Automated STR Data Analysis: Validation Studies

Mark W. Perlin[1], David Coffman[2], Cecelia A. Crouse[3], Felipe Konotop[3], Jeffrey D. Ban[4]

[1]Cybergenetics, Pittsburgh, PA
[2]Florida Department of Law Enforcement (FDLE), Tallahassee, FL
[3]Palm Beach County Sheriff's Office (PBSO), FL
[4]Division of Forensic Science (DFS), Richmond, VA

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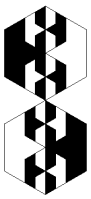

Contact information:

Mark W. Perlin, PhD, MD, PhD
Chief Executive Officer
Cybergenetics
160 North Craig Street
Suite 210
Pittsburgh, PA 15213
USA
(412) 683-3004
(423) 683-3005 FAX
perlin@cybgen.com

Abstract

STR technology has enabled the rapid generation of highly informative DNA data for use in human identification. However, these data must be carefully analyzed. With database samples, there is now an acute shortage of skilled data reviewers. With casework samples (including mixtures), much information is not extracted from the data, despite considerable examiner effort. We are rapidly developing novel computational, mathematical and statistical methods that help overcome these limitations. This report focuses on the collaborative validation of these methods.

Convicted offender DNA databases must be accurate. To minimize error, the original STR data are carefully reviewed by two or more people. Moreover, in a troubleshooting capacity, this review helps to continuously maintain high quality lab data. But there are not enough skilled personnel for this arduous, repetitive task. To alleviate this critical labor shortage, we developed the TrueAllele™ expert system. The computer program automates virtually every human review function, and provides consistent quality assessment and allele designation.

The *TrueAllele validation* began with the original data from 50,000 CODIS genotypes. System parameters were adapted to the instruments (ABI/310, ABI/3700, Hitachi/FMbio) and panels (ProfilerPlus, Cofiler, PowerPlex 1.2) used to generate the data. Computer processing was then done, with automated scoring of the high quality data, followed by limited human review. The computed expert system results were compared against manually scored results. We report here on the relative accuracy and efficiency of the automated approach.

In casework, DNA mixtures are analyzed to assess candidate suspects. When inferred profiles are matched against a convicted offender database, useful leads are generated. When matched against a known suspect, the mixture data can help convict or exonerate. However, data uncertainty leads to inherently complex and ambiguous analysis. We have developed a new technology, Linear Mixture Analysis (LMA), which uses multilocus quantitative data to automatically eliminate this complexity. LMA objectively resolves mixtures into candidate profiles, and provides highly informative statistical measures.

The *LMA validation* involves both synthetic mixtures and actual casework profiles derived from diverse panels and instruments. After quantitative peak analysis (using TrueAllele) on the original data, we applied LMA to automatically determine contributor profiles. Database search validation can be done by assessing the error rates of matching these profiles against existing DNA databases. Casework validation can be done by examining the LMA statistics relative to known suspect profiles. We report here our initial studies on LMA's accuracy and informativeness.

Our presentation describes novel computer-based methods for assuring data quality, automating DNA database review, and analyzing the mixed DNA profiles found in casework. We present here the objective results of our ongoing validation studies, and demonstrate the feasibility of practical automated analysis. Our primary objective is the rapid introduction of validated intelligent data analysis systems for eliminating tedious human STR analysis. This contribution may help free up valuable DNA examiner time for serving justice through forensic science.

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Introduction

STR technology has enabled the rapid generation of highly informative DNA data for use in human identification (1). However, these data must be carefully analyzed for errors to assure forensic usability (2). With database samples, there is a growing shortage of skilled data reviewers for quality assurance (3). With casework samples (including mixtures), much information is not extracted from the data (4, 5), despite considerable examiner effort. We are rapidly developing novel computational, mathematical and statistical methods that help overcome these limitations (6-8). This report focuses on the collaborative validation of these new scientific analysis methods.

We present this paper in three sections:

- Automated STR Analysis. Current STR analysis is a labor-intensive effort performed by expert forensic examiners. It would be highly useful to deploy expert computer systems that could automate much of their work. This section describes the TrueAllele™ expert system for automated STR analysis.
- Databasing Validation. We have performed a validation study of the TrueAllele™ automated databasing solution on a variety of DNA sequencing instruments and STR panels. In this section, we outline our methods and present the main validation results.
- Casework Studies. We have recently initiated casework (mixture and nonmixture) validation studies. In this section, we motivate our casework technology, and present some preliminary mixture validation results.

Automated STR Analysis

We describe here a computer-based solution to automated STR analysis that provides quality assurance, rule-based diagnostics, and runs on most forensic platforms. We provide validation results for this TrueAllele™ expert system in subsequent sections.

Reviewing STR data

Human review (in duplicate) of forensic STR data is currently required to assure that DNA profiles are correctly scored. However, this human analysis step has become a labor-intensive bottleneck impeding the rapid construction of high-quality DNA databases. Any useful scoring approach must address the following issues:

- Computer automation. It would be desirable to have a computer-based analysis technology that can automate most or all of the laborious human data review process.
- Quality assurance. What is the quality of the underlying STR data, including PCR controls and size calibrations? This data quality determines the reliability of the scored genotypes.

- Database integrity. STR databases must be accurate in order to avoid database matching errors. With a false positive match, the wrong person is identified. With a false negative nonmatch (the more likely error), the correct criminal may never be found – even though their (incorrect) profile resides on the database.
- Casework and mixtures. DNA databases of convicted offenders are created primarily for the purpose of identifying suspects later on in casework applications. With mixed or degraded DNA samples, the database match can become inherently ambiguous. Computational methods are needed that can increase the specificity of the match.

In this paper, we explore all of these issues.

Any useful solution to the STR data review problem must achieve these key goals:

- No error. Allele calling errors should be minimized.
- High throughput. The process should rapidly review data, eliminating backlogs.
- Small staff. Personnel numbers should remain constant as throughput increases.

The TrueAllele™ technology, described next, achieves all these goals.

TrueAllele™ technology

The TrueAllele™ process eliminates the STR human review bottleneck (7). Since Cybergenetics has innovated this automation technology for the past decade, its multiple patents protect all aspects of automated STR analysis, including forensic usage, quality scores, PCR stutter handling, and component processing steps (9). Note that the validation study described herein applies solely to the Cybergenetics TrueAllele implementation of these patented expert system processes.

The *input* to the process is the original data generated from a gel, automated or capillary DNA sequencer. The fully automated TrueAllele software then processes the STR data, running on most common computers (Macintosh, Windows, UNIX). Automated processing steps include:

Color separation. TrueAllele can dynamically separate the original channels into their component dye colors.

Image processing. With gel-based sequencers, the software analyzes each dye plane image in two dimensions.

Lane tracking. TrueAllele tracks the size standards to determine the in-lane size calibration. On gels, the program also automatically tracks the lanes.

Signal analysis. The software transforms the signals, and identifies peaks.

Ladder building. TrueAllele automatically analyzes the allelic ladders, and uses these ladders for DNA length determination.

Peak quantification. The technology models every peak, determining the best fit between the set of modeled peaks and the observed signal data. The result is exquisitely accurate estimation of DNA concentration, enabling a highly quantitative allele calling, quality checking and mixture analysis approach.

Allele designation. Using its internal database of DNA sizes and concentrations, TrueAllele designates alleles in DNA length coordinates, using multiple allele calling mechanisms.

Quality checking. TrueAllele computes dozens of quality measures for each genotype experiment, compiling statistics on the different phases of laboratory data generation. The program detects outliers (based on user-determined thresholds), and can focus the user review on just the problematic data.

CODIS reporting. The program automatically generates reports, including CMF files for automated CODIS DNA database submission.

The software *outputs* quality assured STR profiles to DNA databases.

Automated processing

The TrueAllele process begins with automated *input* of the data. A site uses an "auto setup" template that knows the source computer directory of original sequencer data runs. Combining these data together with annotating information (sample lane layout, marker information, DNA sequencer used, etc.), TrueAllele transforms the sequencing data of multiple runs into an instrument-independent "DataDisk" format suitable for downstream processing.

The second step is automated gel *image* or capillary *signal analysis*. On all the sequencer runs, the computer performs data filtering, baseline and primer removal, color separation, lane and size tracking, and extraction of 1D lane (or capillary) signals from pixel into size coordinates. Once the computer has completed its work, users do quality assurance (review, accept, edit, reject, etc.) in interfaces such as "ImageView" (Figure 1A). With good data, a run's Q/A typically takes under two minutes.

In the *allelic analysis* (step three), the computer begins by analyzing the allelic ladders, transforming the data into DNA length coordinates, and then (expending considerable effort) accurately quantitating every DNA event (peak or band) in the data. On every genotype, allele calling uses multiple algorithms, assigns quality scores, and applies dozens of rule checks when looking for possible data artifacts in each phase of laboratory processing. After the computer has allele called and quality checked all the genotypes across all sequencer runs, it navigates the user's quality assurance review (accept, edit, reject) through the potentially problematic genotypes (Figure 1B), showing its associated rule analysis (Figure 3).

In the final *output* step, TrueAllele automatically exports the data in format suitable for automated database (e.g., CODIS) import.

Quality assurance

TrueAllele provides a number of data visualizations and reports that are used primarily for quality assurance. For example, showing the lane data for each marker in size

coordinates provides much information about the relative sizing accuracy and the signal quality (Figure 2A). Also, it is useful to review the size standards and allelic ladders in data views (Figure 2B), overlay plots, with the tracking results shown; large size deviations or low signals can pinpoint potential problems.

Rule system

TrueAllele's automated rule analysis provides detailed quality assurance information at the individual genotype level (Figure 3). Organized by laboratory DNA processing step (extract, amplify, separate), the rules automatically perform a consistent and detailed technical review. Exportable Excel spreadsheets summarize the rule firings, and highlight potential data problems; such information can be very helpful in troubleshooting and for ongoing quality assurance. Applying a lab's standard operating procedures, a site administrator can customize the values of rule thresholds, and determine whether or not to use a rule. TrueAllele's technical documentation gives detailed descriptions and illustrative examples of each rule.

The Hitachi genotype experiment shown (Figure 3) raises interesting quality assurance issues. If data bands have low optical density, but otherwise show every sign of high data quality (no rules fired in this genotype's Rule Analysis window), do these data actually require any human review? In one world view, the user-set rule criteria might say "no," and the data would be automatically accepted. From another perspective, the user's rule criteria might say "yes," with the computer enforcing the human review of all such data. Fortunately, TrueAllele is neutral here: the laboratory has the sole authority (via settable rule parameters) to determine their own data review criteria.

Multi-platform engine

TrueAllele is designed to work with any data, from any DNA sequencing instrument. Results from the Hitachi/FMBio, ABI/310, ABI/3700, and ABI/377 are described in this report. Shown is a TrueAllele run from 96-well plate ABI/3100 16-capillary data (Figure 4A), and the superb signals from Amersham's 96-capillary MegaBACE sequencer (Figure 4B). We work closely with instrument manufacturers to ensure support for all reasonable platforms.

TrueAllele is independent of any instrument or chemistry manufacturer. It works with and displays the actual data. Manufacturer-provided software can always process data extensively for consistently "beautiful" results. TrueAllele, however, shows original STR data, as they have been recorded. We believe that our "never hide the truth" approach is more useful (to prosecution, defense, and society) in a criminal justice setting.

Databasing Validation

The key results of this paper center on our completed scientific study that validates TrueAllele™ for DNA databasing applications. We describe in this section our validation methodology, the rule parameter settings, and accuracy and timing results for the Hitachi FMBio, ABI 310 and ABI 3700 platforms, using both Promega and ABI STR chemistries. We also mention an earlier extensive TrueAllele validation study conducted by the UK Forensic Science Service (FSS) using their SGMplus STR chemistry on an ABI 377 sequencing platform.

Methodology

Our validation was a concordance study between automated computer results, and a subsequent detailed human review that scrutinized every scored genotype. In actual production use, of course, one would not review genotypes that TrueAllele had "accepted" without rule firings, since such extra human effort would be redundant and inefficient. However, for validation purposes, all genotypes were manually reviewed in this study. Our extensive developmental validation should permit forensic databasing labs to use the validated TrueAllele process efficiently – without redundant human review of TrueAllele's "accepted" genotypes.

Obtain the original data. The original data was sent to Cybergenetics on CD from participating laboratories. These STR data included:

- about 2,000 database samples of ABI/310 single capillary data amplified in both ABI ProfilerPlus and Cofiler panels (Coffman, FDLE);
- about 2,000 database samples of ABI/3700 96 capillary data amplified in both ABI ProfilerPlus and Cofiler panels (Coffman, FDLE);
- about 1,000 samples of Hitachi FMBio gel data amplified in the Promega PowerPlex 1.2 panel (Crouse, PBSO); and
- developmental Hitachi FMBio data amplified in Promega STR panels from the Virginia lab and their database vendor (Ban, DFS).

The high quality PBSO Hitachi STR data had been generated for casework applications, rather than for high-throughput database processing. This lane arrangement led to considerable manual set up time to determine the location of the single profile data, preparing the gel data for automated analysis.

Process data in the TrueAllele expert system. In broad overview, automated processing entailed the following steps:

- *auto-setup* The original data for each gel or capillary were put into a DataDisk folder. With ABI capillary instruments, these data are just the individual capillary run files. On the Hitachi platform, these data comprise the raw channel images, along with a proto-layout file which indicates what has been run in each lane. In the AutoSetup process, the computer transforms these raw data formats into a platform-independent representation suitable for downstream TrueAllele processing.

- *process run* Automated TrueAllele processing was done. ImageCall was run on the data (ABI capillary, or Hitachi gel), with the computer automatically processing the signals, removing the primer peaks, separating colors, tracking the sizes and lanes, and extracting the signals in size (bp) coordinates.
- *quality assurance* For Q/A, a human operator reviewed each run (ImageView for gels; CapView for capillaries), performing any necessary editing, with an option to reject the run. On this data set, minimal review (typically several minutes) was needed for each run.
- *call alleles* AlleleCall was run on the 1D electropherogram signals. The module automatically performed peak quantitation, allele calling and quality checking on the data of every genotype. TrueAllele's accurate peak quantitation enabled the use of quantitative allele calling methods and quality checks.
- *apply rules* Several dozen rules were applied to each genotype experiment. The rule thresholds used are described in the next section.
- *check results* The operator then completed a checklist to ensure that all processing steps were properly performed.

The result of this automated processing was the computer's assignment to each genotype experiment a label of "accept," "edit," or "reject."

3. Review all the data. A key concept in the optimized TrueAllele process is "one person, many computers." Using the Timbuktu remote control software (Netopia, Alameda, CA), one computer can control many others over a local area network. Therefore, each TrueAllele operator had available one computer for display, and multiple monitor-less computers for background processing. This way, a person never had to wait for a computer – they could work continuously on their review of the automated processing.

In the TrueAllele process, the computer accepts, rejects or suggests review (with possible editing) of each genotype. Although, the user usually does not examine any computer accepted results, in this validation study all genotypes were reviewed for concordance. The result of this manual review phase was the user's assignment to each genotype experiment a label of "accept," "edit," or "reject."

4. Generate results and statistics. Comparison of the computer and the user labels of "accept," "edit," or "reject" was the core result of the study. Other data were recorded, such as the total human operator time expended on a subset of the runs. The computer generated useful statistics, such as rule firing summaries for every gel or marker; these results helped in assessing the quality of the data.

Rule settings

TrueAllele's rule thresholds are set by the user, preferably using Cybergenetics optimization protocols. We tuned the parameters on data samples different from those used in the study. Settings for the Hitachi gel data and the ABI capillary are shown (Table 1).

After gaining more experience with the Hitachi platform, we determined that 15,000 (rather than 9,000) was a better value for the High Signal rule. Also, the Peak Height Cutoff baseline should have been set to 200 (not 50). We did not change these threshold values during the course of the study. However, we note throughout the paper how using these proper values would have affected important results.

Hitachi/FMBio (PowerPlex)

On the Hitachi FMBio platform, using Promega's PowerPlex 1.2 STR panel, we had TrueAllele process 7,973 PBSO genotypes. The computer performed all gel and allele automated processing. The computer decided that no review was needed for about 80% of these genotypes. Subsequent human review disclosed that all these designations were correct. These results suggest that TrueAllele can eliminate human review of most STR DNA databasing gel data.

The breakdown of the computer vs. human review comparison is shown (Table 2). The first row is for the data that the computer accepts as not requiring any human review; this row provides the most important measures of effectiveness.

- The row's first column gives the *efficiency factor* (72.5%) of the proportion of STR data that is safely scored automatically without requiring human review. That is, both computer "accept" and user "accept" decisions. In fact, the true efficiency was appreciably greater (82.0%) after reprocessing the data with properly tuned Hitachi threshold rule and baseline settings, as indicated above.
- The next two columns measure *accuracy*; specifically, the false negative error rate (0.0%). This critical measure describes how many genotypes might be incorrectly called (computer "accept"), but would actually require human review (user "edit" or "reject"). We want this number to equal (or be very near) zero.

The clear conclusion is that TrueAllele provides both efficient and accurate automated review of STR data for DNA databases on an Hitachi/Promega platform. (Because we used Hitachi data for nonautomation casework, with most human time spent sorting through data rather than actually processing it, there are no useful timings to report.)

ABI/310 (ProfilerPlus & Cofiler)

On the ABI 310 platform, using ABI's ProfilerPlus and Cofiler STR panels, we had TrueAllele process 23,723 FDLE genotypes. The computer performed all capillary and allele automated processing. The computer determined that no review was needed for about 85% of these genotypes. Subsequent human review disclosed that all the properly set up designations were correct. These results suggest that TrueAllele can eliminate human review of most STR DNA databasing capillary data.

The breakdown of the computer vs. human review comparison is shown (Table 3). We look closely at the first row for computer accepted data, i.e., results that should not entail any human review.

- An *efficiency factor* (computer "accept," user "accept") shows that a high proportion (86.4%) of the STR data was safely scored automatically without requiring human review. Another useful efficiency measure is the sum of the second row entries (computer "edit") that the computer asks the user to edit (13.3%).
- The *accuracy* shown in the last two columns of the first row (computer "accept," user "edit" or "reject") gave a zero false negative error rate (0.0%). In fact, because of incorrect STR sizing windows (based on manufacturer guidelines), there were several missized alleles. However, after properly adjusting the sizing windows to actual data, all such computer errors were eliminated.

The human processing time for AlleleView editing (95% of the total effort) was recorded for 43% of the data (10,208 genotypes) as 11.25 hours, or 675 minutes. Reducing this time by the efficiency gained from reviewing only computer "edit" data (13.3%, from the second row), the effective review time was only 90 minutes. Dividing the number (10,208 genotypes) by the time spent (90 minutes) yields an average human review time of 113 genotypes per minute, or *almost 2 reviewed genotypes per second*. This rate far exceeds the data generation capability of current sequencer technology. For example, one would need to run a 16-plex panel over 4 sequencers each having 96 lanes or capillaries every hour to generate 100 genotypes per minute. This data generation rate would be on par with the scoring rate of one TrueAllele-enhanced full-time equivalent (FTE) data reviewer.

When people never wait for computers (i.e., "one person, many computers"), the human operator review time was about two genotypes per second. Properly configured, automatically applying 22 data artifact detection rules to every genotype, no false negative scoring errors were observed in ~24,000 genotypes. That is, TrueAllele provided a thorough, diligent and objective automated process that was accurate and entailed relatively little human effort. These results suggest that the expert system may be useful for automated review of STR data for DNA databases on ABI capillary platforms.

ABI/3700 (ProfilerPlus & Cofiler)

On the ABI 3700 platform, using ABI's ProfilerPlus and Cofiler STR panels, we applied TrueAllele to 17,014 FDLE genotypes. The computer performed all capillary and allele automated processing. The computer decided that no review was needed for about 85% of these genotypes. Subsequent human review disclosed that all the designations were correct. These results suggest that TrueAllele can eliminate human review of most STR DNA databasing capillary data.

The breakdown of the computer vs. human review comparison is shown (Table 4).

- An *efficiency factor* (computer "accept," user "accept") shows that a high proportion (84.9%) of the STR data was scored automatically without requiring human review. The sum of the second row entries (computer "edit") that the computer asks the user to edit was low (14.9%).

- The *accuracy* shown in the last two columns of the first row (computer "accept," user "edit" or "reject") gave a zero false negative error rate (0.0%).

The human processing time was recorded for 35% of the data (5,880 genotypes) as 8.5 hours, or 510 minutes. Reducing this time by the efficiency gained from reviewing only computer "edit" data (14.9%, from the second row), the effective review time was 76 minutes. Dividing the number (5,880 genotypes) by the time spent (76 minutes) yields an average human review time of 77 genotypes per minute, or *over 1 reviewed genotype per second*. These results provide additional support for the use of the TrueAllele as an automated review expert system for DNA databases.

UK FSS: ABI/377 (SGMplus)

The British Forensic Science Service (FSS) has introduced TrueAllele into database production after rigorous evaluation and validation. In the FSS automation process, TrueAllele scores the STR data and assesses data quality. TrueAllele's allele calling results are checked by another expert system (STRess) developed in-house by the FSS. A person then reviews only a fraction of the data, as indicated by TrueAllele's rule firings. The human checked data are then sent to the UK national database.

The UK requires that suppliers to its national database maintain an error rate of under one genotype error per 2,000 samples. This quality is continually checked by double-blind rerunning of ~5% previously processed database samples. Since the FSS validated their TrueAllele process as meeting these quality assurance standards, no "second scorer" review is needed. This approach maintains high database quality and throughput, with reduced personnel requirements.

The FSS validated their TrueAllele process on the ABI/377 gel sequencer platform using the SGMplus panel. They allocated significant resources to this validation project, including:

- *data* – roughly 22,000 genotypes.
- *people* – six managers and six data reviewers.
- *time* – eight weeks of data gathering, and four weeks for reporting.

To summarize some of the key components of their validation study, the FSS:

- correlated the *peak height* between TrueAllele's modeled peaks and their previous GeneScan system;
- established the *baseline height* for error-free allele calling and rule checking;
- measured the *designation accuracy* of TrueAllele relative to their previous manual system;
- determined their *software, hardware and network* computer environment; and
- developed their *quality management system* QMS documentation.

Their main results were finding:

- a *greater data yield* using TrueAllele, and

- *no errors* observed with high quality STR data.

The FSS validation project was done by their Concept Development group (Trident Court, Birmingham, UK), led by Richard Pinchin. The FSS intends to submit their validation methodology and TrueAllele results for scientific publication.

Casework Studies

DNA casework entails the forensic analysis of crime scene evidence. Indeed, convicted offender DNA databases are created primarily to facilitate casework. Mixed DNA samples can present additional complications, particularly when useful statistics are required for court.

We are currently validating a new set of methods for automated casework and mixture analysis based on using quantitative DNA concentration estimates (e.g., "peak heights"). These methods are based on our recently described linear mathematical and statistical models for multi-locus analysis of mixed DNA samples (8). (Appropriate patents have been filed on these and related methods.) The use of quantitative STR data was advocated early on by the FSS (10), with the development of associated statistical descriptions (11).

Our studies are examining data sets in several casework areas, including nonmixture cases, mixed DNA samples, rape kits and their automation, disaster scene analysis, low copy number (LCN) and single nucleotide polymorphism (SNP) mixtures. We describe some of this work in progress.

Statistical information

STR match probabilities, for both single and mixed DNA stains, are based on the allele frequencies at each locus (12). This leads to the probabilities (or likelihoods) of an STR profile, based on the component alleles, under varying contributor assumptions. However, the component alleles are generally assumed to be either present or absent (4). Some forensic DNA examiners might believe that the use of DNA concentration estimates in mixture analysis (i.e., the *degree* to which a peak is present or absent) can affect the genotype probability. They would be correct: the probability of an STR profile in a mixed DNA sample, based on the *quantitative presence* of the component alleles, can be millions of times more informative.

Our linear mixture analysis (LMA) paper described SGMplus STR data for two person DNA mixtures in known proportions (8). For the ten loci used, considering only the typical 2, 3 and 4 allele cases, multiplying the possibilities at each locus implies that there are 100,000,000 theoretically possible STR profiles. However, using our LMA methods, one can compute that for a 30% unknown contributor, there is actually only one statistically feasible STR profile. And, even for a 10% unknown contributor, there

are only about 100 statistically feasible profiles at a 99% confidence level. The comparison of statistically feasible (1 or 100) profiles based on peak heights, to theoretically possible (100,000,000) profiles based on allele presence, shows a factor of one million or more separating common practice from our new methods. It might not serve justice to routinely discard this information. Moreover, providing one name (instead of dozens) from a CODIS match would reduce unnecessary policework.

Validation data sets

The validation data in this study are provided by our collaborators, including government laboratories in Florida, Virginia, New York, Washington DC (FBI), and the UK (FSS), as well as private forensic labs. The data sets are comprised of synthetic DNA mixtures, casework samples, rape kits, and disaster specimens. For each case, the input into our automated LMA software is a small database of quantitated peaks, as automatically exported by TrueAllele. Our study methodologies include direct comparison, concordance with expert examiner results, as well as automation of laboratory data generation, analysis and reporting processes.

Unknown suspect (rape kit)

This problem presents a two person mixture that includes a victim of known genotype, and an unknown suspect. The task is to infer the genotype of the unknown suspect. We created artificial rape kit situations using a set of lab data prepared by the Florida groups (FDLE, PBSO). Six pairs of DNA samples were selected, and mixed in known ratios of 9:1, 7:3, 5:5, 3:7, and 1:9. These data provided 60 synthetic cases (6 pairs x 2 orderings x 5 ratios).

In one representative minor unknown contributor case, the proportions were 70% victim, and 30% unknown suspect. Using LMA, the computer inferred from the data a mixing proportion of 71% victim and 29% unknown suspect (well within pipetting error).

We illustrate the software's operation with some visual examples. Figure 5 shows the original quantitative data for two loci in the SGMplus multiplex, along with the computer's modeling based on inferred mixture proportions and genotypes.

- In the four allele case at locus D5S818, there are (at least) ten theoretically possible genotypes. When the computer performs a multi-locus analysis of peak quantities, only one of the ten possible unknown suspect genotypes is found to be statistically feasible (Figure 5A).
- At locus TPOX, there is inherent data ambiguity in this three allele case, which has six theoretically possible genotypes. Indeed, the computer finds two possible genotype solutions in the confidence set. These solutions are visually shown, along with derived genotype probabilities (Figure 5B).

The result of this higher dimensional quantitative analysis is an exact reporting of the data uncertainty via confidence sets of feasible genotypes.

Disaster data review

We assessed the automated scoring and quality assessment of highly degraded DNA. To do this, we constructed serial dilutions of DNAse (Coriell, Camden, NJ), and used these to digest 9947A control samples (Promega, Madison, WI). We amplified each digest with Promega PowerPlex 16, size separated the PCR product on an ABI/310 (Applied Biosystems, Foster City, CA), and then automatically processed the original data in TrueAllele (Cybergenetics, Pittsburgh, PA). With no DNAse, the full profile appeared; at high DNase concentration, no amplifications were seen. At intermediate DNase levels, more DNase reduced the observed amplification.

With 0.1 unit of DNase, the peak heights of most alleles were under 100 RFU (Figure 6A). However, TrueAllele called the STRs correctly (Figure 6A), with only the "low signal" rule suggesting user review. From the TrueAllele quantitation fit window (Figure 6B), the true alleles appear unambiguously correct. With user confidence in the combined power of the other 20+ Q/A rules, one might set a much lower low signal threshold when working with degraded DNA. This lower setting would eliminate manual review of high-quality, low-signal genotype data.

Conclusion

We have conducted a concordance validation study of the TrueAllele expert system for DNA database applications. We processed 48,710 genotypes using diverse STR panels (Promega PowerPlex, ABI ProfilerPlus and Cofiler) and automated DNA sequencers (ABI/310, ABI/3700 and Hitachi FMBio). We demonstrated that our automated analysis and quality assurance system is accurate, and that it can reduce the human time, error, effort and costs associated with conventional labor-intensive review of CODIS data.

We also presented our ongoing casework validation studies. This preliminary work suggests that it may be feasible to automate much of the current technical review for STR casework, including complex DNA mixtures. Our multi-dimensional mathematical LMA approach is highly specific, and should produce just one suspect from a CODIS match. This greater statistical power would help reduce policework, provide more information for the courts, and decrease the cost to society of DNA-based evidence. Moreover, our automated methods are objective (done by computer) and comprehensive (enabling analysis of all DNA samples), providing for more uniform application of DNA forensic technology.

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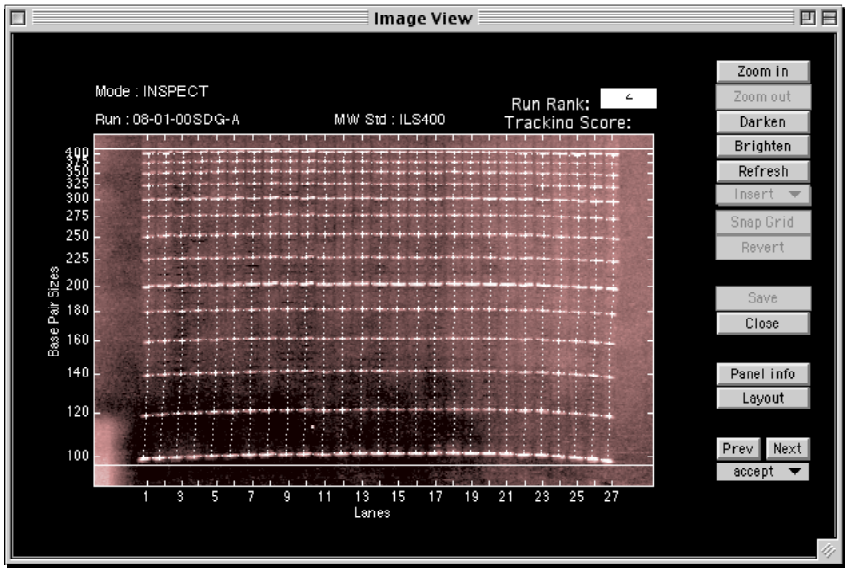
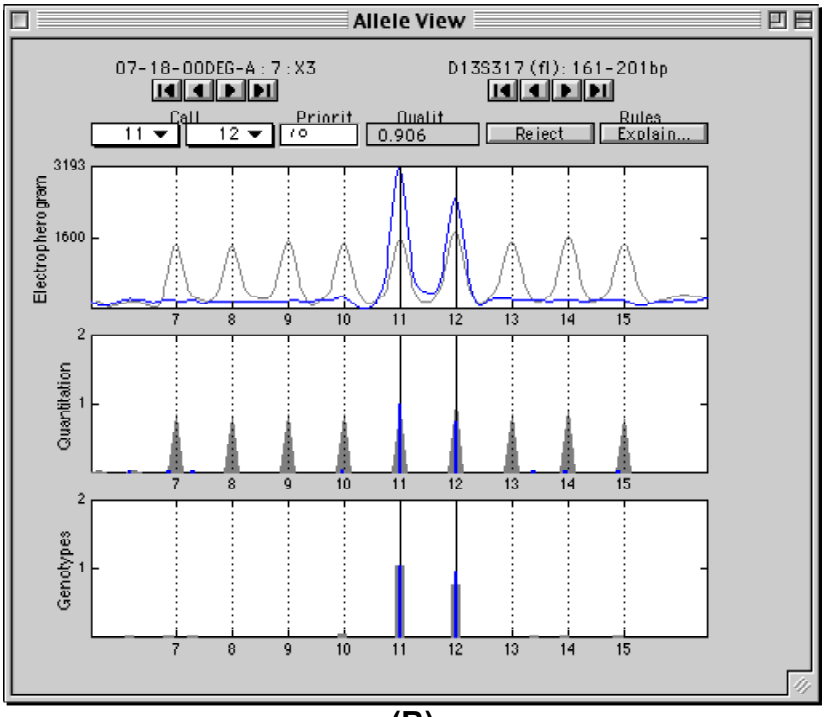
Figure 1. Automated processing. Quality assurance on Hitachi data after automated **(A)** gel analysis (step 2) and **(B)** allelic analysis (step 3).

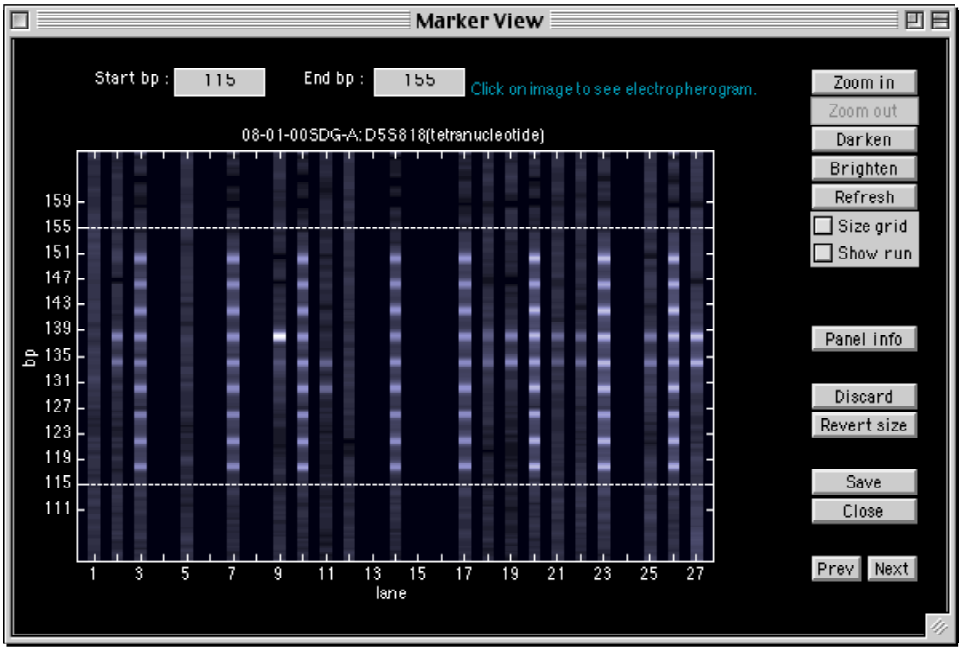


(A)

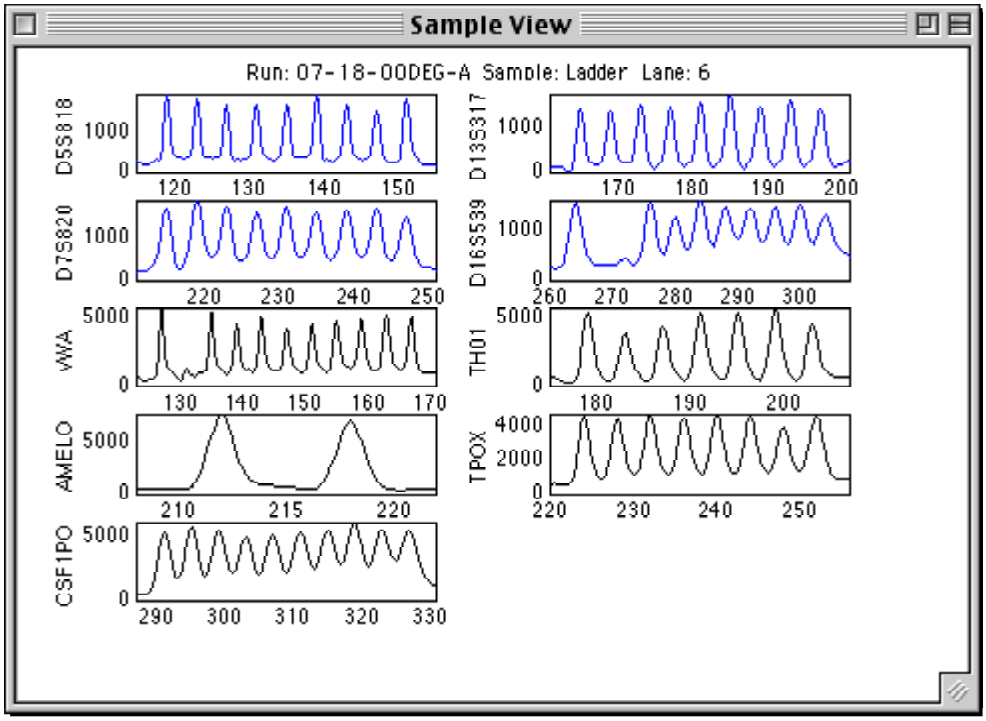


(B)

Figure 2. Quality assurance. Some of TrueAllele's many visualizations for viewing the quality of **(A)** STR data and **(B)** ladder controls.



(A)



(B)

Figure 3. Rule system. The AlleleView navigator, shown together with the Rule Analysis. Although the optical density of the Hitachi bands might be considered "low," the user-set criteria here lead the computer to decide that the data are good (no rules fired), and therefore no human review is necessary. Different user criteria might lead to a different computer decision (e.g., firing a rule). The rule criteria are customized to the needs of the individual laboratory.

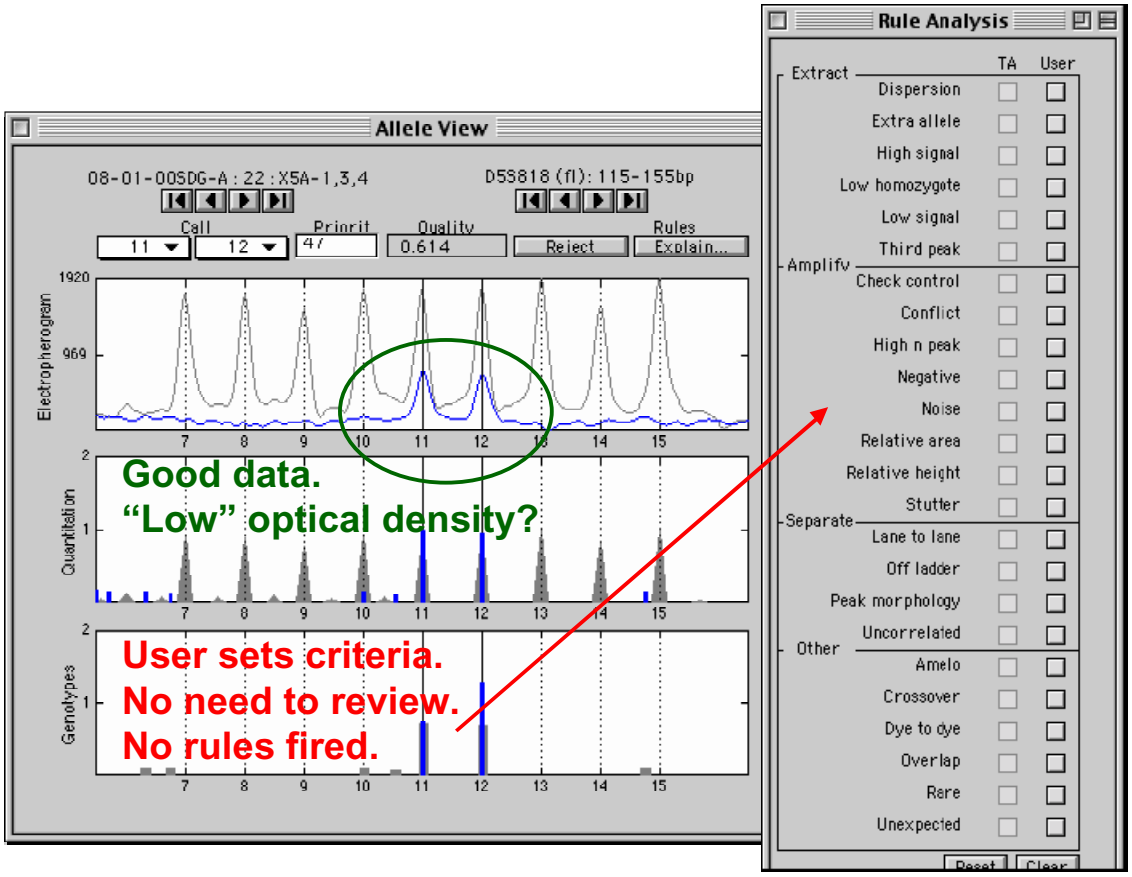
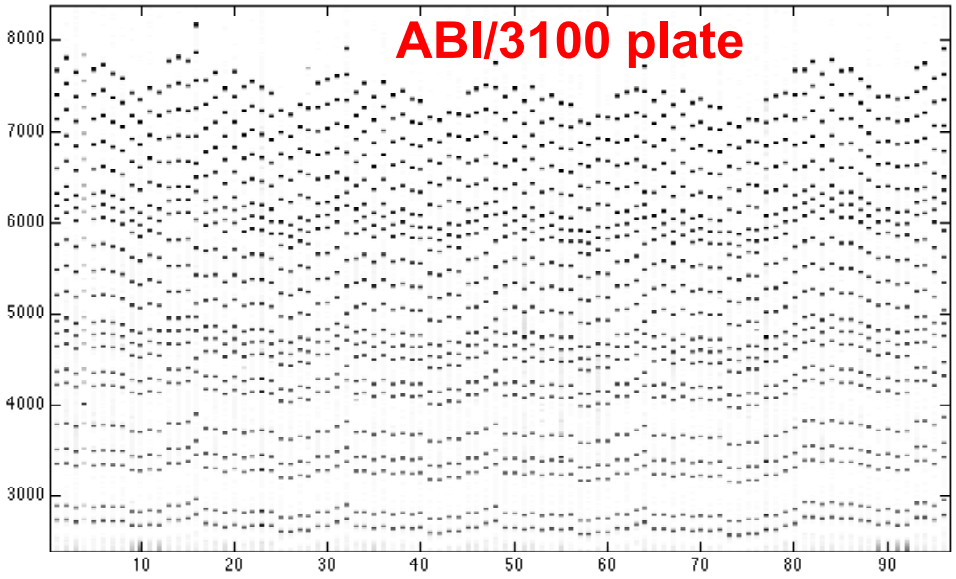
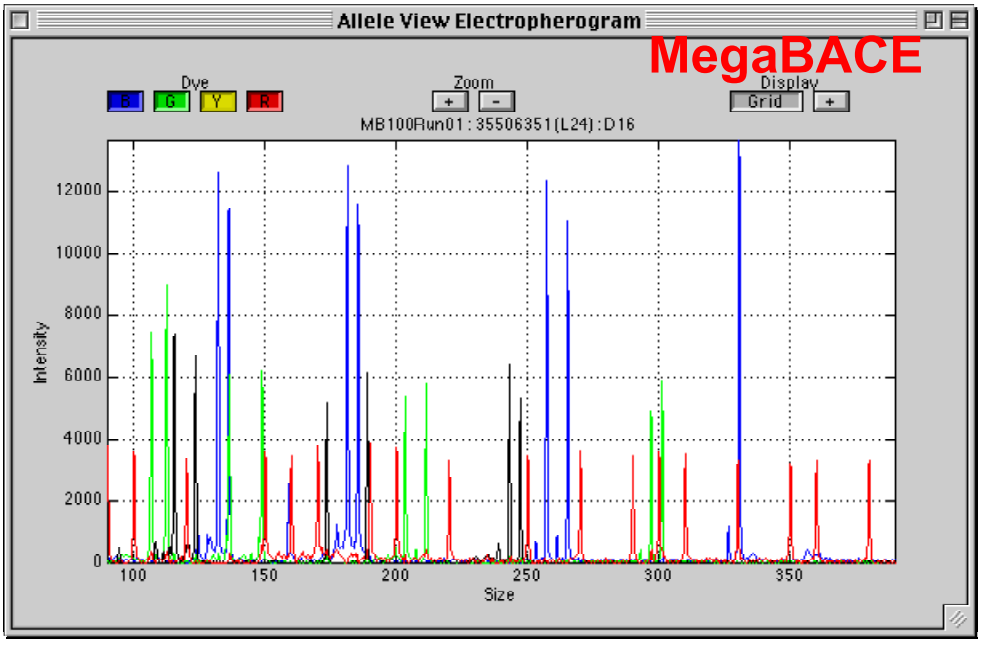


Figure 4. Multi-platform engine. TrueAllele runs on most DNA sequencers, including **(A)** the 16-capillary ABI/3100 (one 96-well plate in six runs) and **(B)** the 96-capillary Amersham MegaBACE.

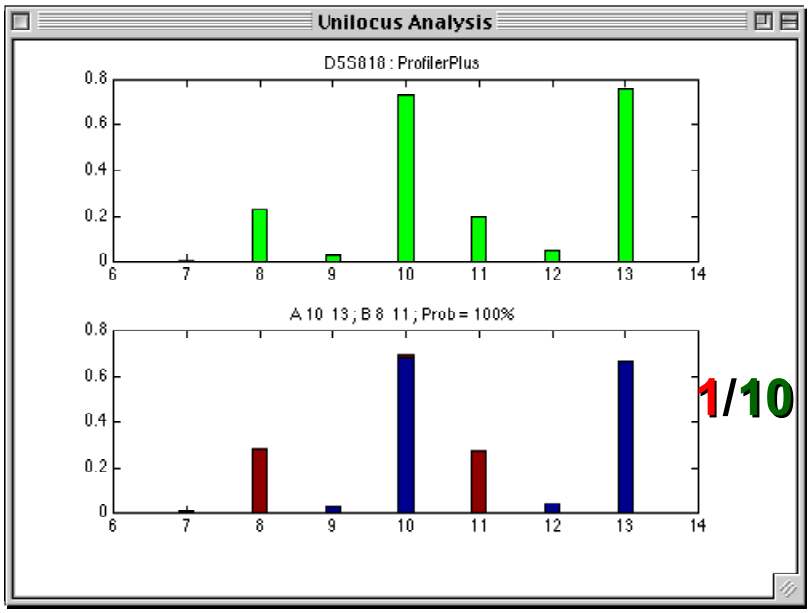


(A)

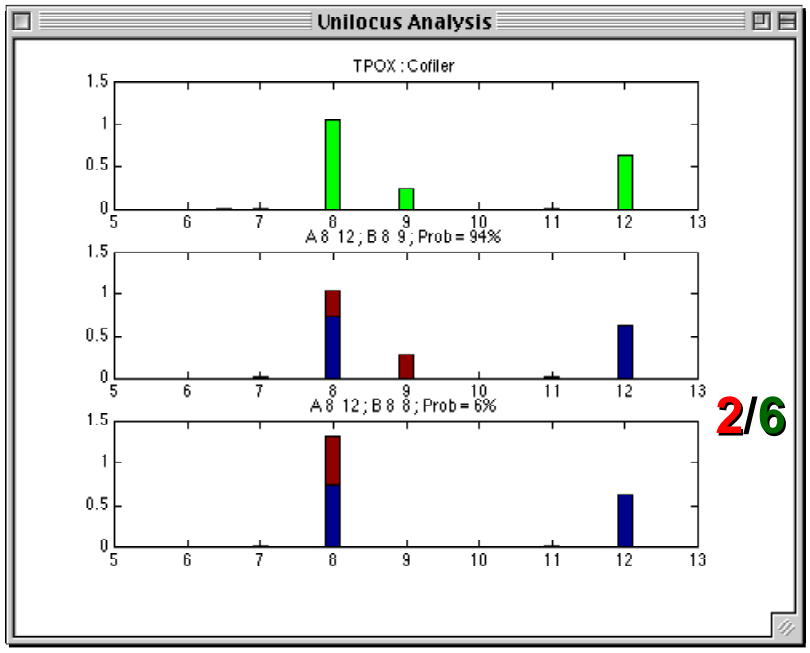


(B)

Figure 5. Unknown suspect (rape kit). Shown are the original quantitative data (green), along with the computer's modeling based on inferred mixture proportions and genotypes. The victim (blue) and suspect (red) contributions are indicated separately. **(A)** When there is a unique solution (D5S818), all but one of unknown suspect genotypes are eliminated. **(B)** When there is inherent data ambiguity (TPOX), there may be more than one possible genotype solution in the confidence set.

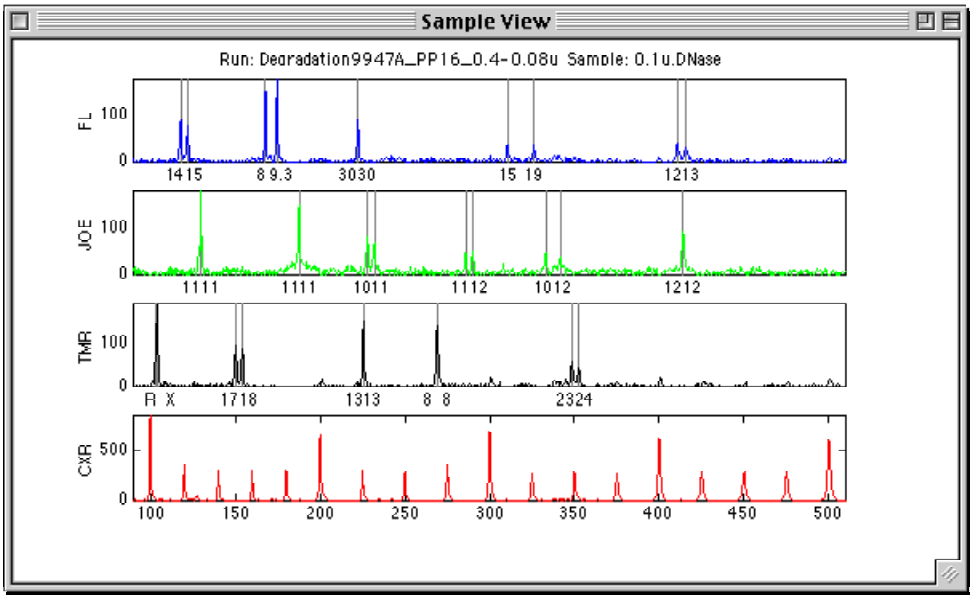


(A)

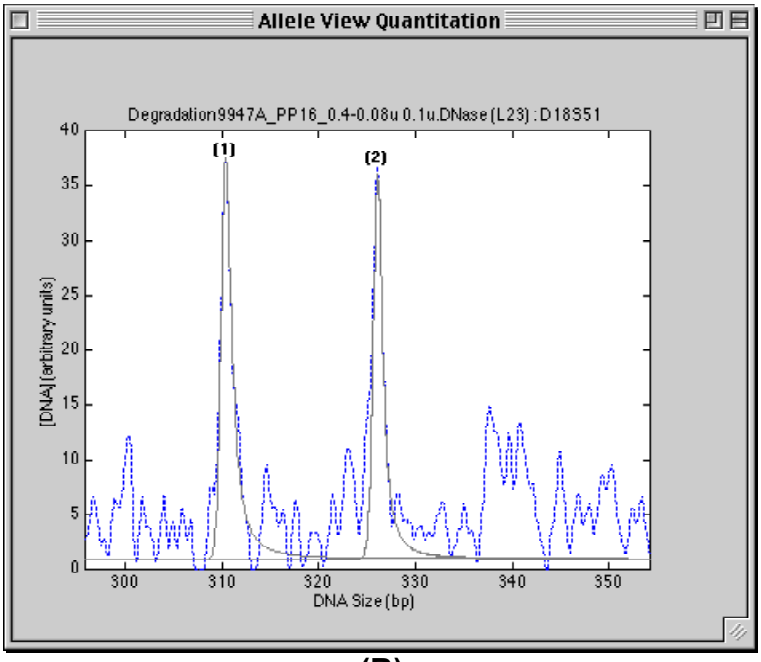


(B)

Figure 6. Disaster data review. With degraded DNA samples, **(A)** peak heights are greatly reduced, but **(B)** automated data scoring is unambiguously correct.



(A)



(B)

Table 1. Rule parameter settings. Rule settings used for the Hitachi gel and ABI capillary platforms.

		GEL		CAPILLARY	
PHASE	RULE	Status	Value	Status	Value
EXTRACT	Dispersion	On	0.6	On	0.8
	Extra Allele	On	0.25	On	0.15
	High Signal	On	9000	On	10000
	Low Signal	On	150	On	300
	Low Homozygote	On	250	On	500
	Third Peak	On	0.23	On	0.15
AMPLIFY	Check Control	On		On	
	Conflict	On		On	
	High n Peak	On	0.3	On	0.3
	Negative	On		On	
	Noise	On		On	
	Relative Area	On	0.5	On	0.5
	Relative Height	On	0.5	On	0.5
	Stutter	On	0.2	On	0.15
SEPARATE	Lane to Lane	Off		Off	
	Off Ladder	On	0.4	On	0.4
	Peak Morphology	On	0.5	On	0.2
	Uncorrelated	On	0.4	On	0.4
OTHER	Amelo	On		On	
	Crossover	On		On	
	Dye to Dye	Off		Off	
	Overlap	On		On	
	Rare	On		On	
	Unexpected	On	0.25	On	0.15

Table 2. Hitachi/FMBio (PowerPlex) results. A table comparing the computer decisions (accept, edit, reject) with the human decisions (accept, edit, reject). *With more appropriate threshold settings, the accept-accept efficiency rate was actually 82.0%.

		HUMAN REVIEW		
		Accept	Edit	Reject
COMPUTER PROCESS	Accept	72.5%*	0.0%	0.0%
	Edit	23.0%	4.2%	0.3%
	Reject	0.0%	0.0%	0.0%

Table 3. ABI/310 (ProfilerPlus & Cofiler) results. A table comparing the computer decisions (accept, edit, reject) with the human decisions (accept, edit, reject).

		HUMAN REVIEW		
		Accept	Edit	Reject
COMPUTER PROCESS	Accept	86.4%	0.0%	0.0%
	Edit	10.9%	2.1%	0.3%
	Reject	0.3%	0.0%	0.0%

Table 4. ABI/3700 (ProfilerPlus & Cofiler) results. A table comparing the computer decisions (accept, edit, reject) with the human decisions (accept, edit, reject).

		HUMAN REVIEW		
		Accept	Edit	Reject
COMPUTER PROCESS	Accept	84.9%	0.0%	0.0%
	Edit	10.8%	3.3%	0.8%
	Reject	0.2%	0.0%	0.0%