** (*Panel Only*)

Indicates which *Bin* the peak is contained in.

**Difference** (*Panel Only*)

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

**Quality** (*Panel Only*)

Assigns a *Pass/Check/Undetermined* quality ranking for each peak with regard to the peak *Score* as set in the Run Wizard *Additional Settings* box ([See Chapter 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 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.

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

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

**OL = Off Ladder**

Peak is outside of the marker range.

**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.

**OB = Out of Bin**

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

**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

**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. **Not** for use in quantitative calculations.

**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.

**SD = Saturation Detected**

Possible saturation is detected based on pull up peaks under the main dye

**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**

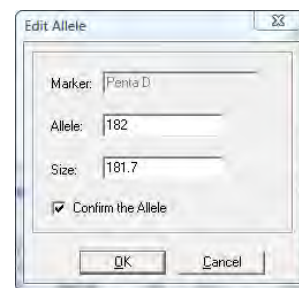
If a peak is given a low quality score, it will receive a *Check* (yellow) or *Undetermined* (red) Quality ranking. To give the peak a *Pass* (green) Quality ranking, right-click the peak center bar and select *Confirm (Hot Key = CTRL+M)*. The peak will be marked *Pass* (green) and receive a "Confirmed" 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**

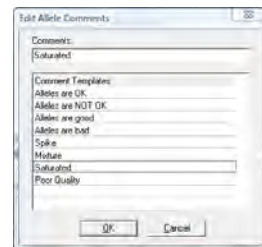
Right-click an allele in the *Electropherogram* or *Peak Table* and select *Edit Allele*. The *Edit Allele* box appears. Add or change the values in the *Allele* and/or *Size* field. The *Allele* field will be blank if no Panel has been applied to the dataset. Check *Confirm the Allele* to automatically 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

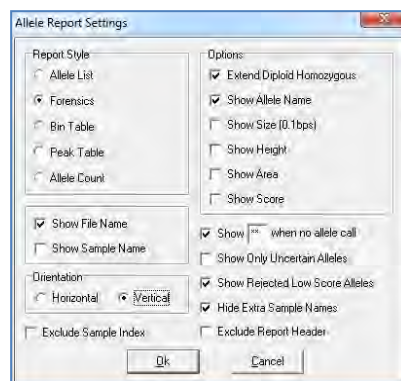
The *Main Analysis* window *Report Table* contains additional information about sample peaks. See [Chapter 6 Reports and Printing](#).

### 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. 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.

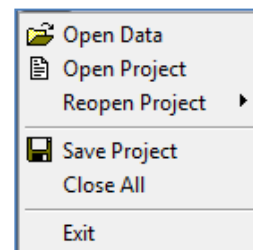
To export only selected cells in the report table, first select the cells by left-mouse drag across the cell range or hold **SHIFT** key and select cells. Right-click on the highlighted cells and select *Copy* (**Hot Key = CTRL+C**). The information is saved to the Windows clipboard and can be pasted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

#### Open Project

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

#### Re-Open Project

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

#### Save Project

Saves a SoftGenetics *GeneMarkerHID* project (.sgf) 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 included in the traces of saved GeneMarkerHID Projects (.SGF files).

#### Close All

Closes a project without exiting the program.

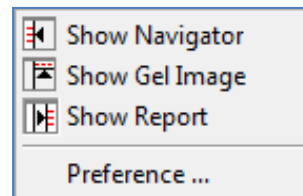
**NOTE:** It is recommended to select *Close All* before exiting the program.

#### Exit

Closes the *GeneMarkerHID* 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 six-tab *Preferences* box. The Import/Export button at the lower left of the box enables laboratories to Export validated settings and Import the Preferences settings .ini file if all workstations should use the same preferences.

## 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

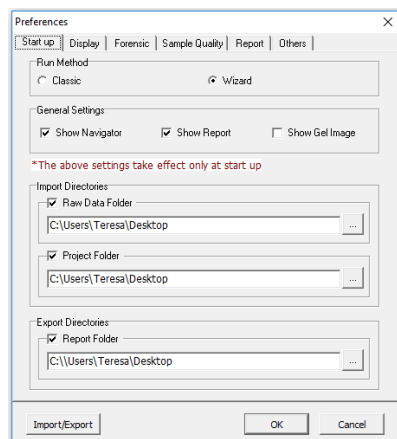
**Show Navigator:** When selected, the *Sample File Tree* will automatically be displayed in the *Main Analysis* window after data processing.

**Show Gel Image:** When selected, the *Synthetic Gel Image* will automatically be displayed in the *Main Analysis* window after data processing. (this is turned off by default and may be displayed automatically by checking the box).

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

**Import Directories:** to set a default folder for import of raw data and project files. This option decreases the amount of navigation needed when folder locations are designated for import of data and projects

**Export directory:** to set a default folder for export of reports.



## 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 1 decimal places for peak size labeling and font size for allele label. Data frames are read as 10 frames per base pair.

**Mark Off-Allele as 'OL':** Select this option to label alleles that are outside of allele ranges as 'OL'. If unchecked the size will be displayed.

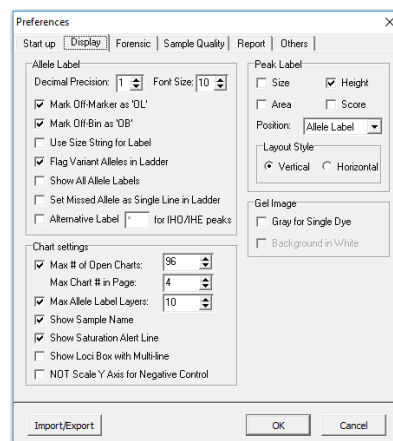
**Mark Off-Bin as 'OB':** Peaks within a marker range, but outside of a bin will be labeled 'OB'. If unchecked the size will be displayed.

**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.

**Font Size:** select the font size of the allele label characters. This increased or decreased 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 unchecked displays allele labels of dye 1 (blue) only when all dyes are overlaid in the electropherogram).



Set Missed Allele as Single Line in Ladder: Allele labels for missed ladder peaks will be placed one row above allele labels of peaks that were called.

Alternative Label [ ] for IHO/IHE peaks: A special options for peaks flagged with IHE or IHO. The allele label will appear at the top of the peak, with the user specified flag on a yellow background.

### 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

NOT Scale Y Axis for Negative Control: Zooming in and out will not affect Y-axis scaling for samples marked as negative controls.

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

### 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.

### Sample Tree

Flag Low Quality ILS as 'SQ': Samples with low quality Size Standard peaks will be flagged with 'SQ'. **See Chapter 4 Fragment Sizing Standards.**

Consider Gender for Flag ?: Impacts multiplex chemistries that contain Y-STR marker(s). N

## Forensic

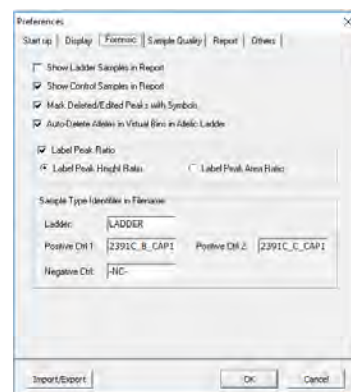
The *Forensic* tab allows the user to determine the display of Ladders and Controls in the *Report Table* and to establish file labeling conventions for Ladders and Controls.

Show Ladder Samples in Report: When selected, the samples designated as Ladders by the *Ladder Identifier* field will appear in the *Report Table*. Select this for export to STRMix software, ensures maximum number of columns.

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  $\chi$  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 when displayed at top of peaks (see Display Settings tab) on the electropherogram



Outline Marker Label if Any Peak is Flagged due to:

Null marker (allows for gender agreement of AMEL and Y-STRs), Red Quality Reason Allele Label Flags, Yellow Quality Reason Allele Label Flags

Ladder Identifier: Enter common filename nomenclature for Ladder samples in the dataset (must be in all capital letters). Upon first analysis, GeneMarkerHID 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). Upon first analysis, GeneMarkerHID will automatically scan the dataset filenames for the *Positive Control Identifier* values and subsequently label the Positive Control samples with a “PC1 and PC2:” and display the sample filename in green font in the *Sample File Tree*.

Negative Control: Enter common filename nomenclature for Negative Control samples in the dataset. Upon first analysis, GeneMarkerHID 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*. See [Chapter 10 Additional Tools – Control Concordance](#).

**Sample Quality**

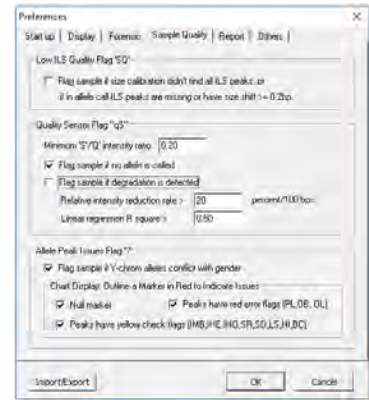
Low ILS Quality ‘SQ’: Samples with low quality Size Standard peaks will be flagged with ‘SQ’. SQ is displayed in the sample name tree, upper left of the electropherogram and in the report table at the right side of the main analysis screen. See [Chapter 4 Fragment Sizing Standards](#).

Quality Sensor Flag “qS” Is designed to provide automated feedback Quality Sensor peaks from chemistries such as Qiagen Investigator®24plex. Select the appropriate settings based on information from the kit manufacturer and the lab’s validation results.

Allele Peak Issues Flag “?” All of the following conditions will result in the file(s) in the sample name tree receiving a ? flag.

*Flag sample if Y-STR alleles conflict with gender results of Amelogenin:* Impacts multiplex chemistries that contain Y-STR marker(s).

Check any of the following to highlight the marker(s) that fired the flag: Null marker, red allele flag(s), yellow allele flag(s).





## 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 GeneMarkerHID 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.

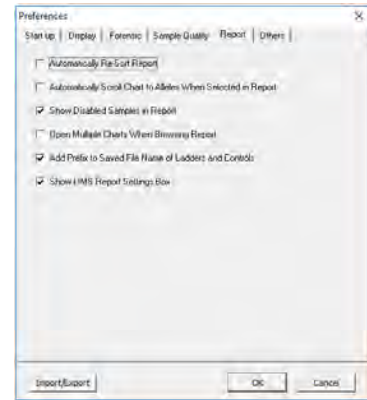
**Automatically Scroll Charts to Alleles When Selected in Report:** You may choose whether to scroll to alleles in the trace when selecting the allele in the report. Leave this feature on to have the software automatically call up alleles in the trace when you double-click on them in the report.

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

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

**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)

**Show LIMS Report Settings Box:** Displays the LIMS report settings box when the LIMS Report option is selected from the report table. **See Chapter 6 Reports and Printing.**



## Others

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

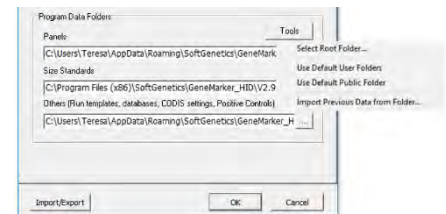
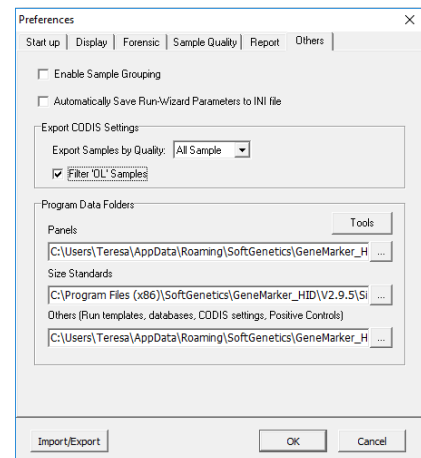
**Enable Sample Grouping:** When selected, activates the *Apply Sample Grouping* option under the *Project* drop-down menu. De-select *Enable Sample Grouping* to inactivate the *Apply Sample Grouping* option. The *Apply Sample Grouping* information is saved and can be recalled by selecting *Enable Sample Grouping*. **See Chapter 8 Additional Tools – Filename Group Editor. File grouping is especially helpful for review of replicate samples and paternity or family testing trios.**

**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.

**Export CODIS Settings:** Designate the appropriate filter settings per the laboratory SOP to limit the genotypes that are automatically checked for export in the CODIS report (See Chapter 6 Reports and Printing).

**Program Data Folders:** The default folders are appropriate for many laboratories; enabling the analysts to access and save Panels, Size Standards, Run templates, databases CODIS settings and Positive Control templates. Depending on the IT requirements, the laboratory manager has the flexibility to use the Tools option to designate other locations for these files.



## 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

GeneMarkerHID will process data using the last set of parameters selected.

### 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.**

### 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*.

### Contamination Check

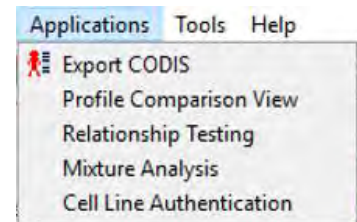
Select the Contamination Check to compare all samples within a project for:

1. lane - to - lane contamination
2. Contamination from profiles saved to a database (for example, the embedded contamination database containing staff genotypes)

**Please see chapter 10, Additional Tools,** for detailed instructions on using the contamination check.

## 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.



### 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, identifies 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.

### Mixture Analysis

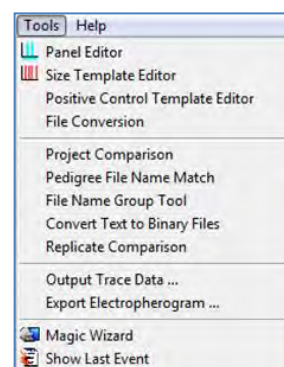
GeneMarkerHID identifies the presence of potential mixture samples, designates allele peaks, and calculates peak area or height ratios in the main analysis screen. The Mixture Analysis Application is activated from the Applications menu in the main analysis screen. Mixture analysis identifies the mixture 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. Note: The analysis template must have Mixture Analysis checked in the third screen of the run wizard to activate the Mixture Analysis application.

### Cell Line Authentication

This application enables users to do a percent match comparison between samples in their current project, and sample genotypes saved in a database (i.e. cell lines), making it ideal for cell line authentication.

### 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 contains 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.

#### Database Manager

This tool allows the user to edit and submit genotypes to the Contamination, Cell Line, and Relationship databases without opening a project. The user may submit genotypes from .txt or .cmf file formats.

#### 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

The *Filename Group Tool* allows users to define file groups and save the group information as a separate text (.txt) file. A Text (.txt) file is exported. [See Chapter 9 Additional Tools.](#) **This tool is required for auto-saving and printing pdf files for each sample or group of samples.**



### 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 *GeneMarkerHID*. [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.

### Validation Assistance

Assistance to determine Analytical Threshold. Export a table with noise peaks (automatically calls noise peaks and does not call data or stutter peaks). Select multiple of std dev and export a table with noise and X x std dev per dye.

### 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

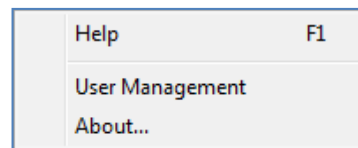
Launches a searchable version of this manual.

#### 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 *GeneMarkerHID* 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*.



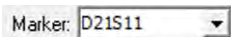
### Save Peak Table

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**  
Can be used in conjunction with the Bin Table Style Allele Report; research application, allows the user to select which bins to include/exclude in the *Report Table*.

## Additional Analysis Options

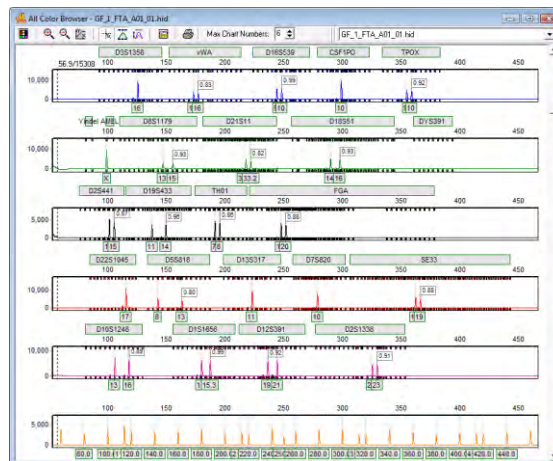
In addition to the *Main Analysis* window, there are two other display options in which the sample data can be viewed: *Browse By All Colors* and the *Profile Comparison View*.

### 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.

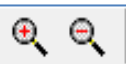
To scroll through samples in the *All Color Browser*, click the drop-down menu in the upper right corner and select a sample from the list. Once a sample is selected in the drop-down menu, you can use the **Up/Down Arrow** keys to scroll through samples.



### Icons and Functions



**Dye Selection** – Use this icon to remove any dye channel from the view.



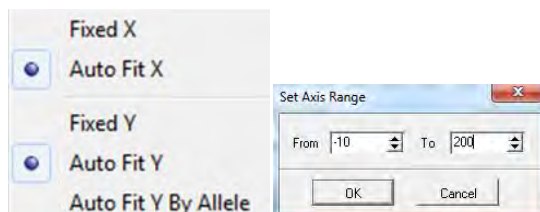
**Zoom In/Out**  
Use these icons to increase/decrease the zoom aspect of the electropherograms.



**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.



**Set Axis**  
The default setting automatically sets the Y-axis according to the maximum peak intensity of the samples. Options include:



Select Fixed Y from this drop-down menu to manually select the same Y axis value for all dyes. To manually select a Y axis value for each dye independently, right mouse click on each dye in the profile, select Set Axis → Fixed Y and enter the desired Y axis for that dye. Repeat for each channel.



### Show/Hide Bin Ranges

When selected, the Bin brackets at the top and bottom of the displayed 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 to print or save the trace display of a single sample from the all color browser with the dye specific Y axis settings

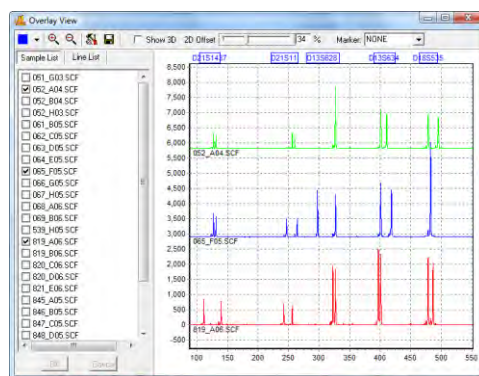
## Profile Comparison View

### 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.

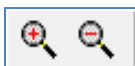


### Icons and Functions



### Dye Color

Single click to scroll through the dye colors - use the drop-down arrow to add or remove dyes from the electropherogram display



### 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

## **Chapter 4 Fragment Sizing Standards**

Size Template Editor

Size Calibration Charts

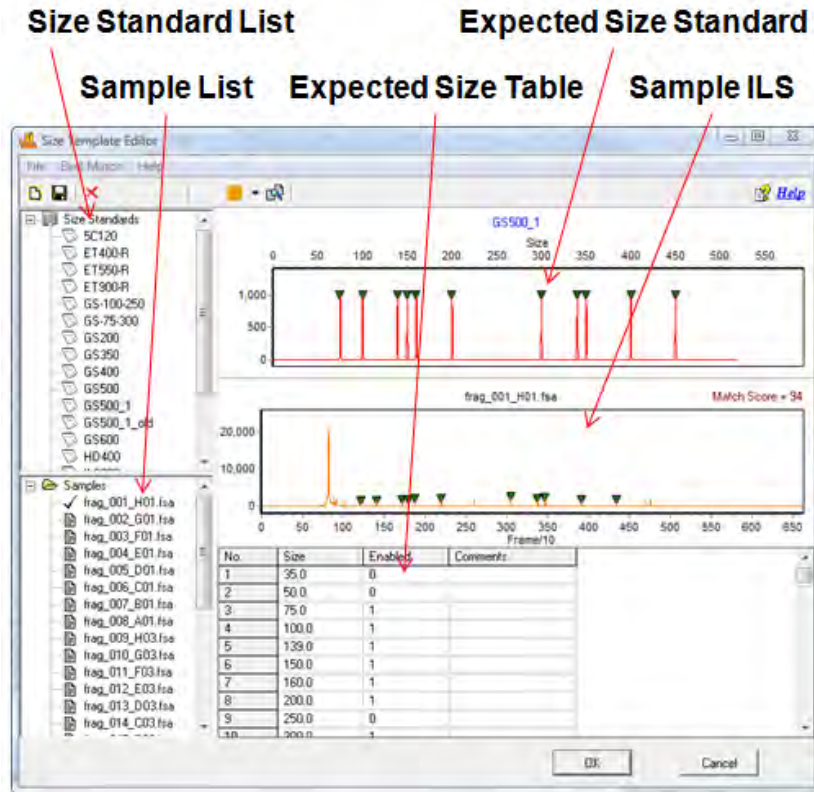
## Size Template Editor

The *Size Template Editor* is a tool in GeneMarkerHID 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:** GeneMarkerHID 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 GeneMarkerHID software.

## Size Template Editor



### 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 GeneMarkerHID 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 GeneMarkerHID 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 GeneMarkerHID 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

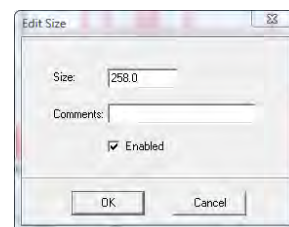
The *Edit Size* box appears. Adjust parameters and click **OK**.

**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. GeneMarkerHID 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. GeneMarkerHID'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. Note: GS500\_1 is commonly used to accommodate data with artifacts in the low mw range and samples where data collection was ended prior to collecting the largest size fragments: the smallest, largest and 250 bp fragments are disabled.

Pre-defined Size Standards include:

5C120	GS500
ET400-R	GS500_1
ET550-R	HD400
ET900-R	ILS500
GS-100-250	ILS600
GS-75-300	Liz120
GS200	Rox1000
GS350	SEQ_600
GS400	550 (BTO)

### Pre-Defined Size Standards

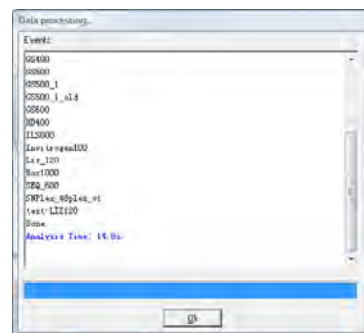
There are two ways to choose a pre-defined Size Standard for the dataset. If the Size Standard name is known, simply single left-click the Size Standard name in the *Size Standard List* and click **OK** in *Size Template Editor*. The selected Size Standard will then appear in the *Size Standard* field 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. GeneMarkerHID 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.

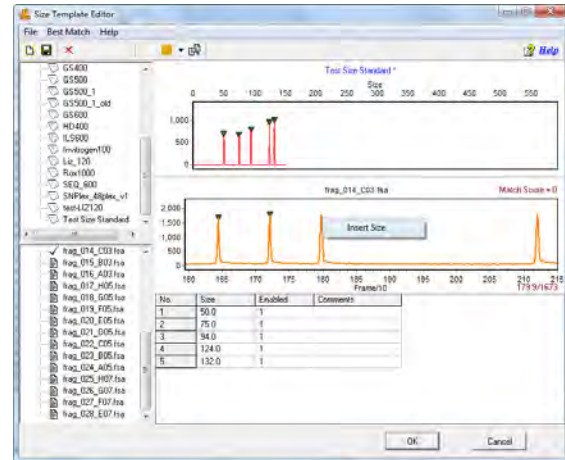
6. Once the Size Standard is chosen, click **OK** in the *Size Template Editor*
7. The selected Size Standard will then appear in the *Size Standard* field of *Run Wizard Template Selection* box and will be used to size the data.



### Custom Size Standard Creation

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

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. GeneMarkerHID 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

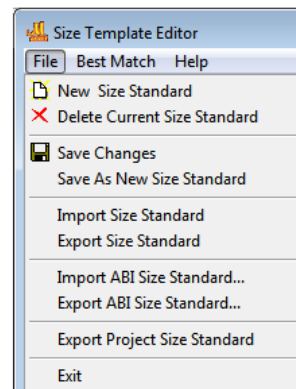
Saves edits and changes to the Size Standard in the SoftGenetics GeneMarkerHID Size Standard directory

##### 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 GeneMarkerHID Size Standard directory.

##### Import Size Standard

Opens a Windows Explorer window to the SoftGenetics GeneMarkerHID 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.

**Export Project Size Standard**

Exports the size standard used to process the current project to the desired folder or directory.

**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 GeneMarkerHID 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.

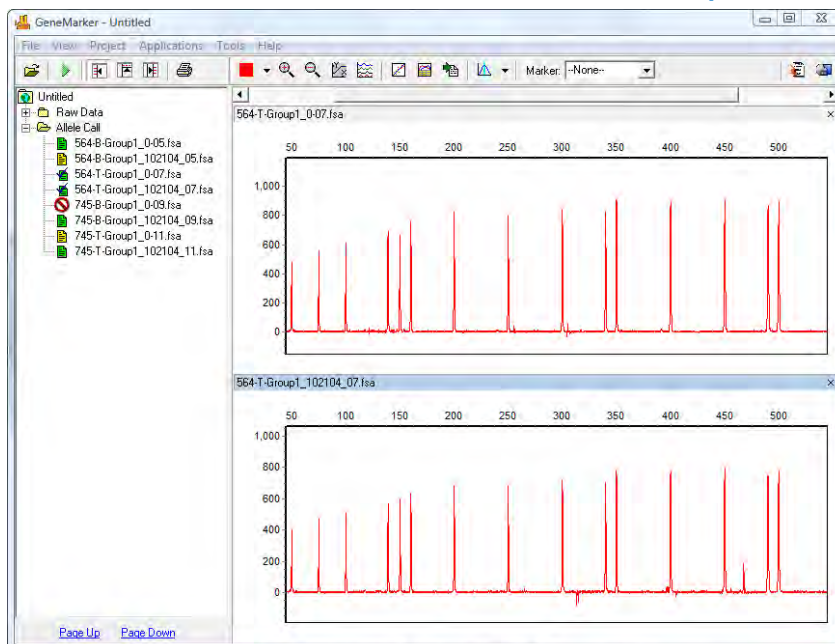
## What to Expect

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 GeneMarkerHID'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*.

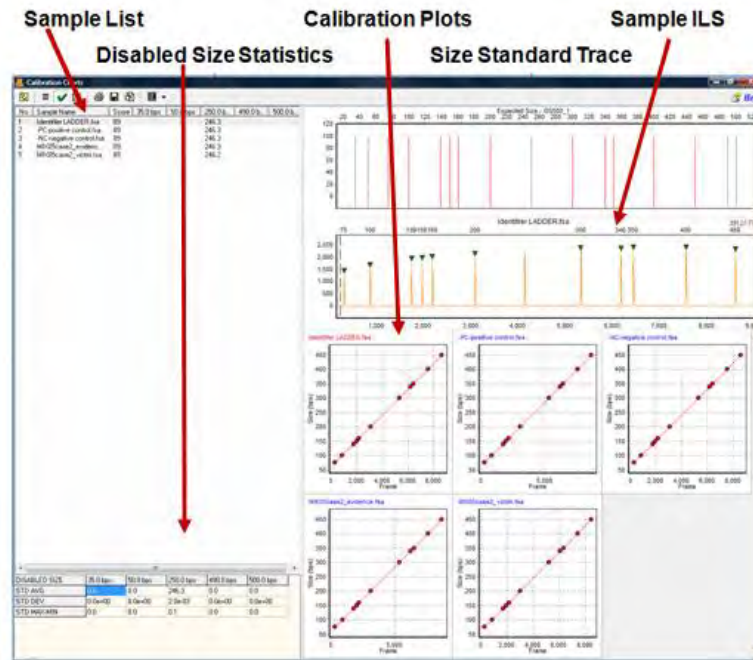
**Score**

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 be 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*.

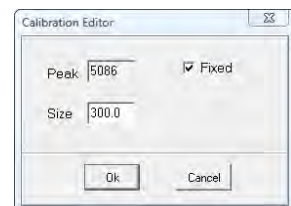
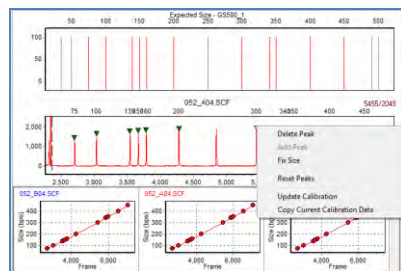
**NOTE:** Deleted peaks will not be recalled when *Reset Peaks* is selected.

### 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

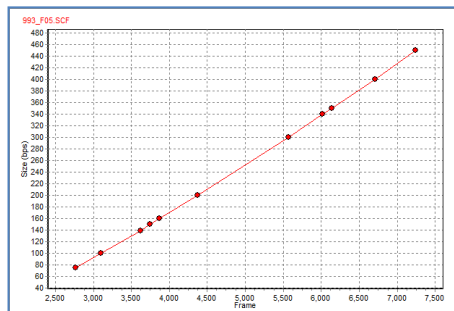
When selected, the frame position and basepair position of the green triangle peak indicators for the selected sample will be copied to the Windows clipboard and can be pasted into a spreadsheet or word processing program such as Microsoft Excel or Word.



## 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.





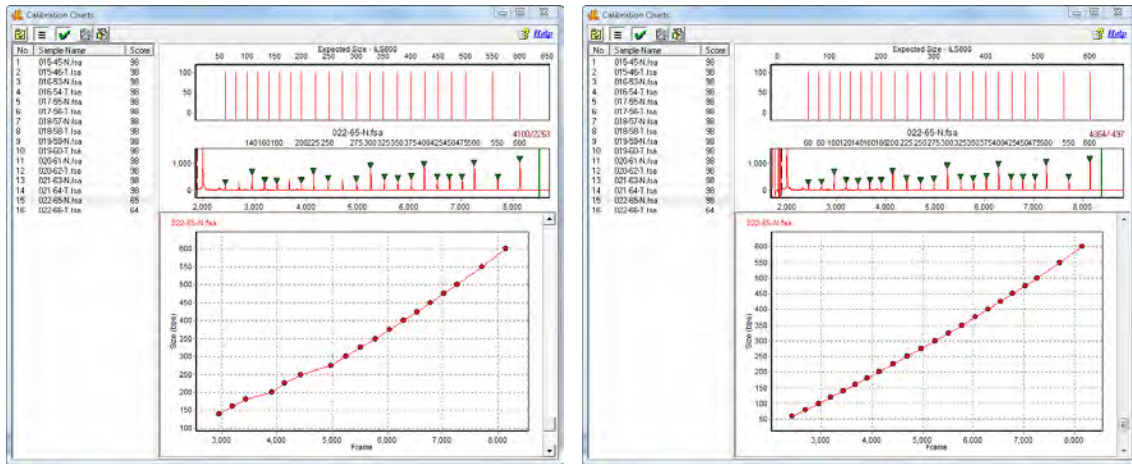
**Manual Calibration**

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.

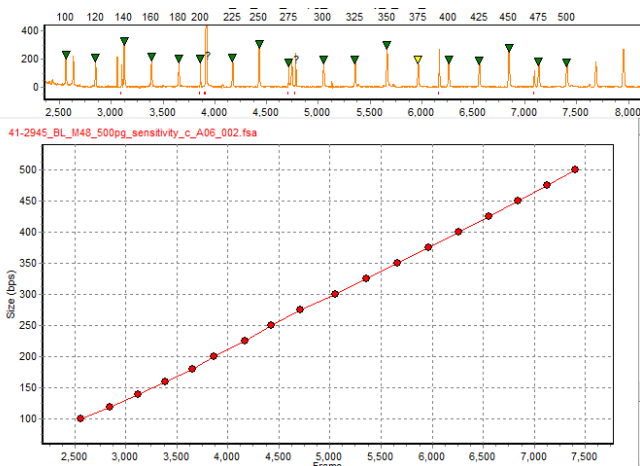
**Before & After Editing Size Call**



**SQ Flagging**

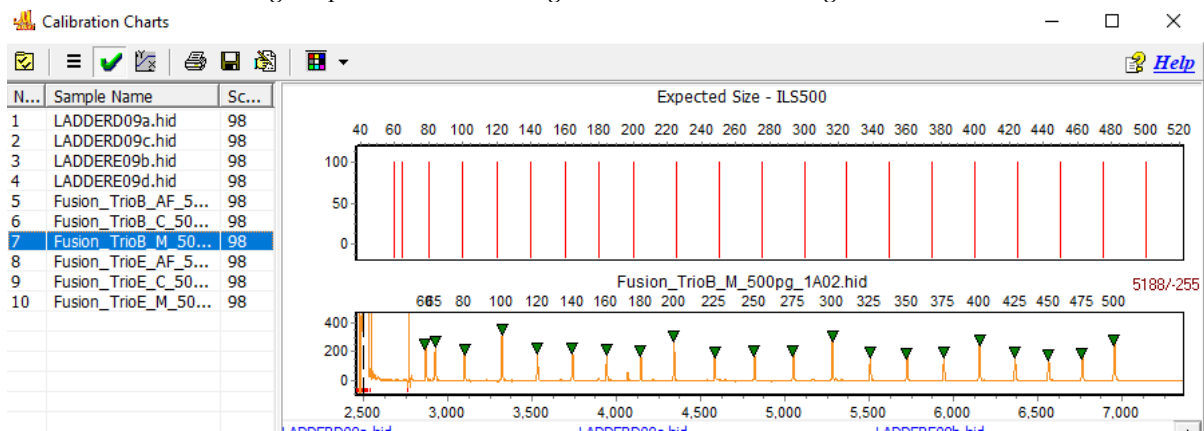
SQ ("Size Quality") flagging is an option that may be selected in the Display Settings tab of the View > Preferences menu. When this preference is selected, samples that have low quality size standard peaks will be designated with a red SQ in the main analysis window. Samples that will receive an SQ indicator include:

Samples where some of the size fragment are not detected in the linear range for size calibration:

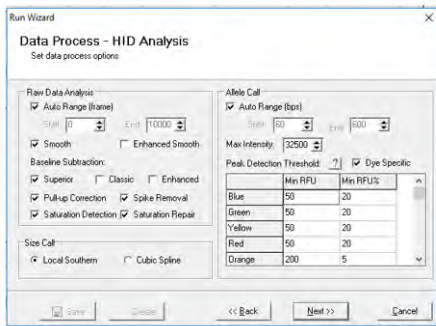


Samples in which all ILS fragments are detected for pattern recognition and are in the linear range – but one or more of the peaks is of low quality and not called during the allele calling in the main analysis window:

Size calibration indicating the pattern of the ILS fragments is in the linear range



However, if the peak is recognized by the calibration algorithm, but the height does not meet the analysis setting some of the ILS peaks in the example below were not called. The sample is flagged SQ in the file name tree, report table and electropherogram. A pop-up message is displayed with mouse-over in the electropherogram.



SQ flagging provides additional Size Standard information to the analyst. SQ flagging may indicate that the size standard has a linear migration but the resolution of the capillary is questionable.

## Chapter 5 Panel Editor

### **Chapter 5 Panel Editor**

Overview

Procedure

Icons and Functions

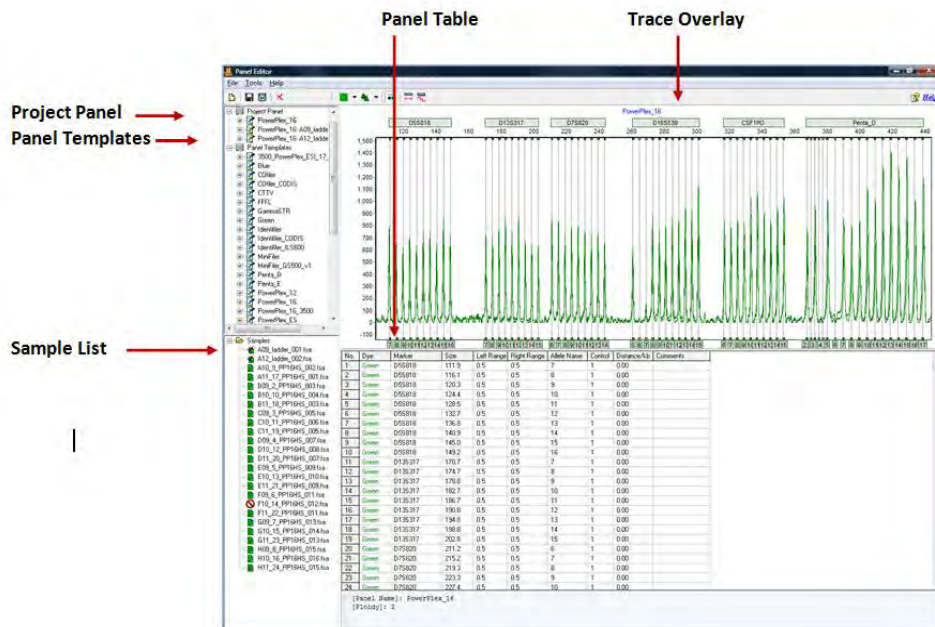
What to Expect

## 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 GeneMarkerHID, 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 GeneMarkerHID, 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. GeneMarkerHID 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 GeneMarkerHID directory. Single left-click on the panel name to display the trace. Single right-click on the Panel name to display the pop-up menu of options. Double-click the Panel name to expand the folder and view the Markers associated with the Panel. Single left-click the Marker name to display that Marker in the *Overlay Trace* frame.

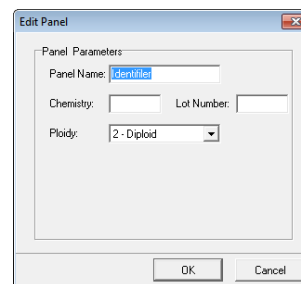
Rename	F2
Edit ...	
Delete	Del
Export	
Reload	
Set As Project Panel	

### 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 GeneMarkerHID 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 GeneMarkerHID 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 GeneMarkerHID program is closed.

**Sample List**

The *Sample List* contains all the samples uploaded to GeneMarkerHID 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

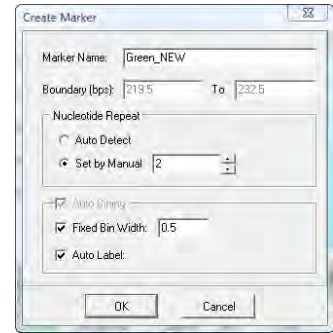
**Auto Detect:** Based on the peaks present in the *Overlay View*, GeneMarkerHID will attempt to detect the number of nucleotides in each repeat unit of the alleles and place Bins at the appropriate interval.

**Set by Manual:** Select this option if the number of nucleotides in the allele repeat unit is known and GeneMarkerHID 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.



Marker Specific Settings: 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.

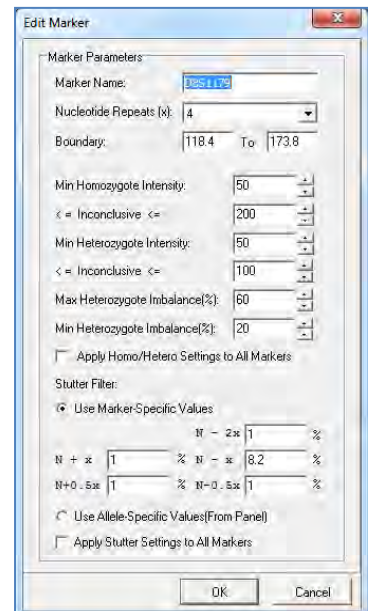
**Nucleotide Repeats:** Use the *Nucleotide Repeats* drop-down menu (1-6) or enter a value into the field to set the number of basepairs expected between each allele in the Marker.

**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 and stretch the marker, hold down **SHIFT** key and mouse-over the edge of the Marker bar until a double-headed arrow appears then right-click and drag the Marker edge to increase or decrease the range. 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. These boundary adjustments are generally used only by research scientists.

### Filtering Parameters

#### Minimum Intensity(RFU) Filter

**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 values 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 (Stochastic Range Flagging):**

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 flagging:**

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 filter:**

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 (including stutter peaks)**. 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.

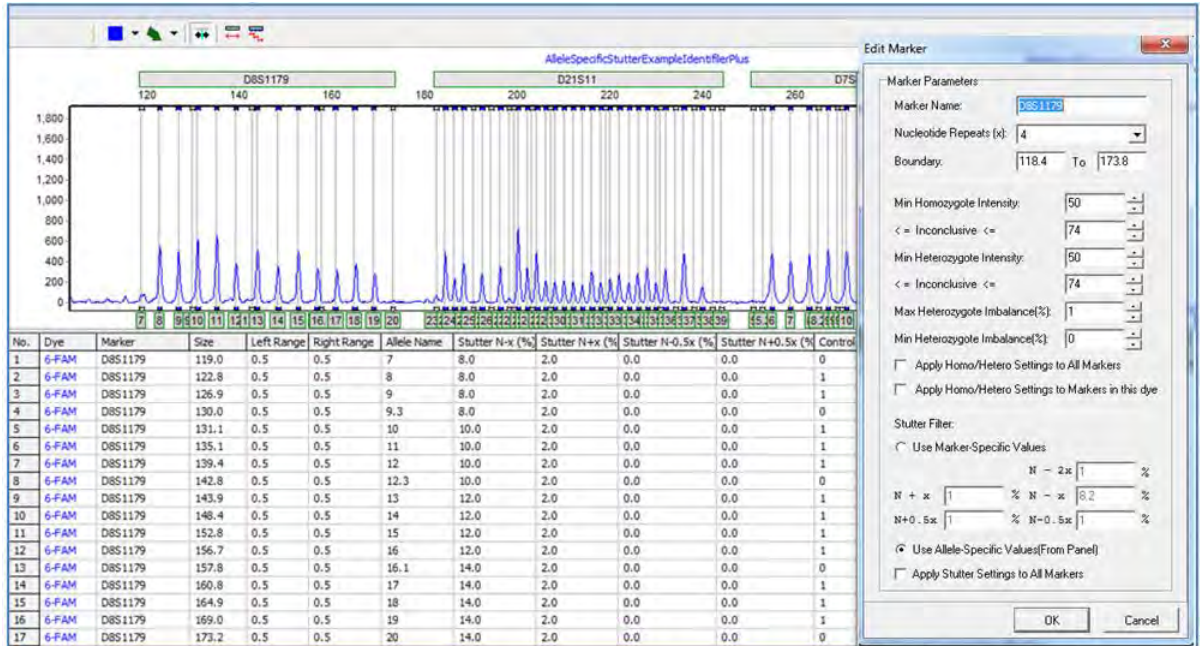
### **Stutter Filter by marker:**

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, N-0.5x and N+0.5x positions that are below the stutter threshold will not be called.

### **Allele Specific Stutter Filter:**

The allele specific stutter filter is activated when Use Allele Specific Values from Panel is selected. The marker stutter values are automatically grayed out. The image below is an example of allele specific settings; D8S1179 N-x position stutter for alleles 7-9.3=8%, alleles 10-12.3=10% and so on. The N+X stutter in this example is set at 2% for all alleles and the N+/- 0.5 stutter is not applied).

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



**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.

Apply Filter to All Markers within this dye: When selected, the values in the filtering parameter fields for intensity, Max het imb flag and Min Het Imb filter will be applied to all markers in the dye. An alternative to marker specific settings is to apply the settings by dye.

### 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*, GeneMarkerHID will attempt to detect the number of nucleotides in each repeat unit of the alleles and place Bins at the appropriate interval.

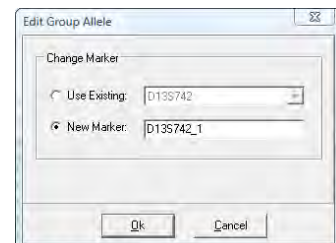
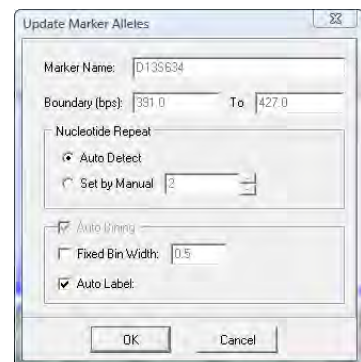
Set by Manual: Select this option if the number of nucleotides in the allele repeat unit is known and GeneMarkerHID will place Bins at the specified interval.

#### 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.

Bin	Dye	Marker	Size	Left Range	Right Range	Allele Name	Control	Distance(AI)	Comments
1	Blue	0.913568	108.3	0.5	0.5	11	0	0.00	
2	Blue	0.913568	112.3	0.5	0.5	12	1	0.00	
3	Blue	0.913568	116.4	0.5	0.5	13	1	0.00	
4	Blue	0.913568	120.4	0.5	0.5	14	1	0.00	
5	Blue	0.913568	124.4	0.5	0.5	15	1	0.00	
6	Blue	0.913568	126.6	0.5	0.5	15.2	0	0.00	
7	Blue	0.913568	128.6	0.5	0.5	16	1	0.00	
8	Blue	0.913568	130.6	0.5	0.5	16.2	0	0.00	
9	Blue	0.913568	132.9	0.5	0.5	17	1	0.00	
10	Blue	0.913568	134.6	0.5	0.5	17.2	0	0.00	
11	Blue	0.913568	137.0	0.5	0.5	19	1	0.00	
12	Blue	0.913568	139.1	0.5	0.5	18.3	0	0.00	
13	Blue	0.913568	141.2	0.5	0.5	19	1	0.00	
14	Blue	0.913568	144.5	0.5	0.5	20	0	0.00	

**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 a 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.

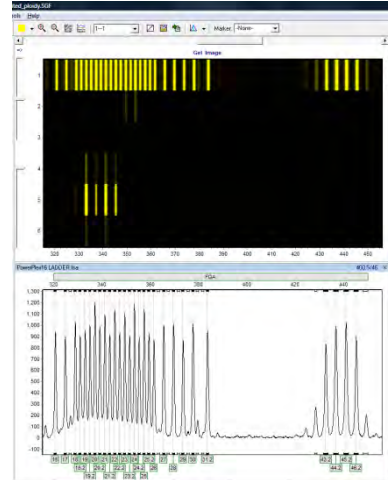
**Distance/kb**

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 GeneMarkerHID, the Panels associated with several commercially distributed human identity kits are included. Examples of some of the pre-defined Panels include Promega’s Powerplex kits and ABI’s Identifiler and Yfiler kits. GeneMarkerHID 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 GeneMarkerHID’s *Panel Editor* tool.

The Panels displayed by default include:

- |                             |                                |
|-----------------------------|--------------------------------|
| PowerPlex® 16               | GenePrint® FFFL                |
| PowerPlex® S5               | GenePrint® CTTv                |
| PowerPlex® Y                | GenePrint®_24                  |
| PowerPlex® Y23              | Globalfiler® AmpFSTR® COfiler® |
| PowerPlex® ESX16 and 17     | AmpFSTR® Identifiler®          |
| PowerPlex® EXI16 and 17     | AmpFSTR® MiniFiler™            |
| PowerPlex® 18               | AmpFSTR® Profiler Plus®        |
| PowerPlex® 21               | AmpFSTR® Profiler®             |
| PowerPlex® Fusion 5C and 6C | AmpFSTR® SEfiler™              |
| DIPplex®                    | AmpFSTR® SGM Plus®             |
| InvestigatorESSPlex®        | AmpFSTR® Yfiler®               |
| InvestigatorHDplex®         | SureID® STRyper-27Y            |
| Investigator24Plex®         |                                |

**Pre-Defined Panels**

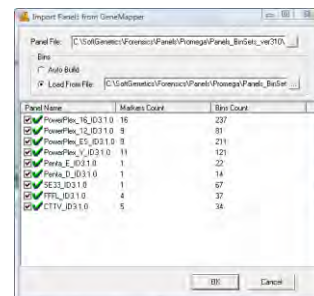
The pre-defined Panels appear in the *Panel List*. Single-click any Panel in the *Panel List* to select it.

Archived data tool – If the laboratory has panel and bin files from ABI that pre-date the current kits, there is a tool to import Panel and Bin files. 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. Note – stutter values and virtual bin designation must be made in the Panel Editor. Please contact [tech\\_support@softgenetics.com](mailto:tech_support@softgenetics.com) if you require a panel not listed in the pre-loaded panels.

**Panels and Bins Files for legacy chemistries**



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**.



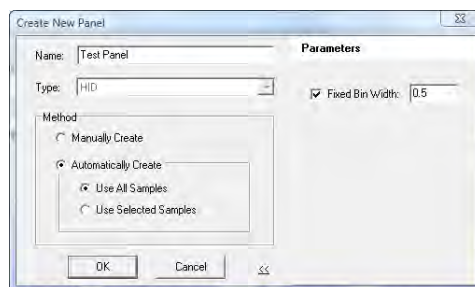
- NOTE:** Select *Bins Auto Build* if a *Bins.txt* file does not exist.
7. Click **OK** in the *Import Panels from GeneMapper* box.
  8. All Panels in the *Panels.txt* file will be uploaded into GeneMarkerHID.
  9. Designate virtual bins by entering a 0 in the control column of the table below the electropherogram.
  10. Select a newly uploaded Panel from the *Panel List*.
  11. Edit the Markers and Bins so that they align with the peaks in the dataset.
  12. Save the edited Panel and close *Panel Editor*.
  13. Click the **Run Project** icon in the *Main Analysis* window.
  14. Select the Panel from the *Panel* field in the *Run Wizard Template Selection* box.
  15. 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 GeneMarkerHID.

### 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 GeneMarkerHID 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**

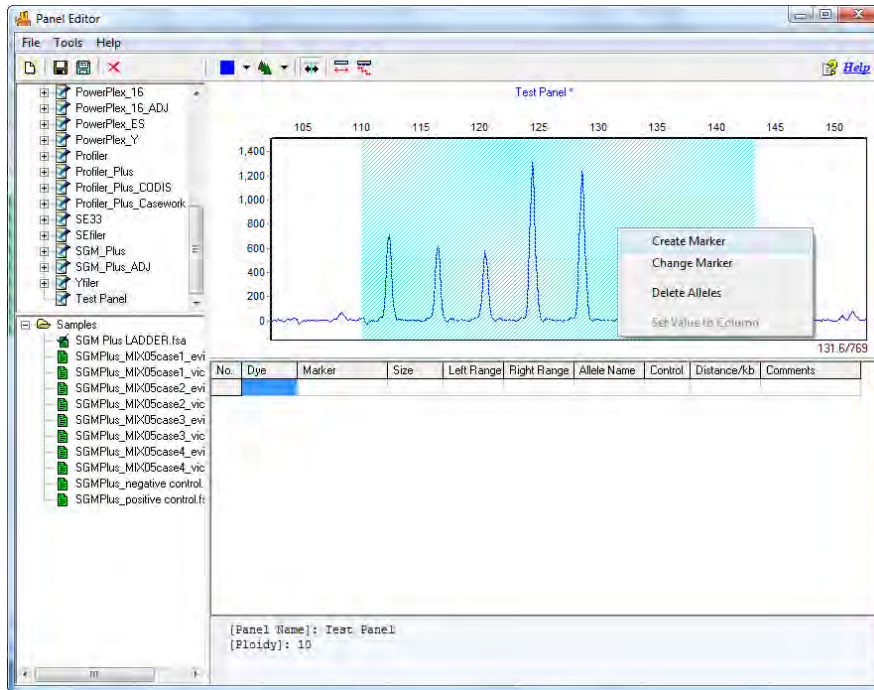
**NOTE:** New Panels are created based on the *Max & Average View Mode*. More intense peaks are given higher priority for Bin placement when peaks do not overlap perfectly.

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

### Manual Panel Creation

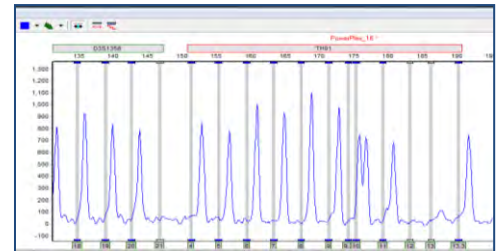
1. In *Panel Editor*, select *File* → *Create New Panel* from the menu bar or click the **Create New Panel** icon.
2. Enter a Panel name in the *Name* field
3. Choose the appropriate *Analysis Type* from the *Type* drop-down menu
4. Select *Manually Create*
5. When finished, click **OK**
6. The Panel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



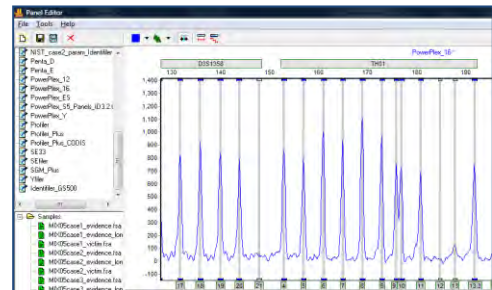
## Adjusting Panels - Manual and Automated Panel Calibration

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

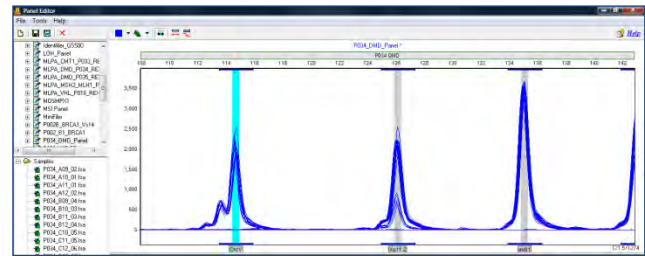
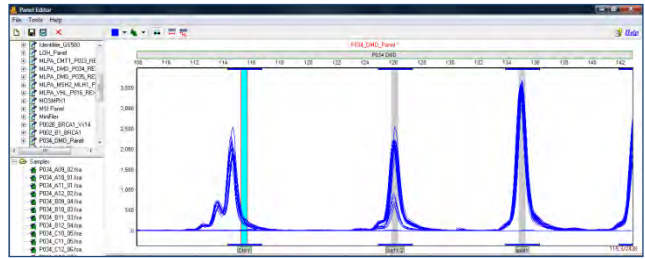
1. Hold down the shift key
2. At the same time place the mouse over the gray marker name bar at the top of the electropherogram
3. The marker rectangle will be outlined in red and the panel name will be in red font when the adjust feature is active
4. Drag the marker to align the bins with the peaks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



## 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 GeneMarkerHID 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 GeneMarkerHID 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 GeneMarkerHID preloaded panels of commonly used commercial chemistries.

##### 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 GeneMarkerHID. Evaluate (and edit if needed) the panel.xml for appropriate designation of virtual bins, N value, AT and stochastic values and stutter settings.

##### 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*. GeneMarkerHID 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 GeneMarkerHID Panel directory.

### Delete Current Panel/Marker

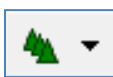
Deletes whichever Panel or Marker is currently highlighted in the *Panel List*. This action is irreversible.



### Show Dye

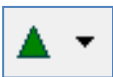
Allows the user to select a single dye color to view in the *Overlay View*. Cycle through the colors by left-clicking on the icon.

### Trace Mode

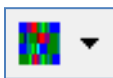


Single left-click to cycle through the options or use the drop-down menu.

*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.



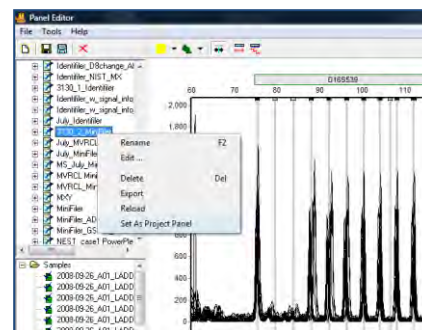
**Major Adjustment of Panel** To be used when a Panel must be adjusted by 1 - 5 basepairs in order to align with the dataset peaks. **For use only with panels that do not contain variant alleles (0 in the control column)**



**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)**

### Project Panel

Once a panel has been aligned and saved 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 it can be applied to the dataset. Save the edited Template Panel 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).

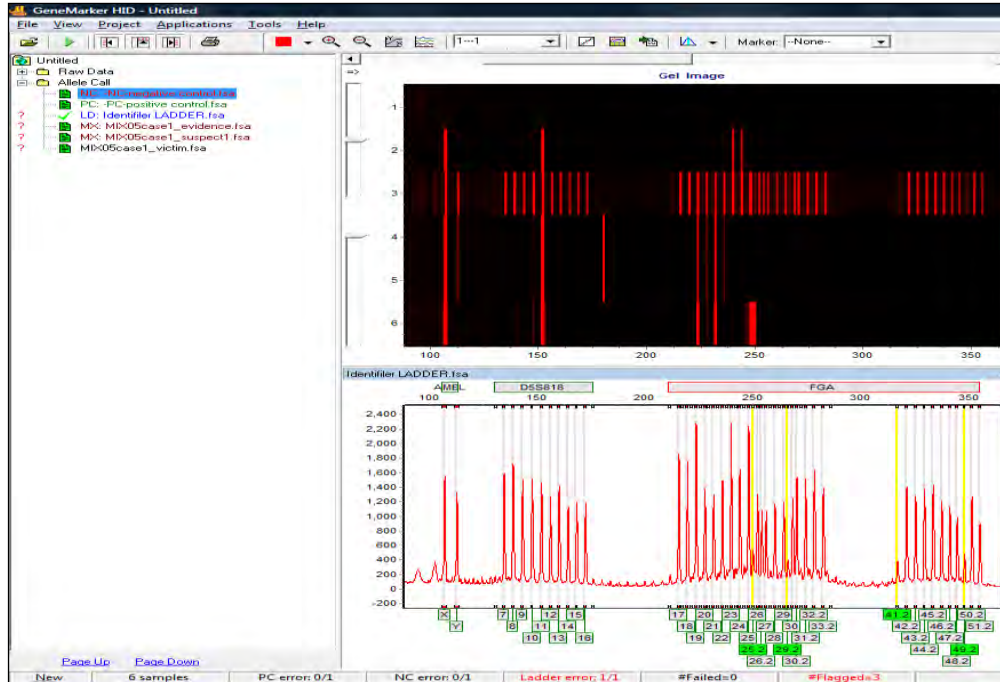


Figure 1 Yellow and green flagging alerts the analyst that although the panel is aligned with the ladder bins, some of the minor peaks in this ladder have a higher RFU than expected. This flagging can be turned off using View - Preferences - Display settings uncheck the box to flag peaks detected in virtual bins.

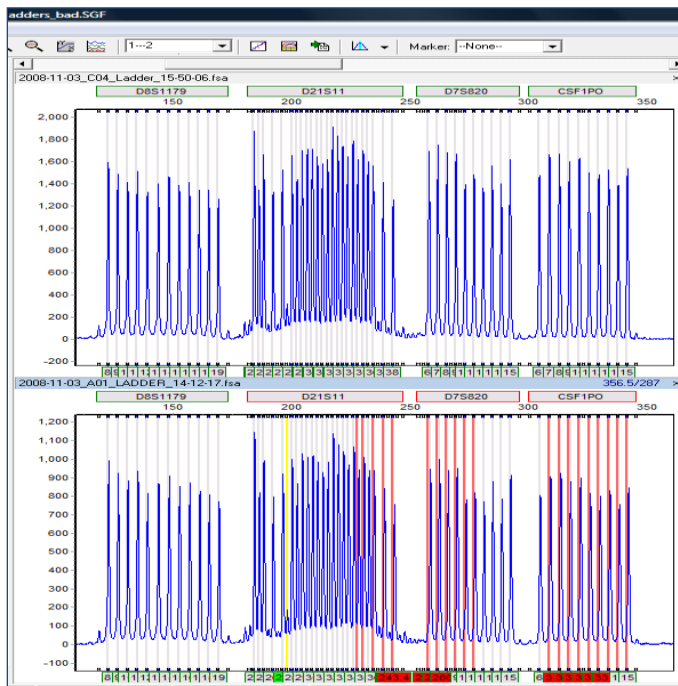
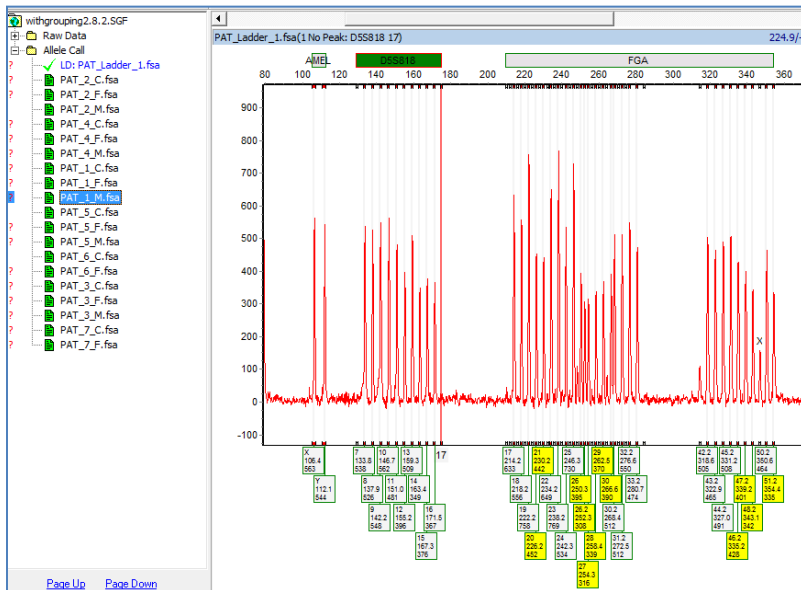


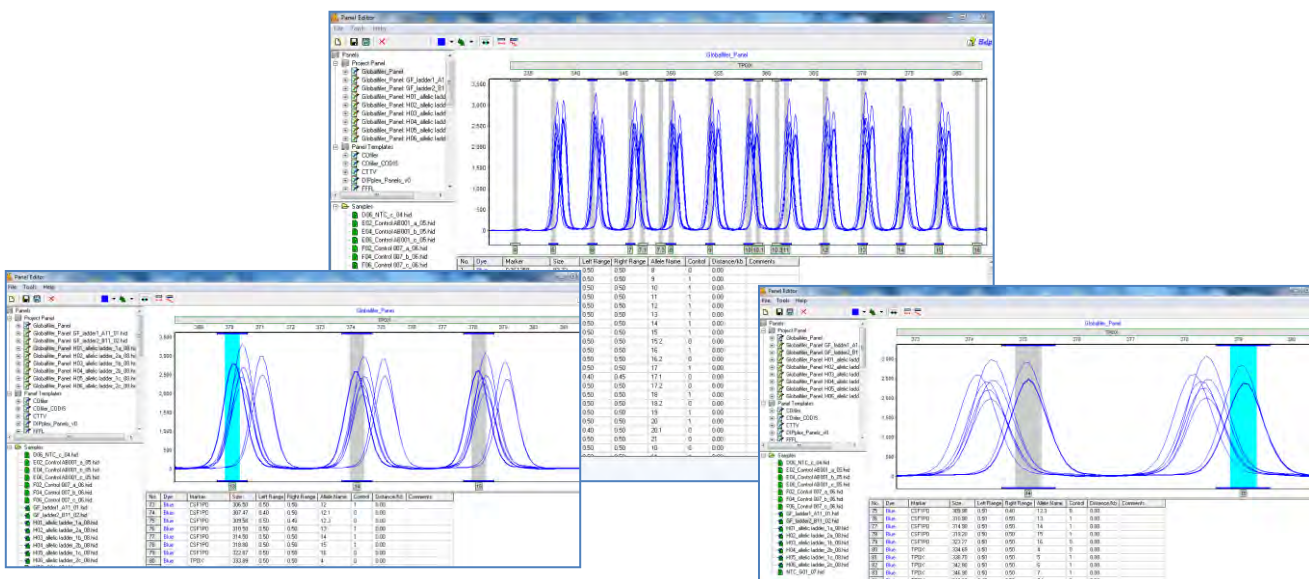
Figure 2 One of the ladder samples in this run has peaks that are out of the bins. These out of bin peaks are flagged with red allele label and red vertical bars



The marker(s) that did not pass the criteria for a passing ladder are dark green. Problematic allele(s) are listed in parentheses above the electropherogram.

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 Panel list under the panel Name. Select the allelic ladder from this list to highlight it in the electropherogram and see the bin alignment.

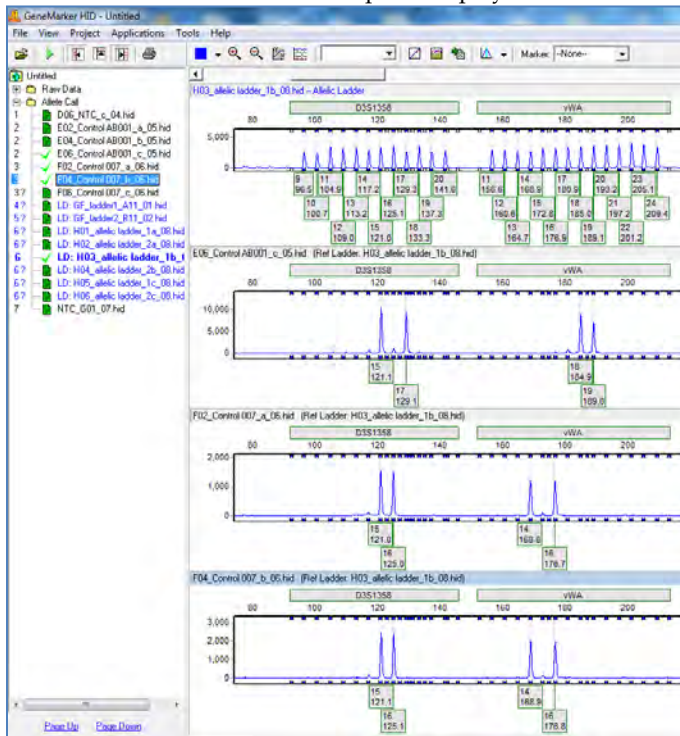
The first two allelic ladders show a migration shift Globalfiler\_Panel\_GF\_ladder1 and ladder 2 (left). When Globalfiler\_Panel\_H01 is selected from the list the electropherogram displays the aligned bins for this ladder file.



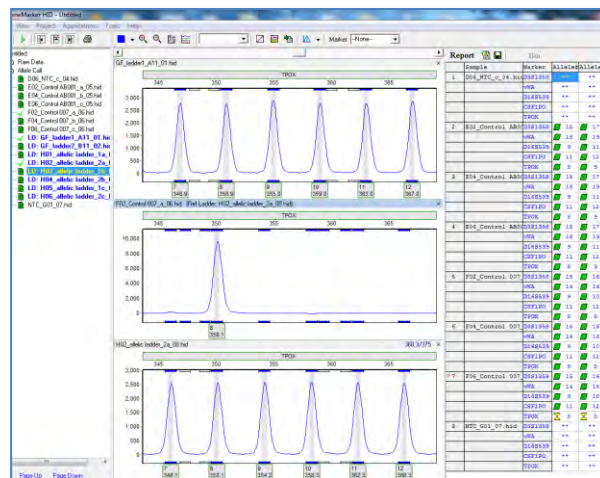
After the Panel is applied to the dataset, the Markers and Bins appear in the *Electropherogram* and *Report Table*. In the *Electropherogram*, the Markers are horizontal grey bars, the Bins appear as dye-colored brackets above and below the trace, and the center of the peaks are marked with a vertical 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





## Chapter 6 Reports and Printing

### **Chapter 6 Reports and Printing**

Report Table  
Print Report  
CODIS Report  
Save Project

## 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.

#### 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 GeneMarkerHID. If the user selects a different report style, the program will display this report style the next time the program is opened.

### MaSTR™ and STRMix™ compatible format:

The settings in the image to the right are used to export tables in a format compatible with STRMix software. Include the allelic ladder to ensure the maximum number of columns are included in each exported table.

**NOTE:** *Forensics* requires that a Panel is applied to the data. See [Chapter 5 Panel Editor](#).

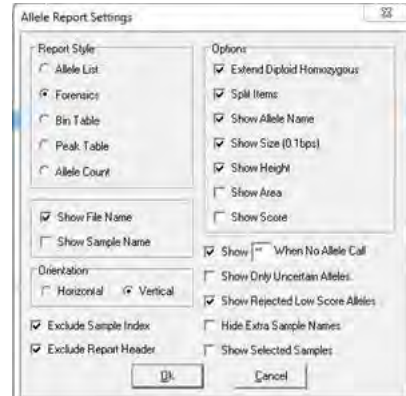
#### Features

##### 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*.



		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	496	OL	120	141
3	052_B04_SCF	327	401	411	479	495	OL
4	052_B03_SCF	411	479	496	OL	OL	178
5	061_B05_SCF	401	403	479	491	OL	124
6	062_C05_SCF	467	479	124	178	182	201
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_I06_SCF	405	415	483	OL	132	136
13	069_J06_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	406	422	483	OL



**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.

**Show File Name check box to display file name column in exported report table.**

**Show Sample Name check box to display sample name column in exported report table.**

**Orientation**

**Horizontal:** Sample names appear on the left in rows and Markers appear at the top in columns.

**Vertical:** Sample names appear in the far left column in rows with Markers listed in the second column. Alleles in order of base pair size appear at the top in columns.

**Exclude Sample Index:** to exclude consecutive numbering column in exported table

		D01011			D198628	
1	D61_G09.SCF	238	242	OL	327	331
2	D62_A04.SCF	257	260		327	327
3	D62_B04.SCF	257	260		327	327
4	D62_B09.SCF	237	240		327	327
5	D62_B09.SCF	247	244		319	319
6	D62_C06.SCF	247	251		323	323
7	D64_D06.SCF	242	247		314	319
8	D64_D06.SCF	247	257		314	327
9	D64_F06.SCF	247	244		297	327
10	D64_G06.SCF	247	244		297	331
11	D67_H06.SCF	251	251		297	323
12	D68_A06.SCF	251	260		297	331
13	D69_B06.SCF	251	254		319	323
14	D39_H06.SCF	247	247		323	331
15	D19_A06.SCF	242	257		323	327
16	D19_B06.SCF	242	257		323	327
17	D20_C06.SCF	247	257		327	331
18	D20_D06.SCF	247	257		327	331
19	D21_E06.SCF	242	254		323	323
20	D46_A06.SCF	247	247		297	331
21	D46_B06.SCF	247	240		297	323
22	D47_C06.SCF	227	247		323	331
23	D48_D06.SCF	227	242		323	331
24	D49_E06.SCF	242	OL	260	323	323
25	D52_F06.SCF	242	242		319	327
26	D53_G06.SCF	242	257		319	327
27	D99_F06.SCF	224	228		289	287

**Show \*\* when no allele call:** Allows user to specify a symbol or short word, up to 4 characters, 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.

**Exclude Report Header:** Beneficial for import into eDNA and some LIMS systems.

**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**

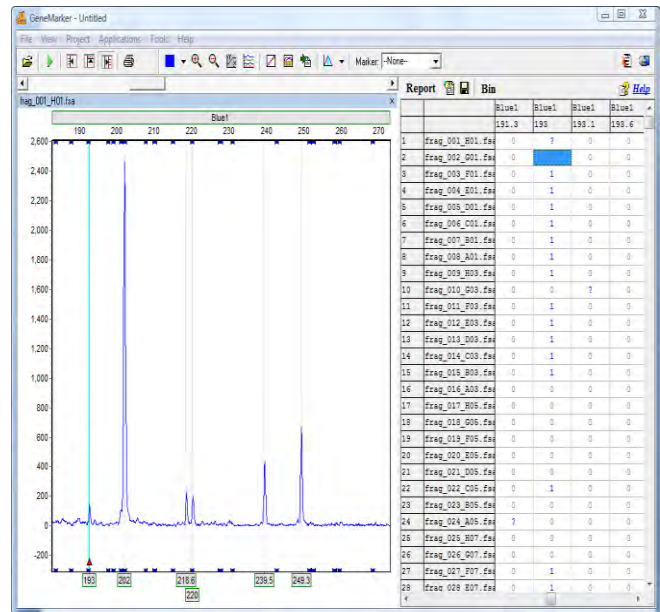
**Horizontal:** Sample names appear on the left in rows and Markers appear at the top in columns.

**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**

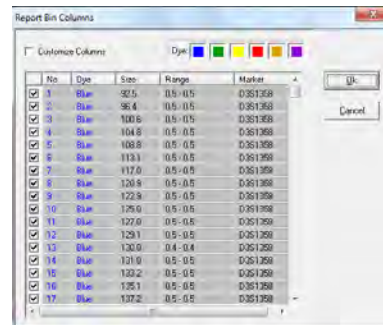
To completely eliminate an entire column in the *Report Table*, left-click any cell within the column then right-click the cell and select *Delete Bin Columns*. When *Vertical Orientation* is selected, the *Report Table* rows which contain the *Bin* information will be deleted (not the columns which 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. Note: Research use only.

**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.

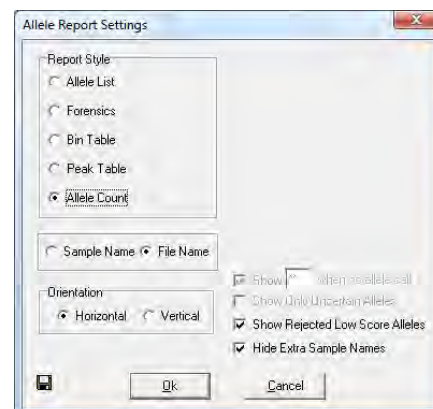
**Hide Extra Sample Names:** When data is displayed in *Vertical Orientation*, the sample names are repeated for each row of data that the sample is associated with. If *Hide Extra Sample Names* is selected, then the sample name will only appear once in the first of the rows it is associated with.

Sample	Marker	Allele#1	Size#1	Height	Area#1	Allele#2	Size#2	Height	Area#2
1 952_F05_SCF	D21S1437	115	115.1	469	4167	135	135.1	407	3003
	D21S11	242	242.3	947	6614				
	D13S628	319	318.6	1021	8787	327	327.0	1051	9178
	D13S634	397	397.2	1473	14559	401	401.1	1389	14114
	D18S536	483	482.8	2528	28522				
	D18S1002	120	119.9	605	3714	128	128.1	587	3612
	D18S391	178	177.9	1653	11898	182	181.9	1526	11004
	D13S742	266	265.7	900	5993	273	273.1	788	6032
	D18S386	351	350.8	776	6923	354	354.5	717	6315
	D13S305	446	446.2	648	7794	450	450.3	609	6589
IFNAR	142	141.9	537	4052	151	150.9	584	4304	
D21S1411	313	312.5	440	3963	325	325.3	383	3335	
2 993_F05_SCF	D21S1437	118	118.0	4790	39306	125	125.1	4906	40647
	D21S11	224	223.5	6237	51755	229	229.7	5804	47590
	D13S628	283	283.2	4711	35329	287	286.9	4341	32289
	D18S1002	156	156.2	7680	52108	160	160.5	7100	45671
	D13S742	257	256.7	2544	35036				
D21S1411	278	278.5	4005	30945	282	282.3	3654	28542	

## 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.

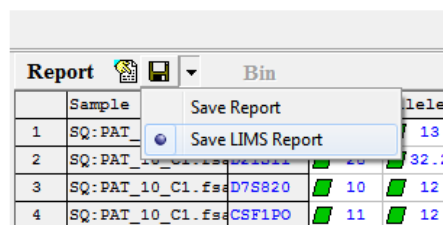
**Hide Extra Sample Names:** This feature is not active for *Allele Count Report Style*.

No.	Samples	D18S386	D13S305	IFNAR	D21S1411	Total Number
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

## LIMS Report

The LIMS report is a specialized function designed for LIMS exporting compatibility. Access the LIMS report settings by clicking the triangle icon to the right of the save icon, and then selecting *Save LIMS Report*.

If the *View > Preference* setting "Show LIMS Report Settings Box" is turned off, the LIMS settings window will be hidden, allowing the user to save the LIMS report without modifying the contents of the report.





Dyes: Select the dye colors to be included in the report.

Sample Name or File Name: Have samples labeled by their external file name, or their internal sample name.

Show Deleted Peaks: Include deleted peaks in the report.

Show [\*\*] When No Allele Call: If no allele calls are present at a specified marker for a given sample, the program will print \*\*. If this option is deselected, the marker will be skipped for that sample instead. Note that you can type a symbol other than \*\*, for example "NA", or "None".

Show Only Uncertain Alleles: Only alleles with red or yellow flagging (i.e. they are in the "check" or "fail" range for at least one parameter) will be exported in the report.

Size Range: Only include allele from the specified size range, otherwise use the default size range.

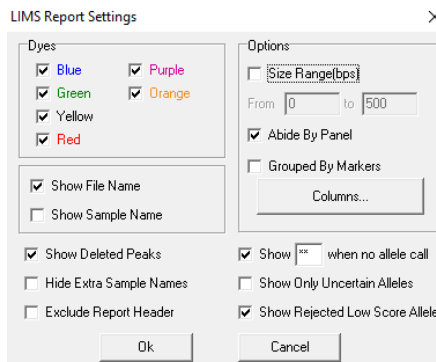
Abide by Panel: Only include peaks that are present in the project Genotyping panel.

Grouped by Marker: If Marker is selected from the Columns list (see below), markers from all samples will be grouped together.

Columns...: Select which values to include in the report. Click on an item and then click "Add". Items in the right window are included in the report, items in the left window are excluded. Items will be displayed according to the order in which they have been added.

Show Rejected Low Score Alleles: Alleles that were rejected because of low score will be included in the report.

Hide Extra Sample Names: The sample name will be displayed for each sample only once, instead of appearing on every row.

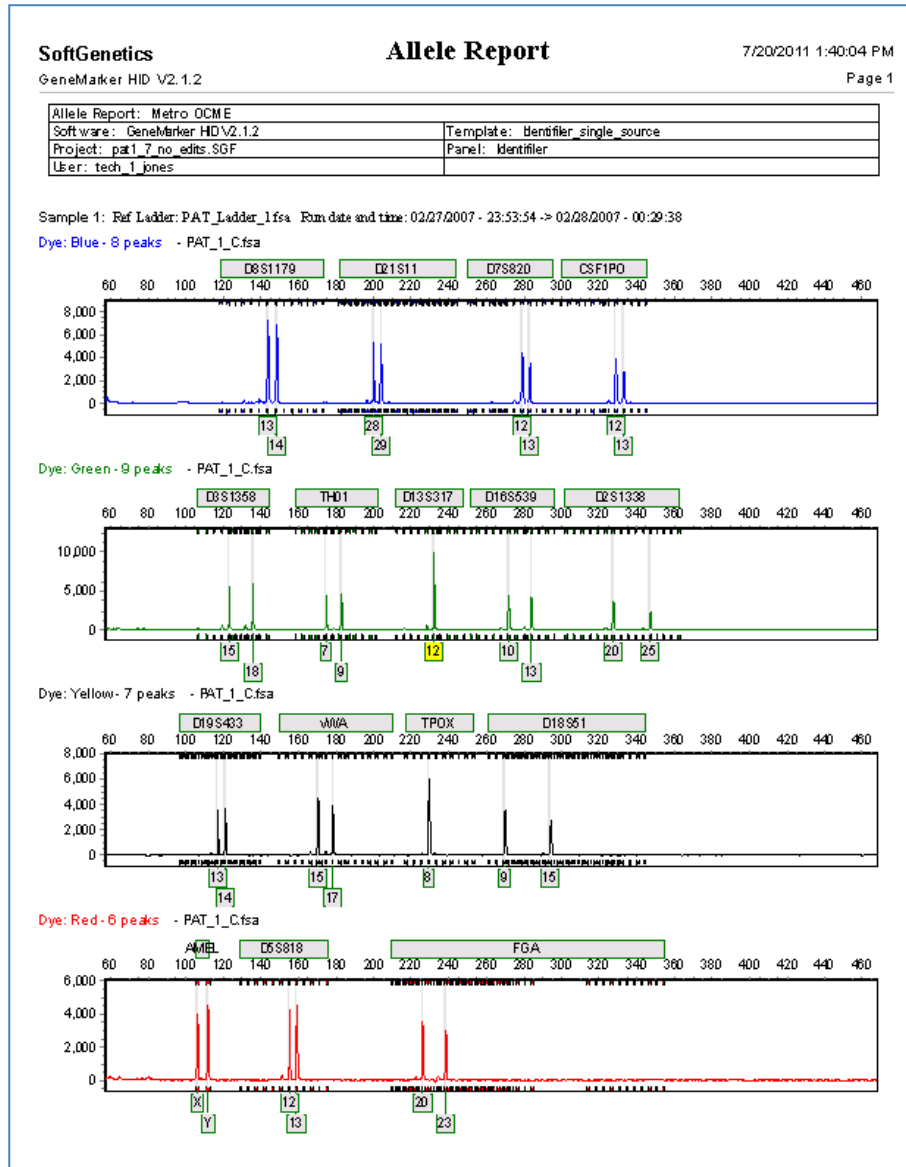


## Print Report

The GeneMarkerHID *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.

# GeneMarkerHID 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 user may also save commonly used print settings as templates. Simply select the desired options, type a Template Name, and then click the save button. Clicking a template will load that template's settings. Standard options can be found under the Standard tab, advanced options under the Advanced tab, and page settings may be found under the Page tab.

### The Standard Tab:

#### 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

Peak Table: Prints the *Peak Table* for each dye color.

Follow Trace Chart: The peak table for each dye channel will directly follow that dye channel's trace.

Start after All Charts Finished: The opposite of the above option - the peak table for each sample will begin below every trace.

Start on Separate Page: The peak table will begin after every trace, but will always start on a new page.

**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 Tab

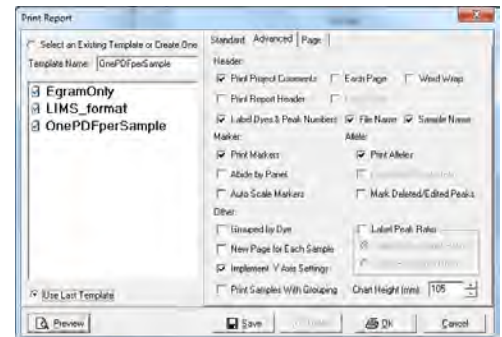
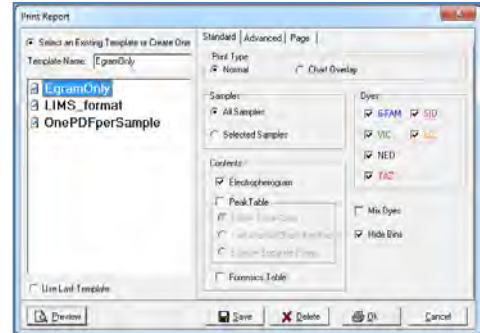
Print Project Comments: Includes the *Project Comments* at the top of the *Print Report*. Select *Each Page* option to display the *Project Comments* on each page in the report. Select *Word Wrap* to engage word wrapping.

Print Report Header: Includes Institution and User ID from User Management and Template, Panel Name, from the Analysis Template. Select *On Each Page* to include the header on each page.

Label Dyes & Peak Numbers: Labels dye color with number of peaks for each electropherogram.

File Name: Labels the file name for each electropherogram.

Sample Name: Labels the sample name for each electropherogram.



Print Markers: The Marker label bars appear above the electropherogram.

Abide by Panel: Prints only alleles within a Panel. Alleles that are outside the Panel are not included in the printed report.

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 Alleles: The Allele Labels appear below the electropherogram.

Print Edited Peak Only: Only peaks that have been edited are included in the peak table.

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

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).

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).

Implement Y Axis Settings: Prints the report using the Y-axis settings the user selected in the *Main Analysis* window *Set Axis* icon.

Print Samples with Grouping: print and/or save samples by group when the grouping tool is applied in the main analysis window. To save a pdf file for each sample: apply sample grouping in main analysis screen, make each sample its own 'group', select print samples with grouping in the print options. When saving, select Save Group Samples as one file.

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.

## The Page Tab

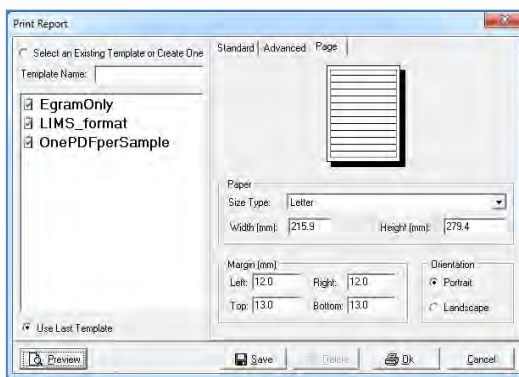
Size Type: Select the desired document format from the dropdown menu. The width(mm) and height(mm) of the selection will be displayed below. Selecting custom will enable the user to manually input the height and width of the document.

Margin(mm): Input the desired margins for the document. Distance is measured from the respective page border.

Orientation: Select either Portrait (vertical) or Landscape (horizontal).

## Creating a New Template and Editing Existing Templates

To create a new print template, simply input a name in the "Template Name field" and then click the Save button at the



bottom-center of the Print Report window. The template saves the current settings in all three tabs - allowing the user to make their specifications once, and then save them for future use.

To update an existing template, simply select the template, make the settings change(s), and then click the Save icon. The template will be immediately updated.

To delete an existing template, select the template, then click the Delete button.

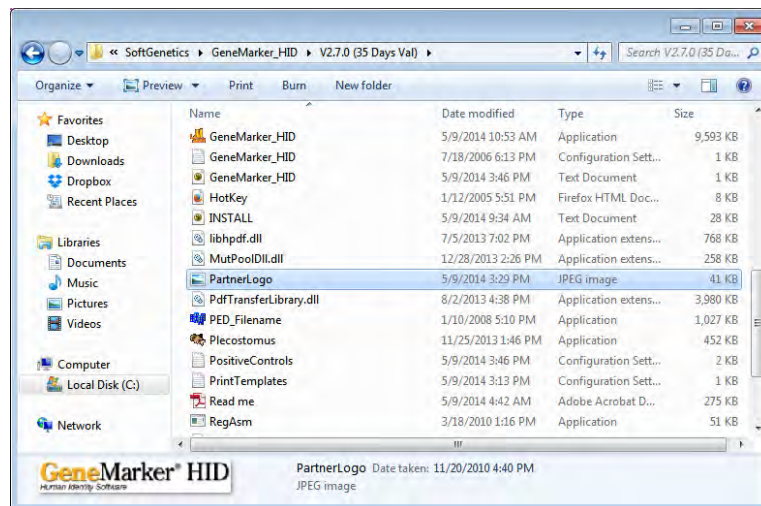
### Print Preview:

Click the print preview button to see a preview of the print report prior to printing or saving it. Once in the print preview you can easily re-open the settings window - allowing the user to experiment with different display options and immediately see updates in the print report. The icons and functions of the print preview are described in more detail below.

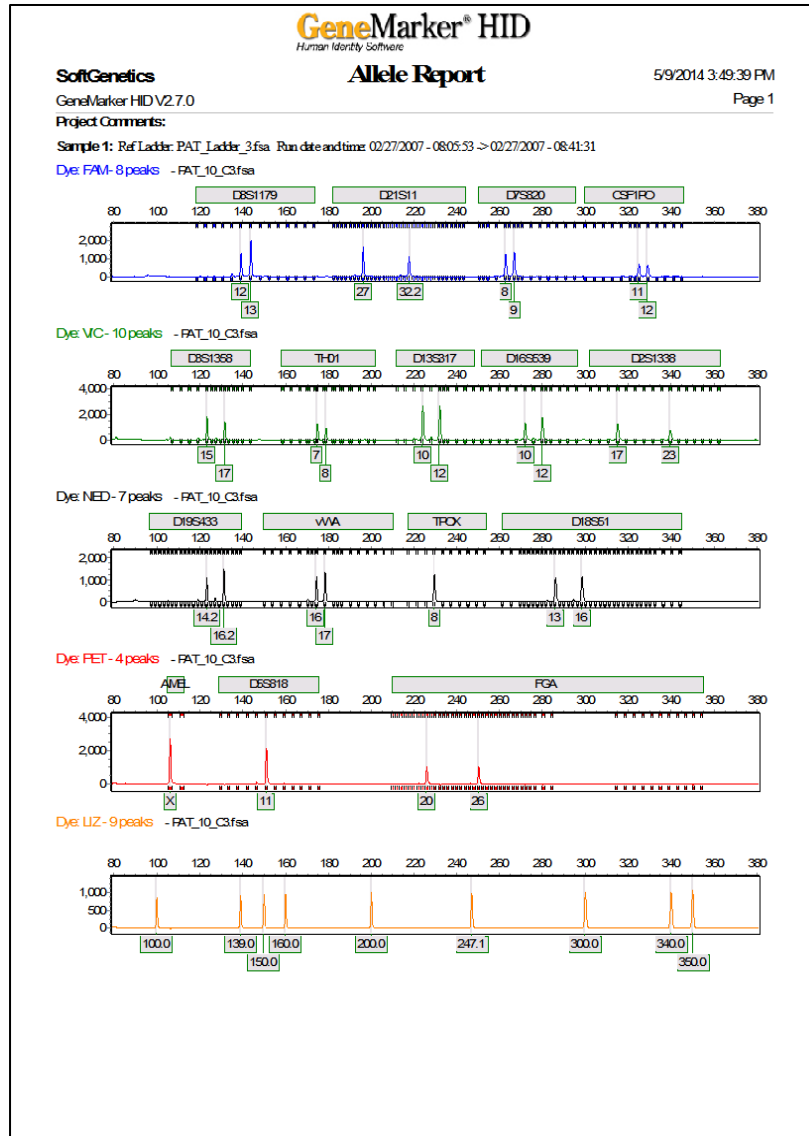
## Include Customizable Logo in the Print Report

GeneMarkerHID allows the user to include a customizable logo in print reports generated from the main analysis screen. The steps for doing this as are follows:

1. Save your lab's logo image file using one of the following image formats: .jpg, .jpeg, .bmp, .ico, .emf, or .wmf (Note: .png is NOT permitted).
2. Move the file to the following directory:  
C:\program files(x86)\SoftGenetics\GeneMarkerHID\[your version number]\
3. Rename the file "PartnerLogo" (no space).



After completing the above steps, your logo will appear on all print reports generated from the main analysis window. For best results, use images that are rectangular. To disable the logo, simply delete the PartnerLogo file, or name it something other than "PartnerLogo".



### Icons and Functions

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



#### Print

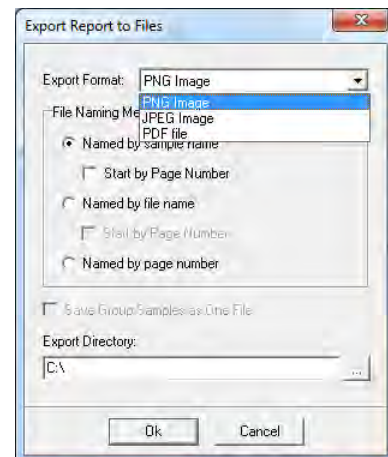
Opens the *Print* options box. Select a printer, the print range and the number of copies.



#### 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. **Note:** to save individual files





as separate PDFs, use sample grouping tool to make each file its own 'group' and then select Save Group Samples as One File. To save paternity trios, group samples by family, include the print option to Group by dye and print samples with grouping.

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

Name	Type
A07_01.jpg	JPEG Image
A07_01_01.jpg	JPEG Image
A08_02.jpg	JPEG Image
A08_02_01.jpg	JPEG Image

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

Pg10_C07_05.jpg	JPEG Image
Pg11_C07_05_01.jpg	JPEG Image
Pg12_C08_06.jpg	JPEG Image

Save Group samples as one file names the file with the file name of the first sample in the group.

Name	Type
PAT_1_F_01.png	PNG image
PAT_1_M.png	PNG image
PAT_2_M.png	PNG image
PAT_3_F.png	PNG image

Named by file name saves each file by the file name.

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

Name	Type
Pg1_PAT_Ladder_1.png	PNG image
Pg2_PAT_Ladder_1_01.png	PNG image
Pg3_PAT_Ladder_1_02.png	PNG image
Pg4_PAT_1_F_01.png	PNG image

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

Name
GeneMarker_Pg1.jpg
GeneMarker_Pg2.jpg
GeneMarker_Pg3.jpg



#### 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*.



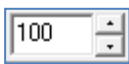
#### Zoom to Fit

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.

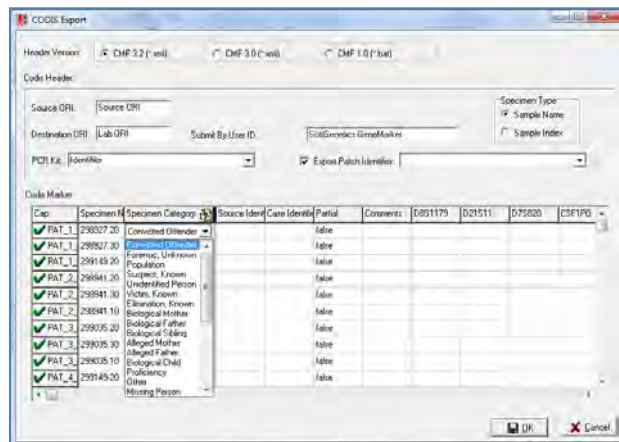
## CODIS Report

### 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]



## Save Project

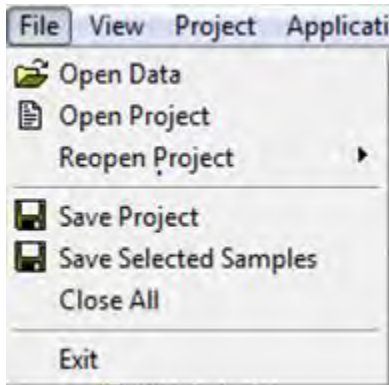
After a dataset is analyzed and edited, the project can be saved as a SoftGenetics GeneMarkerHID Project (SGF). Project files contain the raw, unprocessed data files, the sample files after processing, the process parameters, and all edits. The project file does not contain any custom or modified Panels 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](#).

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 GeneMarkerHID will appear. Navigate to the directory containing the SGF (or SFP) file and click **Open**. Additionally, the last four projects that were opened by GeneMarkerHID 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 GeneMarkerHID.

## Save Selected Samples

This icon saves the currently selected samples, those with a check mark next to the file name in the file name tree. This option is enables labs to provide a project file with only the samples pertinent to a case when discovery is requested.



## Chapter 7 Mixture Analysis

### **Chapter 7 Mixture Analysis**

Overview

Procedure

Icons and Functions

What to Expect

Save and Export Results

Search Database

Mixture Analysis Equations

## 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. GeneMarkerHID 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

GeneMarkerHID 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. LR calculations are displayed for two contributor mixtures. **MaSTR™ Software is available for probabilistic statistics calculated for mixtures of 3 – 5 contributors**

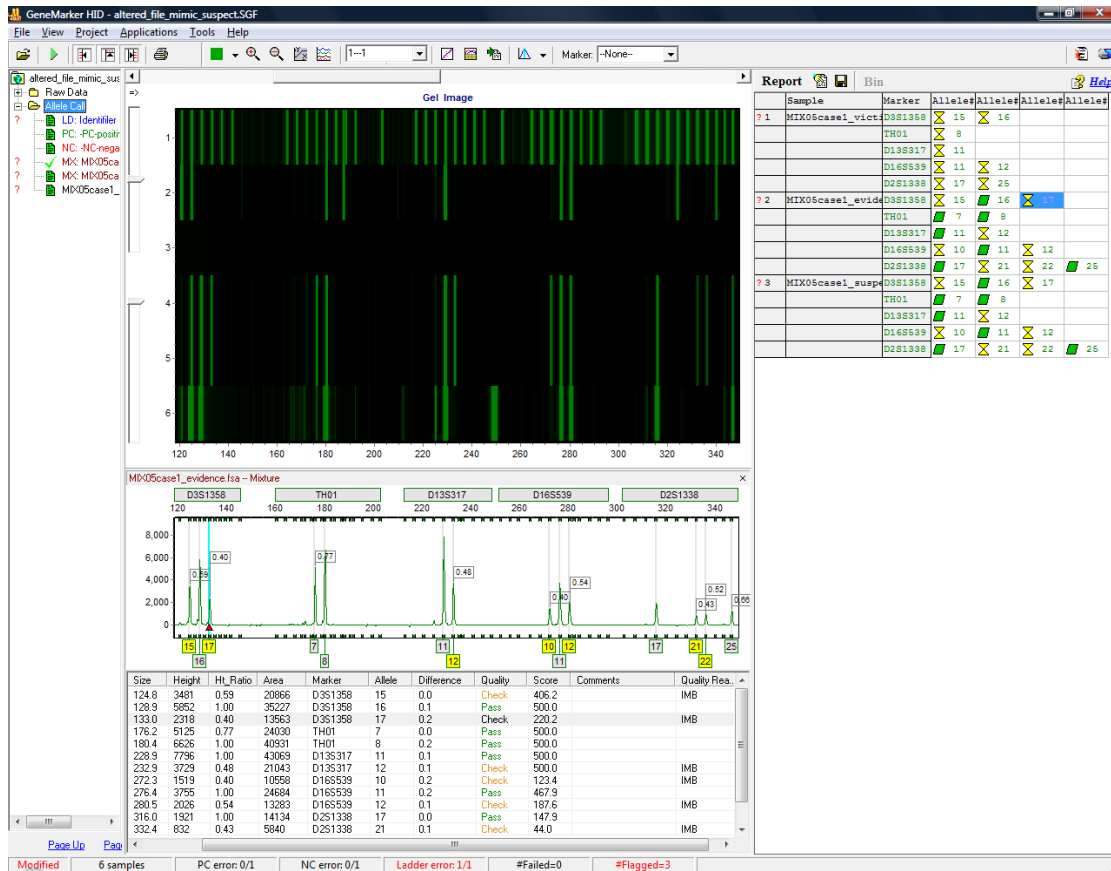
## Procedure

### Identify the Presence of a Mixture

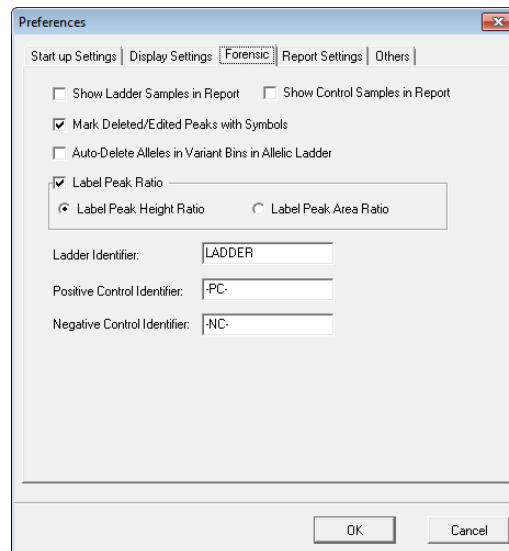
The presence of a mixture is determined by setting the needed parameters in the third plate of the Run Wizard. The criteria are used for identifying potential mixtures include: presence of more than two peaks in for a marker, severe peak height imbalance between alleles of a locus (also see Panel Editor, 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**:

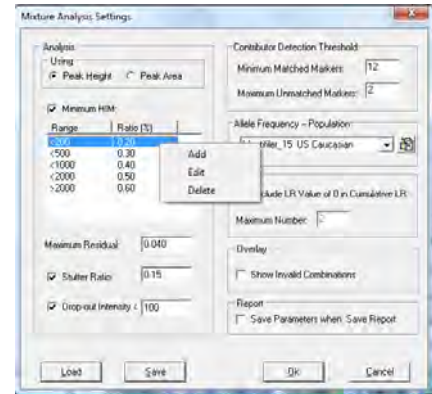


## Mixture Analysis

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 unamplified 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 easily add or remove other population allele frequency .txt tab delimited tables (see chapter 8 for file format template) **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.

- \* 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

### Mixture Calculations Results

File Name Tree

Trace or Report Display Option

The screenshot displays the Mixture Analysis software interface. On the left is a file name tree with a dropdown menu. The central area contains a large table of mixture calculations with columns for No., Mixture, Major Mx, Residual, Major LHM, and Minor LHM. On the right is a 'Trace or Report Display Option' window with a table of Trace Calc. Report data, including columns for No., Mixture, Major Mx, Residual, Major LHM, Minor LHM, and a 'Comment' field.

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)

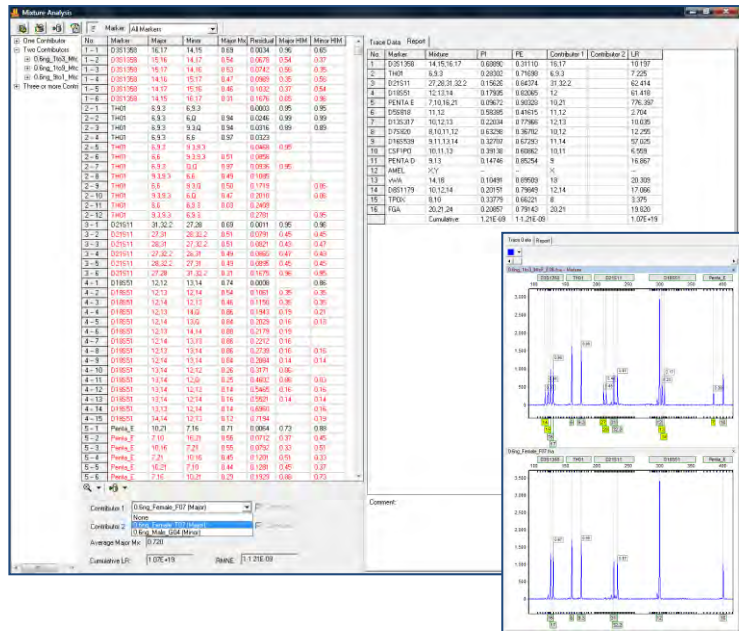
4. Genotypes of contributors
5. Likelihood ratio for selected contributors
6. Combined Probability of Inclusion (CPI)
7. Combined Probability of Exclusion (CPE)

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)

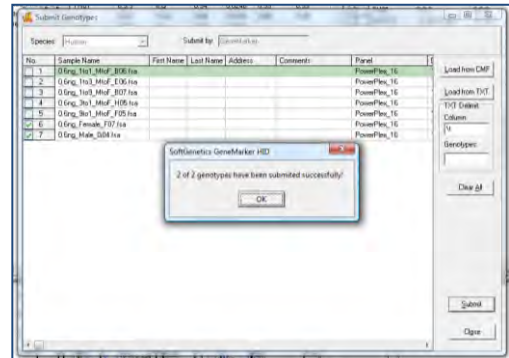
The File Name Tree indicates that two single source files are potential contributors to the evidence mixture. The analyst may select a potential contributor file from the drop-down menu at the bottom of the report. The genotypes combinations and ratio results are displayed in the result table. The Report 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

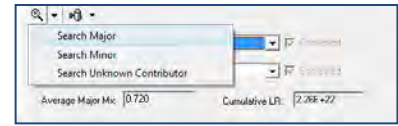
A profile from a reference sample can be submitted directly to the Relationship Testing Database from this mixture analysis screen.

1. Use the **Submit Genotypes to Database** icon
2. **Select** the appropriate file(s)
3. **Submit**
4. **OK**

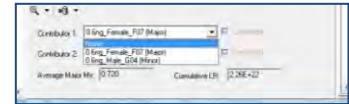


**Search Relationship Testing Database for exact matches**

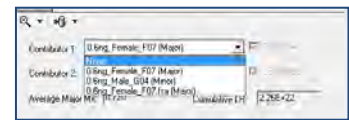
1. Use the Search Database icon to search the database for exact matches
2. Select the desired profile from the drop-down menu
3. File name(s) of matching profiles will be added to the drop-down menu
4. LR s are displayed in the Report Table



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**

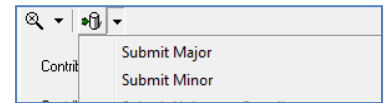
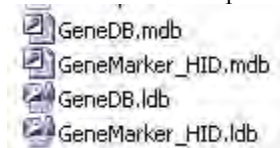
In some cases, only the mixture sample and the victim reference sample are available. For example, DNA isolation from rape cases often has successful male/female cell separation that provides a mixture where the perpetrator is the major contributor and the victim is the minor contributor. Results shown 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.

No.	Name	Locus	Allele	LR	Major	Minor	LR
1	1001179	11	11	0.00	0.000	0.00	0.00
2	1001179	11	12	0.00	0.000	0.00	0.00
3	1001179	11	13	0.00	0.000	0.00	0.00
4	1001179	11	14	0.00	0.000	0.00	0.00
5	1001179	11	15	0.00	0.000	0.00	0.00
6	1001179	11	16	0.00	0.000	0.00	0.00
7	1001179	11	17	0.00	0.000	0.00	0.00
8	1001179	11	18	0.00	0.000	0.00	0.00
9	1001179	11	19	0.00	0.000	0.00	0.00
10	1001179	11	20	0.00	0.000	0.00	0.00
11	1001179	11	21	0.00	0.000	0.00	0.00
12	1001179	11	22	0.00	0.000	0.00	0.00
13	1001179	11	23	0.00	0.000	0.00	0.00
14	1001179	11	24	0.00	0.000	0.00	0.00
15	1001179	11	25	0.00	0.000	0.00	0.00
16	1001179	11	26	0.00	0.000	0.00	0.00
17	1001179	11	27	0.00	0.000	0.00	0.00
18	1001179	11	28	0.00	0.000	0.00	0.00
19	1001179	11	29	0.00	0.000	0.00	0.00
20	1001179	11	30	0.00	0.000	0.00	0.00
21	1001179	11	31	0.00	0.000	0.00	0.00
22	1001179	11	32	0.00	0.000	0.00	0.00
23	1001179	11	33	0.00	0.000	0.00	0.00
24	1001179	11	34	0.00	0.000	0.00	0.00
25	1001179	11	35	0.00	0.000	0.00	0.00
26	1001179	11	36	0.00	0.000	0.00	0.00
27	1001179	11	37	0.00	0.000	0.00	0.00
28	1001179	11	38	0.00	0.000	0.00	0.00
29	1001179	11	39	0.00	0.000	0.00	0.00
30	1001179	11	40	0.00	0.000	0.00	0.00
31	1001179	11	41	0.00	0.000	0.00	0.00
32	1001179	11	42	0.00	0.000	0.00	0.00
33	1001179	11	43	0.00	0.000	0.00	0.00
34	1001179	11	44	0.00	0.000	0.00	0.00
35	1001179	11	45	0.00	0.000	0.00	0.00
36	1001179	11	46	0.00	0.000	0.00	0.00
37	1001179	11	47	0.00	0.000	0.00	0.00
38	1001179	11	48	0.00	0.000	0.00	0.00
39	1001179	11	49	0.00	0.000	0.00	0.00
40	1001179	11	50	0.00	0.000	0.00	0.00
41	1001179	11	51	0.00	0.000	0.00	0.00
42	1001179	11	52	0.00	0.000	0.00	0.00
43	1001179	11	53	0.00	0.000	0.00	0.00
44	1001179	11	54	0.00	0.000	0.00	0.00
45	1001179	11	55	0.00	0.000	0.00	0.00
46	1001179	11	56	0.00	0.000	0.00	0.00
47	1001179	11	57	0.00	0.000	0.00	0.00
48	1001179	11	58	0.00	0.000	0.00	0.00
49	1001179	11	59	0.00	0.000	0.00	0.00
50	1001179	11	60	0.00	0.000	0.00	0.00
51	1001179	11	61	0.00	0.000	0.00	0.00
52	1001179	11	62	0.00	0.000	0.00	0.00
53	1001179	11	63	0.00	0.000	0.00	0.00
54	1001179	11	64	0.00	0.000	0.00	0.00
55	1001179	11	65	0.00	0.000	0.00	0.00
56	1001179	11	66	0.00	0.000	0.00	0.00
57	1001179	11	67	0.00	0.000	0.00	0.00
58	1001179	11	68	0.00	0.000	0.00	0.00
59	1001179	11	69	0.00	0.000	0.00	0.00
60	1001179	11	70	0.00	0.000	0.00	0.00
61	1001179	11	71	0.00	0.000	0.00	0.00
62	1001179	11	72	0.00	0.000	0.00	0.00
63	1001179	11	73	0.00	0.000	0.00	0.00
64	1001179	11	74	0.00	0.000	0.00	0.00
65	1001179	11	75	0.00	0.000	0.00	0.00
66	1001179	11	76	0.00	0.000	0.00	0.00
67	1001179	11	77	0.00	0.000	0.00	0.00
68	1001179	11	78	0.00	0.000	0.00	0.00
69	1001179	11	79	0.00	0.000	0.00	0.00
70	1001179	11	80	0.00	0.000	0.00	0.00
71	1001179	11	81	0.00	0.000	0.00	0.00
72	1001179	11	82	0.00	0.000	0.00	0.00
73	1001179	11	83	0.00	0.000	0.00	0.00
74	1001179	11	84	0.00	0.000	0.00	0.00
75	1001179	11	85	0.00	0.000	0.00	0.00
76	1001179	11	86	0.00	0.000	0.00	0.00
77	1001179	11	87	0.00	0.000	0.00	0.00
78	1001179	11	88	0.00	0.000	0.00	0.00
79	1001179	11	89	0.00	0.000	0.00	0.00
80	1001179	11	90	0.00	0.000	0.00	0.00
81	1001179	11	91	0.00	0.000	0.00	0.00
82	1001179	11	92	0.00	0.000	0.00	0.00
83	1001179	11	93	0.00	0.000	0.00	0.00
84	1001179	11	94	0.00	0.000	0.00	0.00
85	1001179	11	95	0.00	0.000	0.00	0.00
86	1001179	11	96	0.00	0.000	0.00	0.00
87	1001179	11	97	0.00	0.000	0.00	0.00
88	1001179	11	98	0.00	0.000	0.00	0.00
89	1001179	11	99	0.00	0.000	0.00	0.00
90	1001179	11	100	0.00	0.000	0.00	0.00

**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.



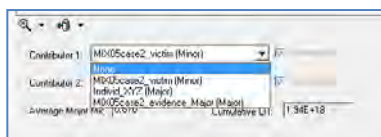
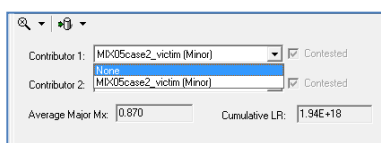
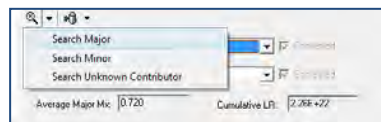
### Search Relationship Testing Database for matching profiles

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

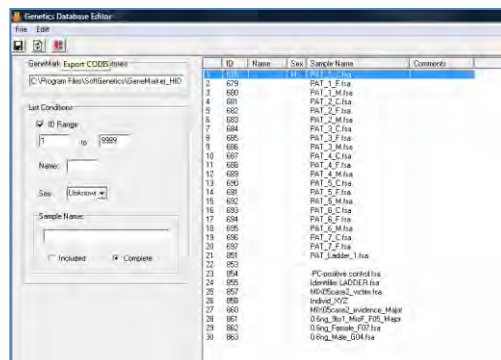


Contributor	No.	Mixture	Major	Minor	Major Mix	Minor Mix	Major LR	Minor LR	Total LR
1-1	085173	11.13	14.14	0.93	0.9005	0.96	0.90	0.96	0.90
1-2	085173	11.13	14.14	0.93	0.9005	0.96	0.90	0.96	0.90
1-3	085173	11.13	14.14	0.93	0.9005	0.96	0.90	0.96	0.90
2-1	029191	28.32	28.32	0.62	0.9996	0.74	0.60	0.60	0.60
2-2	079620	8.10	8.11	0.67	0.9911	0.95	0.99	0.99	0.99
4-1	CS1990	7.10	12.13	0.99	0.9933	0.86	0.73	0.73	0.73
5-1	090199	15.15	15.16	0.99	0.9999	0.99	0.99	0.99	0.99
5-2	079199	15.15	15.16	0.99	0.9999	0.99	0.99	0.99	0.99
6-1	TH01	7.93	6.10	0.89	0.9992	0.74	0.61	0.61	0.61
7-1	012637	12.14	8.9	0.99	0.9992	0.94	0.89	0.89	0.89
8-1	018539	10.11	9.12	0.95	0.9910	0.95	0.95	0.95	0.95
9-1	025138	17.21	16.24	0.99	0.9923	0.94	0.40	0.40	0.40
10-1	019643	13.13	13.14	0.84	0.9993	0.92	0.92	0.92	0.92
10-2	019643	13.13	13.14	0.82	0.9993	0.92	0.92	0.92	0.92
10-3	019643	13.13	14.14	0.92	0.9994	0.92	0.92	0.92	0.92
11-1	w/A	15.15	15.19	0.89	0.9996	0.99	0.78	0.78	0.78
12-1	TPG3	9.10	8.11	0.96	0.9992	0.79	0.50	0.50	0.50
13-1	018991	12.16	12.16	0.94	0.9992	0.93	0.62	0.62	0.62
14-1	AMEL	X,Y	X,Y	0.74	0.9976	0.93	0.63	0.63	0.63
14-2	AMEL	X,Y	X,Y	0.99	0.9991	0.63	0.63	0.63	0.63
15-1	058809	8.11	11.12	0.99	0.9919	0.93	0.66	0.66	0.66
15-2	FGA	20.24	23.23	0.89	0.9998	0.97	0.97	0.97	0.97
16-2	FGA	20.24	22.0	0.96	0.9926	0.97	0.33	0.33	0.33
16-3	FGA	20.24	26.23	0.87	0.9941	0.93	0.63	0.63	0.63
16-4	FGA	20.24	22.24	0.91	0.9985	0.79	0.79	0.79	0.79

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





## Chapter 7 Mixture Analysis

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.

**Mixture Analysis**

Mixture: All Markers

No.	Marker	Major	Minor	Major H1	Residual	Major H1M	Minor H1M
1-1	D8S1178	11.13	11.14	0.92	0.0000	0.99	0.99
1-2	D8S1178	11.13	14.14	0.93	0.0095	0.86	0.95
1-3	D8S1178	11.13	13.14	0.88	0.0091	0.76	0.76
2-1	D21S11	28.32	30.21	0.83	0.0088	0.74	0.60
2-1	D7S320	8.10	9.11	0.87	0.0011	0.85	0.51
4-1	CSF1PO	7.10	12.13	0.90	0.0033	0.86	0.73
5-1	D3S1358	15.15	15.16	0.88	0.0000		
5-2	D3S1358	15.15	16.16	0.94	0.0092		
6-1	TH01	7.8.3	8.10	0.88	0.0092	0.74	0.61
7-1	D13S317	12.14	8.9	0.89	0.0032	0.84	0.91
8-1	D16S1208	10.11	8.12	0.85	0.0010	0.86	0.88
9-1	D2S1338	17.21	16.24	0.90	0.0023	0.84	0.40
10-1	D19S433	13.13	13.14	0.84	0.0003		
10-2	D19S433	13.13	12.14	0.82	0.0053		
10-3	D19S433	13.13	14.14	0.92	0.0054		
11-1	AWA	15.15	16.10	0.80	0.0006		0.78
12-1	TPDX	8.10	8.11	0.85	0.0062	0.79	0.50
13-1	D18S51	17.18	12.15	0.84	0.0012	1.00	0.82
14-1	AMEL	X,Y	X,Y	0.74	0.0078		
14-2	AMEL	X,Y	Y,Y	0.99	0.0021	0.63	0.63
15-1	D5S818	8.13	11.12	0.85	0.0013	0.83	0.86
16-1	FGA	20.24	21.20	0.80	0.0009	0.97	
16-2	FGA	20.24	21.0	0.95	0.0026	0.97	0.30
16-3	FGA	20.24	20.20	0.80	0.0041	0.83	0.10
16-4	FGA	20.24	23.24	0.91	0.0059	0.78	0.79

No.	Marker	Major	PI	PE	Contributor 1	Contributor 2	LR
1	D8S1178	11.13,14	0.9079	0.68271	11.14	11.13	15.027
2	D21S11	28.30,31,32,2	0.3619	0.63481	30.31	28.32,2	37.295
3	D7S320	8.9,10,11	0.64801	0.36289	9.11	8.10	13.536
4	CSF1PO	7.10,12,13	0.40230	0.53470	12.13	7.10	278.420
5	D3S1358	15.16	0.26512	0.73489	15.16	16	14.614
6	TH01	7.8,9,10,11	0.42337	0.57663	8.10	7.8,9	7.146
7	D13S317	8.9,12,14	0.23200	0.76800	9.11	12.14	41.938
8	D16S1208	8.10,11,12	0.68522	0.31378	9.12	10.11	21.855
9	D2S1338	16.17,21,24	0.14376	0.85624	16.24	17.21	86.391
10	D19S433	12.13,14	0.49512	0.50488	12.14	13	16.506
11	AWA	15.15,16	0.17289	0.82711	15.15	16	91.285
12	TPDX	8.9,10,11	0.93045	0.06955	8.11	8.10	74.912
13	D18S51	12.15,17,18	0.23884	0.76116	12.15	17,18	92.178
14	AMEL	X,Y	-	-	X	Y	-
15	D5S818	8.11,12,13	0.79930	0.20070	11.12	8.13	428.095
16	FGA	20.2,24	0.15789	0.84211	20.24	20.24	38.893
				Contributor:	1.97E-07	1.1.97E-07	1.37E-04

Comments:  
 Contestant identifies latent sequence null hypothesis compared to alternate hypothesis that the mixture is from contributor 1 and a random person related to contributor 2

Contributor 1: MD02Scan2\_victim (Male) Contested  
 Contributor 2: MD02Scan2\_evidence\_Maps (Male) Contested  
 Average Mixture: 0.800  
 Contributor LR: 1.97E-07 RMSE: 11.97E-07

Contesting contributor two as a contributor to the mixture

**Mixture Analysis**

Mixture: All Markers

No.	Marker	Major	Minor	Major H1	Residual	Major H1M	Minor H1M
1-1	D8S1178	11.13	11.14	0.92	0.0000	0.99	0.99
1-2	D8S1178	11.13	14.14	0.93	0.0095	0.86	0.95
1-3	D8S1178	11.13	13.14	0.88	0.0091	0.76	0.76
2-1	D21S11	28.32	30.21	0.83	0.0088	0.74	0.60
2-1	D7S320	8.10	9.11	0.87	0.0011	0.85	0.51
4-1	CSF1PO	7.10	12.13	0.90	0.0033	0.86	0.73
5-1	D3S1358	15.15	15.16	0.88	0.0000		
5-2	D3S1358	15.15	16.16	0.94	0.0092		
6-1	TH01	7.8.3	8.10	0.88	0.0092	0.74	0.61
7-1	D13S317	12.14	8.9	0.89	0.0032	0.84	0.91
8-1	D16S1208	10.11	8.12	0.85	0.0010	0.86	0.88
9-1	D2S1338	17.21	16.24	0.90	0.0023	0.84	0.40
10-1	D19S433	13.13	13.14	0.84	0.0003		
10-2	D19S433	13.13	12.14	0.82	0.0053		
10-3	D19S433	13.13	14.14	0.92	0.0054		
11-1	AWA	15.15	16.10	0.80	0.0006		0.78
12-1	TPDX	8.10	8.11	0.85	0.0062	0.79	0.50
13-1	D18S51	17.18	12.15	0.84	0.0012	1.00	0.82
14-1	AMEL	X,Y	X,Y	0.74	0.0078		
14-2	AMEL	X,Y	Y,Y	0.99	0.0021	0.63	0.63
15-1	D5S818	8.13	11.12	0.85	0.0013	0.83	0.86
16-1	FGA	20.24	21.20	0.80	0.0009	0.97	
16-2	FGA	20.24	21.0	0.95	0.0026	0.97	0.30
16-3	FGA	20.24	20.20	0.80	0.0041	0.83	0.63
16-4	FGA	20.24	23.24	0.91	0.0059	0.78	0.79

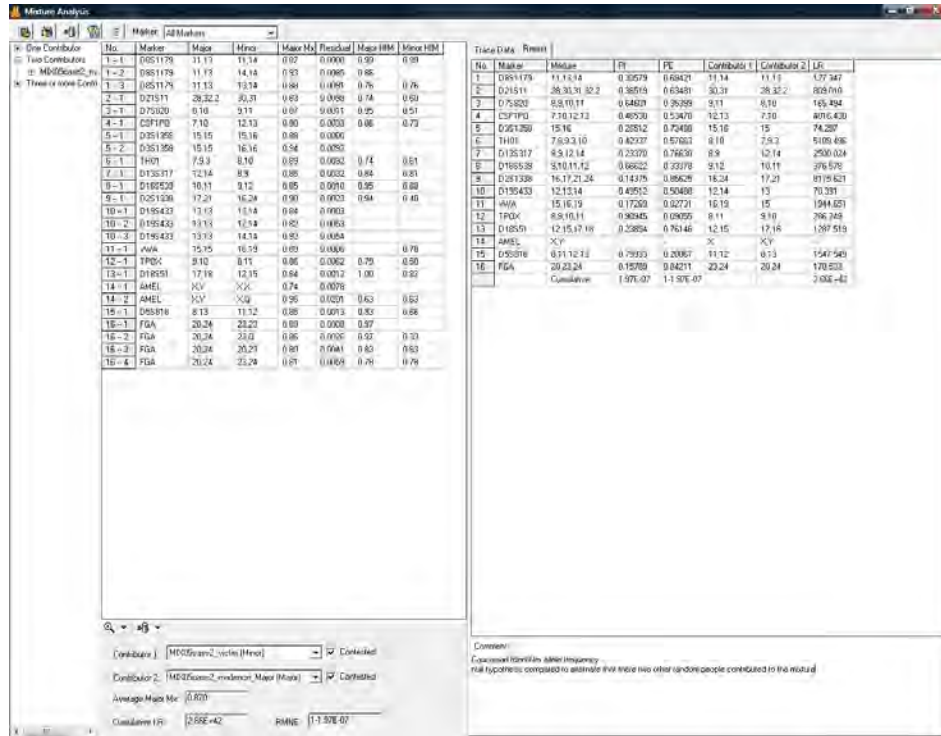
  

No.	Marker	Major	PI	PE	Contributor 1	Contributor 2	LR
1	D8S1178	11.13,14	0.9079	0.68271	11.14	11.13	6.420
2	D21S11	28.30,31,32,2	0.3619	0.63481	30.31	28.32,2	21.715
3	D7S320	8.9,10,11	0.64801	0.36289	9.11	8.10	12.137
4	CSF1PO	7.10,12,13	0.40230	0.59770	12.13	7.10	74.495
5	D3S1358	15.16	0.26512	0.73489	15.16	16	5.904
6	TH01	7.8,9,10,11	0.42337	0.57663	8.10	7.8,9	715.141
7	D13S317	8.9,12,14	0.23200	0.76800	9.9	12.14	59.615
8	D16S1208	8.10,11,12	0.68522	0.31378	9.12	10.11	13.617
9	D2S1338	16.17,21,24	0.14376	0.85624	16.24	17.21	153.288
10	D19S433	12.13,14	0.49512	0.50488	12.14	13	4.915
11	AWA	15.15,16	0.17289	0.82711	15.15	16	23.200
12	TPDX	8.9,10,11	0.93045	0.06955	8.11	8.10	3.647
13	D18S51	12.15,17,18	0.23884	0.76116	12.15	17,18	34.677
14	AMEL	X,Y	-	-	X	Y	-
15	D5S818	8.11,12,13	0.79930	0.20070	11.12	8.13	3.607
16	FGA	20.2,24	0.15789	0.84211	20.24	20.24	5.803
				Contributor:	1.98E-16	1.1.98E-07	1.98E-16

Comments:  
 Contestant identifies latent sequence null hypothesis compared to alternate hypothesis that the mixture is from contributor 2 and a random person not related to contributor 1

Contributor 1: MD02Scan2\_victim (Male) Contested  
 Contributor 2: MD02Scan2\_evidence\_Maps (Male) Contested  
 Average Mixture: 0.800  
 Contributor LR: 1.98E-16 RMSE: 11.98E-07

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

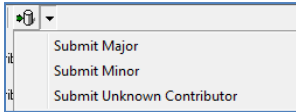
Allows the user to select all colors to view, hide all colors, or choose a single dye layer in the trace view. Choose a single dye by single left mouse clicking on the icon.

Marker:

### Marker

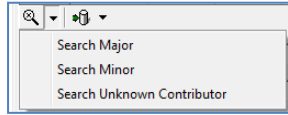
Select a Marker or Locus to view in the *Electropherogram Charts*





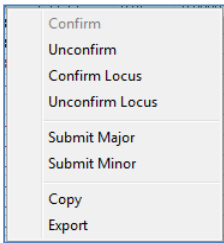
**Submit to Relationship Testing Database**

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.

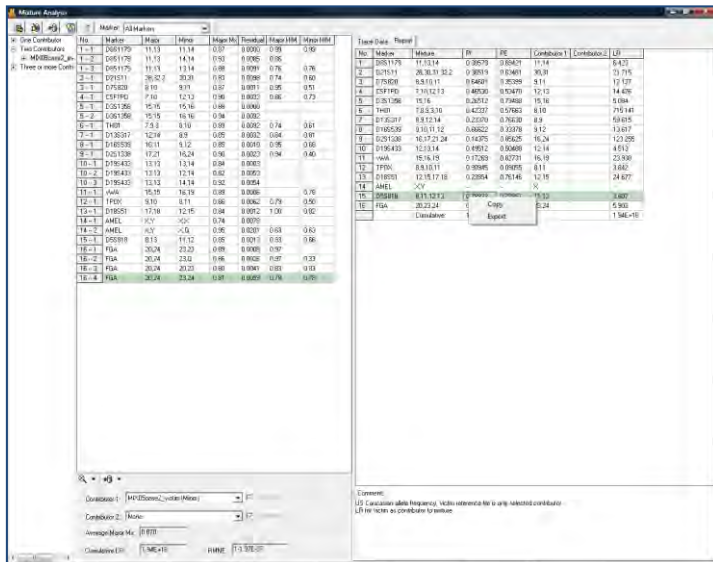


**Report Options**

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

$$\text{Major Mx} = (A + B) / (A + B + C + D)$$

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:

$$\begin{aligned} \text{Mx} &= (972 + 932) / (972 + 932 + 343 + 526) \\ &= 0.69 \end{aligned}$$

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

No.	Marker	Major	Minor	Major Mx	Residual	Major HIM	Minor HIM
1-1	D3S1358	16.17	14.15	0.63	0.0034	0.96	0.65
2-1	TH01	6.93	6.93	0	0.0003	0.96	0.96
2-2	TH01	6.93	6.0	0.94	0.0246	0.99	0.99
2-3	TH01	6.93	9.24	0.34	0.0216	0.69	0.69
2-4	TH01	6.93	6.6	0.97	0.0323		
3-1	D21S11	21.22	27.29	0.68	0.0011	0.95	0.95
4-1	D16S51	12.12	13.14	0.74	0.0008		
5-1	Fenta_E	10.21	7.36	0.71	0.0054	0.73	0.68

$$\text{Residual} = \sum (pa_{\text{observed}} - pa_{\text{expected}})^2$$

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) Average Major Mx is determined from a series of iterations.

No.	Size	Height	Ht_Ratio	Area	Ar_Ratio	Marker	Allele	Quality	Quality Res...
1	118.0	343	0.35	2840	0.37	D3S1358	14	Check	IMB
2	122.1	526	0.54	4231	0.55	D3S1358	15	Check	IMB
3	126.3	972	1.00	7736	1.00	D3S1358	16	Pass	
4	130.3	932	0.96	7566	0.98	D3S1358	17	Pass	

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

$$\text{Major HIM} = 932/972 = 0.96$$

$$\text{Minor HIM} = 343/526 = 0.65$$

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

Combination	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	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	(1-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	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. GeneMarkerHID 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)

$$PI = (p_1 + p_2 + \dots + p_i)^2$$

### Probability of Exclusion (PE)

$$PE = 1 - (p_1 + p_2 + \dots + p_i)^2$$

### Combined Probability of Inclusion (CPI)

$$CPI = PI_1 \times PI_2 \times \dots \times PI_i$$

### Combined Probability of Exclusion (CPE)

$$CPE = 1 - [(1-PE_1) \times (1-PE_2) \times \dots \times (1-PE_i)]$$

**PI, CPI, PE, CPE examples**

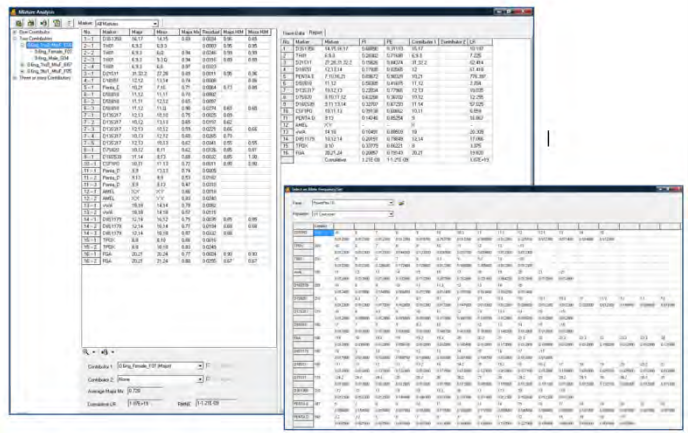
PI for D3s1358  
 $= (0.1404 + 0.2463 + 0.2315 + 0.2118)^2$   
 $= 0.6889$  or 69% of population are included based on this one marker

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.

$$CPE = 1 - [(1-PE_1) \times (1 - PE_2) \times \dots \times (1 - PE_i)]$$



**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

Chapter 7 Mixture Analysis

Examples

D3S1358 Alleles Frequency

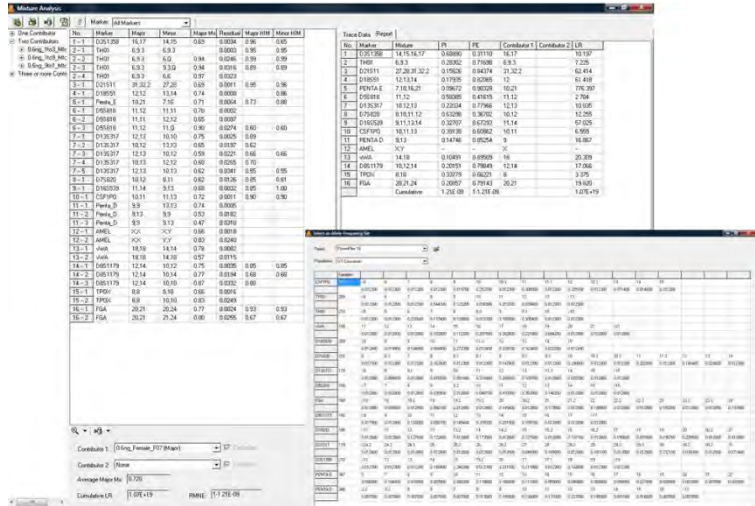
16	0.2315
17	0.2118

$LR = 1 / (2 \times 0.2315 \times 0.2118) = 10.197$

D18S51 Allele Frequency

12	0.1276
----	--------

$LR = 1 / (0.1276 \times 0.1276) = 61.42$



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

Scenario 2

Null Hypothesis, Mixture is from A and B

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)

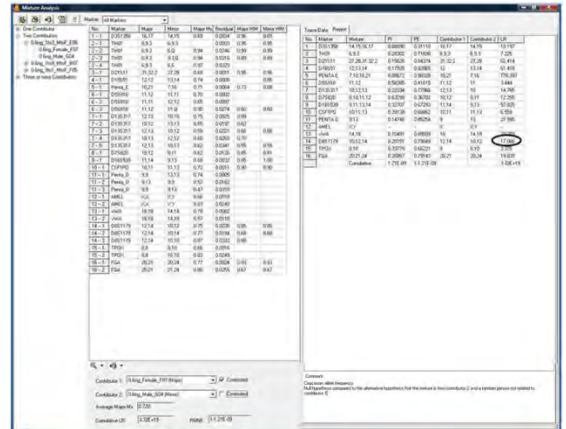
Example for D8S1179

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.

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

$$LR = 1 / (2 \times 0.1454 \times 0.2015) \times ((0.1020 \times 0.1020) + (2 \times 0.2015 \times 0.1020) + (2 \times 0.1454 \times 0.1020))$$

$$LR = 210.245$$





## Chapter 8 Relationship Testing

### Chapter 8 Relationship Testing

Kinship Analysis using Identity by Descent

Data Base Search: Finding Nearest Relatives

Automated Pedigree Diagrams

Paternity Index using American Association of Blood Banking (AABB) trio and single parent equations

## 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

GeneMarkerHID 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 y

Identity by Descent (IBD  $\Phi_2, \Phi_1, \Phi_0$ ) for each relationship category:

	$\Phi_2$	$\Phi_1$	$\Phi_0$
Parent - Child	0	1	0
Siblings	0.25	0.5	0.25
Half-siblings	0	0.5	0.5
Cousins	0	0.25	0.75
Uncle/nephew	0	0.5	0.5
Grandparent/Grandchild	0	0.5	0.5

Kinship Formula Transition Matrices

$$I = \begin{matrix} & \begin{matrix} A_1A_1 & A_1A_2 & A_2A_2 \end{matrix} \\ \begin{matrix} A_1A_1 \\ A_1A_2 \\ A_2A_2 \end{matrix} & \begin{vmatrix} 1 & 0 & 1 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{vmatrix} \end{matrix}$$

$$T = \begin{matrix} & \begin{matrix} A_1A_1 & A_1A_2 & A_2A_2 \end{matrix} \\ \begin{matrix} A_1A_1 \\ A_1A_2 \\ A_2A_2 \end{matrix} & \begin{vmatrix} p_1 & p_2 & 0 \\ 0.5 p_1 & 0.5(p_1 + p_2) & 0.5 p_2 \\ 0 & p_1 & p_2 \end{vmatrix} \end{matrix}$$

$$O = \begin{matrix} & \begin{matrix} A_1A_1 & A_1A_2 & A_2A_2 \end{matrix} \\ \begin{matrix} A_1A_1 \\ A_1A_2 \\ A_2A_2 \end{matrix} & \begin{vmatrix} p_1^2 & 2 p_1 p_2 & p_2^2 \\ p_1^2 & 2 p_1 p_2 & p_2^2 \\ p_1^2 & 2 p_1 p_2 & p_2^2 \end{vmatrix} \end{matrix}$$

From C C Li and Sachs, 1954

**Relationship Testing**  
**Kinship Formula:**  
 $[P_2(xy) \times \Phi_2] + [P_1(xy) \times \Phi_1] + [P_0(xy) \times \Phi_0]$   
 Where:  
 $\Phi_2, \Phi_1, \Phi_0$  = 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.<sup>5</sup>  
 $P_A, P_B, P_C, P_D$  = 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 GeneMarkerHID *Pedigree* module:

Pre/Ped files

SGF

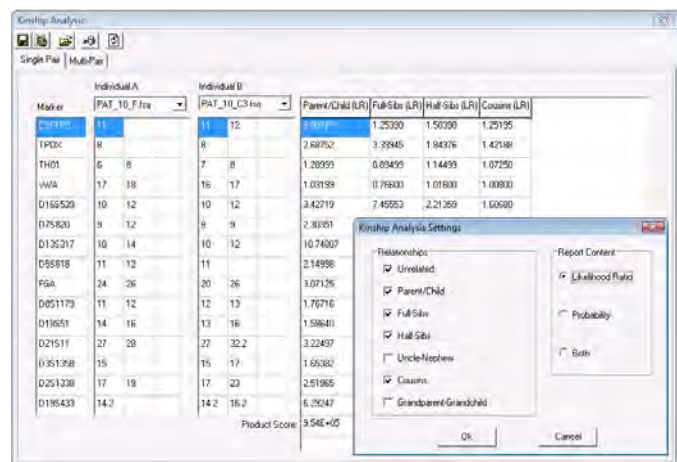
TXT

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



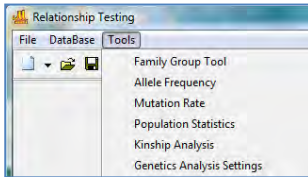
Probabilities for the occurrence of the genotypes within the population having a specific relationship or being unrelated for each locus and all loci combined are displayed in table form.

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 (Budowle 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.



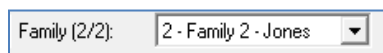
### Show Conflict

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



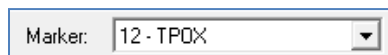
### Show Genotype

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

Marker	Individual A	Individual B	Parent/Child I.R.	Full Sibs I.R.	Half Sibs I.R.	Cousins I.R.
TH01	8	17	0.142857	0.52381	1.17647	1.08571
vWA	15	17	2.29510	2.24990	1.64405	1.52225
D16S539	10	13	2.27273	1.38036	1.71138	1.35548
D7S820	12	13	13.15786	6.62095	1.83036	1.31818
D13S317	12	12	2.86854	2.39901	1.83477	1.36726
D5S818	12	13	1.02281	0.76146	1.01146	1.00573
FGA	20	23	2.00000	1.25000	1.50000	1.25000
D8S1179	13	14	2.25022	1.37931	1.62511	1.31256
D18S51	9	16	1.49870	0.89885	1.24895	1.12480
D21S11	28	29	1.16225	0.62713	1.08112	1.04056
D3S1358	15	19	0.86028	0.60329	0.93023	0.86515
Product Score			2.34E+04	1.02E+03	3.52E+02	7.49E+01

**Multi-Pair Kinship Analysis**

The kinship analysis tool also supports Multi-Pair analysis - enabling the user to make multiple comparisons simultaneously, as opposed to selecting each pair individually. To navigate to the Multi-Pair kinship analysis module, simply open the Kinship Analysis tool and click the Multi-Pair tab.

Sample Selected:

- PAT\_10\_C1.fsa
- PAT\_10\_C2.fsa
- PAT\_10\_C3.fsa
- PAT\_10\_F.fsa
- PAT\_11\_C.fsa
- PAT\_11\_F.fsa
- PAT\_11\_M.fsa
- PAT\_12\_C.fsa
- PAT\_12\_F.fsa
- PAT\_12\_M.fsa
- PAT\_13\_C.fsa
- PAT\_13\_F.fsa
- PAT\_13\_M.fsa

Calculation Statement:

Using Specific Sample

Only do analysis with clean genotype

Allele Frequencies:

Panel Name: Identifier16\_Bullet2003

Population: US African American

Mutation Rate: AABB 2000

Kinship Analysis:

Marker	PAT_10_C1.fsa	PAT_10_C2
CSF1PO	11	12
TPDX	8	8
TH01	7	8
vWA	16	18
D16S539	12	10
D7S820	10	12
D13S317	12	14
D5S818	11	11
FGA	20	26
D8S1179	11	13
D18S51	14	13
D21S11	28	32.2
D3S1358	15	15

The Multi-Pair module offers two methods of analyzing the data, depending on whether or not the *Using Specific Sample* option is selected.

**Procedure When *Using Specific Sample* is **not** Selected**

In this case, simply select that samples that you would like to compare, and click the refresh button. The results will appear in the window to the right. Comparisons will be made between each sample in the selection. For example, if samples 'A', 'B', and 'C' were selected, results will be shown for AB, AC, and BC.

**Procedure When *Using Specific Sample* **is** Selected**

Selecting the option *Using Specific Sample* will allow the user to select a sample from the dropdown menu in the upper-left quadrant of the screen. This method differs from that described above in that the selected sample (from the dropdown) will be compared to each selected sample in the file list. For example, if sample 'A' is selected from the dropdown, and samples 'B', 'C', and 'D' are selected in the sample list, results will be shown for AB, AC, and AD.

*Only do analysis with clean genotype* With this option selected, only samples with zero flagging will be included in the calculations, whether or not they have been selected.

## 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 HIID 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.

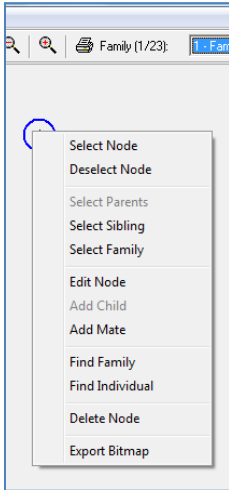
### Procedure

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

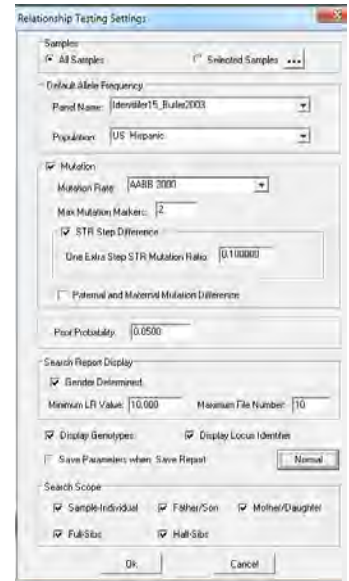
#### *Individual sample*

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



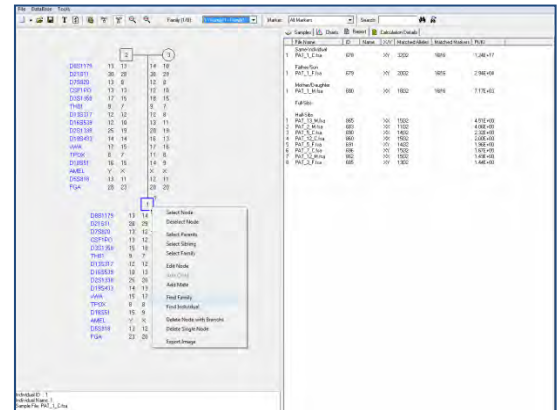


File Name	ID	Name	X/Y	Matched Alleles	Matched Markers	PI/KI
Same-Individual						
PAT_1_C.fsa	678		XY	3232	1616	1.24E+17
Father/Son						
PAT_1_F.fsa	679		XY	2032	1616	2.94E+04
Mother/Daughter						
PAT_1_M.fsa	680		XX	1832	1616	7.17E+03
Full-Sibs						
Half-Sibs						
PAT_13_M.fsa	865		XX	1532		4.51E+00
PAT_2_M.fsa	863		XX	1132		4.08E+00
PAT_5_C.fsa	690		XX	1432		2.32E+00
PAT_12_C.fsa	860		XX	1532		2.00E+00
PAT_5_F.fsa	691		XY	1432		1.98E+00
PAT_7_C.fsa	696		XY	1532		1.67E+00
PAT_12_M.fsa	862		XX	1532		1.49E+00
PAT_3_F.fsa	685		XY	1332		1.44E+00



**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

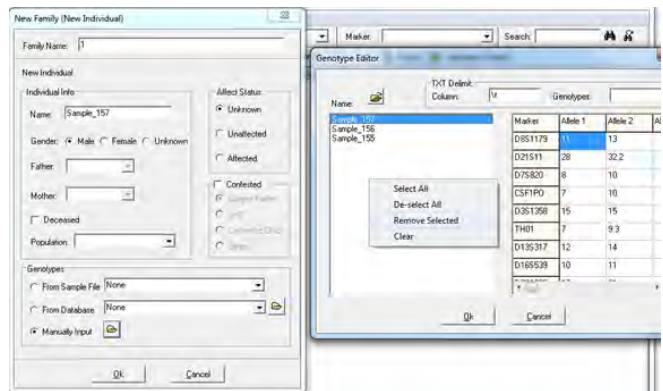


**Search Database for .txt file genotype**

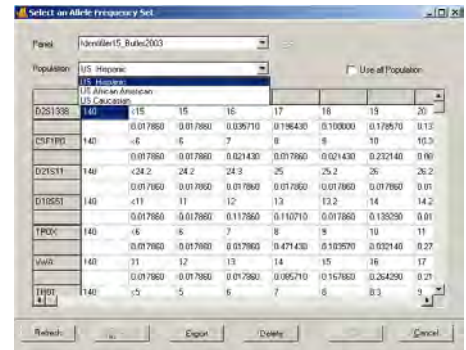
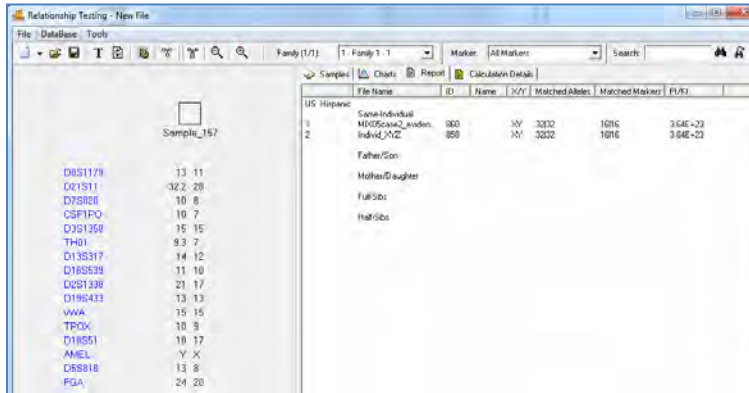
Use this function if the genotype is only available from an archived file -- the .fsa or .hid data file is not available. Format the .txt tab delimited file as in the example below -- the exact format is required for the program to recognize the genotype. The file must be saved as .txt tab delimited.

AID	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).



- 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

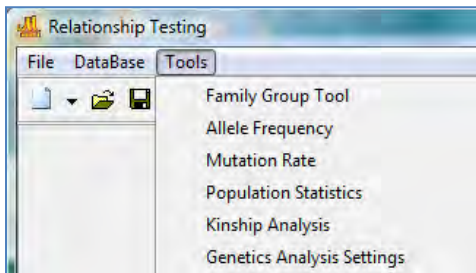


## Icons and Functions



**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**Hill, C.R., Duewer, D.L., Kline, M.C., Coble, M.D., Butler, J.M. (2013) *Forensic Sci. Int. Genet.* 7: e82-e83 (Supplemental Material Table 2).

### **CODIS 13\_2001\_Budowle et al.**

Budowle B, Shea B, Niezgodna 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](http://www.fsigenetics.com/article/S1872-4973(10)00071-2/abstract)

**GlobalFiler** Hill, C.R., Duewer, D.L., Kline, M.C., Coble, M.D., Butler, J.M. (2013) *Forensic Sci. Int. Genet.* 7: e82-e83 (Supplemental Material Table 2).

**Fusion**

Hill, C.R., Duewer, D.L., Kline, M.C., Coble, M.D., Butler, J.M. (2013) *Forensic Sci. Int. Genet.* 7: e82-e83 (Supplemental Material Table 2).

**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](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 probability.



**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 (2/2):

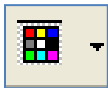
### Family

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

Marker:

### 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

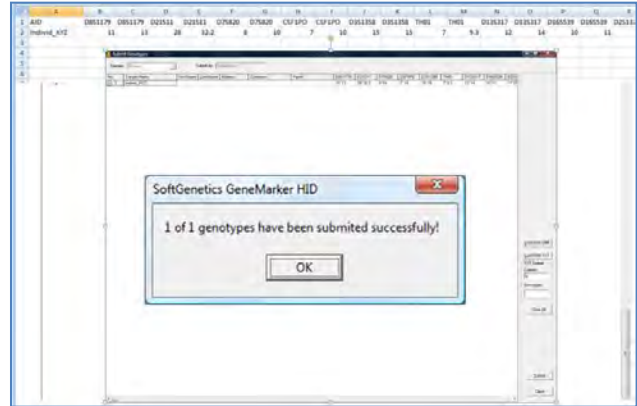
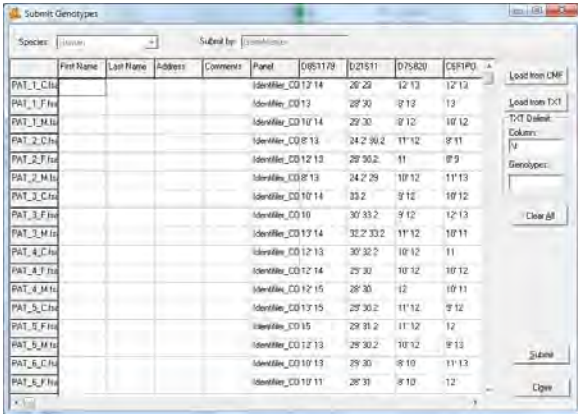
The Relationship Testing application in GeneMarkerHID has the allele frequencies for major USA populations and the mutation rates specified by AABB pre-loaded. Customization with population specific allele frequency and mutation rates is easily accomplished using the open folder icon of the allele frequency 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:

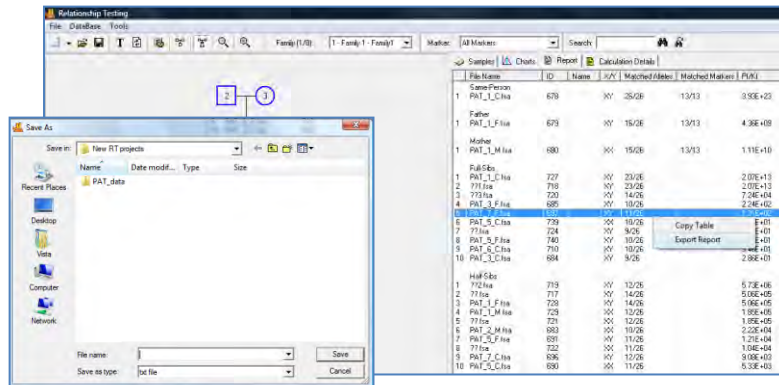
AID column = sample identification

Row 1 B – The marker names (case sensitive) repeated as below

AID	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	D2
Sample_157	11	13	28	32.2	8	10	7	10	15	15	7	9.3	12	14	10	11	
Sample_156	12	13	27	28	7	8	8	11	15	14	7	7	12	12	10	11	
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



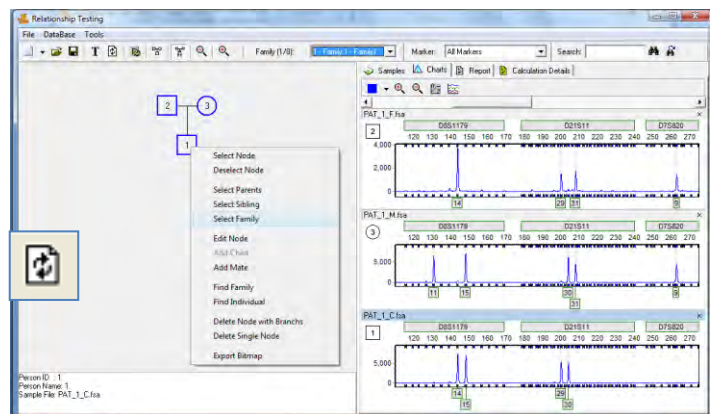
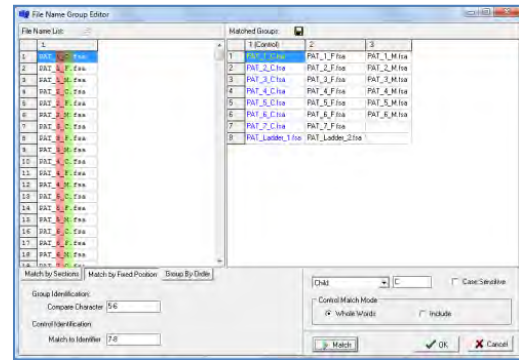


## Automated Pedigree Trio Diagrams and Analysis using Family Group Tool

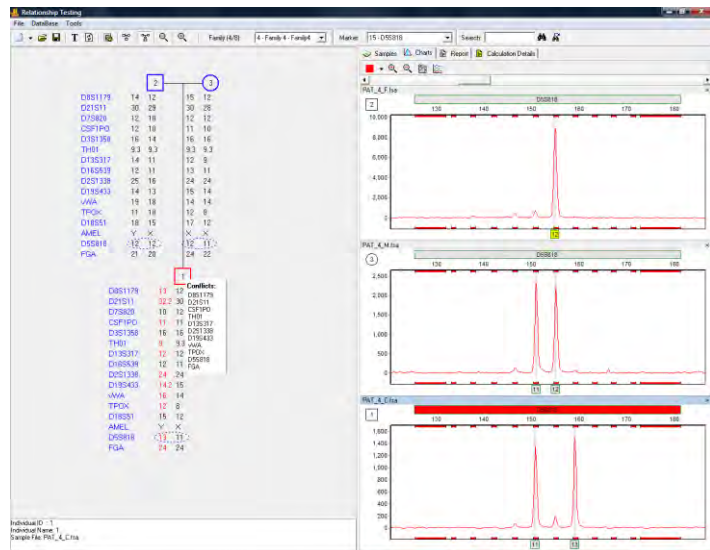
When a naming convention is followed the Family Group Tool enables matching of files into family groups. Prior to starting, GeneMarkerHID has the option to automatically name the nodes with the file name or sample name. To use this feature, Select Tools – Auto Name Individual and select sample name or file name. If edits are made to the file selection for anode after the pedigree auto-draw, make sure the clip icon next the sample name is selected.

### 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.




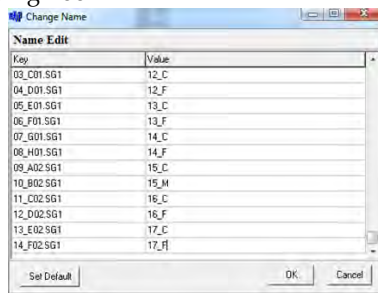


**Renaming Tool:**

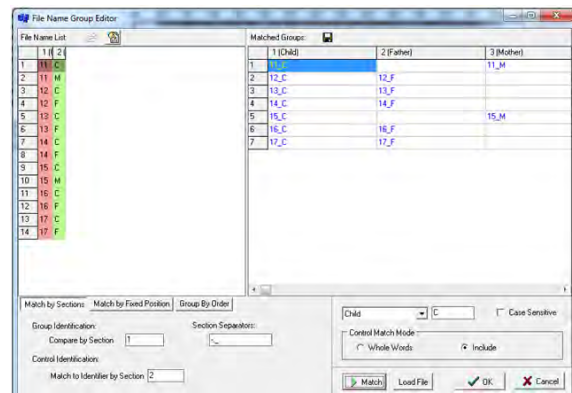
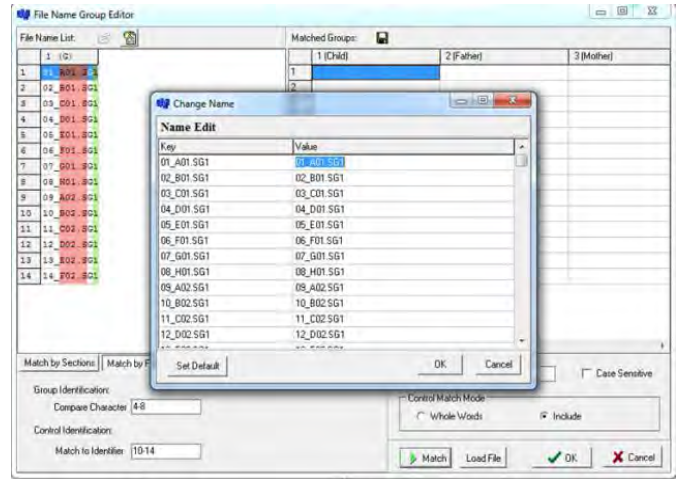
If the file names are not adequate for use in the family group editor, the renaming tool provides transient naming conventions and allows the user to use the automated pedigree drawing instead of manually diagramming the pedigree.

Procedure:

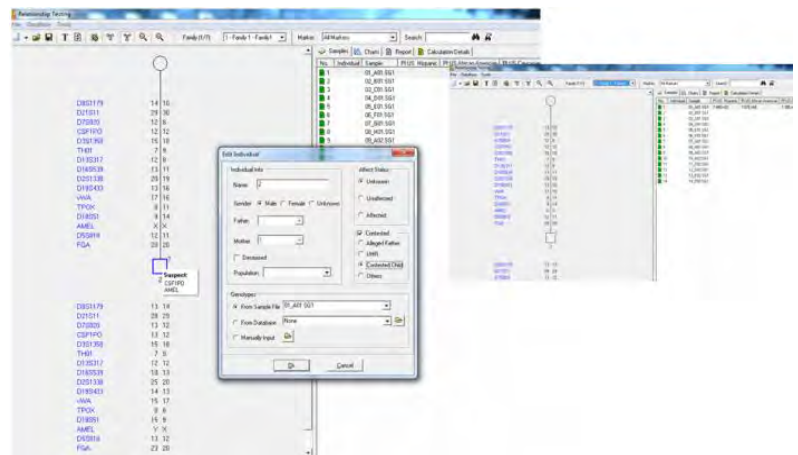
1. Click on the  icon to activate the renaming tool
2. Enter values that have a family identifier file\_relationship field
3. C = Child, F=Father, M=Mother – for several children in a family group, number them C1, C2, C3...
4. Enter Group Identification and Control Identification values as previously 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.

	Father	Missing Mother	child 1	child 2	child 3
D8S1179	12 11	<u>13</u> <u>13</u>	11 13	11 13	12 13
D21S11	27 28	<u>32.2</u> <u>32.2</u>	28 32.2	27 32.2	27 32.2
D7S820	9 12	<u>10</u> <u>10/8</u>	12 10	9 10	9 8
CSF1PO	11 11	<u>13</u> <u>12</u>	11 12	11 13	11 12
D3S1358	15 15	? <u>17</u>	15 15	15 17	15 17
TH01	8 6	<u>7</u> <u>7</u>	8 7	8 7	8 7
D13S317	10 14	<u>12</u> <u>12</u>	14 12	14 12	10 12
D16S539	10 12	<u>13</u> <u>12</u>	12 12	10 13	10 12
D2S1338	17 19	<u>20</u> <u>23</u>	17 23	17 20	17 23
D19S433	14.2 14.2	<u>14</u> <u>16.2</u>	14.2 16.2	14.2 14	14.2 16.2
vWA	17 18	<u>16</u> <u>16</u>	18 16	17 18	17 16
TPOX	8 8	? <u>8</u>	8 8	8 8	8 8
D18S51	16 14	<u>14</u> <u>13</u>	14 14	16 13	16 13
AMEL	X Y	<u>X</u> <u>X</u>	X X	X X	X X
D5S818	11 12	<u>11</u> <u>11</u>	11 11	12 11	11 11
FGA	26 24	<u>25</u> <u>20</u>	26 20	24 25	26 20

## 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

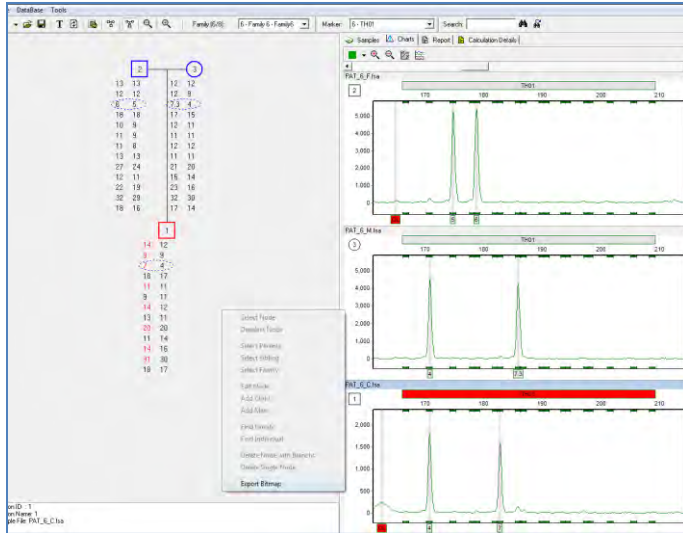
Select the individual's sample file from the drop-down list. Any sample that was previously saved to the database will be available.

## 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



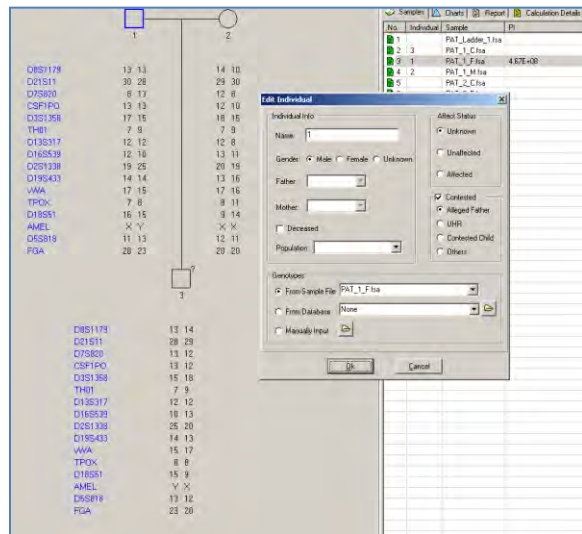
## Paternity Index Calculations

### 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 Frequency 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



### Reverse Parentage

Reverse parentage is calculated by right mouse click on the node of the child and selecting contested. The calculation is that of the AABB Standards for Relationship Testing; often used in cases of unidentified human remains when potential mother and father genotypes are available.

## 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.

The screenshot displays a software interface with a network diagram on the left and a data table on the right. The network diagram shows three nodes (1, 2, 3) connected by lines. Node 1 is a square, node 2 is a circle, and node 3 is a circle with a question mark. Various sample IDs are listed next to the nodes. The data table on the right has the following structure:

	PI	POP	PE	1	2	3	Numerator	Denominator	
Total	3.70E+13	99.99%	1.0000000						
CSFIPO	9.56E+00	90.52%	1.00E+00	10.11	7.12	7.11	2.50E-01	2.62E-02	
TPDX	6.67E+00	86.95%	1.00E+00	8.9	6	6.8	5.00E-01	7.50E-02	
TH01	7.90E+00	88.76%	1.00E+00	7.9.3	8.9	9.9.3	2.50E-01	3.16E-02	
vWA	1.04E+01	91.22%	1.00E+00	16.18	14.17	14.18	2.50E-01	2.40E-02	
D165539	2.01E+00	66.76%	1.00E+00	10.11	9.10	9.11	2.50E-01	1.24E-01	
D75820	1.60E+00	61.46%	1.00E+00	8.11	8.10	8.10	2.50E-01	1.57E-01	
D135317	2.67E+00	72.72%	1.00E+00	11.15	9.11	11	2.50E-01	9.38E-02	
D55818	1.08E+01	91.54%	1.00E+00	12.13	8.11	8.13	2.50E-01	2.31E-02	
FGA	1.10E+01	91.65%	1.00E+00	21.22	21	21.22	5.00E-01	4.55E-02	
D8S1179	8.25E+00	89.19%	1.00E+00	13.16	13.14	13.16	2.50E-01	3.03E-02	
D18551	4.91E+00					15.16	2.50E-01	5.09E-02	
D21S11	3.70E+02					33.2	24.2.33.2	2.50E-01	6.76E-04
D3S1358	2.47E+00	71.15%	1.00E+00	15	16.17	15.16	5.00E-01	2.03E-01	
D2S1338	2.12E+01	95.43%	1.00E+00	19	24.26	19.24	5.00E-01	2.36E-02	
D195433	8.88E+00	89.88%	1.00E+00	12.13	13	12.13	5.00E-01	5.63E-02	

A context menu is open over the table, showing 'Copy Table' and 'Export Calculation Details' options.





# Chapter 9 Cell Line Authentication - Percent Same Genotype Search

## Chapter 9 Cell Line Authentication - Percent Same Genotype Search

Building a Database  
Searching the Database  
Exporting Results

## Cell Line Authentication

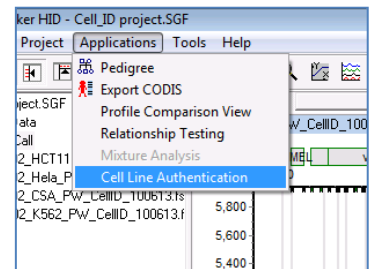
### Overview

Cell line authentication is the process of using genetic markers to determine if the cells in a culture sample are in fact the expected type. Cell line authentication has become a necessity in many fields, thanks in part to revelations that a significant number of cell lines are either contaminated or misidentified entirely.

Typically, a cell culture is sampled and amplified using a commercially available qfPCR kit. The genotype obtained from this amplification is then compared to a database of known genotypes to determine the most probable match. GeneMarkerHID's Cell Line Authentication module provides database and searching functionality, both of which are described in detail below.

### Opening the Cell Line Authentication Application

To open the Cell Line Authentication Application simply navigate to Applications > Cell Line Authentication. Like other GeneMarkerHID applications, you must have a current project loaded in order to enter this application.



### Icons and Features



**Save Icon:** Launches the "Save As" dialog box. Search Results may be exported as either an excel (.xls) or text (.txt) file. (Also: File > Save Report.)



**Submit to Database:** Opens the "Submit Genotypes" dialog, which enables the user to save genotypes to GeneMarkerHID's Cell Line database. Genotypes can be uploaded from samples in the current project, or from samples saved in a text file. (Also: File > Submit to Database.)



**Database Editor:** Launches the "Genetics Database Editor" from which the user can manage, edit, and delete genotypes in the Cell Line Database. (Also: File > Database Editor.)



**Refresh:** Used to refresh search results. This is most commonly needed after changing the percent match threshold.

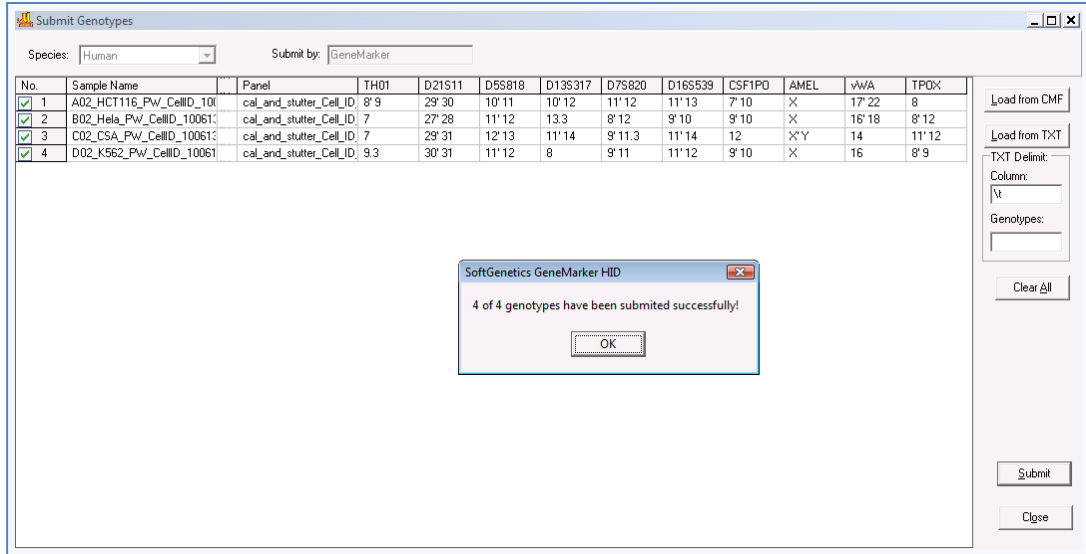
## Building Your Database

GeneMarkerHID provides a built in database to which the user can save Cell Line genotypes. (**Note** that the Cell Line database is unique and separate from the Relationship Testing database. The user has two methods of populating the database: importing sample genotypes from the current project, or importing genotypes saved in a text file.

### Importing Genotypes from the Current Project

1. Enter the Cell Line Authentication Application (Applications > Cell Line Authentication).
2. Navigate to the "Submit Genotypes" dialog box by either clicking the "Submit to Database" icon, or by navigating to File > Submit to Database.
3. Sample Genotypes derived from samples in your **current project** will be displayed in the Submit Genotypes window. Use the checkbox in front of each sample to choose whether or not so submit it to the database.
4. After making your selections click **Submit**. You should receive a confirmation that the genotypes were uploaded successfully. The sample genotypes are now saved in the database.

Chapter 9 Cell Line Authentication – Percent Same Genotype Search



*Importing Genotypes from a Text File*

This option is more commonly used to transfer a large, preexisting database into GeneMarkerHID. It's also useful for submitting genotypes from cultures for which samples are no longer available.

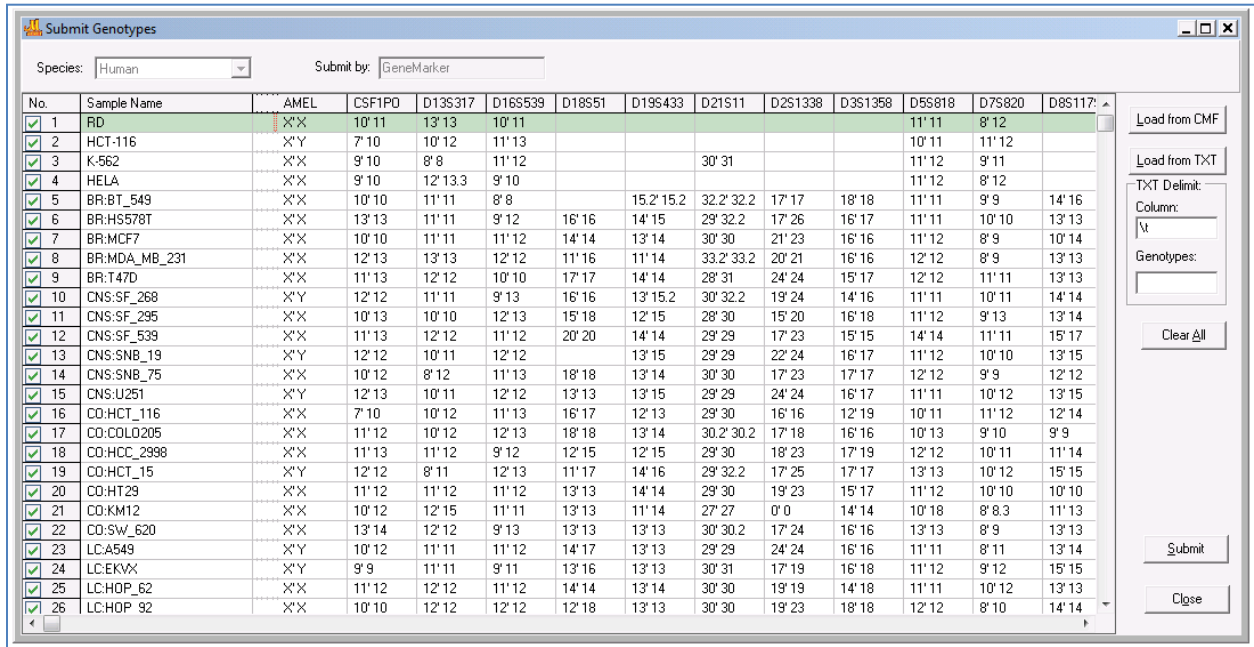
1. Begin by formatting your database in Microsoft Excel or another spreadsheet program. Use the exact formatting shown below

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	AID	AMEL	AMEL	CSF1PO	CSF1PO	D13S317	D13S317	D16S539	D16S539	D18S51	D18S51	D19S433	D19S433	D21S11
2	HCT-116	X	Y	7	10	10	12	11	13	**	**	**	**	**
3	K-562	X	X	9	10	8	8	11	12	**	**	**	**	30
4	HELA	X	X	9	10	12	13.3	9	10	**	**	**	**	**
5	BR:BT_54	X	X	10	10	11	11	8	8	**	**	15.2	15.2	32.2
6	BR:H5578	X	X	13	13	11	11	9	12	16	16	14	15	29
7	BR:MCF7	X	X	10	10	11	11	11	12	14	14	13	14	30
8	BR:MDA_X	X	X	12	13	13	13	12	12	11	16	11	14	33.2
9	BR:T47D	X	X	11	13	12	12	10	10	17	17	14	14	28
10	CNS:SF_2_X	X	Y	12	12	11	11	9	13	16	16	13	15.2	30
11	CNS:SF_2_X	X	X	10	13	10	10	12	13	15	18	12	15	28
12	CNS:SF_5_X	X	X	11	13	12	12	11	12	20	20	14	14	29
13	CNS:SNB_X	X	Y	12	12	10	11	12	12	**	**	13	15	29
14	CNS:SNB_X	X	X	10	12	8	12	11	13	18	18	13	14	30
15	CNS:U251	X	Y	12	13	10	11	12	12	13	13	13	15	29
16	CO:HCT_1	X	X	7	10	10	12	11	13	16	17	12	13	29
17	CO:COLO2	X	X	11	12	10	12	12	13	18	18	13	14	30.2

Specifically, the phrase "AID" should be written in the upper left-hand corner of the spreadsheet. List each marker name twice (once for each allele). Use \*\* with sample and markers for which no data is available. No cell should be left blank.

2. After formatting the table, save it as a tab-delimited text file (.txt).
3. Enter the Cell Line Authentication Application (Applications > Cell Line Authentication).

4. Navigate to the "Submit Genotypes" dialog box by either clicking the "Submit to Database" icon, or by navigating to File > Submit to Database.
5. Click **Load From Text**, and then navigate to your saved text file.
6. You should see the contents of the text file displayed in the Submit Genotypes dialog box.

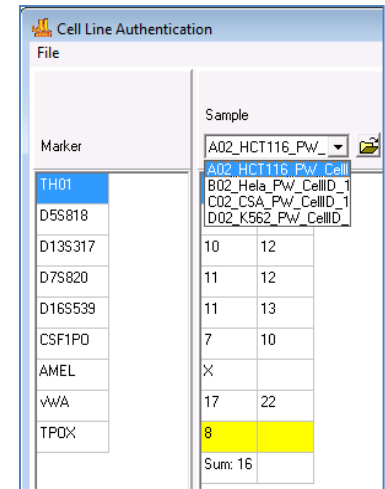


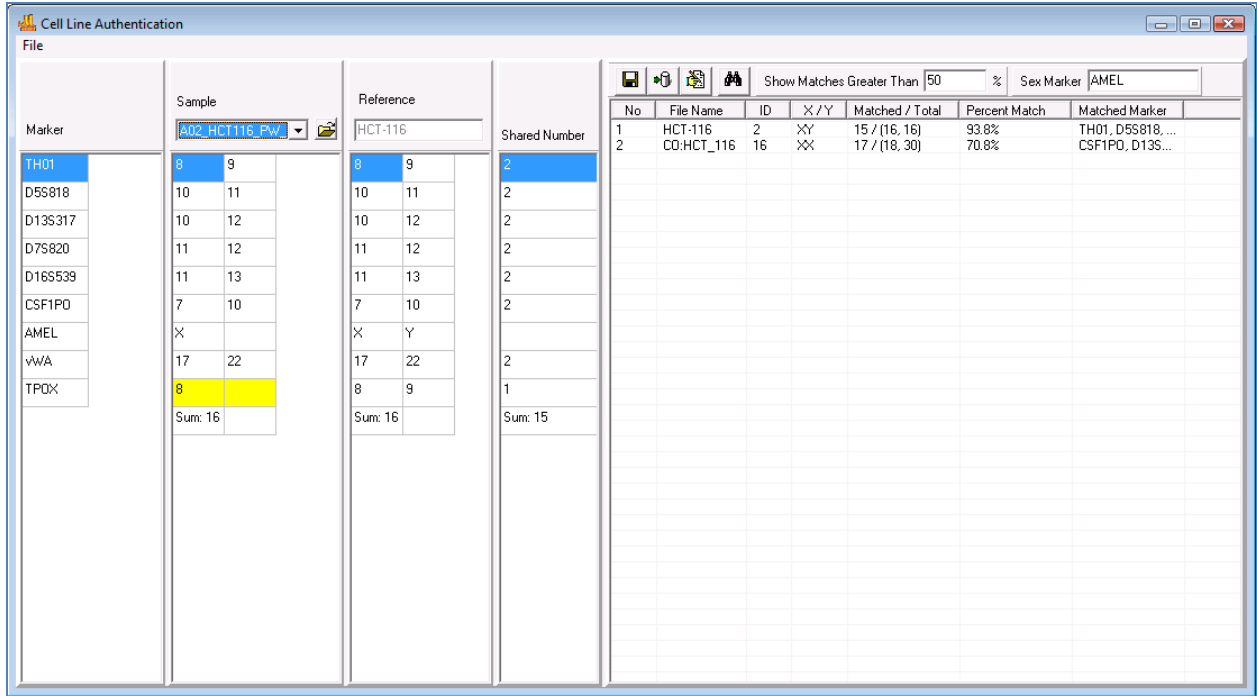
7. Click **Submit** to save the genotypes to the database.
8. You should receive a confirmation that the genotypes were successfully saved.

## Searching the Database

Once you have established your database, you can use the Cell Line Authentication Application to query your samples against the database and search for the closest matches.

1. Load your raw data files, process them, and make any necessary edits. (See [Chapter 2: General Procedure for more information.](#))
2. Enter the Cell Line Authentication application (Applications > Cell Line Authentication).
3. Use the dropdown menu (in the column labeled "**Sample**") to select a sample file from your current project.
4. When you use the dropdown to select a sample, two things will happen: (1) That sample's genotype, *obtained from your current project*, will appear in the sample genotype table directly below the dropdown. (2) The program will automatically search your Cell Line database for samples that most closely match the selected sample.
5. Results are displayed in right half of the Cell Line Application. Select a result to display that sample's genotype in the **Reference** column. Differences are highlighted in yellow.





*Adjusting the Match Percentage*

When searching the database, GeneMarkerHID uses a percent match calculation to compare genotypes. The user may set a **Percent Match Threshold**, such that only the matches meeting this threshold are displayed. Next to the words "Show Matches Greater Than" is a field in which the user may input their desired match threshold (0 - 100%). Only matches great than or equal to this number will be displayed. Click the **Refresh Icon** (binoculars) to update the results.

*Sex Marker*

The marker name typed in the "Sex Marker" field is ignored during the search, and is not used to calculate the percent match value.

**Managing Your Database**

*Overview*

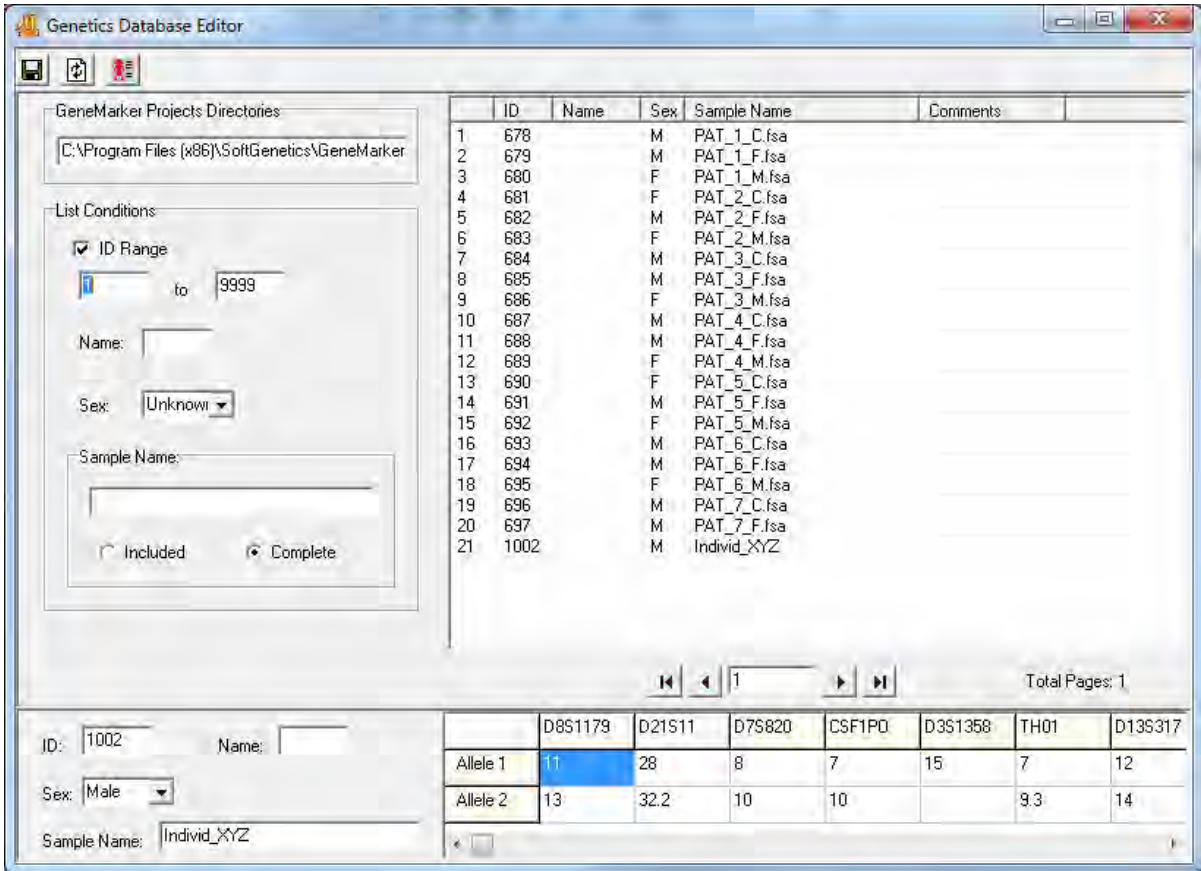
You can manage your database from the Cell Line Authentication Application by navigating to File > Database Editor, or by clicking the Database Editor icon. Either method will open the Genetics Database Editor tool.

Samples saved in the database are listed in the main window. Use the < > buttons to page forward and backwards, or use the | < > | buttons to move directly to the first and last page, respectively. Simply click on an entry to display that sample's genotype in the lower table. Double click on a cell to edit the value. Other details relating to the selected sample, e.g. sex, ID number, and sample name, are displayed in the lower left corner of the window.

*List Conditions*

The options in the "List Conditions" section allow the user to search for a specific sample, or restrict the collection of samples that is being displayed.

Check the ID range box and input the desired range to show only samples with IDs that fall within that range. Select a Sex to only display samples of that sex. Finally, input a sample name to display samples that share that name. Click the refresh icon to see that changes take effect.



*Other Icons and Features*



**Save Icon**

Save any changes that have been made to a sample.



**Refresh Icon**

Update the display. Use the refresh icon to search for a sample using the "Sample Name" field, or to implement any other "List Conditions".



**Export Codis**

Launches the CODIS Export tool, which can be used to export a genotype or genotypes in CODIS format.



## Chapter 10 Additional Tools

### **Chapter 10 Additional Tools**

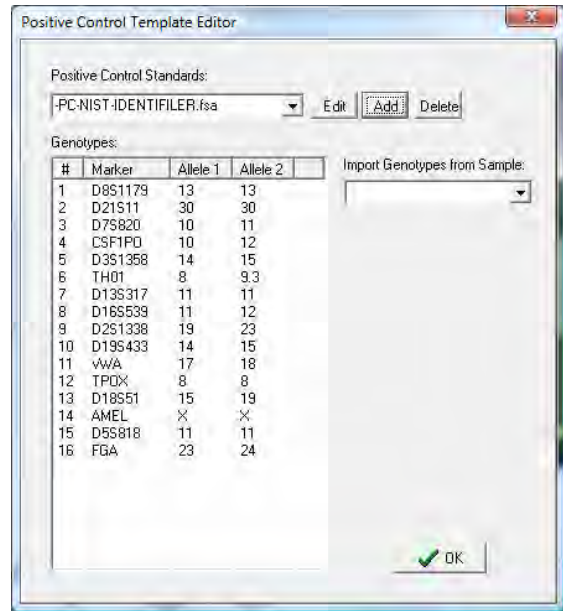
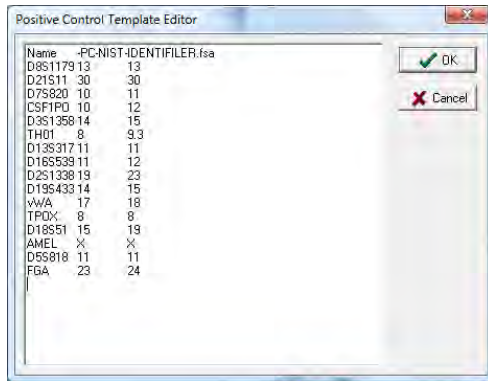
Automated Control Concordance  
Contamination Check / Elimination Database  
Filename Group Editor  
Output Trace Data  
Project Comparison  
Convert TXT to Binary  
Export Electropherogram  
Quality Sensor Evaluation

## Automated Control Concordance

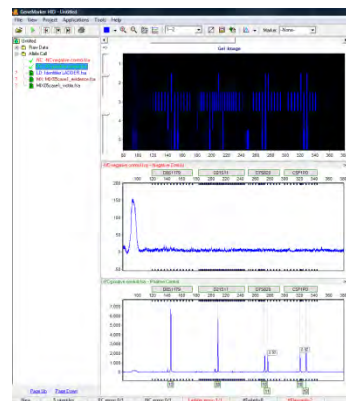
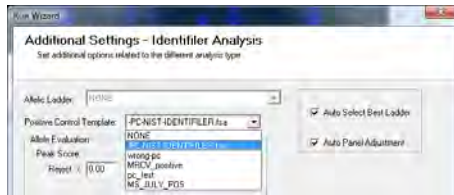
Control samples in a project can be identified by either of the two methods described in Chapter 3. If the chemistry use has Y-STR markers, use male DNA for the positive control(s).

### Positive Control Template Editor

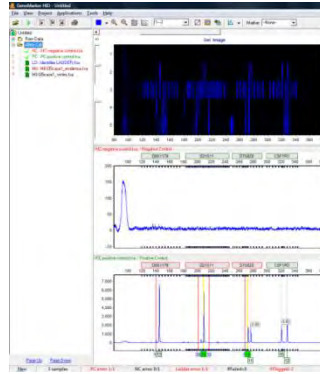
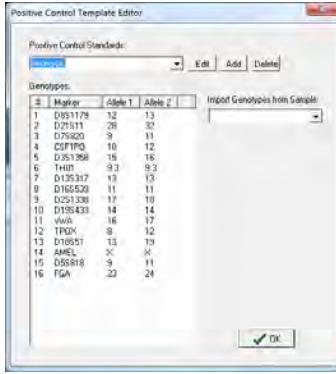
1. Tools → Positive Control Template Editor to launch dialog box
2. Import Genotypes from samples using dropdown menu
3. Select from functions to Add new positive control samples, Edit or Delete files



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.



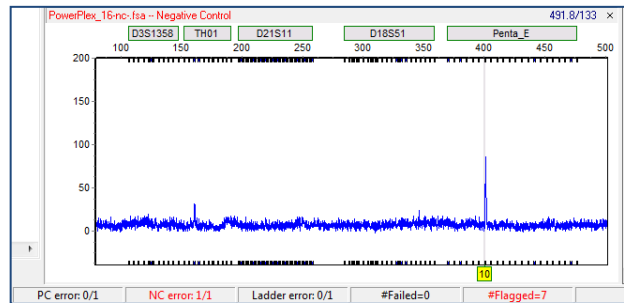
5. If the positive control samples are not in agreement with the template the message will be PC error 1/1 and red lines will indicate the peaks in error.



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



## Contamination Check and Elimination Database

This link enables analysts to automatically detect any lane-to-lane contamination within a project and compare each sample of a project to a staff database to detect potential contamination from someone who has handled the samples. In order to search the staff database, it is necessary to first save the desired genotypes to a contamination database. This database is separate from the relationship testing (Chapter 8) and cell line authentication (Chapter 9 percent same genotype search).

### Icons and Features



**Save Icon:** Launches the "Save As" dialog box. Search Results may be exported as either an excel (.xls) or text (.txt) file.



**Submit to Database:** Opens the "Submit Genotypes" dialog, which enables the user to save genotypes to GeneMarkerHID's Contamination database. Genotypes can be uploaded from samples in the current project, or from samples saved in a text file.



**Database Editor:** Launches the "Contamination Database Editor" from which the user can manage, edit, and delete genotypes in the Contamination Database.



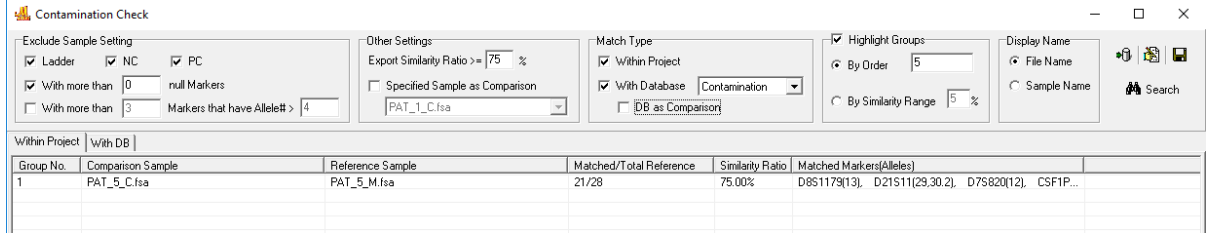
**Refresh:** Used to refresh search results. This is most commonly needed after changing the Similarity Ratio threshold.

**Display Name:** Choose between displaying sample names or file names (default).

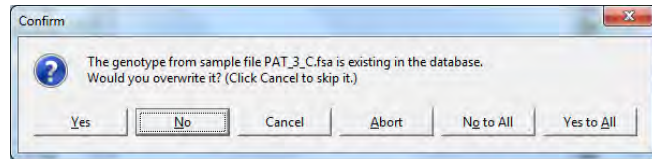
**Highlight Groups:** Choose to highlight samples by order or by similarity range (%).

**Saving reference genotypes to the contamination database**

1. Main tool bar - Project - Contamination Check.
2. Click on the Database icon at the right side of the screen The dropdown box at With Data base is empty at this point. It will be available from the dropdown menu after files have been added to the database.



3. Submit genotypes to the Contamination Check Database
  - a. If the samples are in the current project, submit to database by clicking the Submit button in the lower right (Figure 2 below)
  - b. To submit archived genotypes from CODIS format .cmf files, click the Load from CMF - Add - select the file(s) -- OK
  - c. To submit archived genotypes from .txt files, format the .txt file exactly as shown in Figure 1 below. Row 1 must have AID (accession ID number) in the first cell followed by the marker names. Marker names are case specific and must match the marker names of the genotyping panel. Click the Load from TXT - Add - select the file - OK
  - d. Samples with the same name but a different kit was used can be submitted to the database.
    - i. When submitting the second sample with the same name, select "No" when the error message below is displayed:



	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	D2
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	

Figure 1: Txt file format required to save genotypes to the database from a .txt file.

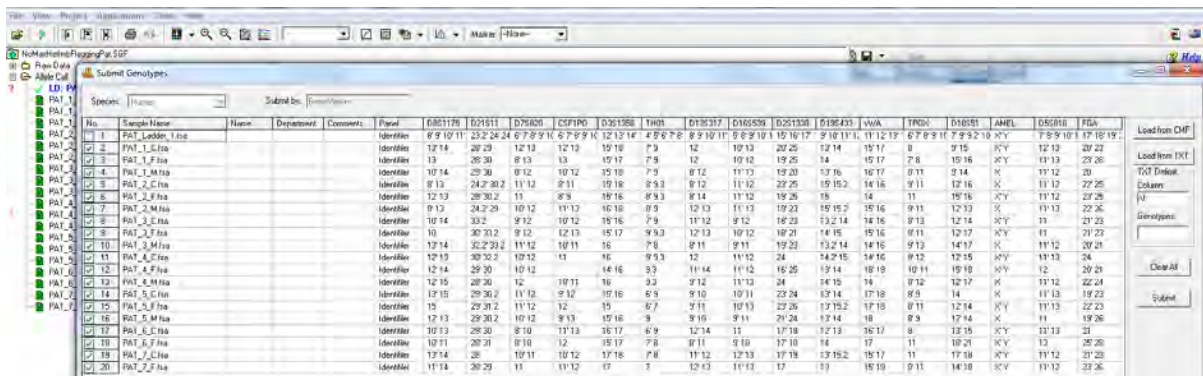


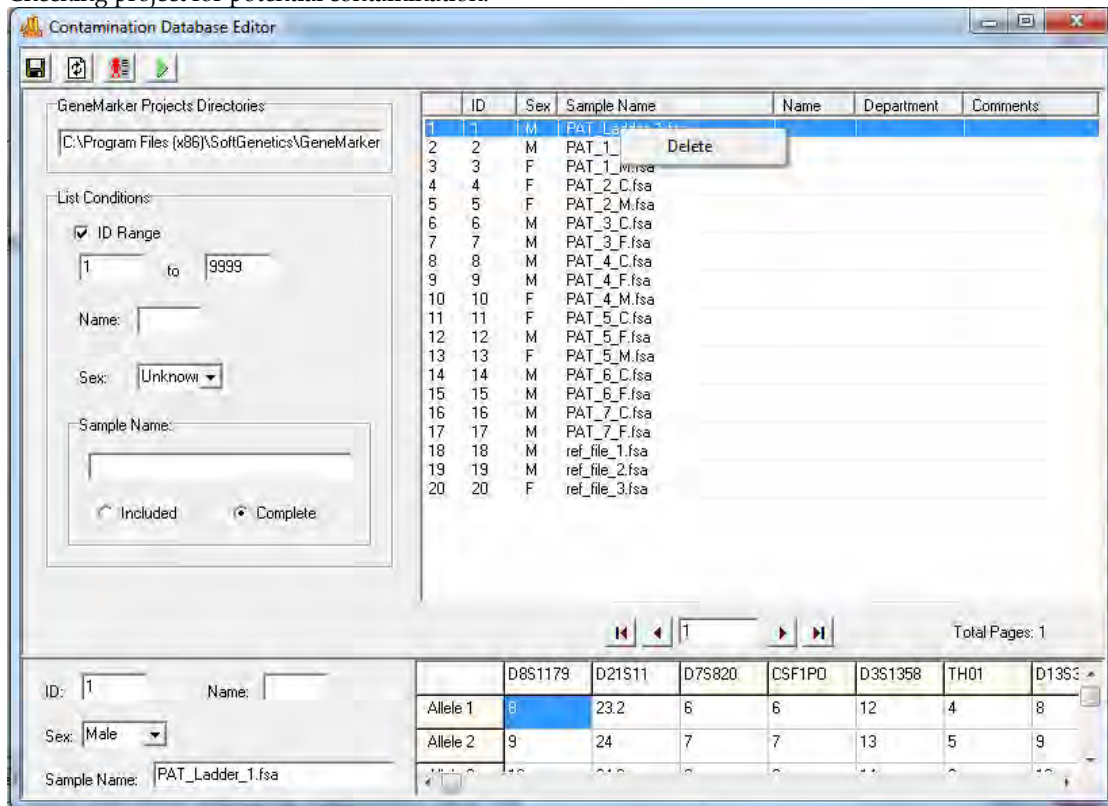
Figure 2: Submit Genotypes screen. When submitting profiles to the contamination database from a current project, name, department and short comments identifiers can also be included with the profile in the contamination database by typing them in the appropriate cell before clicking the Submit button.

To remove profiles from the contamination database



1. Select the Contamination Database Editor icon
2. Right mouse click on the profile and select Delete
3. Hold down the Ctl key and click with the mouse to select several samples, or the Shift key and mouse select consecutive samples, right mouse click on the highlighted samples and delete all highlighted profiles from the database
4. If the database is large, use the List Conditions and refresh icon to locate samples to delete using the ID Range or the sample name.

Checking project for potential contamination:



A project with data from MX2005 study (<http://www.cstl.nist.gov/strbase/interlab/MIX05.htm>) is used below to demonstrate the contamination check process. The samples are a two-person mixture and a single source file; all of the allele calls of the single source file are included in the mixture. We use these to simulate lane-to-lane contamination where one of the genotypes is contained within the other. Table 1 displays the allele call and peak heights for the two samples. Note that all allele calls have a green flag (there are no allele calls with quality reason flags). It is recommended to resolve any quality reason flags before entering the contamination check. The percent similarity is calculated using only the green labeled alleles (alleles that have passed all analysis parameters or that have been confirmed by the analyst in the main analysis window). If a sample has any quality reason flagged peaks, the sample is used for the contamination check, however, the flagged alleles will not be used in calculating the Similarity Ratio.

$$\text{Similarity Ratio} = \frac{\text{\# of reference sample alleles included in the comparison sample}}{\text{Total \# of reference sample alleles}} \times 100$$



Contamination check parameters include: Excluding control files or files with null markers, the similarity ratio value, option to use one sample as the comparison profile, compare all samples in project to each other and/or compare all samples in the project to genotypes in a database. Samples with more null markers than specified in the Exclude Sample Settings will be excluded from the contamination check (Figure 3). Check boxes for Match Type are used to specify lane-to-lane similarity of genotypes (Within Project) and/or samples of the project compared to genotypes within a database (With Database).

Select the preferred settings at the top of the screen. Results for sample to sample comparison are displayed when the Within Project tab is selected (Figure 1). The results from the Contamination Database are displayed at the With DB tab (Figure 2). Columns can be reordered by clicking on the column header.

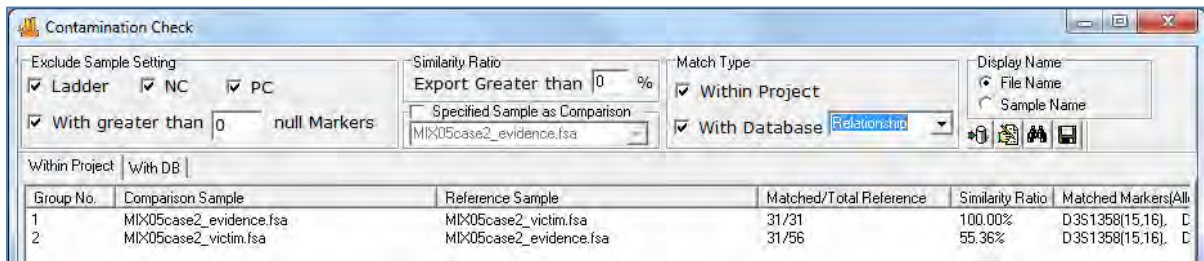


Figure 1: In Group 1, 100% of the Reference sample alleles are included in the comparison sample. In Group 2, 55% of the alleles in the reference sample are included in the comparison sample. The last column of each row contains the shared genotype. The results can be saved as a .txt or .xlsx, .xls file from the disk icon.

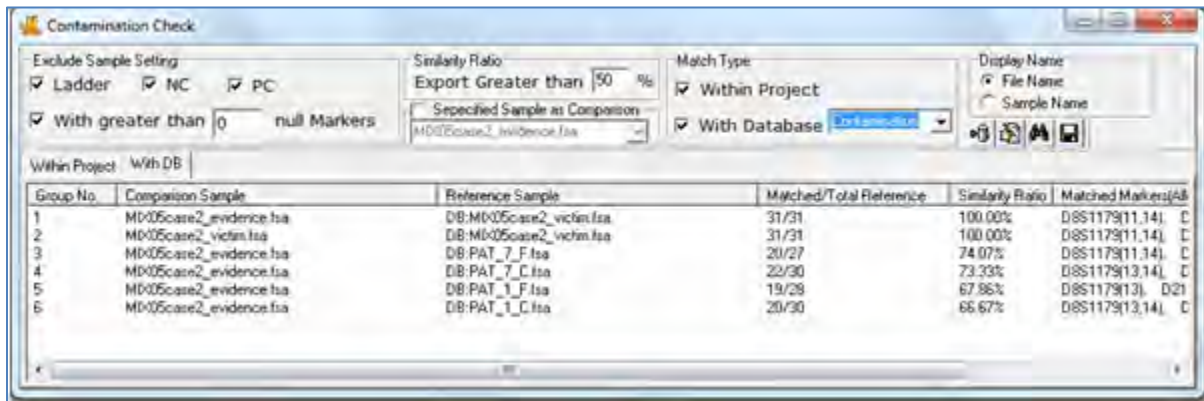


Figure 2: The With DB tab displays the results comparing samples from the project to reference samples in the database. The single source file from the case and several other single source files were previously saved to the database to provide matches to the two samples of the project for this demonstration. The results indicate that 100% of the allele calls in MX05case2\_victim sample are included in the comparison samples 1 and 2. Four samples from the database (Reference Samples) have greater than 50% similarity to the Control Sample MX05case2\_evidence.fsa of the current project. Note also that reference samples retrieved from the database are designated with DB:filename in the Reference Sample column.



MIX05case2_evidence.fsa	D8S1179	11 (6224)	13 (5354)	14 (822)		MIX05case2_victim.fsa	11 (4504)	14 (4954)
D21S11	28 (4418)	30 (960)	31 (580)	32.2 (3277)		30 (2826)	31 (2689)	
D7S820	8 (1975)	9 (400)	10 (2077)	11 (202)		9 (1878)	11 (1406)	
CSF1PO	7 (1590)	10 (1364)	12 (141)	13 (194)		12 (1145)	13 (1471)	
D3S1358	15 (8555)	16 (567)				15 (3830)	16 (3859)	
TH01	7 (5255)	8 (770)	9.3 (6887)	10 (1264)		8 (9818)	10 (8341)	
D13S317	8 (763)	9 (945)	12 (5339)	14 (4487)		8 (5535)	9 (5243)	
D16S539	9 (555)	10 (2592)	11 (2723)	12 (376)		9 (3662)	12 (2600)	
D21S1338	16 (381)	17 (2491)	21 (2337)	24 (152)		16 (3060)	24 (2019)	
D19S433	12 (1192)	13 (8502)	14 (721)			12 (3450)	14 (3323)	
vWA	15 (5643)	16 (313)	19 (400)			16 (2378)	19 (1830)	
TPOX	8 (712)	9 (3629)	10 (2877)	11 (363)		8 (2995)	11 (3817)	
D18S51	12 (143)	15 (175)	17 (823)	18 (819)		12 (1365)	15 (1068)	
AMEL	X (3010)	Y (1786)				X (2753)	X (2753)	
D5S818	8 (1599)	11 (232)	12 (351)	13 (1719)		8 (1645)	12 (1268)	
FGA	20 (1270)	23 (303)	24 (1231)			23 (1390)	24 (1119)	

Table 1: Genotypes of the two samples used in Figures 1 and 2 as examples of positive matches for the contamination tool.

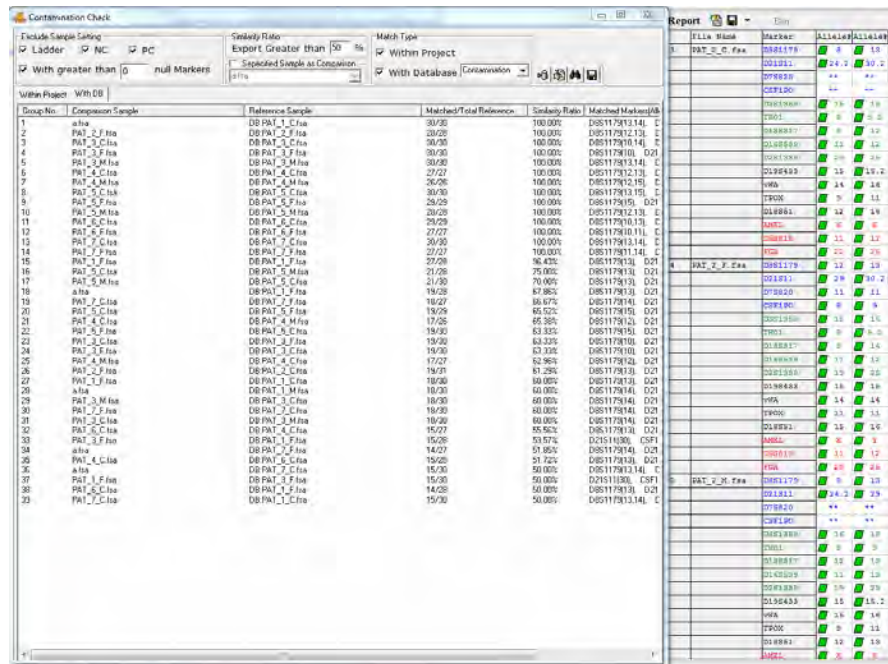


Figure 3 A: When the Null Marker allowance is set to 0 any sample with null markers is excluded from the comparison. In this example Samples PAT\_2\_C and PAT\_2\_M have two null markers. They are not included in comparison.

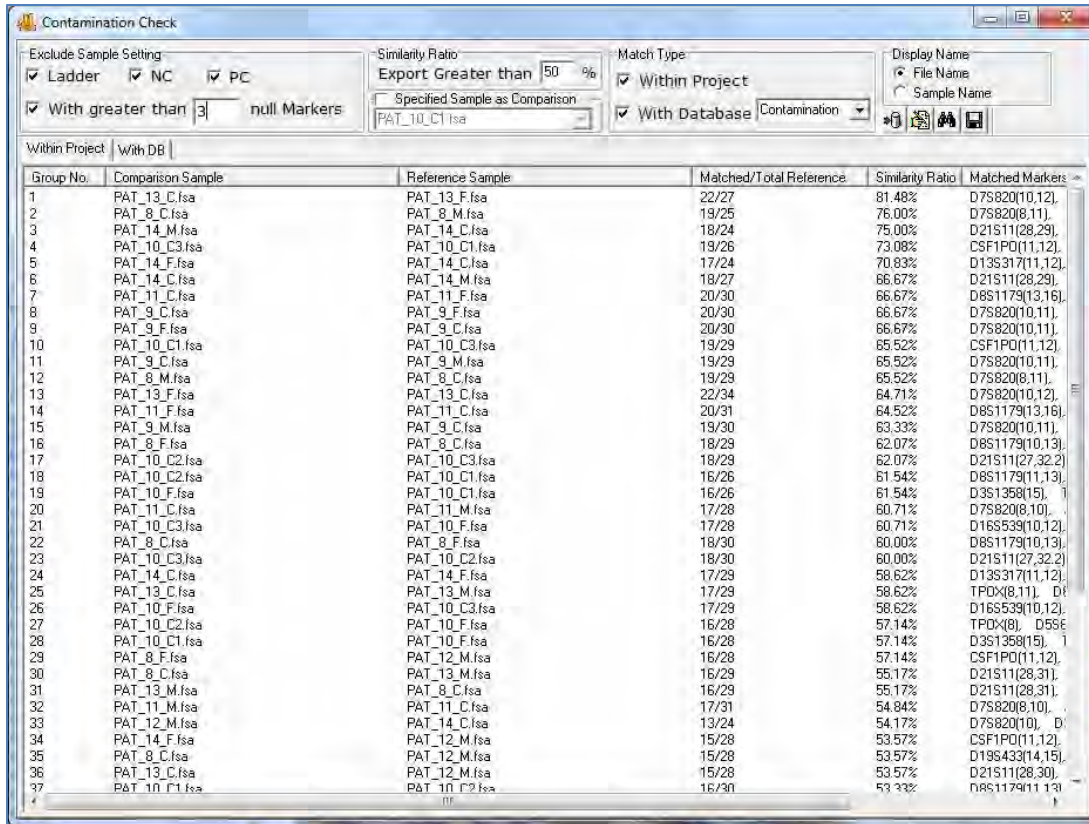


Figure 3B: To include samples with null markers, increase the null Markers setting to the desired amount and click the search (binoculars) icon to refresh the table. Lines 19 and 20 show the results for these samples in the refreshed table.

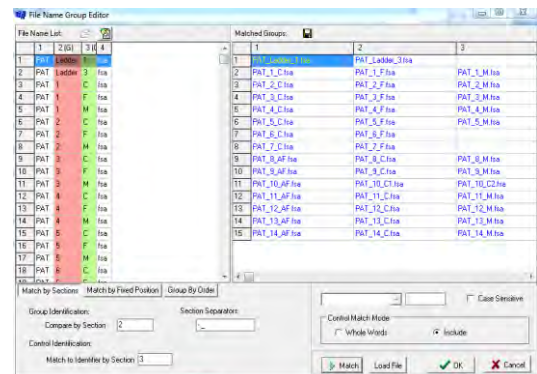
## Filename Group Editor

### Project → Apply Sample Grouping

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*.

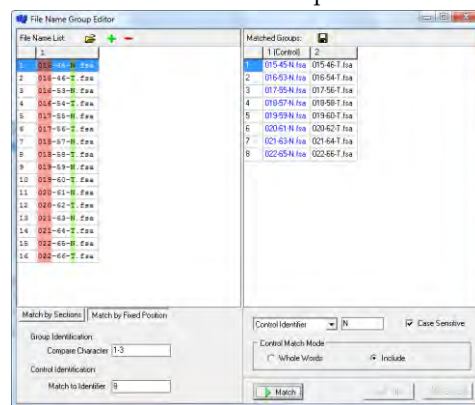
**Control Identification:** Identifies which section of the filename contains the reference vs. sample information based on the section number entered in the *Match to Identifier by Section* field. The section of the filename specified will be highlighted green in the *File Name List*.

## 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.

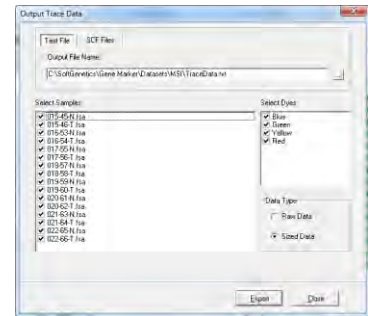
## Output Trace Data

### 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

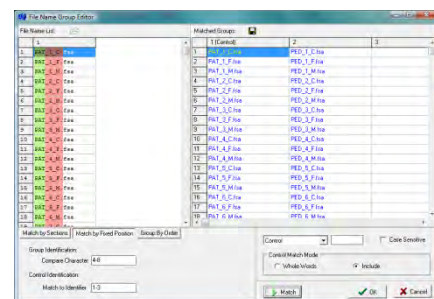
The *Project Comparison* tool can serve three functions. First, it can be used to compare two independent analysts' analyses. Second, it can be used as a validation tool to determine differences in allele calls based on analysis parameters or instrument runs. Third, it can be used to compare the projects 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 GeneMarkerHID 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 GeneMarkerHID. The first project in GeneMarkerHID will be considered the Reference project and the project uploaded to the *Project Comparison* tool will be considered the Sample project.



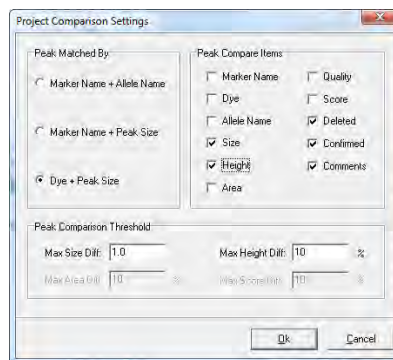
#### Project Comparison Settings

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 GeneMarkerHID 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.



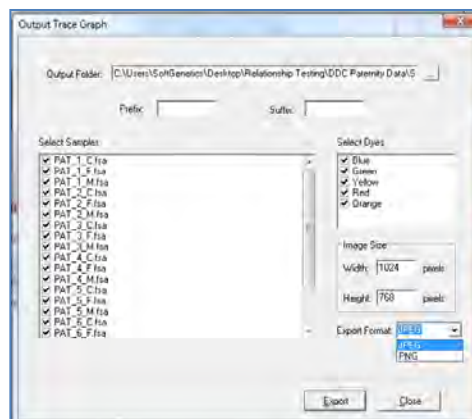
## Export Electropherogram

### 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.

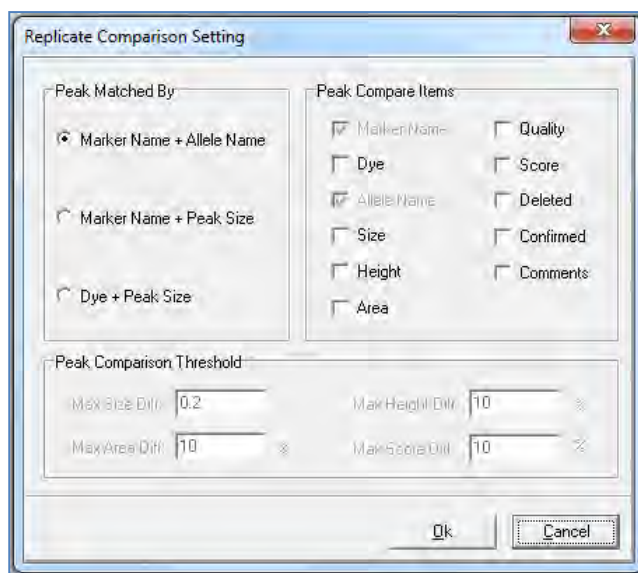


## Replicate Comparison Tool

### 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.

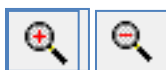
As an example, suppose the height of a peak was 1000 RFU, and the height of the corresponding peak in a replicate was 1050 RFU. If the *Max Height Difference* was set to 10%, this conflict would not be flagged, as it is a difference of only 5%.

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		
TPDX	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.

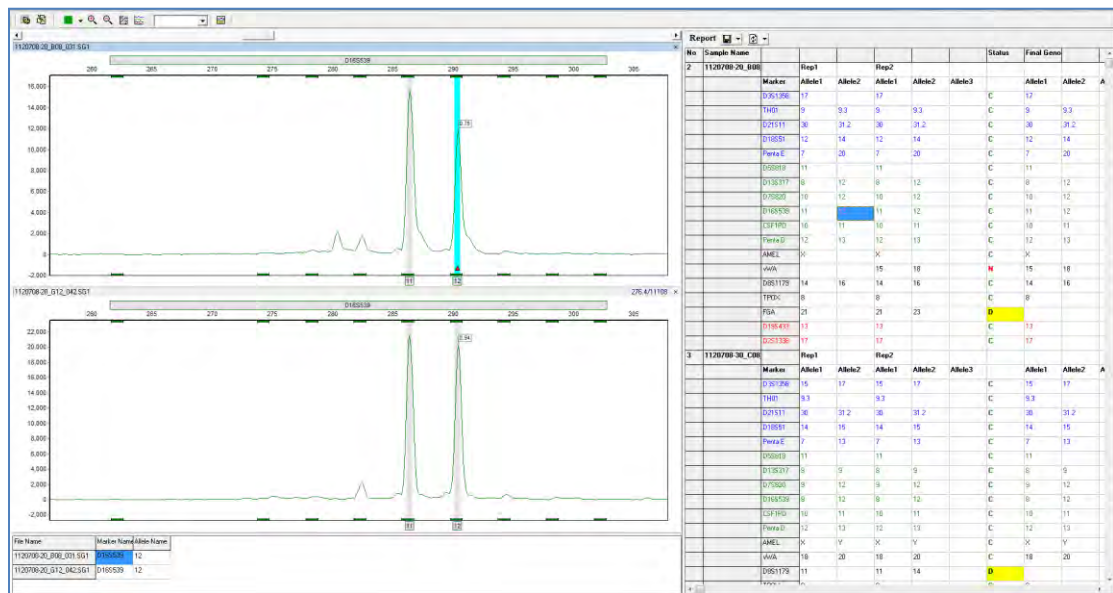
Selecting the consensus genotype:

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 11
	D5S818	9	11	9	11	C		
	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.

No	Sample Name	Marker	Allele1	Allele2	Allele3	Status	Final Gene
	D2S1288	16	17	15	16	17	D
	TH01	6	7	6	7		C 6 7
	D21S11	30	33.2	28	29	30	D
	D18S51	14	15	14	15		C 14 15
	Prnka E	5	12	12			D
	D18S217	11	11	11	12		D
	D18S217	10	11	10	11	12	D
	D7S820	8	11	8	10	11	D
	D6S509	9	12	9	10	12	D
	CSF1PO	10	11	10	11	12	D
	Prnka C	9	10	9	10		C 9 10
	AMEL	X	X	X	X		D
	vWA	16	18	16	18		D
	D8S1179	10	13	10	11		D
	TPDc	8	11	9	11		C 8 11
	FGA	20	22	20	22		C 20 22
	D19S432	14	14				C 14
	D2S1288	16	17	15	16	17	D
1	1120708-10_A08	Rep1	Rep2				
	Marker	Allele1	Allele2	Allele3			Allele1 Allele2 Allele3

No	Sample Name	Marker	Allele1	Allele2	Allele3	Status	Final Gene
	D2S1288	16	17	15	16	17	D
	TH01	6	7	6	7		C 6 7
	D21S11	30	33.2	28	29	30	D
	D18S51	14	15	14	15		C 14 15
	Prnka E	5	12	12			D
	D6S509	9	12	9	10	12	D
	D18S217	10	11	10	11	12	D
	D7S820	8	11	8	10	11	D
	D6S509	9	12	9	10	12	D
	CSF1PO	10	11	10	11	12	D
	Prnka C	9	10	9	10		C 9 10
	AMEL	X	X	X	X		D
	vWA	16	18	16	18		D
	D8S1179	10	13	10	11		D
	TPDc	8	11	9	11		C 8 11
	FGA	20	22	20	22		C 20 22
	D19S432	14	14				C 14
	D2S1288	16	17	15	16	17	D
1	1120708-10_A08	Rep1	Rep2				
	Marker	Allele1	Allele2	Allele3			Allele1 Allele2 Allele3
	D2S1288	16	17	15	16	17	C 17

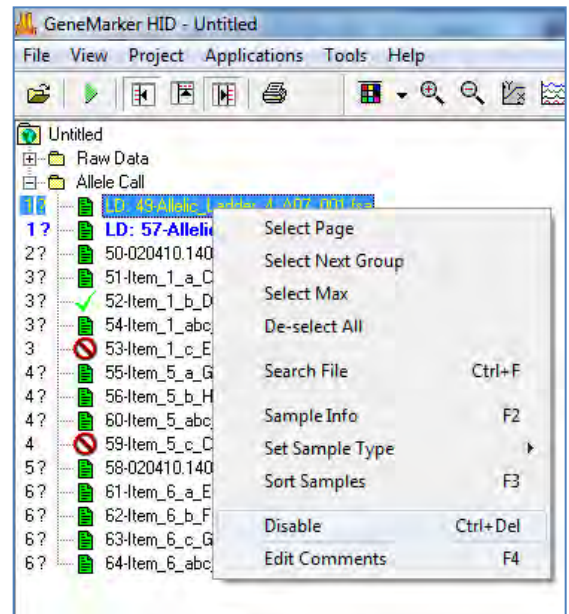
### Disabling Allelic Ladders

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.

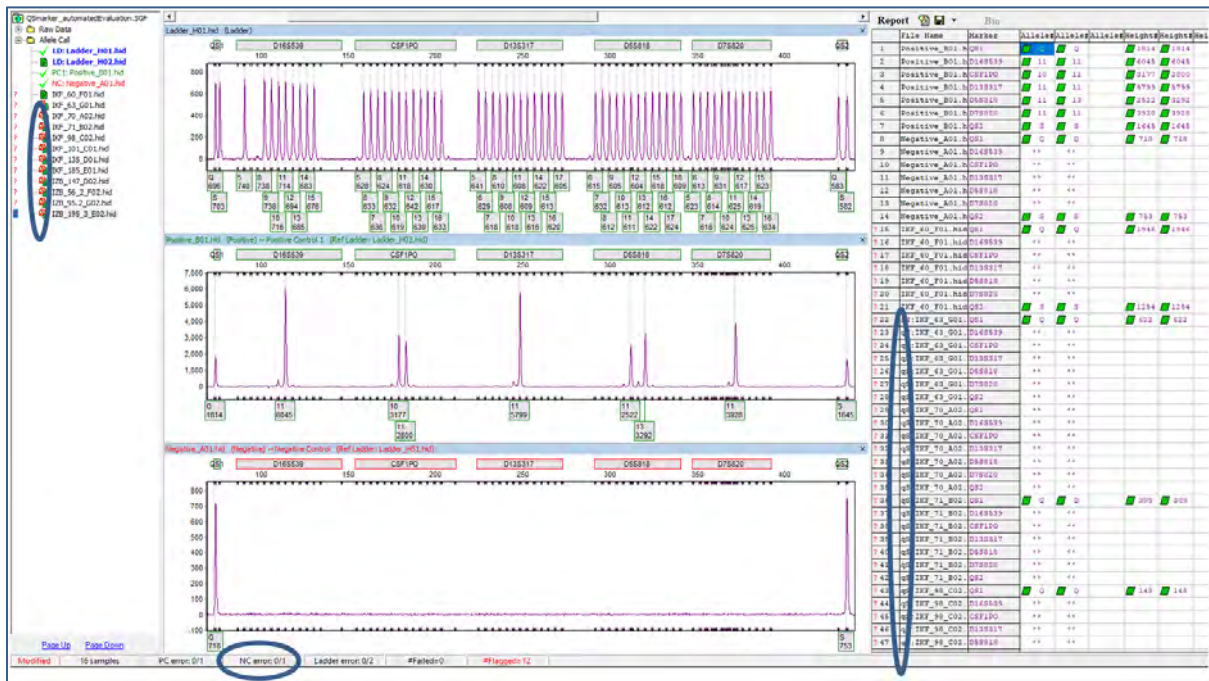
File grouping must be done **after** allelic ladder samples have been disabled. Otherwise, the ladders will be included in the replicate comparison window.



## Quality Sensor Evaluation (QS)

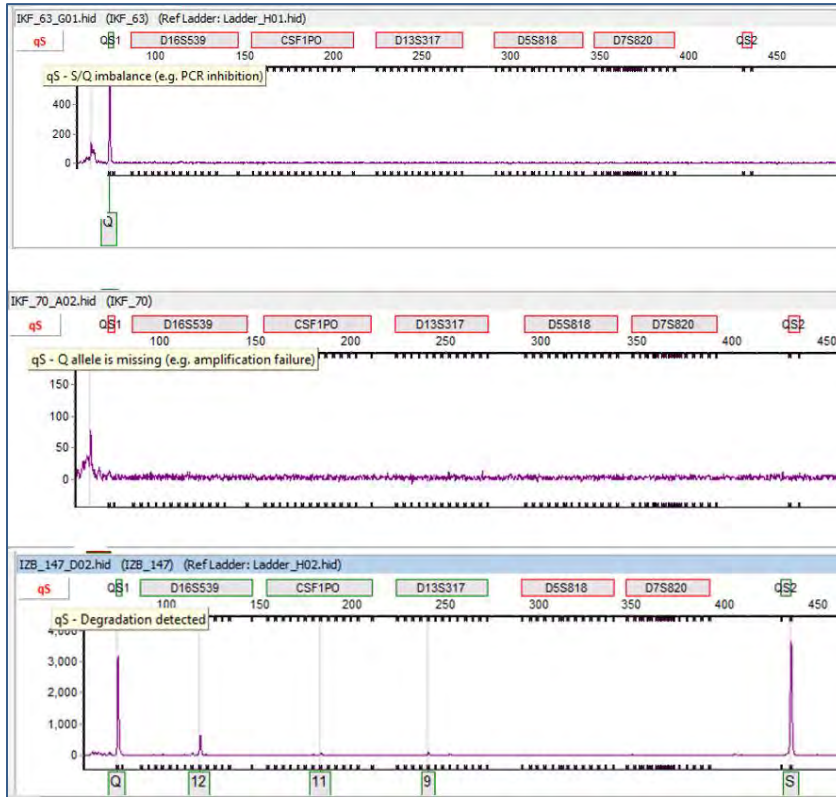
Rules for automated evaluation for quality sensor peaks are applied in the analysis template (run wizard third screen per example in chapter 2 and in the View – Preferences – Sample Quality Tab, per the example in chapter 3.

Overview of the main analysis screen in GeneMarkerHID software: Positive and negative control concordance (note the **negative control has no error** in the summary bar at the bottom of the screen when Q and S fragments are detected), qS flagging of appropriate samples in the file name tree at left, the electropherogram and the report table that is exported to the laboratory LIMS system.

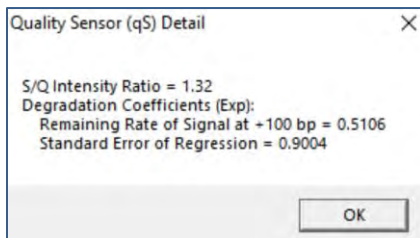




Electropherograms of samples that have the QS rule(s) fired are flagged with **qS** in the upper left corner. A mouse-over will display a pop-up message with the rule fired (see image below).



To display peak evaluation details, mouse click on the **qS** - per this sample electropherogram D02 example with degradation detection:





## Chapter 11 User Management

### **Chapter 11 User Management**

Procedure

User Manager

History

Settings

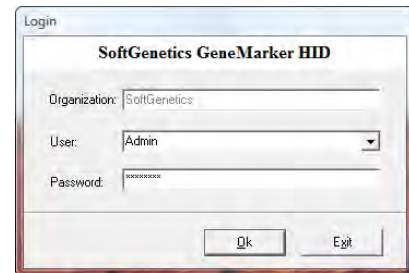
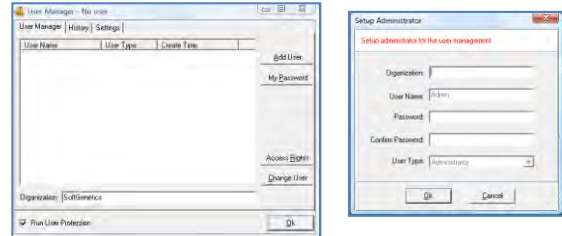
Edit History/Audit Trail

## Overview

User management may be implemented after installation of GeneMarkerHID. 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 GeneMarkerHID 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 GeneMarkerHID 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.

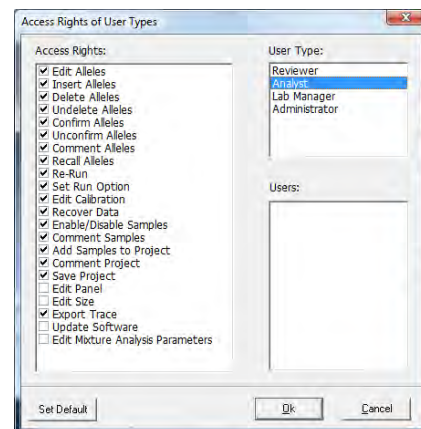
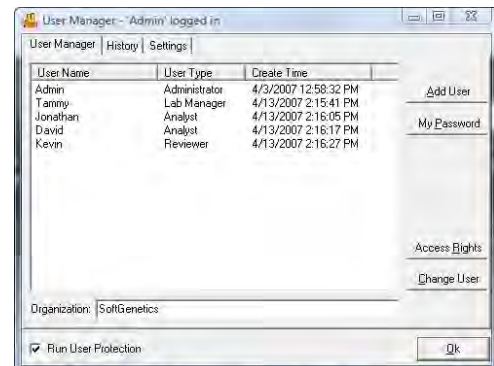
### Access Rights

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

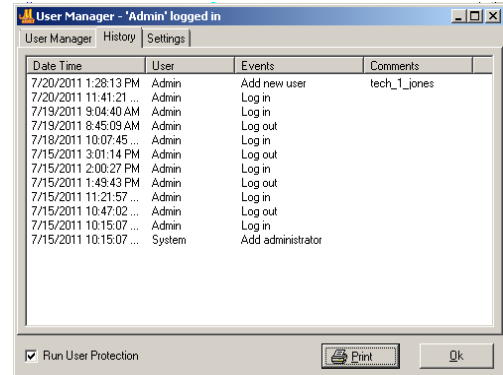
Identifies the username of the person that performed the activity.

### 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		User Management History		7/20/2011 1:28:16 PM
GeneMarker HID V2.1.2				Page 1
Date/Time	User	Events	Comments	
7/20/2011 1:28:13 PM	Admin	Add new user	tech_l_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

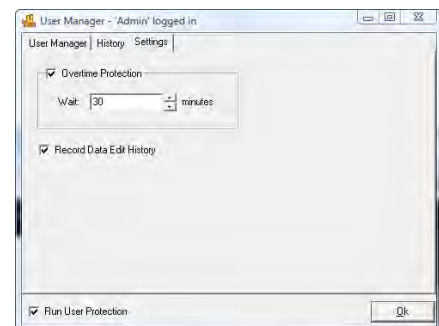
The *User Manager Settings* tab contains additional options for the *User Management* function.

### Overtime Protection

When selected, GeneMarkerHID 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.

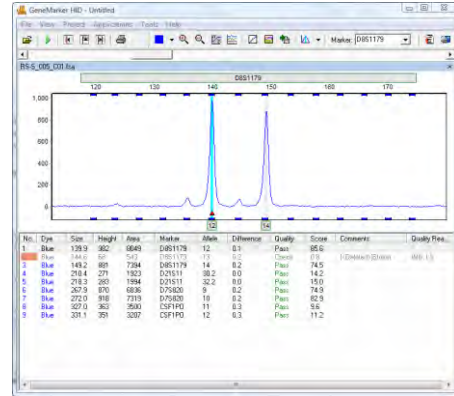


## Edit History/Audit Trail By Sample

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

	Dye	Size	Height	Ht_Ratio	Area	Ar_Ratio	Marker	Allele	Difference	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 pulled	IMB
Old Value	Blue	143.8	567	0.41	3727	0.45	D8S1179	13	0.0	Check	56.7	143.3	144.3		IMB

	Edit Time	Organization	User	Operation	Module	Recover
2	07/20/2011 - 13:19:28	Metro OCME	Admin	Comment Allele	AlleleChart	
1	07/20/2011 - 13:18:03	Metro OCME	Admin	Confirm Allele	AlleleChart	

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

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.

No.	Edit Time	Organization	User	Operation	Module	Recover
1	07/20/2011 - 13:18:03	Metro OCME	Admin	Confirm Allele	AlleleChart	
2	07/20/2011 - 13:19:28	Metro OCME	Admin	Comment Allele	AlleleChart	

## By Project

Select **View - View Project History** to save/print the audit trail of all edits for a project.

Index

**2**

2D OFFSET..... 44  
 2ND DERIVATIVE TRACE..... 14

**A**

ABI IDENTIFIER ..... 64  
 ABIDE BY PANEL ..... 82  
 ACCESS RIGHTS ..... 146  
 ACCOUNT AND PASSWORD .....7, 9  
 ADD FAMILY MEMBERS ..... 117  
 ADD FOLDER..... 12  
 ADD INDIVIDUAL..... 116  
 ADD SAMPLES..... 28  
 ADD SAMPLES TO PROJECT ..... 39  
 ADD USER..... 146  
 ADJUSTING PANELS ..... 66  
 ADMINISTRATOR..... 146  
 AFFECTED STATUS ..... 117  
 AGAROSE GEL ..... 46  
 ALLELE BOUNDARIES..... 68  
 ALLELE CALL ..... 20  
 ALLELE CALL FOLDER ..... 26  
 ALLELE COMMENTS ..... 33  
 ALLELE COUNT REPORT STYLE ..... 78  
 ALLELE EVALUATION ..... 21  
 ALLELE LABEL ..... 35  
 ALLELE LIST ..... 74  
 ALLELIC LADDER..... 21  
 APPLY FILTER TO ALL MARKERS ..... 62  
 APPLY SAMPLE GROUPING ..... 38  
 AUDIT TRAIL..... 148  
 AUTO FIT Y ..... 54  
 AUTO IDENTIFY..... 37  
 AUTO PANEL ADJUSTMENT..... 22  
 AUTO PULL-UP REMOVAL ..... 14  
 AUTO RANGE.....18, 20  
 AUTO RUN ..... 39  
 AUTO SCALE MARKERS.....44, 82  
 AUTO SELECT BEST LADDER ..... 22  
 AUTOMATED PEDIGREE DRAWINGS ..... 103  
 AUTOMATIC PANEL CREATION ..... 65  
 AUTOMATICALLY RE-SORT REPORT..... 38  
 AUTOMATICALLY SCROLL CHARTS TO ALLELES WHEN SELECTED IN  
 REPORT ..... 38

**B**

BASILINE SUBTRACTION..... 14, 15, 18, 19  
 BC = BIN CONFLICT ..... 31  
 BESTMATCH..... 48  
 BESTMATCH MATCH ALL ..... 50  
 BESTMATCH MATCH SELECTED..... 50

BIN BOUNDARY.....63  
 BIN OPTIONS.....63  
 BIN RANGE.....60  
 BIN TABLE .....75  
 BINNING ..... 60, 62  
 BINS.....71  
 BROWSE BY ALL COLORS.....42, 107, 112  
 BROWSE BY ALL COLORS .....43

**C**

CALIBRATION PLOTS .....54  
 CALL ALLELE .....42  
 CALL ALLELE ICON .....23  
 CHANGE PASSWORD.....146  
 CHANGE USER .....146  
 CHART OVERLAY.....81  
 CHART SETTINGS .....36  
 CHART SYNCHRONIZE .....54  
 CHECK RANGE IN EDIT.....68  
 CLASSIC.....35  
 CLIENT COMPUTER ..... 8  
 CLOSE ALL .....34  
 CMF 1.0 ..... 39, 85  
 CMF 3.0 ..... 39, 85  
 CODIS REPORT ..... 39, 85  
 COLOR MATRIX.....15  
 COMBINED PROBABILITY (CPI).....98  
 COMBINED PROBABILITY OF EXCLUSION (CPE) .....98  
 CONFIDENCE LEVEL .....21  
 CONFIRM PEAKS.....34  
 CONFIRM/UNCONFIRM ALLELE .....32  
 CONTACT US .....10  
 CONTAMINATION CHECK.....129  
 CONTROL CONCORDANCE ..... 127, 128  
 CONTROL GENE .....63  
 CONTROL IDENTIFICATION ..... 134, 135  
 CONTROL IDENTIFIER .....135  
 CONTROL MATCH MODE .....135  
 CONVERT MACINTOSH FILE FORMATS ..... 6  
 CONVERT TEXT TO BINARY .....137  
 CONVERT TEXT TO BINARY FILES .....41  
 CREATE BIN.....63  
 CREATE MARKER .....59  
 CREATE NEW PANEL .....65, 67, 68  
 CREATE NEW SIZE STANDARD .....50  
 CUBIC SPLINE METHOD .....20  
 CURRENT/OLD VALUES .....148  
 CUSTOM PANEL CREATION .....65  
 CUSTOM SIZE STANDARD CREATION .....49  
 CUSTOMIZE BIN COLUMN .....43

**D**

DATA FILE LIST .....12

DATA PROCESSING .....	21, 22
DATABASE SEARCH .....	93, 103, 108
DECIMAL PRECISION .....	35
DEDUCING MISSING PARENT GENOTYPE .....	116
DELETE BIN .....	63
DELETE INDIVIDUAL .....	116
DELETE MARKER.....	63, 67
DELETE PANEL.....	59, 67
DELETE PEAKS .....	34
DELETE SIZE.....	47
DELETE SIZE STANDARD .....	47, 49
DELETE/UNDELETE ALLELE .....	32
DIFFERENCE .....	31
DISABLE SAMPLES .....	28
DISABLED SAMPLES .....	38
DISABLED SIZE COLUMNS .....	52
DISPLAY SETTINGS.....	35
DISTANCE/KB.....	64

**E**

EDIT ALLELE.....	32
EDIT BIN.....	63
EDIT BINS .....	62
EDIT HISTORY .....	148
EDIT HISTORY LIST.....	148
EDIT INDIVIDUAL.....	116
EDIT MARKER BINS .....	62
EDIT PANEL .....	59
EDIT SIZE .....	47
EDITING PEAKS.....	32, 33
EDITING SIZE CALL.....	53
ELECTROPHEROGRAM.....	28
ELECTROPHEROGRAM TRACE DISPLAY.....	29
ENABLE SAMPLE GROUPING.....	38
ENHANCED BASELINE SUBTRACTION .....	19
ENHANCED SMOOTH.....	18
EVENT LOG.....	43
EXIT .....	34
EXPORT ABI SIZE STANDARD .....	50
EXPORT CALIBRATION DATA .....	53
EXPORT CODIS.....	39, 85
EXPORT ELECTROPHEROGRAM .....	41
EXPORT ELECTROPHEROGRAM .....	138
EXPORT PANEL .....	59, 67
EXPORT SIZE STANDARD .....	47, 50
EXPORT THE PROJECT PANEL .....	68
EXPORT TO SCF.....	137
EXPORT TO SG1 .....	137
EXTEND DIPLOID HOMOZYGOUS .....	74

**F**

FAMILY .....	106, 112
FAMILY NAME.....	116
FATHER .....	117
FILE LABELING CONVENTIONS .....	36
FILE NAME GROUP .....	134

FILE NAME GROUP TOOL .....	40, 134, 138
FILE TYPES .....	12
FILENAME GROUP EDITOR.....	134
FILTERING PARAMETERS .....	60
FIX SIZE.....	53
FIXED BIN WIDTH .....	62
FORENSIC PREFERENCES TAB.....	36
FORENSICS REPORT STYLE .....	74
FRAGMENT MOBILITY .....	46
FRAME .....	18

**G**

GEL IMAGE .....	68
GEL IMAGE DISPLAY .....	29
GEL IMAGE DISPLAY SETTINGS.....	36
GENDER .....	117
GENEMARKER HID LOCAL VERSION.....	6, 8
GENEMARKER HID NETWORK VERSION.....	8
GENERAL SETTINGS .....	35
GROUP IDENTIFICATION.....	134, 135
GROUPED BY DYE .....	82

**H**

HETEROZYGOUS IMBALANCE (HIM) .....	98
HI = HIGH INTENSITY .....	32
HIDE EXTRA SAMPLE NAMES .....	74
HIDE TOGGLES .....	42
HORIZONTAL MOVEMENT .....	28
HORIZONTAL REPORT TABLE .....	75

**I**

IHE = INCONCLUSIVE HETEROZYGOUS .....	32
IHO=INCONCLUSIVE HOMOZYGOUS .....	32
ILS STATISTICS .....	52
IMB = HETEROZYGOTE IMBALANCE .....	32
IMPLEMENT Y AXIS SETTINGS.....	82
IMPORT A PANEL .....	17
IMPORT ABI PANELS .....	65, 67
IMPORT ABI SIZE STANDARD .....	50
IMPORT DATA FILES .....	12
IMPORT PANELS .....	67
IMPORT PANELS FROM GENEMAPPER .....	65
IMPORT PRE-DEFINED PANELS .....	67
IMPORT SIZE STANDARD .....	49
INDIVIDUAL ID .....	117
INSERT ALLELE .....	32
INSERT SIZE .....	47
INSTALLATION .....	6
INSTALLATION WIZARD .....	6, 8
INTENSITY .....	20
INTERNAL LANE STANDARD (ILS) .....	46

**K**

KINSHIP ANALYSIS.....	103, 104
-----------------------	----------



KINSHIP ANALYSIS EQUATIONS ..... 104

## L

LABEL DYES & PEAK NUMBERS..... 81  
 LADDER IDENTIFIER.....22, 37  
 LADDER LABEL..... 37  
 LANE SCORE ..... 52  
 LINE LIST ..... 44  
 LINKED USER MANUAL..... 41  
 LINUX ..... 6  
 LO = LOW INTENSITY ..... 31  
 LOCAL SOUTHERN ..... 20  
 LOGIN ..... 146  
 LOW TEMPLATE FLAGGING ..... 61  
 LS = LOW SCORE ..... 31

## M

MACINTOSH ..... 6  
 MAGIC WIZARD .....41, 43  
 MAJOR ADJUSTMENT OF PANEL..... 69  
 MAJOR MX..... 98  
 MANUAL CALIBRATION..... 55  
 MANUAL PANEL CREATION..... 65  
 MANUAL PULL-UP CORRECTION..... 14  
 MANUAL SELECTION OF RANGE..... 20  
 MARK OFF-ALLELE AS 'OL' ..... 35  
 MARKER BOUNDARY ..... 60  
 MARKER DROP-DOWN MENU ..... 42  
 MARKER OPTIONS ..... 59  
 MARKER PARAMETERS ..... 60  
 MARKERS ..... 71  
 MATCH BY FIXED POSITION ..... 135  
 MATCH BY SECTIONS ..... 135  
 MATCH LADDER ..... 68  
 MATCH SCORE ..... 48, 50, 51, 52, 53, 54  
 MATCHED GROUPS ..... 135  
 MAX # OF OPEN CHARTS ..... 36  
 MAX & AVERAGE ..... 68  
 MAX ALLELE LABEL LAYERS..... 36  
 MAX CHART # IN PAGE ..... 36  
 MAXIMUM IMBALANCE ..... 61  
 MENU OPTIONS ..... 34  
 MERGE BINS..... 77  
 MINIMUM COMPUTER REQUIREMENT ..... 6  
 MINIMUM HETEROZYGOTE INTENSITY ..... 60  
 MINIMUM HOMOZYGOTE INTENSITY..... 60  
 MINIMUM IMBALANCE..... 61  
 MINOR ADJUSTMENT OF PANEL..... 69  
 MIX DYES ..... 81  
 MIXTURE ANALYSIS ..... 40, 87, 90  
 MIXTURE ANALYSIS DATABASE SEARCH..... 92  
 MIXTURE ANALYSIS EQUATIONS ..... 98  
 MIXTURE ANALYSIS LIKELIHOOD RATIOS..... 99  
 MIXTURE ANALYSIS OVERVIEW ..... 88  
 MIXTURE ANALYSIS PROCEDURE ..... 88  
 MIXTURE ANALYSIS RESULTS ..... 91

MODIFY SIZE STANDARD .....48  
 MOTHER .....117

## N

NAVIGATION .....28  
 NEGATIVE CONTROL .....37  
 NEGATIVE CONTROL CONCORDANCE .....129  
 NEW PAGE FOR EACH SAMPLE .....82  
 NEW PEDIGREE FILE ..... 106, 111  
 NEW SIZE STANDARD CREATION .....49  
 NEW TEMPLATE .....17  
 NUCLEOTIDE REPEAT ..... 60, 62  
 NUCLEOTIDE REPEATS .....61

## O

OFF LADDER PEAKS .....55  
 OFF LADDER PEAKS .....71  
 OFFLINE REGISTRATION ..... 7, 9  
 OL = OFF LADDER .....31  
 ONLINE REGISTRATION ..... 7, 9  
 OPEN DATA ..... 34, 42  
 OPEN DATA FILES .....12  
 OPEN PEDIGREE FILE ..... 106, 111  
 OPEN PROJECT..... 34, 86  
 OUTPUT TRACE DATA ..... 41, 136  
 OVERLAY TRACE .....59  
 OVERTIME PROTECTION .....147

## P

PANEL .....17  
 PANEL CREATION .....64  
 PANEL EDITOR ..17, 20, 22, 31, 32, 40, 57, 58, 59, 64, 65, 67,  
 68, 69, 74, 78, 86, 109  
 PANEL LIST.....58  
 PANEL TABLE .....63  
 PANELS AND BINS FILES .....64  
 PASSWORD .....146  
 PATERNITY INDEX (PI) .....118  
 PEAK COMPARE ITEMS .....137  
 PEAK COMPARISON THRESHOLD .....137  
 PEAK DETECTION THRESHOLD .....20  
 PEAK INFORMATION .....34  
 PEAK LABEL.....36  
 PEAK MATCHED BY .....137  
 PEAK SATURATION .....19  
 PEAK SCORE .....21  
 PEAK TABLE ..... 30, 148  
 PEAK TABLE COLUMNS .....77  
 PEAK TABLE REPORT STYLE.....77  
 PEDIGREE .....39  
 PEDIGREE ANALYSIS .....121  
 PEDIGREE DRAWING AUTOMATION .....114  
 PEDIGREE FILE NAME MATCH .....40  
 PEDIGREE PARAMETERS..... 106, 111  
 PERCENTAGE .....20

Index

PL = BEYOND PLOIDY ..... 31  
 POSITIVE CONTROL..... 37  
 POSITIVE CONTROL TEMPLATE EDITOR..... 40, 128  
 PRE-DEFINED PANELS ..... 64  
 PRE-DEFINED SIZE STANDARDS..... 48  
 PREFERENCES BOX ..... 35  
 PREPROCESS RAW DATA ..... 54  
 PRINT ..... 84  
 PRINT ALLELES ..... 82  
 PRINT MARKERS..... 82  
 PRINT PAGE SETUP ..... 85  
 PRINT PROJECT COMMENTS..... 81  
 PRINT REPORT ..27, 35, 39, 41, 42, 44, 73, 79, 80, 81, 82, 84, 85, 87  
 PRINT REPORT CONTENT OPTIONS ..... 81  
 PROBABILITY OF EXCLUSION (PE) ..... 98  
 PROBABILITY OF INCLUSION (PI)..... 98  
 PROFILE COMPARISON VIEW..... 39, 44  
 PROJECT COMMENTS ..... 39  
 PROJECT COMPARISON ..... 40, 136  
 PROJECT COMPARISON SETTINGS ..... 136  
 PROJECT PANEL ..... 58  
 PROMEGA POWERPLEX..... 64  
 PULLUP CORRECTION ..... 15

**Q**

QUALITY..... 31  
 QUALITY REASONS ..... 31  
 QUALITY SENSOR EVALUATION ..... 143

**R**

RAW DATA ANALYSIS..... 18  
 RAW DATA FOLDER ..... 26  
 RAW DATA MAIN ANALYSIS WINDOW ..... 13  
 RAW DATA TRACES ..... 13  
 RE-ANALYZE INDIVIDUAL SAMPLES..... 23  
 RE-ANALYZE WITH RUN WIZARD ..... 22  
 RECORD DATA EDIT HISTORY ..... 147  
 RECOVER OLD VALUE..... 148  
 REFRESH PEDIGREE..... 106, 111  
 REGISTRATION..... 7  
 REGISTRATION ID .....7, 9  
 RELATIONSHIP TESTING..... 39  
 RELOAD PANEL..... 59  
 RELOAD SIZE STANDARD ..... 47  
 REOPEN PROJECT ..... 86  
 RE-OPEN PROJECT ..... 34  
 REPLICATE COMPARISON..... 41  
 REPLICATE COMPARISON TOOL ..... 138  
 REPORT SETTINGS ..... 43  
 REPORT SETTINGS ICON ..... 33  
 REPORT SETTINGS PREFERENCES TAB ..... 38  
 REPORT STYLE ..... 74  
 REPORT TABLE ..... 33, 74  
 REPORT TABLE LAYOUT ..... 75  
 REPORT TABLE ORIENTATION ..... 75

RESIDUAL .....98  
 RMNE .....98  
 RUN..... 39, 41  
 RUN METHOD .....35  
 RUN PROJECT ..... 17, 42  
 RUN USER PROTECTION .....146  
 RUN WIZARD ..... 17, 35  
 RUN WIZARD ADDITIONAL SETTINGS .....21  
 RUN WIZARD DATA PROCESS .....18  
 RUN WIZARD TEMPLATE SELECTION .....17

**S**

SAMPLE FILE TREE.....26  
 SAMPLE GROUPING .....28  
 SAMPLE ILS ..... 48, 53  
 SAMPLE INFORMATION.....27  
 SAMPLE SCORE .....52  
 SAMPLE SEARCH OPTIONS.....27  
 SAMPLE SORTING OPTIONS .....27  
 SATURATED PEAK CORRECTION .....16  
 SATURATION CORRECTION .....14  
 SAVE AS NEW PANEL .....67  
 SAVE AS NEW SIZE STANDARD .....49  
 SAVE CHANGES .....50  
 SAVE CHANGES TO PANEL .....68  
 SAVE PEAK TABLE ..... 32, 42  
 SAVE PEDIGREE FILE ..... 106, 111  
 SAVE PROJECT ..... 34, 86  
 SAVE REPORT .....43  
 SAVE REPORT TABLE.....34  
 SAVE RUN WIZARD PARAMETERS .....38  
 SCF FILE.....137  
 SCORE .....31  
 SECOND DERIVATIVE TRACE.....16  
 SERVER COMPUTER ..... 8  
 SET AXIS ..... 42, 43, 107, 112  
 SET SAMPLE TYPE .....37  
 SG1 FILE .....137  
 SGF FORMAT.....86  
 SHOW 3D.....44  
 SHOW CHART/TABLE.....42  
 SHOW COLOR ..... 42, 96, 107, 112  
 SHOW COLUMNS .....30  
 SHOW CONFLICT..... 106, 111  
 SHOW CONTROL SAMPLES IN REPORT .....37  
 SHOW DISABLED SAMPLES IN REPORT .....38  
 SHOW GEL IMAGE.....35  
 SHOW INDIVIDUAL NAME ..... 106, 111  
 SHOW LADDER SAMPLES IN REPORT .....36  
 SHOW LAST EVENT .....41  
 SHOW NAVIGATOR .....35  
 SHOW ONLY UNCERTAIN ALLELES .....74  
 SHOW REJECTED LOW SCORE ALLELES .....74  
 SHOW REPORT .....35  
 SHOW TOGGLES .....42  
 SHOW TYPE SYMBOL .....75  
 SHOW/HIDE ICONS .....26

## Index

SIZE CALIBRATION .....	42, 54
SIZE CALIBRATION CHARTS .....	51
SIZE CALL.....	20
SIZE CALL METHODS .....	20
SIZE EDITOR ACTION HELP.....	50
SIZE MATCH .....	50
SIZE STANDARD.....	18
SIZE STANDARD .....	86
SIZE STANDARD LIST .....	46
SIZE STANDARD STATISTICS.....	52
SIZE TEMPLATE EDITOR.....	18, 40, 46, 50
SMOOTH .....	14, 15, 18
SOFTGENETICS WEBSITE .....	41
SOFTWARE VERSION .....	41
SP = SATURATED (PULL- UP) .....	31
SPIKE REMOVAL .....	14, 16
SR = SATURATED (REPAIRED) .....	31
STANDARD COLOR.....	18
START UP SETTINGS .....	35
START YOUR PROJECT .....	41
START/END.....	31
STUTTER .....	61
STUTTER FILTER .....	61, 62
SYNTHETIC GEL IMAGE .....	28
SYSTEM REQUIREMENTS .....	6

## T

TECHNICAL SUPPORT.....	41
TEMPLATE NAME .....	17
TRACE MODE.....	68

TRACE OVERLAY .....	68
---------------------	----

## U

UNIX.....	6
UPDATE CALIBRATION.....	53
USB KEY .....	6, 8
USB PORT .....	6, 7, 9, 10
USE LAST TEMPLATE.....	17
USE SIZE STRING FOR LABEL.....	35
USER ID .....	7, 9
USER MANAGEMENT .....	41, 146
USER MANAGER HISTORY .....	147
USER MANAGER SETTINGS .....	147

## V

VERTICAL REPORT TABLE .....	75
VIEW HISTORY .....	33, 148
VIEW MODE.....	54
VIRTUAL OR VARIANT ALLELES IN PANELS .....	64
VIRTUAL PANEL.....	68
VPANEL_PANELNAME.....	68

## Z

ZOOM IN .....	42, 107, 112
ZOOM IN/OUT .....	28
ZOOM OUT .....	42, 107, 112