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**PAPER**

**CRIMINALISTICS**

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# GeneMarker® HID: A Reliable Software Tool for the Analysis of Forensic STR Data

**ABSTRACT:** GeneMarker® HID was assessed as a software tool for the analysis of forensic short tandem repeat (STR) data and as a resource for analysis of custom STR multiplexes. The software is easy to learn and use, and includes design features that have the potential to reduce user fatigue. To illustrate reliability and accuracy, STR data from both single-source and mixture profiles were analyzed and compared to profiles interpreted with another software package. A total of 1898 STR profiles representing 28,470 loci and more than 42,000 alleles were analyzed with 100% concordance. GeneMarker HID was also used to successfully analyze data generated from a custom STR multiplex, with simplified and rapid implementation. Finally, the impact of the user-friendly design features of the software was assessed through a time scale study. The results suggest that laboratories can reduce the time required for data analysis by at least 25% when using GeneMarker HID.

**KEYWORDS:** forensic science, short tandem repeat analysis software, high throughput laboratory analysis

Examiners in forensic DNA laboratories are faced with the analysis of an ever increasing volume of short tandem repeat (STR) data, both from casework and convicted offender databanking efforts. Estimates suggest that backlogs of more than 100,000 forensic cases and 500,000 convicted offender samples exist on a continual basis in crime laboratories across the United States; data from the President's DNA Initiative and the National Forensic DNA Study Report. Therefore, every possible time-savings tool should be developed and considered when running a forensic DNA laboratory. While automation and the development of advanced techniques and methodologies have certainly supported this process over the past 10–20 years (for example, [1–5]), areas remain that could be addressed. For example, the time necessary to analyze STR data can represent more than one quarter to one half of a forensic examiner's workload in the laboratory. Therefore, the availability of software tools that reduce user fatigue and shorten analysis times would be of great value.

There are a limited number of commercially available software packages for the routine analysis of STR data in forensic laboratories. The software most commonly used is GeneMapper ID from Applied Biosystems (Foster City, CA) (6). While this is certainly a proven and reliable software package, the software can be difficult to learn, the limited number of user-friendly features can greatly increase analysis times, and the cost of the software is relatively high. Steps as simple as scaling of data or printing electropherograms are cumbersome and contribute to user fatigue. While not used on a widespread basis, other software tools such as the Forensic Science Service's $I^3$ software suite (I-STRess, I-STReam and I-Integrity) (http://www.cstl.nist.gov/div831/strbase/pub_pres/Dolph_AAFS2008poster.pdf [accessed October 20, 2009]) and the Cybergenetics TrueAllele System 3 (http://www.promega.com/geneticidproc/ussymp16proc/abstracts/perlin.pdf [accessed October 20, 2009]) (7) are excellent as expert systems, but lack a user-friendly interface to support routine analysis of STR data. In addition, while the new version of GeneMapper (*ID-X*) is considered an expert system and has addressed some of the user-based concerns, it is still a relatively difficult software package to use. To address these concerns, GeneMarker HID from SoftGenetics, Inc. (State College, PA) was assessed as an analysis tool for STR data. In particular, the software was tested to determine its reliability, user-friendliness, and potential time savings for the typical laboratory performing forensic DNA analysis. The parent version of the software, GeneMarker®, was developed in 2004 as a research-based fragment analysis software package, allowing for the analysis of data generated from a variety of laboratory techniques, including multiplex ligation-dependent probe amplification (MLPA), amplified fragment length polymorphism (AFLP) analysis, and single nucleotide polymorphism (SNP) analysis (8–10). In addition, the software has modules for trisomy detection, microsatellite instability, loss of heterozygosity, and phylogeny studies (11–13). While the flexibility of the original GeneMarker program was useful for diagnosticians and genetic researchers, forensic requirements are unique and required the development of a dedicated version of the software. As a result, in 2006, the original GeneMarker program was used as a platform for the development of GeneMarker HID. Forensic rules were used to guide the development process, and the software was put through a series of rigorous tests to assess its use as an STR data analysis tool for forensic casework, convicted offender databanking, paternity or relationship testing, and as a resource for the analysis of custom STR multiplexes.

An evaluation of the ability of GeneMarker HID to accurately and reliably analyze single-source STR data was conducted first. This was accomplished through comparison of profile results analyzed with GeneMarker HID, with those analyzed with GeneMapper ID.

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Reproducibility and reliability were further assessed through replicate analysis of both single-source and mixture profiles by multiple users. Interestingly, these criteria were explored at an entry level, as the users were all newly trained and inexperienced (i.e., undergraduate and Master's level students), eliminating any potential bias from qualified examiners. In addition, a time scale study was conducted with experienced examiners to assess GeneMarker HID's performance and user-friendliness in relation to GeneMapper ID. Finally, the ability of GeneMarker HID to be used as an analysis tool for custom STR multiplexes was evaluated on a multiplex developed by the Institute of Legal Medicine in Innsbruck, Austria (14). The results of these studies are presented here and illustrate the usefulness of GeneMarker HID as a reliable and effective tool for the routine analysis of STR data generated in forensic and paternity laboratories.

## Methods

### *Sample Profiles*

All STR profiles analyzed at Penn State University were generated using the Amp*Fl*STR® Identifiler PCR Amplification kit from Applied Biosystems (AB) and run on the AB 3130xl instrument. These profiles were used for all the studies conducted, with the exception of those studies performed on the custom STR multiplex. Those profiles analyzed at the Institute of Legal Medicine in Innsbruck were generated using a custom developed miniSTR multiplex kit (14). Amplicons were run on an AB 3100 instrument following the manufacture's recommendations except for the use of POP6 in place of POP4.

### *Accuracy, Reliability, and Reproducibility*

A total of 212 single-source STR profiles were analyzed with both the GeneMarker HID and GeneMapper ID software packages. In addition, to assess the reproducibility of replicate analysis, 35 single-source and 15 mixture profiles were analyzed with GeneMarker HID by 26 newly trained users (students in the Forensic Science Program at Penn State University). These studies were conducted over a period of 15 months, and all data were compared for concordance. Therefore, a total of 1512 STR profiles were analyzed and compared, representing 22,680 loci and more than 34,000 alleles.

### *Time Scale Study*

Four individual data sets containing a total of 386 STR profiles (*c*. 70–75 different profiles per data set) were analyzed by three trained scientists over a period of 3 weeks. Each data set contained both single-source and mixture profiles, and all profiles were generated using the Identifiler kit and run on the 3130xl instrument. Each scientist analyzed data sets 1 and 2 with GeneMarker HID and data sets 3 and 4 with GeneMapper ID during the first week. After a 1-week break, data sets 3 and 4 were analyzed with GeneMarker HID and data sets 1 and 2 with GeneMapper ID during the third week of the study. This approach was taken in an attempt to eliminate any potential bias from seeing the data a second time. Each data set was expected to take between 45–90 min to analyze depending on the user. The data generated in this exercise provided an additional assessment of reproducibility and accuracy, bring the total number of comparative analyses to 1898 STR profiles, representing 28,470 loci and more than 42,000 alleles.

### *Custom Multiplex*

A custom panel for the miniSTR multiplex was created using the GeneMarker HID panel editor tool following the instructions of the software operation manual. Markers and pins were added manually on the basis of the allelic ladder profile. The loci included in the miniSTR multiplex were D2S1338, D16S359, D18S51, TH01, and FGA. See ref. (14) for a detailed description of the multiplex development and amplification conditions.

### *GeneMarker® HID Software*

GeneMarker HID is compatible with the Windows® PC operating system up to and including VISTA® OS and can be run on an Intel®-based Macintosh® system with Parallels™ Desktop or Apple® Boot Camp®. Raw data files from the Applied Biosystems 3100 and 3130xl capillary electrophoresis instruments (.fsa files) were imported directly into the software. However, other file formats are compatible with the software, e.g., .abi, .ab1, .scf, .rsd, .esd, .smd, .smr, and .txt. The first user interface allows the data to be displayed as an unanalyzed synthetic gel image and/or in electropherogram format. The raw data traces are saved within the project file and can be recalled at any time during analysis. As with GeneMapper ID, the original raw data files are not permanently modified in any way during the analysis.

When pull-up occurs, a simple matrix calculation is applied by the software. The major peak is identified, and the height ratio of the other color peaks under the major peak is calculated. The software takes the height and area calculation for the pull-up peak and adds it to the top of the saturated peak so that the apex is smooth and the peak is a representative height. Electrophoretic spikes are eliminated by creating a first derivative trace of the data and then removing the outliers (3–5$\sigma$, sigma) which contain the spikes. A minimum peak detection threshold and percentage filter can be set by the software, including an intensity parameter that is applied to peaks outside of the marker ranges as set in the allelic ladder panel. The global percentage parameter is a noise filtering option that is also applied to peaks outside of the loci in the panel. The height of all peaks within the dye color is calculated, and 95% of that value is used as the ratio calculation for the global percentage. Peaks that fall below the global percentage, times 95% of all peaks in the dye color, are not called.

The user defines a peak score range for the software to Reject, Check, or Pass individual allelic peaks. The peak score is calculated based on signal-to-noise ratio and peak shape or morphology. Lower peak scores indicate a poorer quality peak. If a peak's score is below the Reject threshold, it is not to be called unless the peak falls within the pre-defined allelic bin. If the peak falls within the bin, the peak is called, but marked with a red highlight, demanding user attention. Peaks with scores between the Reject and Pass thresholds are in the Check range and are highlighted yellow for further inspection by the user. Quality scoring ranges can also be set to highlight only those peaks that fall into the Reject and Pass categories, bypassing the use of the Check function.

Additional filtering parameters are associated with the individual loci. A minimum relative fluorescence units (RFU) peak threshold for both homozygote and heterozygote profiles can be defined per locus, as well as local percentage calculations similar to the global thresholds described earlier. The threshold of allelic peak imbalance can also be set to flag those peaks that fall below the threshold. A threshold associated with peaks located at the typical stutter positions (e.g., N−4, N−8, N + 4) can also be defined per locus. Even before peak thresholds are applied, the raw data must be converted

from frame (or time) on the *x*-axis to base pair sizes. The internal lane standard (ILS) associated with each sample is used as the size calling guide.

GeneMarker HID allows for sizing of data using either the *Local Southern* or *Cubic Spline* methods. A trace comparison method is used to match the representative size standard peak with peaks in the ILS for each sample. A lane score is generated based on how successful the software was in identifying all expected peaks, and if necessary, manual changes can be made to the position of individual sizing fragments in the ILS. While a customizable feature for laboratories running GS500 ILS from Applied Biosystems, a tool is currently available in the software that determines the difference in the maximum and minimum size values for the ~250 bp ILS peak within a set of samples. Using this tool allows the user to quickly determine whether the mobility of each ILS in a data set has run within an expected ±1 bp window (see Fig. 1) and saves considerable analysis time, especially for large data sets.

Once the data is sized, the allelic ladder samples in the data set are automatically identified by the user-defined filename and the selected panel is applied. Environmental factors surrounding the CE instrument, and chemistry, often result in varying degrees of migration patterns for different data sets. For this reason, an *Automatic Panel Adjustment* feature is included in the software. The original panel of loci selected by the user contains known peak size and peak height ratio information which is then used to identify specific peaks in the ladders. The markers and bins are then "adjusted" to match the data set's ladders, and the adjusted allelic ladder panel is verified prior to analysis of sample data. It is important to note that ladders with higher intensity peaks are more influential in the positioning of the adjusted bins. This can sometimes force the user to spend more time on ladder refinement. However, it is a design feature that is being further developed by the company (SoftGenetics) to allow for all viable ladders to contribute equally to the creation of bins in the adjusted, to create an actual virtual ladder.

GeneMarker HID's reporting options include export of the peak table or report table in Microsoft Excel format or tab-delimited text format for easy import into most Laboratory Information Management Systems (LIMS). In addition, the data can be exported in CMF format (v3.2) for Combined DNA Index System (CODIS) upload; CODIS CMF versions are updated and compliant with CODIS CMF Spec and Validator per FBI CODIS Program Manager, SAIC.

Finally, new Panels can be created using the Automatic Panel Creation feature or by manually adding Markers and Bins and can be used by laboratories developing new STR multiplexes or creating their own fragment analysis kits. The Institute of Legal Medicine in Innsbruck, Austria used this feature to evaluate a new mini-STR multiplex kit developed for analyzing degraded DNA (14).

## Results

With GeneMarker HID, each individual peak is scored, and if it does not meet certain user-defined thresholds, it is flagged with yellow or red highlights in the electropherogram and peak table. When a peak is flagged yellow or red, the *Quality Reasons* column in the peak table gives coded indications as to why the peak was flagged. The user can right-click the peak in the electropherogram

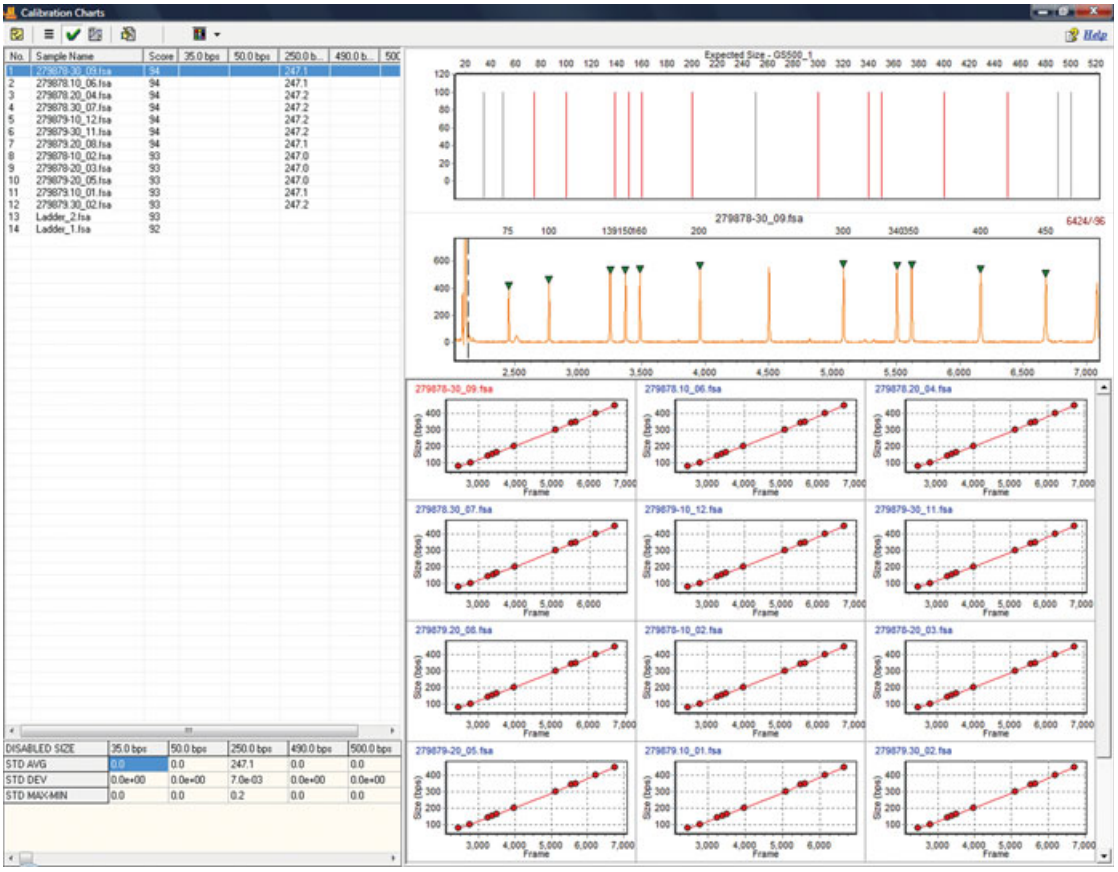


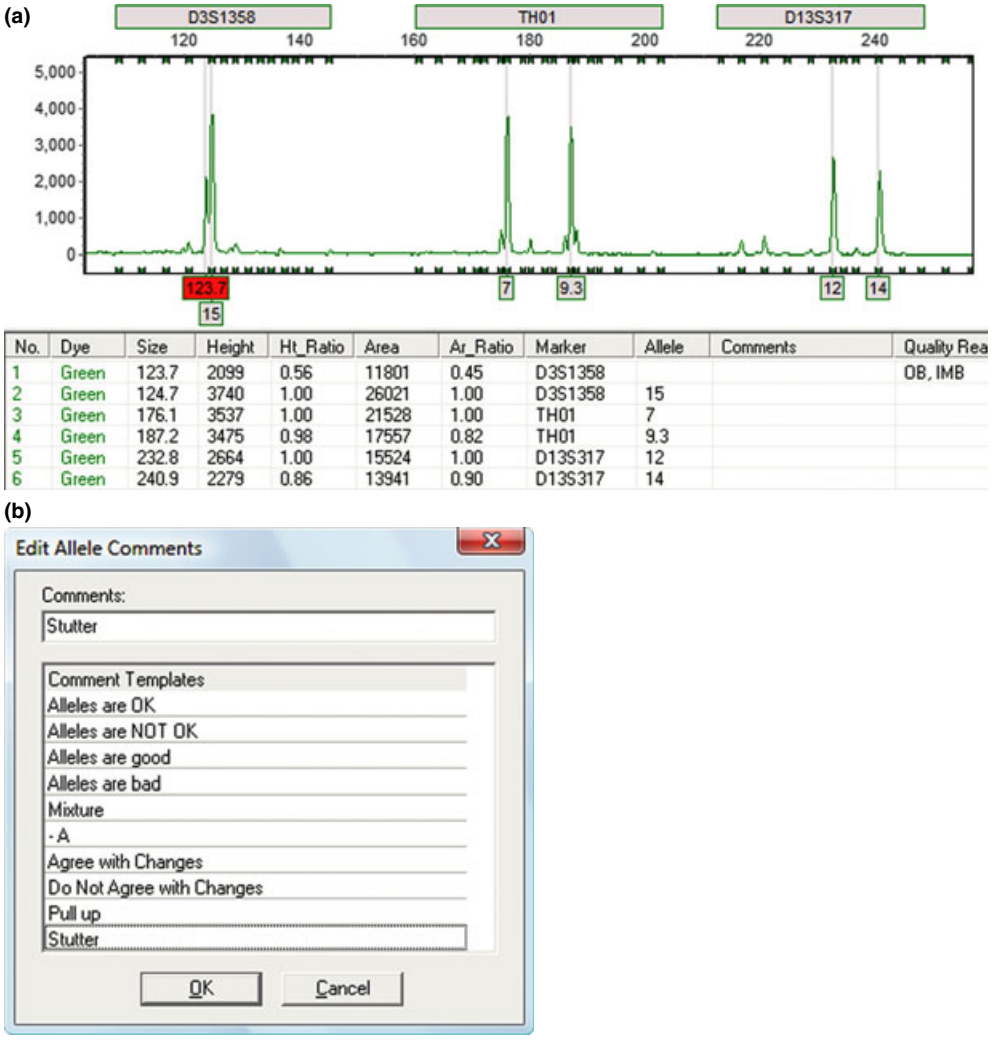

FIG. 1—*Size Calibration Chart displaying expected size trace versus individual sample internal lane standard (ILS) traces. The STD MAX-MIN value for this data set is 0.2 bps, which means that the ~250-bp ILS peak for each sample is no more than 0.2 bps in size away from any other 250-bp peak.*

or any cell in the peak table to Add, Delete, Confirm, and/or enter a Comment. Figure 2 is an example of an electropherogram and peak table highlighting alleles which require some form of user-introduced interpretation. All 1898 sample profiles for this study were analyzed using this approach.

GeneMarker HID allows for bulk printing of the electropherograms and associated reports. Prior to printing, the software allows the user to auto-scale the RFU axis for each locus within the electropherogram. This eliminates the need to blow up specific loci when documenting results in hard-copy case files. Figure 3 illustrates the auto-scaling feature. A project compare feature within the software allows the technical reviewer to identify the differences between independent analyses of data sets. While other, home-brewed software packages have been developed to compare data from different analysis projects, no software that we know of will allow for a complete project comparison, to include all edits, comments, and differences between the projects. All differences are flagged by the software, and the two projects will open to the same point of interest by simply double-clicking on the difference. This feature significantly reduces user fatigue when conducting technical review and results in decreased time necessary for the review process. Figure 4 illustrates the results of a project comparison.

A total of 212 single-source STR profiles were analyzed with both the GeneMarker HID and GeneMapper ID software packages. Simple comparisons were made to determine the level of concordance. In addition, to assess the reproducibility of replicate analysis, 35 single-source and 15 mixture profiles were analyzed with GeneMarker HID by 26 newly trained users (students in the Forensic Science Program at Penn State University). These studies were conducted over a period of 15 months, and all data were compared for concordance. Therefore, a total of 1512 STR profiles were analyzed and compared, representing 22,680 loci and more than 34,000 alleles. There were no discrepancies identified between individual analyses.

Four individual data sets containing a total of 386 single-source and mixture profiles (*c*. 70–75 profiles per data set) were analyzed by three trained scientists over a period of 3 weeks. Each data set was expected to take between 45–90 min to analyze depending on the user. On average, data analyzed with GeneMarker HID took 48 min to complete, and data analyzed with GeneMapper ID took 65 min to complete. Therefore, the time necessary to analyze STR profile data sets was decreased by ~25% when using GeneMarker HID. In addition, the data generated in this exercise provided a further assessment of reproducibility and accuracy, bringing the total




| No. | Dye | Size | Height | Ht_Ratio | Area | Ar_Ratio | Marker | Allele | Comments | Quality Rea |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Green | 123.7 | 2099 | 0.56 | 11801 | 0.45 | D3S1358 | | | OB, IMB |
| 2 | Green | 124.7 | 3740 | 1.00 | 26021 | 1.00 | D3S1358 | 15 | | |
| 3 | Green | 176.1 | 3537 | 1.00 | 21528 | 1.00 | TH01 | 7 | | |
| 4 | Green | 187.2 | 3475 | 0.98 | 17557 | 0.82 | TH01 | 9.3 | | |
| 5 | Green | 232.8 | 2664 | 1.00 | 15524 | 1.00 | D13S317 | 12 | | |
| 6 | Green | 240.9 | 2279 | 0.86 | 13941 | 0.90 | D13S317 | 14 | | |

FIG. 2—*Electropherogram (Panel A) and peak table (Panel B) as displayed in GeneMarker® HID's main analysis window. Previous comments are stored in the Edit Allele Comments box and can be recalled and applied to new peaks. The values flagged in red are −A peaks at the D3S1358 locus of the Identifiler short tandem repeat (STR) multiplex.*

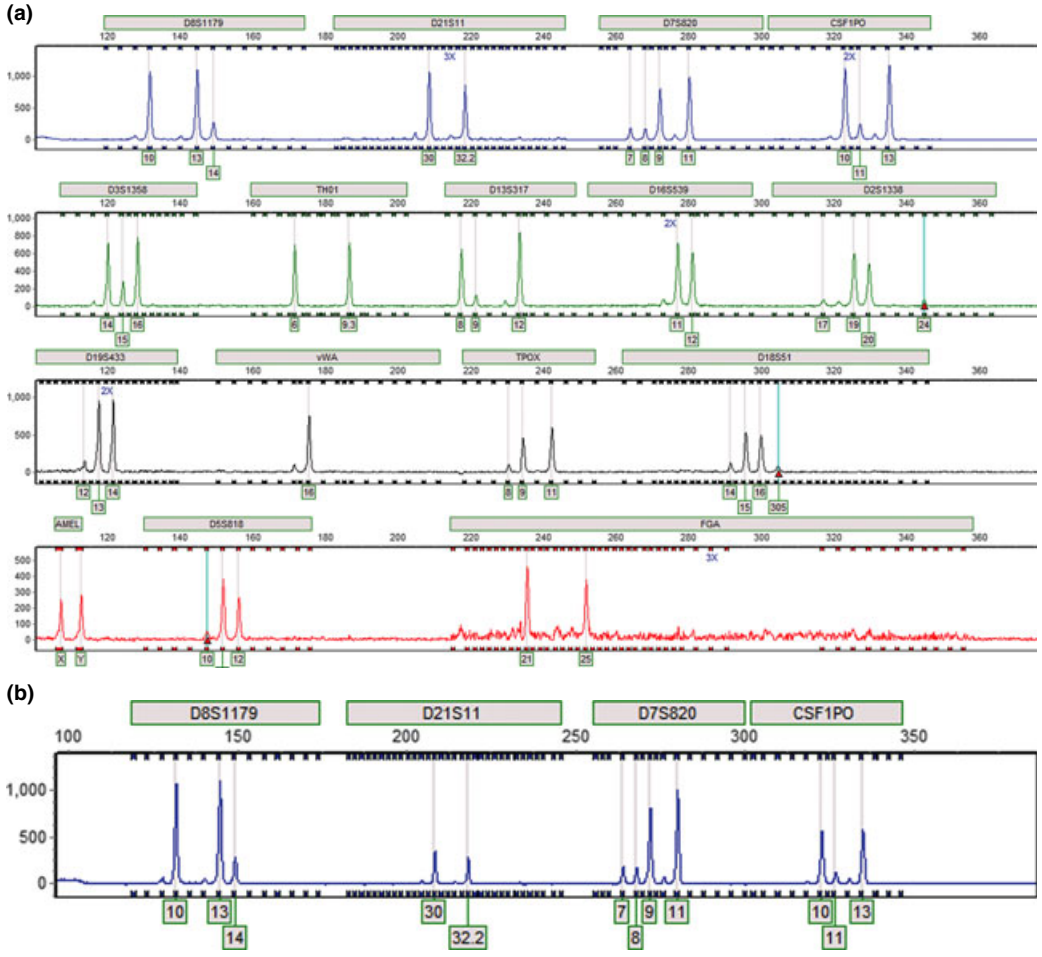



FIG. 3—*Panel A: Auto-scaling of low-level mixture data across an electropherogram allows the user and reviewers to easily view data within the same printout. The specific auto-scaling levels are identified at each locus: D21S11, CSF1PO, D16S539, D19S433, and FGA. For example, the peak intensities at the D21S11 locus have been amplified threefold to bring the intensity of those peaks in line with other loci. Panel B: Illustrates the printed electropherogram in the blue channel without auto-scaling.*

number of comparative analyses to 1898 STR profiles, representing 28,470 loci and more than 42,000 alleles.

The miniSTR profiles of 13 samples were analyzed using the Genotyper software from Applied Biosystems using a customized macro for allele designation. The samples were also typed with SGMplus (Applied Biosystems), and overlapping loci confirmed the allele calling of the GeneMarker HID custom panel.

## Discussion

Based on the results of our study, GeneMarker HID is a reliable, accurate, and user-friendly software tool for the analysis of STR data generated in forensic casework, convicted offender databanking, and paternity/relationship testing. The thousands of Identifiler STR profiles analyzed with GeneMarker HID produced accurate allele calls, including those allele calls verified through comparison to GeneMapper ID results. The design features of GeneMarker HID have the potential to reduce user fatigue and to shorten forensic STR analysis times by *c*. 25% (when compared to GeneMapper ID). A reduction in this magnitude could significantly increase productivity in larger forensic DNA laboratories; the impact would be equivalent to three forensic DNA analysts generating the work of four. Included in the elements that contribute to a reduction in user fatigue, GeneMarker HID allows the DNA analyst to view the sample list, electropherograms, reported data table, and allele tables at the same time on one screen; or any of the three can be hidden from sight. For those individuals that like to view the data in a gel format, this feature is also available. Most importantly, the different views are linked by double-clicking on an element of the data; for example, double-clicking on an allele value in the data table or a band in the gel will immediately direct the electropherogram window to the allelic peak. The data table can be viewed in different formats as well, to include only the alleles that the software has flagged as requiring additional analysis. This allows the DNA analyst to focus on the data requiring the most attention first, reducing the chance of error as user fatigue increases throughout the analysis process. For paternity laboratories, a pedigree analysis tool can reduce user fatigue as well. The pedigree is linked directly to the STR data, as a single right-click will display conflicts between child and parents, or child and siblings; clicking on the locus header brings up the applicable section of the electropherogram on the same screen.

The ability to zoom in-and-out of the electropherogram window with a click of the mouse, and a scroll feature that auto-scales the allelic peaks as the user moves through the data, is far superior to any other software available for forensic DNA analysis. Drawing a box around the data of interest will immediately zoom in on that data. Drawing a box in the opposite direction zooms out to the

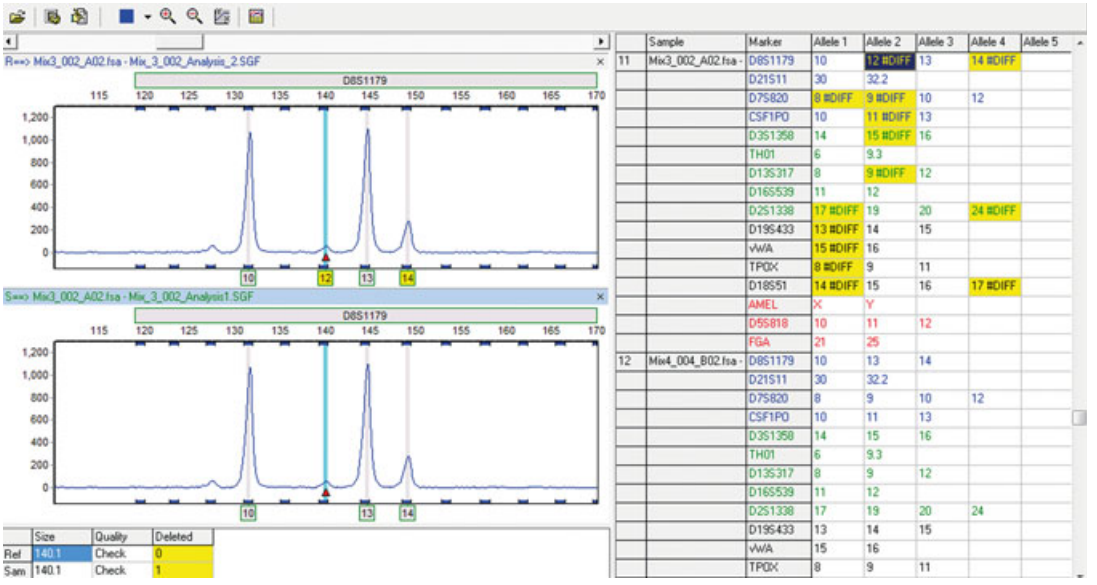



FIG. 4—*The project comparison feature of GeneMarker® HID allows for easy technical or administrative review of short tandem repeat (STR) data.*

original full-view screen. No matter what the view may be, however, the software automatically scales the data to adjust for the intensity of the highest and the lowest peaks in the window. These features significantly reduce the time necessary to analyze the data for each STR profile (especially for low copy number and mixture profiles), saving valuable time having to resize and reposition data. In addition, these features all positively impact the quality review process when interpreting data, allowing the DNA analyst to focus on the evaluation of data and not on how to navigate through the software.

Once the data analysis process is completed, the DNA analyst may want to export the data and print electropherograms. GeneMarker HID provides a dropdown menu that allows for easy export of data in CODIS format, or the data can be exported out of the project in Excel (.xls) or tab-delimited Text (.txt) file formats. In addition, printing can be performed on selected electropherograms or on all electropherograms in a project with a single command, with options to print individual color channels, allele tables and with different allele labels, such as allele number, base pair size, and/or peak height. Electropherograms can be printed without modification, or an auto-scaling feature can be applied that artificially adjusts peaks heights at loci that are of lower intensity so that the data is easily reviewed once printed (see Fig. 3). This eliminates the need to print out rescaled, individual loci. Finally, for those interested in using STR data in reports and/or presentation materials, screen-shots of the STR profiles or any other forms of the data can be captured and copied into reports, PowerPoint presentations, or any other electronic document format. The figures used for this manuscript were all captured using the imbedded screen-shot feature.

For those laboratories interested in developing custom multiplexes, GeneMarker HID includes a flexible interface to allow for the creation of ladder panels and user-defined parameters necessary to handle any type of STR (or SNP) multiplex. To illustrate this, GeneMarker HID was used to analyze data generated from a custom-made STR miniplex developed by a team of scientists at the Institute of Legal Medicine in Innsbruck, Austria (14). The loci included in the miniplex were D2S1338, D16S539, D18S51, TH01, and FGA. Because redesigned primer pairs were synthesized for this multiplex, GeneMarker HID was used to create a custom ladder panel that allowed the researchers to successfully analyze their data.

As the forensic science community moves forward, it will continually be searching for tools to expand capabilities and improve on quality and throughput. Solutions such as the GeneMarker HID software package should help crime laboratories reach these goals, as the software not only has the essential features of being fast and accurate for the analysis of STR data, but it also provides a sophisticated user interface that has the potential to significantly reduce analysis time and user fatigue.

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