NIJ Grant Final Report

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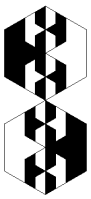
December 12, 2003

Grant Number: 2001-IJ-CX-K003
Title: Automated STR Mixture Analysis
Grantee: Cybergenetics, Pittsburgh, PA
PI: Dr. Mark W. Perlin



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Abstract

Complex DNA evidence (e.g., mixed, degraded, or small amounts of DNA) can confound straightforward approaches to interpretation and reporting. Reporting simplicity here has four components: (1) rapid turnaround time to the police or prosecutors who need DNA answers, (2) high information content that provides a useful discriminating power (DP), (3) understandable results that can be presented to the layperson, and (4) admissibility in court that reflects the underlying reliability of the scientific methods employed.

We have developed a fully automated expert computer system that interprets complex DNA evidence based on mathematical models of the STR process, and a hyper-modern statistical assessment of certainty. After taking several minutes to consider thousands of numerical variables, the computer can present the results of its rigorous deliberations as ordinary probabilities that are understandable to juries. This report describes the collaborative study design, data generation, analytic interpretation, and scientific results obtained in validating our expert system.

The validation study was designed collaboratively with crime labs in Florida, Maryland, and Virginia. The "mock rape kit" approach analyzed a set of two contributor "sperm fraction" DNA mixtures (10%, 30%, 50%, 70% and 90% proportions; at 1.0, 0.5, 0.25 and 0.125 ng dilutions), along with their "victim" reference samples. NIST prepared the mixed DNA stock solutions in known proportions for two individual pairs, and sent these materials to the ten participating forensic laboratories. Each lab followed detailed study protocols, generated PCR data for 56 templates, and sent their original sequencer data to Cybergenetics for automated computer interpretation. In addition, each lab also provided about 100 single-source samples that were used for calibrating their PCR process and artifacts (stutter, preferential amplification, peak variation, etc.). All STR chemistries and DNA sequencers in current forensic use were represented.

Cybergenetics ran their sequencer-independent TrueAllele® program on the original lab data to generate a database of quality checked, quantitated peaks (under five minutes of human time per gel). The interpretation expert system was then applied to these data (no human time). The calibration produced graphs of stutter and pref amp for each marker. The automated mixture analysis for each lab's 40 unknown suspect cases (mixture and reference data, but no suspect data) yielded an estimate of the mixing proportion, the genotype confidence set at each locus, and quantitative bar graphs that permitted rapid visual comparison between the observed data and the best genotype model. These computer results were reviewed by each laboratory.

The DNA templates and the computer interpretation were the same throughout the study, so we could compare the amount of information present in each laboratory's data. Our key information measure was discriminating power – the probability that a randomly selected person from the population matches the inferred unknown suspect profile. Unlike conventional measures (e.g., CPE, likelihood ratios), our computed DP is an understandable probability result that reflects the computer's detailed consideration of all feasible genotypes, and captures all relevant information. We (1) found that DP is an accurate measure of laboratory data quality, (2) established a quantitative relationship between mixture proportion and template dilution relative to information content, and (3) showed how the computer could combine multiple mixture samples at low DNA concentration without any reference sample to derive very high unknown suspect DP.

The legal admissibility of our scientific approach was established by the reliability demonstrated in our collaborative validation study. All points of Daubert were addressed: testability, error rate, peer review, and general acceptance. These admissibility issues are detailed in this report. We have validated an expert computer approach to the interpretation of complex DNA evidence that is objective, unbiased, reproducible, reliable and admissible. Moreover, the system generates simple and understandable results that are useful in court.

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Introduction

Justice helps protect society by exonerating the innocent, and apprehending and convicting the guilty. One task of the criminal justice system is to identify the people who were present at a crime scene. In the past decade, DNA identification has become an objective way to obtain such scientific evidence (1). This section describes each of the concepts introduced in the title of this report.

Simple reporting of pristine, single source DNA data is understandable, and readily achieved. The data in Figure 1 show such a clean DNA profile. At each locus, there are exactly 1 or 2 major peaks, and each peak directly corresponds to an allele. Interpretation here is easy – at each locus, look at the peaks, and report the genotype as the pair of alleles that is present.

With complex DNA evidence, however, the underlying interpretation may not be immediately apparent. Figure 2 shows a mixed DNA profile comprised of two contributors. At a locus with three peaks, it is unclear which alleles come from which contributors. There are many such data issues that are common in DNA casework: low peak height, varying peak size, PCR stutter artifact, preferential amplification, mixed DNA stains, low amounts of DNA, degraded DNA, interpreting many samples together, handling multiple underlying contributors, determining the number of contributors, background noise, random peak variation, and low copy number signals. Thus, peaks no longer directly correspond to alleles. Moreover, as society's expectations increasingly demand DNA for every routine case, such fuzzy data will become the future of DNA evidence.

Fortunately, automated computer interpretation can help satisfy these expectations. For every data artifact, scientists can model the phenomenon with mathematics, and use statistics to assess the uncertainty. Although these models can become quite complex, entailing thousands of interacting equations, they are readily solved by computers. The result is that even the most complex DNA evidence can be reported as simply as clean single source data. For each contributor, at each locus, the computer simply reports the genotypes. With unambiguous data, this genotype will be unique; highly ambiguous data may lead to multiple genotype possibilities.

Background

The last decade has witnessed many important revolutions. We focus here on just four.

- Science. In molecular biology, the amplification power of polymerase chain reaction (PCR) (2), and its application to short tandem repeat (STR) polymorphisms (3), have revolutionized scientists' ability to study trace amounts of DNA. And, the complementary automated fluorescent DNA sequencer (4) (which sequenced the human genome) provided a painless way to quantitatively characterize STR fragments.

- Technology. For the last four decades, the ubiquitous computer has exponentially increased each year in power and compactness (5). Whether used in cell phones, automobiles, DNA sequencers, or the Internet, computer intelligence pervades and facilitates our daily productivity.
- Law. The 1993 Daubert decision revolutionized the role of science in the courtroom (6). Instead of relying on accepted practice, judges are now the gatekeepers of scientific evidence, applying objective criteria to assess scientific reliability.
- Statistics. In the past decade, using new mathematics and modern computers, statisticians have moved beyond the null hypothesis, and now apply powerful methods to determine computational truth (7). These new techniques have been used in hundreds of applications (8).

Cybergenetics TrueAllele® expert system automates the interpretation of STR data. An expert system is a computer program that replicates (or transcends) human expertise (9); this is what the TrueAllele system does for allele calling. The TrueAllele project started over ten years ago, with the concept that the PCR stutter artifact in dinucleotide repeat markers was reproducible, and could be mathematically removed by computer. The resulting STR analysis, interpretation and reporting technology led to a series of papers (10, 11), patents (12) and genetic applications (13). Five years ago, the TrueAllele System 2 software was adapted for forensic databasing applications (14-16). Over the last four years, we have been developing TrueAllele System 3, an intelligent system for forensic casework (17). This past year, we designed, built and tested version 15, a System 3 program specifically created for solving complex casework that contains lower quality data.

Validation

Scientific evidence is admissible in court only if it is reliable. Rule 702 of the United States Federal Rules of Evidence states that all of these components must be reliable: the data, the method used, and the application of the method to the data. The older 1923 Frye standard assessed this reliability based on general acceptance. The more modern 1993 Supreme Court Daubert ruling provided three additional prongs for determining the reliability of each component (data, method, application):

(1) Testable. The component should be capable of being tested, and have actually been tested.
(2) Error Rate. The component should have an error rate, which has actually been determined.
(3) Peer Review. The component (and its reliability) should be disseminated in the relevant scientific community.

The usual approach to demonstrating these admissibility criteria is to establish reliability through a validation study.

Our validation study was designed to assess interpretation efficacy for two contributor mixture cases. The experimental design has three axes: varying mixture ratios, serial DNA dilutions, and different contributor pairs. As shown in Table 1, the mixture weights

were 10%, 30%, 50%, 70% and 90%. The DNA amounts were 1 nanogram, 0.5 ng, 0.25 ng and 0.125 ng, standardized to a 25 microliter volume. DNA from four different individuals was used to create two distinct sets of two contributor mixtures.

Premixed DNA templates were prepared by the National Institute of Standards and Technology (NIST). There were 14 stock solutions, 7 for each mixture set, which included the two individuals and the five mixed DNA samples. NIST sent these stock solutions to each of the 10 participating DNA laboratories, located in Florida, Maryland, New York, Ohio, Pennsylvania, Virginia and the United Kingdom. Using detailed laboratory protocols that we had prepared for this study, each lab diluted their stock solutions into the 56 PCR templates of the experimental design. Each laboratory then followed its usual casework protocols to generate electronic DNA sequencer files. A diverse set of STR panels (Promega PowerPlex 1, 2, 16; ABI ProfilerPlus, Cofiler, SGMplus, Identifiler) and DNA sequencers (Hitachi FMBio; ABI 377, 310, 3100, 3700) were used in the STR data generation. Each lab forwarded its completed DNA sequencer files to Cybergenetics for further processing in the study.

Technology

The TrueAllele® Technology automates the three cognitive tasks that follow STR data generation: analysis, interpretation and reporting. Starting from the original sequencer data file, the TrueAllele Analysis program automatically transforms the file into a quality checked peak height database. This is shown in the upper half of Figure 3. The computer independently performs all steps of the analysis process, including image and signal processing, background subtraction, dye color separation, lane tracking, peak detection, peak sizing, ladder derivation and comparison, coordinate transformation, peak quantitation, artifact detection, and quality assurance of the sequencer run and its controls (e.g., positive, negative, sizing and allelic ladders). The human task is then reduced to simply checking the computer's automated quality checking, which takes about 3 minutes of operator time per sequencer run.

In our earlier TrueAllele System 2 for DNA databanking, after the peak analysis there is additionally the interpretation of the single source data (16). The computer uses over 20 rules, applied to each genotype, to identify potentially problematic allele calls. The user then reviews just these problematic genotypes (typically, about 10% of the total), and decides whether to except, reject or edit the allele calls. This computer-based, streamlined STR review process has led to considerable efficiencies in DNA databank construction, particularly in the United Kingdom.

The UK Forensic Science Service (FSS) adds 350,000 STR profiles every year to the UK National DNA Database, using Cybergenetics' TrueAllele expert databank system to automate their data review. The FSS identified several key TrueAllele process improvements relative to their previous semi-automated manual process. These improvements included (a) reduced turn-around time from one week to an eight hour shift, (b) adaptive capacity that could meet or exceed the throughput requirements of

30,000 samples per month, using two desktop computers, (c) reduced manpower requirements from 75 individuals to 2 people per shift, with elimination of associated space, computers and software, (d) a high quality process, leading to greater confidence in the accuracy of the data review results and automated troubleshooting, and (e) standardization of the databank review process, making it reproducible and objective, with automated quality assurance and audit trails.

For casework interpretation, the TrueAllele Interpretation program downloads relevant data from the quality-checked quantitative peak database, automatically interprets the data, and then uploads its results to the reporting database. This is shown in the lower half of Figure 3. When interpreting the data, the TrueAllele program applies the mathematical and statistical models of data behavior described above, and infers the genotypes of each of contributor, along with other useful formation, such as mixture weights and discriminating power. This process takes no human time, since the interpretation is done entirely by computer.

Example

To understand how the TrueAllele interpretation system works, it is useful to step through its problem solving on a straightforward no suspect, mock sexual assault example. The STR data used in this example were amplified in the Cybergenetics laboratory using 1 ng NIST DNA templates with a Promega PowerPlex 16 STR panel, and then detected on an ABI 310 automated fluorescent DNA sequencer. In this mock case, there are two contributors: (A) the victim, and (G) the unknown perpetrator. Two mock evidence data samples are interpreted together:

- A1, the victim control sample, and
- C1, a DNA mixture comprising a 70% contribution from victim A, and a 30% contribution from the unknown perpetrator G.

For objectivity, the computer only evaluates the case evidence, and does not consider any suspect profiles (e.g., perpetrator G) when it performs its interpretation. Suspect matching can be done after the computer has completed all of its processing.

The victim control A1 data are shown in Figure 1 – a clean, single source, easily interpreted DNA profile, where each peak corresponds directly to an underlying allele. The data peaks for mixture C1, shown in Figure 2, have more complex patterns. In some of the four peak patterns (e.g., TH01), one can visually pick out the two minor peaks that probably belong to the unknown minor contributor. However, in the two and three peak patterns, it is more difficult to derive the minor contributor genotype possibilities.

The TrueAllele interpretation computer solves this problem using two inputs: a quantitative peak database for the peaks shown in the two data figures, and an interpretation request that tells the computer where to find the two DNA sequencer lanes used in this case. Using SQL database queries to look into the PostgreSQL relational database (18) that underlies TrueAllele System 3:

- Table 2 shows some of the peak information recorded for a few peak records. Each peak table record corresponds to one data peak, and describes its chain of custody (e.g., laboratory, DNA sequencer, STR panel), peak size information (e.g., pixel, designation), and peak quantitation information (e.g., height, area). All peaks with heights greater than zero are recorded in the database, since statistically reliable interpretation requires all peaks to be considered.
- Table 3 shows the request table that connects each specimen in this case with its DNA sequencer capillary or lane location. This information is provided by a forensic scientist, or by a laboratory information management system (LIMS), in order to specify the scientific interpretation question. The request can include specimens from one case, from many cases, or from one focused part of a case.

The actual processing of this case was demonstrated live during the conference presentation on an Apple G4 PowerBook laptop computer. The computer downloaded the peak and request information from the database, set up the problem, interpreted the data by considering thousands of variables (including all feasible genotypes and mixture weights, each peak and its variation, PCR stutter, preferential amplification, and background noise), and then uploaded its results back to the database. The entire process took 29 seconds.

The TrueAllele report for this case includes results of interest to both scientists and laypeople. The results were queried from the underlying TrueAllele relational database:

- Table 4 shows the mixture weights, which can be used as evidence of contact between biological materials. The victim sample A1 corresponds to the first contributor, since the victim's DNA has a weight of 1 for the first contributor, and a weight of 0 for the second contributor. The probative specimen C1 is a mixture of the two contributors, with a weighting that combines about 70% of the first victim contributor, and 30% of the unknown second contributor. These computer inferred weights accurately correspond to the experimentally created proportions in DNA templates A1 and C1.
- Table 5 shows the genetic identity of the unknown second contributor. (The computer also infers the first contributor victim's genetic identity, which is straightforward since it has available reference sample A1.) In this mock case, even though the unknown is a 30% minor contributor, the TrueAllele computer infers a unique (and correct) genetic identity at the 99% confidence level at each STR locus. This mathematical conclusion is possible because the laboratory data contain sufficient information to support a high level of statistical precision.

These results satisfy the reporting simplicity goals stated in the Abstract.

(1) Time. The total interpretation and reporting time for this minor contributor mock sexual assault case was less than one minute.

(2) Information. As detailed in the next section, the unique inferred genetic profile has full discriminating power for identifying (hence apprehending and convicting) the unknown suspect, and excluding innocent men.

(3) Understandable. The results for the triers of fact were presented as the genotype at each locus of the unknown contributor. (Had there been greater data ambiguity, a

locus might have reported multiple genotypes.) This mixture result is as easy to understand as the reporting of clean, single source DNA profiles.

(4) Admissible. These results are reproducible and scientifically reliable, as reported here and in forthcoming scientific publications. In particular, one can test a computer interpretation system, and determine its error rate, as mandated by Rule 702 under Daubert.

There are 1440 minutes in each day; there are many laptop computers in this world. And yet there is a backlog of hundreds of thousands of no suspect sexual assault cases and convicted offender profiles (19). Clearly, with the TrueAllele technology, the analysis, interpretation and reporting components of this case backlog can be fully addressed within several months. It may be advantageous for society to identify perpetrators, and prevent them from committing further violent crimes, using fast computer-based interpretation methods .

Information

Discriminating power is a good measure of DNA information. Although each person has a unique genetic profile, crime scene evidence can have varying degrees of clarity. Think of a photograph: a sharp picture of someone's face may be uniquely identifying, but a blurry image taken from a distance on a rainy night might match millions of individuals. A similar range of identification power is found with DNA evidence, where a unique genotype may have a discriminating power of one in quadrillion, whereas complete ambiguity yields no discriminating power at all.

Consider the illustrative three peak example in Figure 4. A typical "conservative" interpretation might report six possible genotypes at locus, offering relatively little discriminating power. A less conservative "cautious" group might consider data at other loci or specimens, and eliminate some possibilities in order to increase discriminating power. Alternatively, a mathematically powered, statistically enabled computer solution might scientifically derive the "exact" solution, thereby realizing the greatest discriminating power. Sharper discriminating power provides greater scientific truth to the criminal justice system.

As part of our ongoing TrueAllele concordance studies, we are comparing computer and human discriminating powers on the same cases. In a no suspect case, where no match is performed, discriminating power can provide an objective comparison measure. Table 6 compares the genetic profile results in the example case for the TrueAllele computer interpretation and a double reviewed forensic laboratory human interpretation. The computer accurately designates all 26 of the 26 possible alleles, with population frequencies generating a discriminating power of 3.6×10^{16} (rarer than one in quadrillion). The more conservative human review designates only 18 of the 26 alleles, yielding a discriminating power of 6.1×10^{11} (commoner than one in a trillion). The almost five order of magnitude computer improvement in derived information becomes increasingly important as DNA case data become more ambiguous.

Comparisons

Having discriminating power as an information metric permits useful comparisons between different experimental variables. In this section, we compare data quality between different laboratories, look at the effect of mixture weight and DNA concentration on information content, and examine the effect of extreme data conditions. In these comparisons, we used TrueAllele System 3, version 14, across a range of laboratories, mixture weights, DNA concentrations, and contributor pairs.

Since discriminating power ranges over many orders of magnitude, we use the negative logarithm of discriminating power as a measure of identification information. For example, with a discriminating power of one in a trillion, or 10^{-12}, the information measure is 12, since the negative exponent $-\log_{10}(10^{-12})$ is $\log_{10}(10^{12})$, which equals 12. This use of probability logarithms is a standard method used in information theory.

Data quality

Assume a fixed total amount of DNA. As the mixture weight of the unknown contributor decreases, the amount of contributor DNA decreases as well. Therefore, as the unknown contributor weight decreases from 100% down to 10%, the contributor's peak heights decrease by an order of magnitude, and one would expect a corresponding decrease in identification information.

Figure 5a shows the discriminating power information of five collaborating laboratories at different mixture weights at a 1 ng DNA concentration. Each solved case includes the known victim reference A, and a mixed DNA sample containing contributors A and G. The mixture weights of G shown are 10%, 30%, 50%, 70% and 90%, corresponding respectively to the mixed specimens B, C, D, E and F. To standardize the comparison, we restricted the comparison to only those labs using the ABI/310 DNA sequencer on STR panels that included the 13 core CODIS loci. For a meaningful cross laboratory comparison, the discriminating information shown uses just the 13 core CODIS loci.

First, we look at the average information produced by the laboratories as mixture weight varies. In a 1 ng sample having a 90% unknown contributor (sample F1), all labs produce the full information of 17 (corresponding to a one in 10^{17} discriminating power). As the proportion of unknown contributor decreases, the identification information decreases as well, reaching 12 (1 in a trillion) at 30% unknown (sample C1), and 6 (1 in a million) at 10% unknown (sample B1).

Next, we look at the variation in information produced by the different laboratories. All laboratories start with the same DNA template material provided by the NIST. The analysis, interpretation and reporting is done by the same version of the TrueAllele software. Therefore, the only source of experimental variation is in the laboratory process itself, generating STR data by PCR amplification and DNA sequencer detection. The variation seen in Figure 5a suggests that some laboratory processes

yield more information than others, based on the quality of their STR data. For example, at the 30% unknown suspect level (sample C1), the data of lab 1 has an identification information of 14, relative to an average information of 12.

This difference in laboratory data quality is even more pronounced in Figure 5b. This chart shows the information of the laboratories at different mixture weights (at a 1 ng DNA concentration) for cases that include the known victim reference H, and a mixed DNA sample containing contributors H and N. The mixture weights of N shown are 10%, 30%, 50%, 70% and 90%, corresponding respectively to the mixed specimens I, J, K, L and M. Note that at the 30% unknown suspect level (sample J1), the data of lab 1 has information at 15 log units, while lab 5 has a marked diminution in data quality, with information only at 7 log units -- an 8 order of magnitude discrepancy. This data quality problem is seen again with lab 5 at the 10% unknown suspect level (sample I1) and 50% unknown suspect level (sample K1).

Since the TrueAllele information analysis had indicated a data quality problem on this data set, we asked lab 5 to repeat its experiments for the second contributor pair H and N. These updated results are shown in Figure 5c. Comparing the values of lab 5 and lab 5r ("r" for repeat), we see a restoration of full data quality (as measured by discriminating information) in the repeated experiments. These results suggest that the TrueAllele technology can provide a useful objective measure for monitoring casework data quality.

DNA quantity

With reduced quantities of DNA, the peak heights are smaller and the data variation is greater. Figure 6 shows low-level STR data from DNA specimen C4. The mixture sample C has two contributors, weighted in a 70:30 mixture ratio. The 4th serial dilution C4 has just 0.125 ng DNA concentration, compared with the full 1 ng DNA concentration of C1 (see Figure 2). Observe that many of the loci in Figure 6 (e.g., CSF1PO, TH01, vWA) show informative peaks with all heights less than 100 RFU.

Statistical theory predicts that as the amount of DNA decreases, so too will the information content. Theory also predicts that serial reductions in DNA quantity should produce a linear reduction in information (i.e., decreasing logarithm of discriminating power). Our data experiments confirm these predictions.

Figure 7a shows the computed discriminating information at different mixture weights for four different DNA concentrations – 1, 1/2, 1/4 and 1/8 ng. Each solved case includes the known victim reference A, and a mixed DNA sample containing contributors A and G. The mixture weights of G shown are 10%, 30%, 50%, 70% and 90%, corresponding respectively to the mixed specimens B, C, D, E and F. The study data used for this comparison were generated in the Cybergenetics laboratory.

The information content decreases with decreasing DNA quantity. This is seen in Figure 7a with decreasing mixture weight of the unknown contributor G at each fixed

DNA concentration, echoing the pattern previously observed in Figure 5a. Moreover, at a given mixture weight, the serial reduction in DNA quantity produces a linear reduction in information. For example, at the 50:50 mixture ratio for sample D, the serial dilutions of 1, 1/2, 1/4 and 1/8 ng (corresponding respectively to templates D1, D2, D3 and D4) show an information reduction of about 1.25 information units (discriminating power log units) per serial dilution.

This observed concordance with statistical theory does not depend on the particular pair of DNA contributors. Figure 7b is analogous to Figure 7a, but uses instead the contributor pair H and N, with mixed specimens I, J, K, L and M. These data similarly demonstrate the predicted pattern of information loss with reduced DNA quantity, as well as the quantitative linear information reduction for serial dilutions.

Complex cases

The TrueAllele computational system can integrate data in ways that are not possible with human analysts. Consider a case that has several low-level mixed DNA stains, but includes no single-source samples. In current human review practice, peak height cutoffs would be applied to most of the data, and little information would remain. However, a sophisticated mathematical model can consider unifying contributor genotypes across all these data, and use statistical modeling to delimit the certainty of its results.

From our study data, we constructed such complex test cases. We gave the computer data from three samples: C, D and E, corresponding respectively to 70:30, 50:50 and 30:70 mixtures of contributors A and G. This TrueAllele interpretation was done at four DNA concentrations – 1 ng (C1, D1, E1), 0.5 ng (C2, D2, E2), 0.25 ng (C3, D3, E3) and 0.125 ng (C4, D4, E4). Representative electropherogram data of these mixed DNA samples is shown for adequate amounts of DNA (specimen C1 in Figure 2, a 70:30 mixture at 1 ng), and for low amounts of DNA (specimen C4 in Figure 6, a 70:30 mixture at 0.125 ng).

The results are shown in Figure 8. The right hand side of this figure shows the information (logarithm of discriminating power) obtained for the unknown suspect G. At the 1 ng level, combining the three mixtures C1, D1 and E1 without any single source reference material the computer produces a unique genotype result for the unknown contributor G having full information – 17, or one in 10^{17} discriminating power.

At the 0.125 ng level, the computer can integrate all the data, rather than discarding peaks. Combining all the noisy peak data for the three low-level mixtures C4, D4 and E4 (without any single source reference), the TrueAllele interpretation yields a genotype set for the unknown contributor G that has an information value of 12, i.e., one in a trillion discriminating power. This information would be more than sufficient to identify, apprehend and convict a true perpetrator, or to exonerate an innocent man.

Specific Aim Results

This section describes the results of the TrueAllele linear mixture analysis validation project in the context of the specific aims of the original proposal.

(1) Provide quantitative DNA mixture analysis on a wide range of data.
(1a) Derive individual DNA profiles from STR mixture data.
(1b) Determine the quality and utility of the derived profiles.
(1c) Operate on STR data generated from different platforms.

This study validated the TrueAllele System 3 casework interpretation system. The system derives individual DNA profiles for each contributor from STR case data. By representing the statistical confidence of each locus of each contributor as a set of feasible genotypes, the quality and utility of the derived profiles can be determined by the size of the set. A unique genotype indicates high-quality, useful information; a large confidence set of possible data types suggests lower quality information. Through our large network of collaborating laboratories, the study used STR data from every panel and sequencer currently in forensic use.

(2) Handle known STR artifacts and adapt to new marker technologies.
(2a) Account for PCR stutter artifacts.
(2b) Modify experimental parameters for robust analysis.
(2c) Determine applicability to SNP markers.

TrueAllele System 3 includes statistical models for many known data artifacts, including PCR stutter and relative amplification. The primary experimental parameters that permit robust analysis are the quality and quantity of STR data. As demonstrated in the preceding section, higher quality data (e.g., based on the laboratory process and the amount of DNA) can be objectively shown to produce more information than lower quality data. Also, the more STR data experiments conducted, the greater the produced information. For example, we showed that combining multiple low-level DNA experiments can produce highly useful DNA information. Software experiments on simulated SNP data showed that the linear mixture analysis method applies to quantitative SNP markers (results not shown).

(3) Interact well with users, including DNA analysts and reporting officers.
(3a) Devise strategies for effective court presentation of results.
(3b) Determine how to best train people in the use of the software.
(3c) Provide dynamic computer visualizations for effective communication.

In the course of the study, much attention was given to software training and to dynamic computer visualizations. We presented computer interfaces that showed how the peak data could be modeled by appropriate computer parameters (e.g., genotype, weight, stutter). However, we also learned that the ultimate gatekeeper and triers of fact are laypeople who are quite comfortable with the "random man" presentation of simple, pristine, single-source DNA genotype results. And that most people (including many

DNA analysts) do not appreciate the complexities of current DNA mixture interpretation. Our conclusion, therefore, is that the best presentation for the ultimate users is simply describing the locus results for each contributor in terms of genotypes – a solution that the sophisticated mathematical and statistical TrueAllele System 3 computational engine can provide. Related information, such as discriminating power and match results, are also simple and useful numerical results. Since the TrueAllele interpretation system is entirely automated, there is no interactive software that would require user training.

Application

There are many ways to deploy the TrueAllele interpretation technology into a laboratory process. The approach taken depends entirely on the laboratory's workflow, speed and accuracy requirements, and re-engineering objectives. Representative scenarios include using the TrueAllele automation:

- to initially screen the data and organize the case, prior to human review;
- as a second scorer that reviews the case data after an initial human review;
- for post-conviction DNA testing (e.g., in an innocence project) as a way to screen DNA case evidence in-depth, prior to involving a defense expert;
- as a DNA assistant for the forensic scientist that can perform the detailed review of DNA evidence;
- for cleaning up inferred DNA case profiles before uploading them to a forensic crime database, by reducing the set of possible genotypes to just those that are scientifically feasible;
- to reduce from many to one (or none) the number of scientifically feasible DNA suspect profiles obtained from an ambiguous convicted offender database search – this can reduce considerably the police effort of tracking down all these suspects;
- to review the DNA data in large-scale mass disasters;
- for solving serial crime by combining DNA evidence from multiple cases;
- to detect terrorist activity by inferring genetic profiles from low-level DNA evidence; and
- for quality assurance, and in the real-time troubleshooting of automated DNA production lines.

The forensic scientist integrates diverse types of scientific evidence into a unified, coherent presentation. An advanced TrueAllele computer that can reliably interpret DNA data can free the scientist from a task better done by high-powered calculators. Computer interpretation would give the forensic scientist time to work more closely with police, prosecutors and the courts – time to synthesize and communicate information in ways that only people can. By offloading some of the computational DNA burden to the computer, there would be more human time for productive, creative scientific thought.

Conclusion

This research introduced an automated computer system for DNA casework interpretation. The system is:

- objective, inferring genetic identity results in complex mixture cases without ever seeing a suspect DNA profile;
- unbiased, using only quantitative DNA peak information on the experiments of interest;
- reproducible, consistently generating the same results given the same data;
- reliable, producing scientifically sound answers that are useful to the criminal justice system;
- admissible, based on scientific studies that test reliability and determine error rates; and
- available, as the TrueAllele System 3 software, version 15.

The system uses its mathematical power to generate genetic identity reports that are easy to understand, and simple to explain to nonspecialists. The results are analogous to the jury-tested reporting of single source DNA samples. Our computational approach differs from current mixture reporting methods, whose inherent complexity may confuse the issues or mislead the jury.

We are currently conducting a number of concordance and validation studies with both government and private DNA forensic laboratories. Our concordance studies on no-suspect sexual assault cases use discriminating power as a measure of useful information. When perpetrators have been identified, then match results can be used to assess interpretation accuracy. In our ongoing studies, we expect to analyze up to one thousand cases in the coming year; we will report on these results in the scientific literature.

The goal of this report was to show how simple reporting of complex DNA evidence could be achieved through automated computer interpretation. As described above, we have established this result. Moreover, we presented an actual problem solving case example that illustrated our simplicity criteria of turnaround time, genetic information, layperson understandability, and legal admissibility. As intelligent DNA interpretations systems come into general use, we expect society to benefit from the more rapid and accurate application of DNA evidence in our criminal justice system.

Acknowledgements

I would like to thank our collaborating forensic DNA laboratories in Florida, Maryland, New York, Ohio, Pennsylvania, Virginia, and the United Kingdom. Dr. Margaret Klein at NIST prepared and distributed the mixed DNA templates for this study. Dr. Jay Kadane at Carnegie Mellon University collaborated in the statistical development. Jeff Ban, Dr. Robin Cotton and Dr. Cecilia Crouse were instrumental in designing the study. At

Cybergenetics, the software developers, quality process team, and DNA laboratory all contributed significantly to the study.

This research was supported in part under Award number 2001-IJ-CX-K003 from the Office of Justice Programs, National Institute of Justice, Department of Justice. Points of view in this document are those of the authors and do not necessarily represent the official position of the U.S. Department of Justice.

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Figures

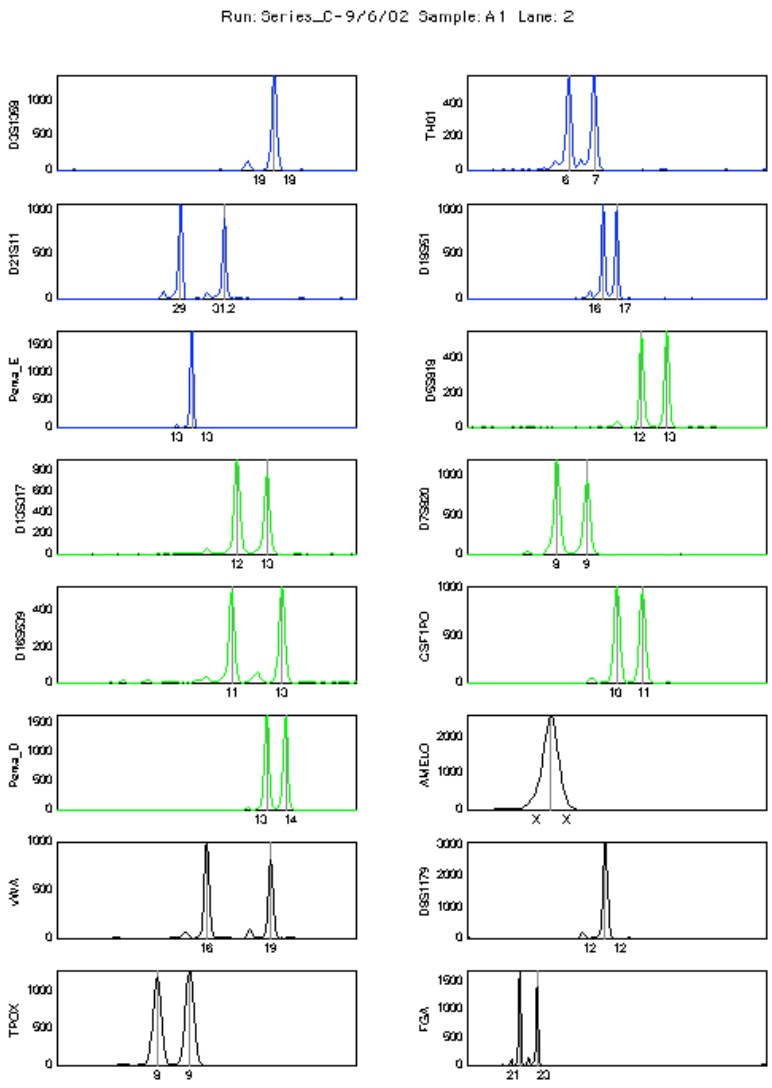


Figure 1. The pristine STR data from single source victim control sample A1.

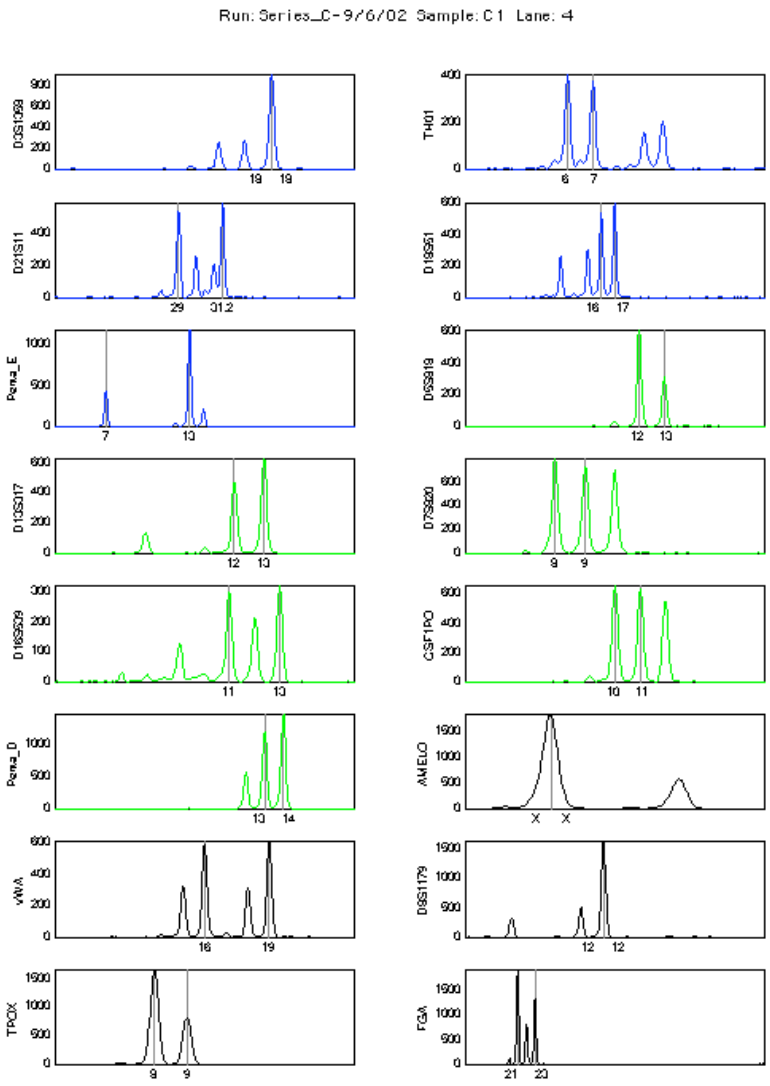


Figure 2. The STR data from the mixed DNA specimen C1. There are two contributors, weighted in a 70:30 mixture ratio.

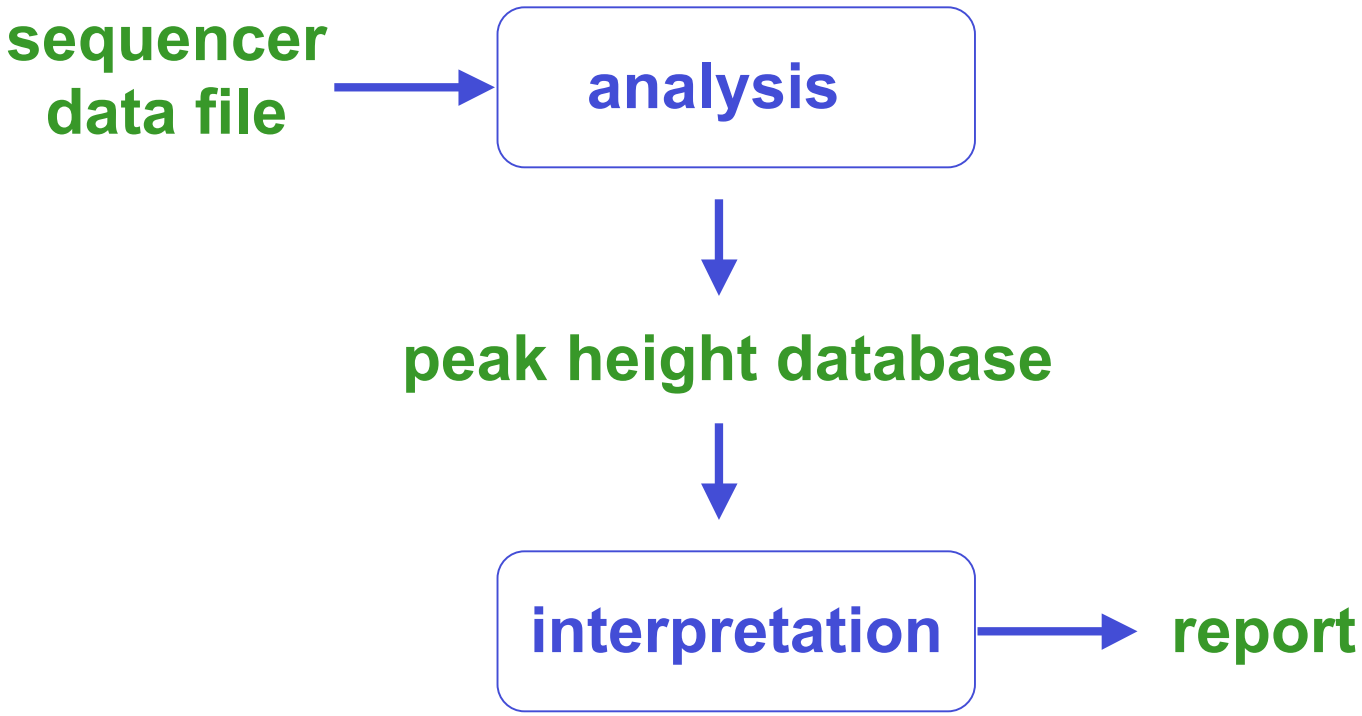
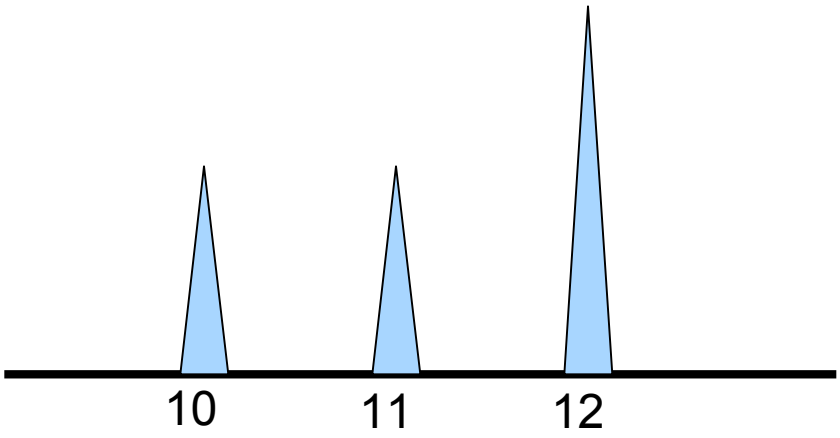


Figure 3. The TrueAllele® process flow for automated analysis and interpretation.



Conservative		**Cautious**		**Exact**	
10	10	10	12	10	12
10	11	11	12		
10	12	12	12		
11	11				
11	12				
12	12				

Figure 4. A simplified three peak mixture example. Different degrees of interpretation lead to different levels of discriminating power.

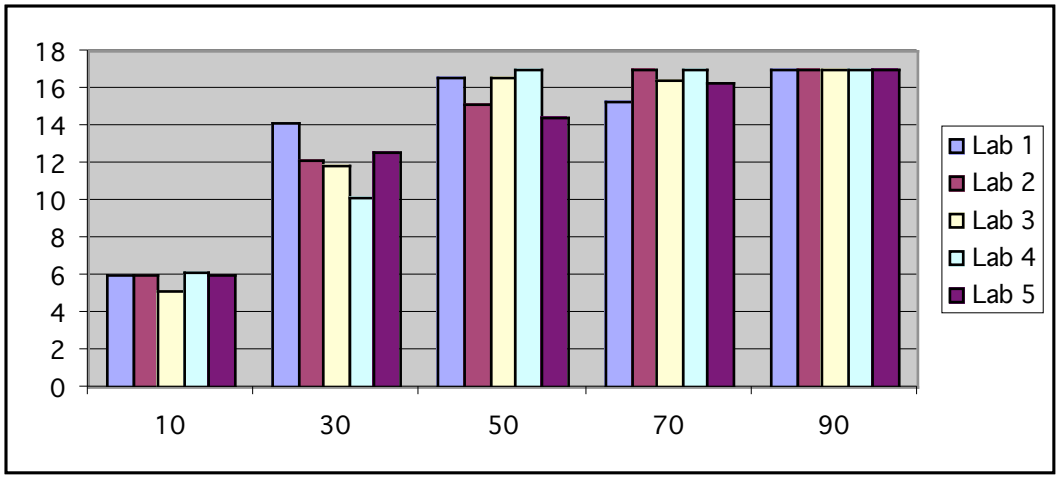


Figure 5a. The discriminating power of five labs at different mixture weights.

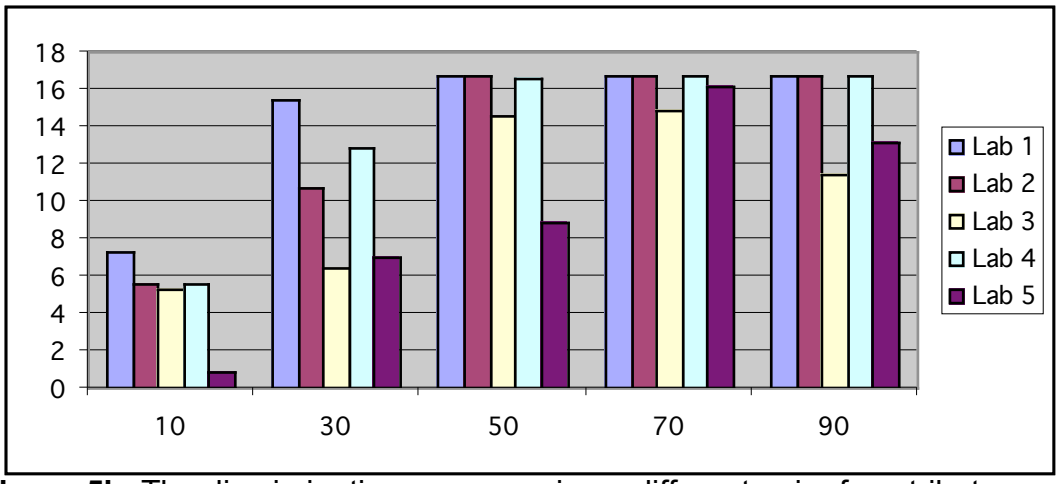


Figure 5b. The discriminating powers using a different pair of contributors.

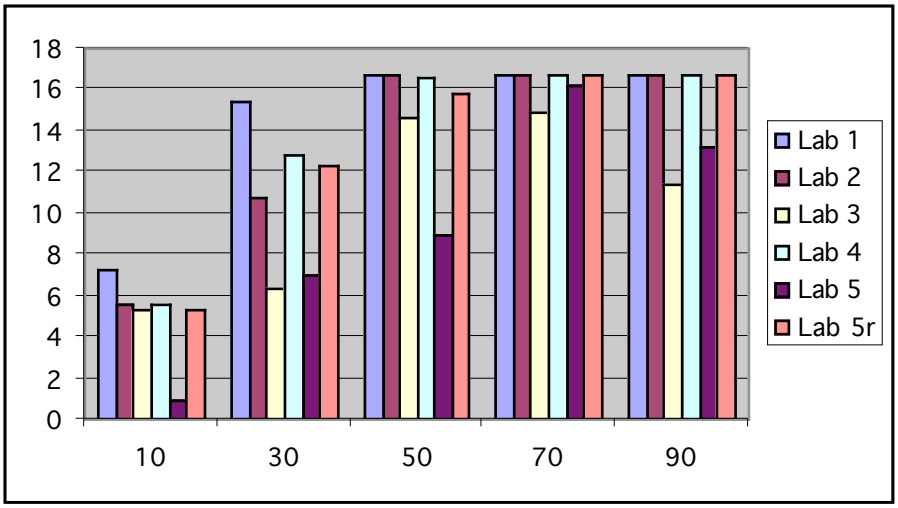


Figure 5c. The discriminating powers shown for lab 5 after repeating the experiments.

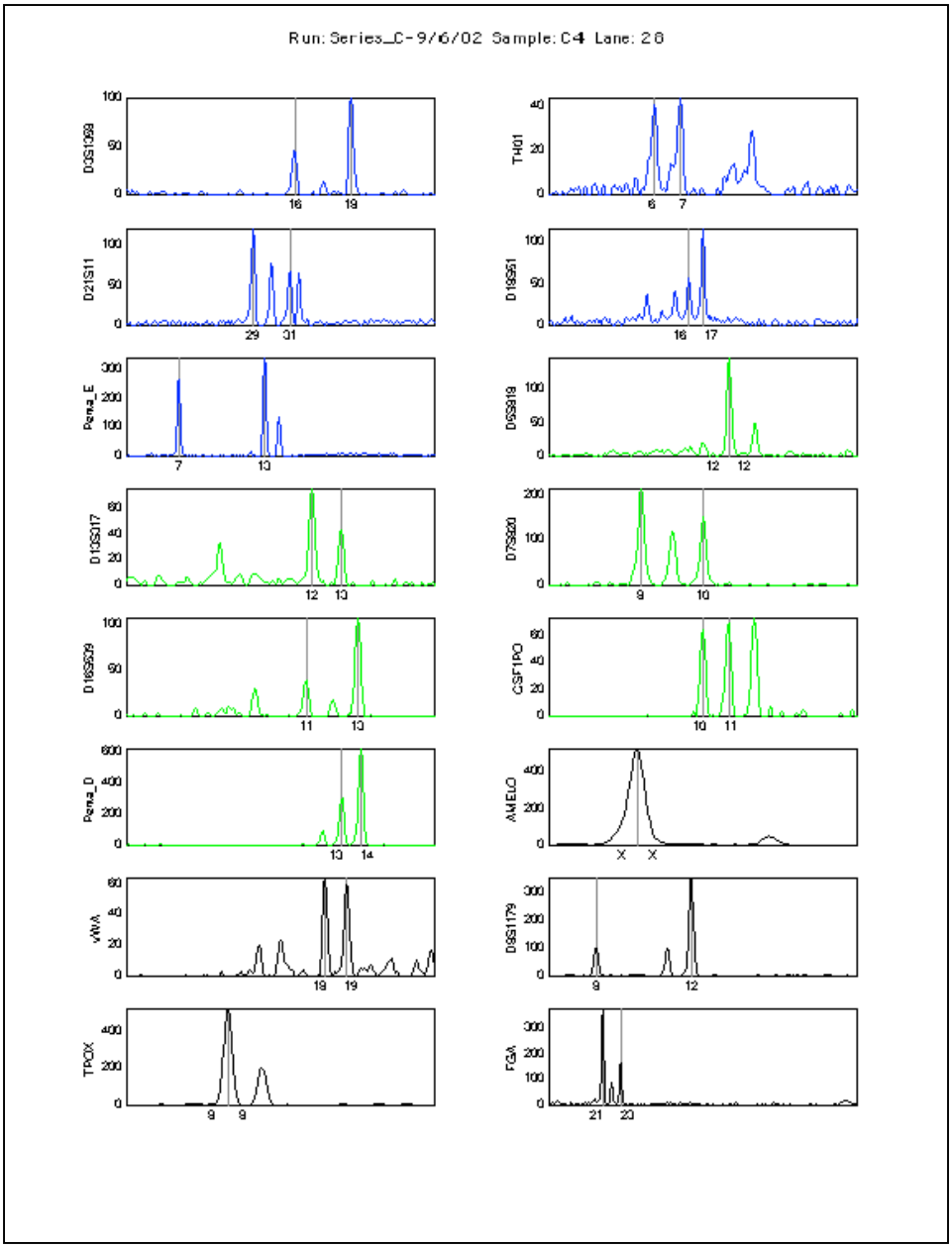


Figure 6. The low-level STR data from the mixed DNA specimen C4, which has an 0.125 ng DNA concentration. There are two contributors in C4, weighted in a 70:30 mixture ratio. Compare with the higher level data for C1 (Figure 2), which has a full 1 ng DNA concentration.

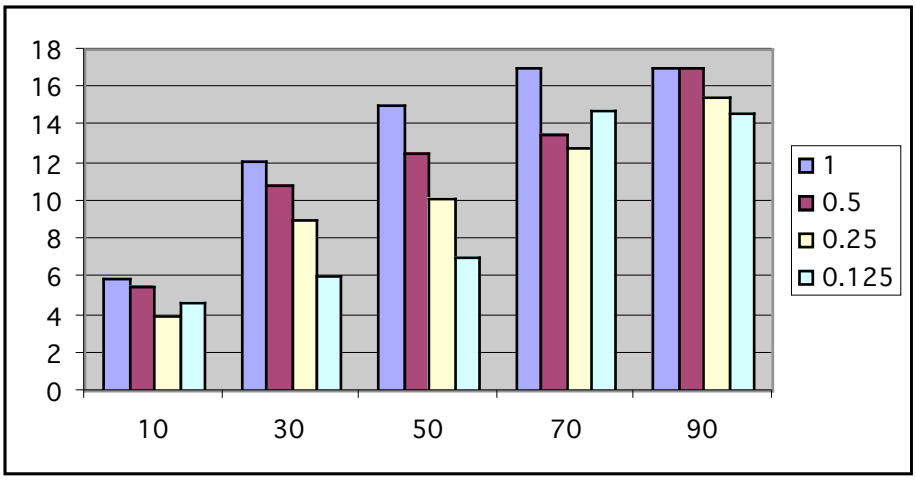


Figure 7a. The behavior of discriminating power as a function of mixture weight and DNA template amount for contributors A and G.

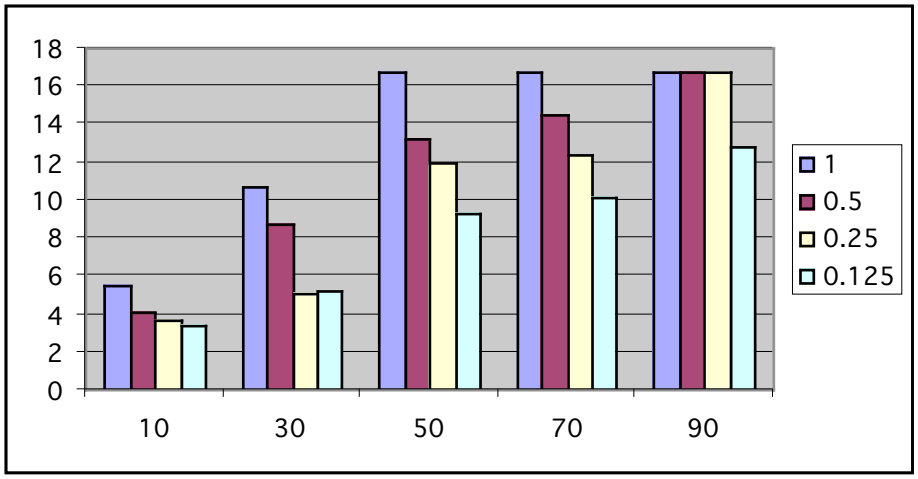


Figure 7b. The behavior of discriminating power as a function of mixture weight and DNA template amount for contributors H and N.

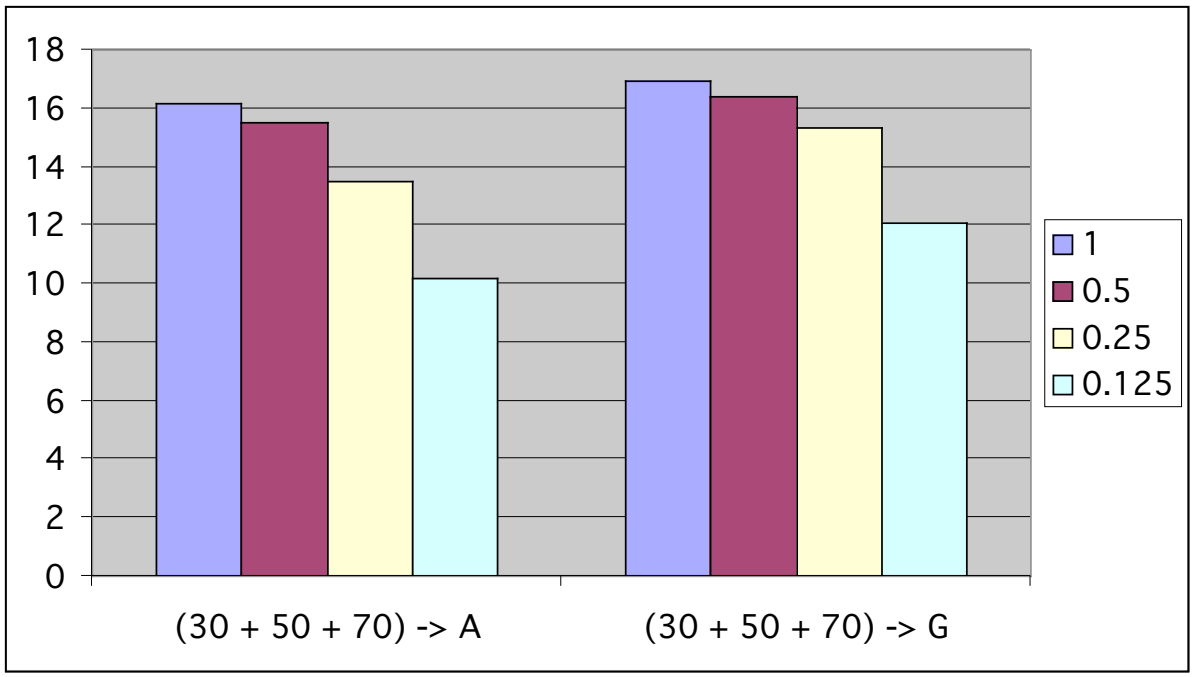


Figure 8. The discriminating power for contributors A and G with decreasing DNA quantity when only mixed stains are used and no reference samples are provided.

Tables

Set 1	ng amplified			
Mixture ratio	1	0.5	0.25	0.125
10:0	A1	A2	A3	A4
9:1	B1	B2	B3	B4
7:3	C1	C2	C3	C4
5:5	D1	D2	D3	D4
3:7	E1	E2	E3	E4
1:9	F1	F2	F3	F4
0:10	G1	G2	G3	G4

Set 2	ng amplified			
Mixture ratio	1	0.5	0.25	0.125
10:0	H1	H2	H3	H4
9:1	I1	I2	I3	I4
7:3	J1	J2	J3	J4
5:5	K1	K2	K3	K4
3:7	L1	L2	L3	L4
1:9	M1	M2	M3	M4
0:10	N1	N2	N3	N4

Table 1. The TrueAllele® validation study design. The three validation dimensions are mixture ratio, serial dilution and contributor pair.

lab	seq	gel	lane	sample	panel	locus	pixel	desig	height	area
CYB	ABI310	Series_C	4	C1	PowerPlex16	CSF1PO	5348	7.2	6.48	2.09
CYB	ABI310	Series_C	4	C1	PowerPlex16	CSF1PO	5363	8.0	10.30	3.79
CYB	ABI310	Series_C	4	C1	PowerPlex16	CSF1PO	5391	9.0	38.84	20.38
CYB	ABI310	Series_C	4	C1	PowerPlex16	CSF1PO	5407	9.2	19.23	9.18
CYB	ABI310	Series_C	4	C1	PowerPlex16	CSF1PO	5420	10.0	659.11	361.34
CYB	ABI310	Series_C	4	C1	PowerPlex16	CSF1PO	5449	11.0	645.33	353.79
CYB	ABI310	Series_C	4	C1	PowerPlex16	CSF1PO	5478	12.0	542.75	306.56

Table 2. Some data in the TrueAllele® analyzed 'peak' database table. Each record shows source, sizing and quantitation information for one peak.

lab	name	specimen	cutting	prep	pcr	seq	gel	lane
CYB	A1C1	A1	1	standard	standard	ABI310	Series_C	2
CYB	A1C1	C1	1	standard	standard	ABI310	Series_C	4

Table 3. Two specimen lanes in the user's 'request' database table. These records specify the data used in the mixture case example.

		template	
		A1	C1
contrib	1	1.00	0.68
	2	0.00	0.32

Table 4. The results of querying the 'weight' database table. Each PCR template *column* shows the ratio of underlying contributors in the specimen. Conversely, a contributor *row* shows how much that contributor is contained within each specimen.

locus	allele1	allele2	probability
CSF1PO	12.0	12.0	1.00
D13S317	9.0	13.0	1.00
D16S539	9.0	12.0	1.00
D18S51	13.0	15.0	1.00
D21S11	30.0	31.0	1.00
D3S1358	16.0	17.0	1.00
D5S818	12.0	12.0	0.99
D7S820	10.0	10.0	1.00
D8S1179	8.0	11.0	1.00
FGA	21.0	22.0	1.00
Penta_D	12.0	14.0	1.00
Penta_E	7.0	14.0	1.00
TH01	9.0	9.3	1.00
TPOX	8.0	8.0	1.00
vWA	15.0	18.0	1.00

Table 5. The results of querying the 'genotype' database table after the TrueAllele® system interprets the example case data. The genotypes (pairs of designated alleles) and probabilities are shown for each locus. With ambiguous results, a locus would display more than one row. In this case, at the 99% level, the genotype results are unique, so there is only one row for each locus.

	TrueAllele® Interpretation		**Human Interpretation**	
locus	**allele 1**	**allele 2**	**allele 1**	**allele 2**
CSF1PO	12	12	12	
D13S317	9	13	9	
D16S539	9	12	9	12
D18S51	13	15	13	15
D21S11	30	31	30	31
D3S1358	16	17	16	17
D5S818	12	12		
D7S820	10	10	10	
D8S1179	8	11	8	11
FGA	21	22		22
TH01	9	9.3	9	9.3
TPOX	8	8		
vWA	15	18	15	18

Table 6. A comparison of computer and (doubly reviewed) human reporting on the example case. The computer designates all 26 alleles, while the conservative human review designates only 18 of the alleles. While both answers are correct, the conservative approach loses much information, as measured in discriminating power.