# GeneMarker<sup>®</sup> HID STR Human Identity Software

- NDIS Approved Expert System
- Fast, Accurate, and User-friendly
- ~ Documented time savings of up to 40%<sup>1</sup>
- ~ Up to 70% less analyst intervention<sup>2</sup>
- User Management with Audit Trail
- Mixture Analysis
- Relationship Testing
  - ~ Kinship

Genetic Analysi

PowerTools for

Software

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- ~ Paternity
- Database Search Tools:
  - ~ Missing Persons Search
  - ~ Mass Disasters

State DNA Index System (SDIS) Search

- Quality Control and Validation Tools
- Compatible with all Major CE systems, Rapid instruments, and Human ID Chemistries.

# You will see the difference!



**GeneMarker®HID Software is an NDIS approved expert system** designed for human identification and forensics applications. GeneMarkerHID Software offers several advantages including time savings<sup>1</sup>, ease of use<sup>2</sup>, and a suite of integrated post genotyping applications, thereby making it an excellent alternative to Genotyper<sup>®</sup>, GeneMapper<sup>®</sup>, GeneMapper<sup>®</sup>IDX, and other fragment analysis programs.

#### **Features:**

NDIS Approved expert system Accurate Size/Allele Calls Linked Navigation Running Audit Trail Automated Control Concordances All Major Chemistries

#### **Project Review Tools:**

All Color Browser Panel Editor Replicate Comparison Tool Project Comparison Tool CODIS Compatible Export Contamination Check/Elimination Database Search

#### **Human ID Applications:**

Allele Specific Stutter Filters Database Searching Mixture Deconvolution Percent Match Search Kinship Analysis Paternity Testing

GeneMarkerHID Software is compatible with all major commercially available Human Identity chemistries, as well as CE instruments, and Rapid Systems (Including 6 dye chemistries). The program's built-in panel editor tool also provides extensive support for custom chemistries. GeneMarkerHID Software can be run on Windows<sup>®</sup> Vista, 7, 8, and 10 Operating Systems.



# **User Operation**

Thanks to its linked navigation and 'point and click' interface, GeneMarkerHID Software is easy to use for experts and beginners alike. The "Run Wizard" simplifies parameter setting by guiding the user through three simple steps, thereby making repetitive analyses quick, easy, and accurate. The user may select one of several preloaded templates, or create and save their own. GeneMarkerHID Software's patented pattern recognition technology automatically corrects for most chemistry errors, including peak saturation, noise, bleed-through, instrument spikes, and stutter, thereby reducing user intervention by up to 70%<sup>2</sup>.



The three pages of the Run Wizard allow the user to customize the analysis parameters for each project. Groups of settings can be saved as templates for future analyses.

#### **Main Analysis Window**

After Data Processing, results are displayed in the Main Analysis Window. Colored icons on the left indicate the success of size calling, while colored icons in the report table indicate the strength of a given allele call. Flags alert the user to any fired rules. Flexible reporting options to meet laboratory LIMS requirements and export to probabilistic software programs.

The main analysis window: Colored icons indicate the success of size calling (left) and allele calls (right). The linked electropherogram in the center of the screen allows the user to edit, delete, and confirm calls.

Linked navigation allows the user to quickly and easily survey the results. A simple right-click allows

the user to insert, delete, and edit alleles – actions that are all recorded by the software. Display options allow the user to customize what information is shown. The Main analysis window also serves as a portal to other tools and post-genotyping applications discussed below.

### All Color Browser

The All Color Browser is another viewing option that allows the user to see every dye channel simultaneously. This view is particularly useful for detecting pull up and other artifacts, as well as making edits or comments.



#### **Automated Panel Calibration**

During data processing, GeneMarkerHID Software automatically calibrates the selected genotyping panel using Allelic Ladder samples that have been uploaded into the project. If multiple ladders have been uploaded, the program selects the best ladder for each sample.



The Panel Editor tool allows easy visualization and organization of genotyping panels. During panel calibration, panel bins are automatically aligned to ladder peaks, increasing accuracy.

### **Marker-Specific Parameters**

In addition to the analysis settings discussed in the Run Wizard,GeneMarkerHID Software fully supports Marker-Specific analysis thresholds including:

- Homo and Heterozygote RFU thresholds
- Inconclusive (Stochastic) Ranges
- . Heterozygous Imbalance thresholds
- Stutter Filters

These settings allow the user to fully customize their analysis parameters for each marker and save them for future analyses.

# Allele Specific Stutter Capability

Allows incorporation of Allele specific stutter values from validation studies. Especially beneficial for markers with complex repeat patterns and LUS effects.

> Marker specific settings for SE33 Select to apply specific stutter filters. Marker stutter is grayed out when allele specific is selected.

#### **User Management and Audit Trail**

GeneMarkerHID Software is equipped with an extensive user management system and built-in audit trail. User management provides control of user access rights and generates an edit history for each sample profile in the project. Access rights for each user are set by the administrator – thereby providing assurance that unauthorized individuals don't accidentally modify or alter a project or settings. User Management is also linked with reports – report headers contain the analyst, date, institution name, and project information. Password control is also available, requiring users to log in with their password before opening the program.

#### **Reporting Options**

GeneMarkerHID Software supports numerous electronic and print reporting options, including:

- Complete PDF Reports
- Excel/Text table Reports
- CODIS Compatible Exports
- Importation to LIMS system

GeneMarkerHID Software's extensive reporting customization options allow a vast array of different reporting formats and layouts. Print templates enable the user to save groups of settings under a single template name, enabling effortless printing of common reports.

Above is one of many possible print reports that may be generated in GeneMarkerHID Software. Detailed print settings (right) allow extensive customization of reports. Templates can be created and recalled for frequently used formats.







# **Linked Applications**

#### **Mixture Analysis Tool**

GeneMarkerHID Software comes preloaded with a Mixture Analysis Tool. The tool can completely deconvolute mixtures with two contributors, and can calculate the probability of inclusion for mixtures with three contributors. In addition, GeneMarkerHID Software's mixture analysis tool automatically:

- . Identifies the number of potential contributors in each mixture
- Displays all possible allele combinations at each locus
- Performs all PI, PE, LR, and RMNE calculations
- . Ranks the most likely genotype combinations (with or without single source reference profiles)
- Deconvolutes major and minor contributors from mixture
- Searches database for consensus contributor genotypes

Using the 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, the mixture analysis application automatically determines the most likely genotype combination at each locus, and then determines the most probable contributor genotypes (major and minor).

If no files in the current project match a contributor genotype, the user may query GeneMarkerHID Software's built-in relationship testing database for possible matches. In addition, consensus contributor genotypes may be uploaded to the database for future searches.



In the main analysis window, sample names are colored according to the sample type. Mixture profiles are displayed in olive-green, negative controls in red, positive controls in green, and ladder profiles in blue.

In the Mixture Analysis Application, peaks are visually displayed in the trace comparison, while the peak table contains all data including PI, PE, LR, and RMNE. The peak table may be exported as .txt tab delimited or excel file. All results are presented in report tables that may be saved or copy/pasted into existing documents.

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The mixture analysis application: Possible genotype combinations are shown on the left, and PI, PE, and LR values are calculated on the right. The dialogs in the bottom left allow the user to change which individuals are being contested.

### **Database Searching**

GeneMarkerHID Software's linked, searchable database enables hundreds of thousands of profiles to be imported into the program and saved for future searches. Genotypes can be imported from the current project, from a text file, or from CODIS .cmf files. This lends itself to numerous applications, including:

- Missing Persons Search
- SDIS Search
- Mass Disaster Identification
- Crime Scene Suspect or Familial Search
- . Kinship Analysis Statistics for Three Generations

Using the Relationship Testing application, the user may query a sample against the database for potential matches across different populations at varying relationship levels. For each potential match, an Identity by Descent (IBD) calculation is used to determine the likelihood ratio of each possible relation to the test sample. Calculation details and the electropherogram are easily accessible for analyst review.

Genotypes can be imported into the database directly from the current project, limiting data transfer; they may also be imported from CODIS or text files.

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The Database Search Tool: Profiles may be tested against a database of known genotypes. Likelihood Ratios (LR = PI/KI) are displayed for the most likely candidate at each relationship level.

The linked kinship analysis tool allows direct comparison of two individual profiles on a variety of different relationship levels: siblings, parent-child, uncle-nephew, and so on. Likelihood ratios and probabilities are available for analyst review and can be saved as a text file, an excel file, or may be copy/pasted.

The built-in Kinship Analysis tool allows the user to calculate probabilities and likelihood ratios across three generations. Report tables may be saved as .txt tab-delimited files, or may be copy/pasted into an existing document.

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VWA.	16	18	15	18	1.81249	1.05624	1.30624	1.39624	1.15/12	1.30624
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# **Paternity Testing**

GeneMarkerHID Software's paternity testing application is a versatile tool with many capabilities, including:

- Automated (and manual) Pedigree Drawing
- Identity By Descent (IBD) Calculations
- AABB paternity trio and motherless case calculations
- Reverse Parentage Testing
- Easy visualization of results
- Separate Kinship Analysis tool

The relationship testing module is embedded within GeneMarkerHID Software, allowing the user to proceed directly from data processing to paternity and/or kinship analysis. Profiles from the current project are used to automatically construct pedigrees and family trees. Conflicting genotypes are automatically flagged and linked to an electropherogram comparison for user review.



An example pedigree demonstrating exclusion: Conflicting alleles are highlighted in red. The pedigree is linked to an electropherogram, allowing the user to review potential conflicts in more detail.

The user may select one of several preloaded allele frequency tables or create their own to contest individuals within their pedigrees. Paternity Index (PI), Probability of Exclusion (PE), and Probability of Paternity (POP) calculations are available for this purpose. A wide variety of settings allow the user to customize the procedure, and take into account variables such as mutation rates and step-wise mutations.

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An example pedigree demonstrating inclusion: PI, POP, and PE scores are calculated for each marker. The table can be copied directly or exported as a text file.

# **Percent Match and Cell Line Authentication Tool**

The percent match tool uses a percent-similarity calculation to compare profiles in the current project to a database of known genotypes. This lends itself to several applications including the following:

#### Cell Line Authentication

Results are ranked in order of their percent match score, with differences highlighted in yellow. Results can be exported in the form of an excel or text file.

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01/5549	10	13	2	1						
01/5833	11	11	2	1						
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The Percent Match Tool: After querying a sample against the database, results will be ranked on the right according to their percent match score. Here the tool is being used to demonstrate that all peaks from a Y-STR profile are included in a mixture sample.

#### **Contamination Check/Elimination Database Search**

- Y-STR Comparison
- Database Search when allele frequencies are not available

Improvements to the sensitivity of the polymerase chain reaction (PCR) have necessitated increased diligence in detecting possible contaminants. Contamination may jeopardize data analysis and can originate from many common sources, particularly from the skin or hair of a staff member. GeneMarkerHID software solves this problem by enabling the user to create a database of staff profiles as an elimination database. This database can then be compared against profiles in the current project to search for any contamination. Profiles of the current project may also be compared to one another to detect possible profile-to-profile contamination.

Exclude Sample Setting   Image: Ladder Image: NC Image: PC   Image: With greater than 0 null Markers	Similarly Ratio Export Greater than 60 % Specified Sample as Comparison	Match Type Within	Project atabase Contamination 🔄	Display Name File Name Sample Name		
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The contamination check tool provides several options for customization. The user can choose to exclude different sample types, including negative controls, postive controls, ladders, and samples with a user-specified number of null alleles. The user may also choose to perform the comparison between all samples, or a specific sample. Finally, the user can choose to compare profiles to profiles within the project, to samples within a database, or both.

# **Quality Control and Validation Tools**

# **Project Comparison Tool**

The project comparison tool compares two completed projects to one another, enabling easy identification of any inconsistencies. This can provide significant time savings when comparing projects completed by two different analysts, or when performing a validation between two different procedures.

Related projects are opened in a single screen; differences detected in the parameters of interest are highlighted in the report table and linked to the electropherogram. The tool reduces time required for SOP and analysis system validation.

The Project Comparison Tool: Projects are displayed side by side with differences flagged in yellow.



# **Replicate Comparison Tool**

The Replicate Comparison Tool automates replicate comparison and determination of consensus genotypes. This tool is a valuable companion to genotyping applications, and any chemistry that has elevated risk of allele dropout and other artifacts. The tool groups replicates together, and automatically compares each replicate based on a number of factors, including peak call, size, height, and score.

Flagging alerts the user to any discrepancies, at which point the user may manually enter the desired allele call, or select none if a consensus cannot be determined. Otherwise, if each replicate has the same call, the program automatically selects that call as the consensus genotype. Linked navigation and sorting features allow the user to quickly and efficiently navigate through the results, which can be exported as a text or excel file. The replicate comparison tool can also accommodate larger projects with up to 5 replicates per sample.

The replicate comparison tool: A peak comparison is displayed on the left, while sample genotypes are displayed on the right. Flagging indicates whether or not the calls are concordant based on user defined parameters, and warns of possible mixtures or contaminants.



# **Automated Control Concordances**

Any control samples are flagged in the file name tree and in the summary bar at the bottom of the main analysis screen if they differ from the expected genotype.

#### **Positive controls**

Customize the analysis parameters with up to 2 different positive control genotypes. The setting accomodates SOPs that use one DNA source for an amplification positive control and a different DNA source for the extraction positive control.

Negative controls will be flagged if any peaks are detected above the analytical threshold.



#### **Quality Sensor Evaluation**

Settings are available to automatically evaluate the Quality Sensor fragments to assist labs in trouble-shooting problematic samples. Samples are flagged qS in the file name tree and electropherogram if the Quality Sensor fragments fire any of the rules indicating potential 1) amplification failure, 2) inhibition or 3) degradation.



The figure illustrates typical results of the automated quality sensor evaluation. To display a pop-up message of the rule fired, mouse over the qS at the electropherogram. The first electropherogram is a passing negative control, the only peaks detected are the Q and S alleles. The middle electropherogram has Q/S peak imbalance, indicating potential inhibition. The third electropherogram has balanced Q/S fragments but an exponential decrease in total peak height with increasing fragment size, indicating potential degradation.

Please open disc to review your applications of interest and to install a 30-day trial of GeneMarkerHID Software.

#### **Disk Contains**

Applications Notes 30-day free GeneMarkerHID Trial User Manual Demonstration Data

#### **Minimum Hardware Requirements**

#### PC

32 or 64 bit Windows® Operating System: 7, 8, or 10 Processor: Pentium III, 1GHZ RAM: 128MB 20GB Minimum available hard disk space.

### Intel Powered Macintosh Computer

Virtual Windows Machine running on OS: 10.4.6 or higher. RAM: 2GB 20GB Minimum available on hard disk

#### **References**

- 1) Holland, Mitchell and Walther Parson. GeneMarkerHID: A Reliable Software Tool for the Analysis of Forensic STR Data. J Forensic Sci. 2011. 56:29-35.
- 2) Schmidt, Ronald. Evaluation of GeneMapper ID-X and GeneMarker HID for use in Forensic DNA Analysis. 2012; http://www.marshall.edu/forensics/files/2012/09/Schmidt-Ron-Poster-New.pdf.





Software PowerTools for Genetic Analysis

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