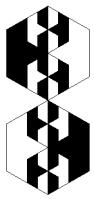
Australia TrueAllele® Validation Report

Cybergenetics

September 2011

CONFIDENTIAL
DO NOT DISTRIBUTE

DRAFT



Abstract

DNA evidence resides at the center of modern criminal justice, and it is used to help apprehend, convict and exonerate suspects. An ideal DNA system would provide identification information with speed, accuracy and objectivity. These desirable features are already found in the data generation process, in which a DNA laboratory transforms biological specimens into quantitative computer signals. However the second phase – data interpretation – is still largely conducted by a human review process.

With pristine DNA data (e.g., reference samples), human review can work well. But DNA casework evidence is usually not pristine. Extracted under real-world conditions, DNA evidence is often mixed (having multiple contributors), damaged (by heat or bacteria) or low level (thus hard to discern with any certainty).

Uncertain DNA data may suggest multiple genotype possibilities, thereby reducing identification information. Human review of uncertain DNA can be a time-consuming process that does not fully elicit all the information that the data contain. Moreover, human comparison of DNA evidence and suspect may not be entirely objective.

Computer interpretation of DNA evidence can overcome these issues. Specifically, it is:

- *Fast*, with parallel computers turning out solutions every few minutes;
- *Accurate*, able to employ mathematical models that fully preserve all of the identification information residing in the DNA data; and
- *Objective*, interpreting evidence without ever seeing a single suspect genotype.

Such computer processing can effectively handle the mixed, damaged and low level DNA evidence that currently consume much of the human review effort.

To properly use such a computer system, it is essential to know its capabilities and limitations. For example, how well does it handle two, three or more unknown contributors? How damaged or low level can the DNA be? Can independent evidence be mathematically combined to make a more informative identification?

This validation study helps determine the applicability of Cybergenetics TrueAllele® Casework, a commercial computer system for the mathematical interpretation of DNA evidence. Cybergenetics collaborated with the New South Wales Police (NSWP), who designed the study, generated the laboratory data and reviewed the results.

Table of Contents

Project

Interpreting uncertain DNA evidence

A definite genotype can be determined when a person's DNA produces clean data. However, when the data signals are less definitive, or when there are multiple contributors to the evidence, uncertainty arises. This uncertainty is expressed in the resulting genotype, which may describe different genetic identity possibilities. Such genotype uncertainty may translate into reduced identification information when comparison is made with a suspect.

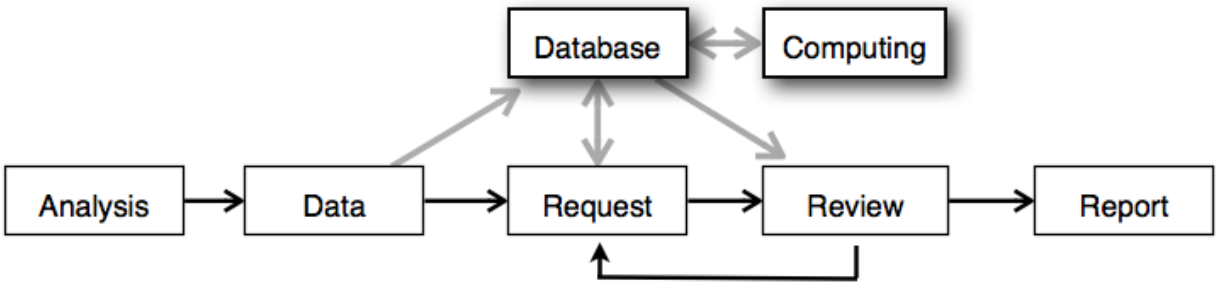
The DNA identification task can thus be understood as a two-step process:

1. objectively *inferring genotypes* from evidence data, accounting for their uncertainty using probability, and
2. subsequently *matching genotypes*, comparing evidence with a suspect relative to a population, to express the strength of association using probability.

The match strength is reported as a single number, the likelihood ratio (LR), which describes the gain in identification information produced by having examined the DNA evidence.

The TrueAllele® system is a computer implementation of this two-step objective genotype approach. TrueAllele infers genotypes from DNA data through mathematical modeling (1, 2). To capture all the identification information present in the data, the system represents genotype uncertainty using probability. These uncertain genotypes are stored on a TrueAllele database so that they can be compared with suspects for investigative and evidential identification. The TrueAllele user asks interpretation questions of DNA data, visually reviews the computer's answers, and generates match reports to use in court.

The TrueAllele technology



Cybergenetics TrueAllele technology for automated interpretation and reporting of DNA evidence is based on biology, mathematics and computation. The many differentiating features of the unique TrueAllele system are described in the Sole Source Justification document (Appendix). This section describes the TrueAllele workflow from a system and user perspective.

Analysis

The DNA interpretation process requires quality-checked quantitative data. The TrueAllele Analysis computer starts with the laboratory's original electronic DNA files, and works with the user to check and quantify these raw data signals, in order to produce interpretation-ready data. For each 96-well plate of DNA samples and controls, Analysis applies multiple rules to the signals to ensure that good data move forward on to interpretation. The computer gives the lab feedback about any data issues that it finds. The process is fast, taking a few minutes of user time for a typical DNA plate.

To assess the DNA data signals in Analysis, a user opens a folder of electronic DNA sequencer files. He then asks the TrueAllele computer to check the DNA sizing calibration data, and looks for any problems with these (and other) control samples. Man-machine communication is exchanged visually, with the user pointing his mouse at the screen to explore an issue, and the computer responding by rendering a data image or figure that focuses on the user's question. After the computer has processed the peak events in the DNA data signals, the user has a data file of quality-checked quantified peaks ready for the database.

Data

After peaks are quality checked in the Analysis phase, we can view them in the TrueAllele Data interface. This gives the user another opportunity to review the peaks before interpretation. The TrueAllele computer can signal the presence of any possible artifacts in the data, so that the user can evaluate the peak and take action upon it if necessary. Once the quality-checked peaks have been reassessed, they are ready for upload to a TrueAllele database, and then used in TrueAllele interpretation.

To upload quality-checked quantified peaks into a database, the user opens a "Visual User Interface for easy review" (VUIer™) Data window. He first connects to a TrueAllele database that will store the data. After opening the file created in the Analysis phase, the data peaks appear on the screen as intuitive visually rendered signals. Each data injection is shown within its own track. The user can ask the computer to show possible lingering data artifacts, along with pertinent data information. This annotating information is stored with the peak file on the database. When the data review is complete, the user uploads the peak data to the database, making it available for creating TrueAllele interpretation requests.

Database

The uploaded DNA data reside on a TrueAllele PostgreSQL relational database. The database is like an electronic filing cabinet that permits information retrieval simultaneously from multiple file folders. The database provides persistent and secure storage for all the information needed by the TrueAllele user and system. The (over seventy five) database tables provide quantitative DNA data, TrueAllele interpretation questions, the computed results, and supporting

information, such as population frequencies. The database also helps administer system activities, and supports the monitoring expert system that coordinates the system.

The user logs on to a TrueAllele database to initiate processing or to review results. The user works through the (VUIer) software. This database client exchanges DNA case data with the database, and presents information visually on the computer screen. All the user modules (e.g., Data, Request, Review, Report) automatically generate database queries and DNA visualizations through the VUIer. Typical displayed case information includes DNA data, genotype probability, mixture weight distribution and match rarity likelihood ratio values.

Request

Once the data are on the database, we can ask DNA interpretation questions that the TrueAllele computer can solve for us. Each question involves one or more DNA evidence items, and can be run under different problem solving conditions. (Example conditions are how many unknown contributor genotypes to find, how much computer time to use, or whether to account for degraded DNA.) While a victim reference may be optionally included in a question, for total objectivity a suspect genotype is never used. Questions can be asked one at time, in duplicate for reproducibility, or in batches of a hundred or more. Regardless, once a question has been posed, the statistical calculating is done entirely by computer.

To ask interpretation questions in a case, the user opens a VUIer Request window. After connecting to her evidence database, she selects the DNA data that she wants to use. These data images appear visually in the interface, with each signal in its own track. She then forms visual DNA items (each corresponding to an evidence sample) from the track signals. Finally, she makes each case interpretation request by indicating one or more DNA items, and setting optional problem solving parameters. Once she is satisfied with her questions, the user uploads her interpretation requests to the TrueAllele database for computer processing.

Computing

After the user has posed DNA interpretation questions to the TrueAllele computer, the data for each request is retrieved from the database. The computer uses all the data to infer a genotype distribution and mixture weight for each contributor. To infer a genotype distribution, the computer explores various peak patterns to statistically model the data. Throughout this modeling process the computer considers several different variables, such as genotype, mixture weight, stutter and preferential amplification. As a result, the reported genotype distribution reflects how well a set of proposed patterns fit the data. Patterns that closely fit the data receive higher probabilities, and patterns that do not receive lower probabilities. The computer then matches the inferred genotype distribution against provided references, and calculates a likelihood ratio statistic.

The TrueAllele parallel compute servers can process multiple requests at the same time. For example, solving a DNA interpretation question in duplicate creates two independent

calculations, establishing statistical reproducibility. We routinely run 24 parallel TrueAllele processes on our system, each one working on a different case. A typical DNA mixture takes about an hour or so to solve, so the overall throughput can be quite high (e.g., over 300 cases a day). When the problem solving is done, the computer stores its results (inferred genotype distributions, mixture weights, likelihood ratios, etc.) on the database for downstream review.

Review

Once the requests have finished processing, we can review the computer interpretation results. During this review process, we can see several aspects of the DNA case. For example, we can examine a contributor's genotype probability distribution, either visually or in a table. It is this key genotype variable, and its probability uncertainty, that establishes genetic identity. With multiple DNA contributors, we can visually review mixture information with informative pictures of mixture weight probability. The quantitative match information can be seen visually at the different genetic loci.

The user first opens a VUIer Review window, and selects a request from the database. A Profile window appears, visually displaying computed genotype probability distributions. From here, the user can navigate to other windows, including ones for the original Data and the Mixture separation. When TrueAllele finds a match between an evidence contributor and a suspect, the Match window and tables show quantitative LR match information. An Explain window visually explains the computer's reasoning. A user can always ask more questions by exiting Review and returning to the Request module, where he can create new TrueAllele interpretation questions.

Report

After the interpretation requests have been processed by the computer and reviewed by the analyst, we are ready to generate reports for court presentation. TrueAllele generates the customizable report automatically based on user selected options. A typical report consists of an evidence interpretation summary, lab information, a match rarity statement and detailed locus results. The reported match statistic incorporates appropriate population allele frequencies, and can set a coancestry coefficient (theta) for a statistic with population substructure.

For automatic report generation, the user opens the VUIer Report window. After connecting to a TrueAllele database, the user downloads genotypes of interest: evidence contributors, suspect references, and population frequencies. The probability distributions of each genotype are displayed together visually in the VUIer Report window. The user can review different matches of evidence contributors to suspect references, and generate a report for any match. She can export her report from VUIer as a text document, and import it into a spreadsheet program.

Validation materials

The validation will be done on a set of one, two and three unknown DNA mixtures. Some of these DNA samples will also be tested as dilutions. The NSWP will provide the DNA samples and generate the STR data. The data has been designed by the NSWP (Appendix) to specifically address the following questions:

- What are the lower limits of *PCR product* that TrueAllele will detect and type correctly from single-source material?
- What are the lower limits of *template DNA* that TrueAllele will detect and type correctly from single-source material?
- In 2-person mixtures of varying ratios of *PCR product*, what is the limit at which TrueAllele can detect and resolve accurately the 2 profiles?
- In 2-person mixtures of varying ratios of *template DNA,* what is the limit at which TrueAllele can detect and resolve accurately the 2 profiles?
- In 2-person mixtures of varying ratios of template, what is the limit at which TrueAllele can detect and resolve accurately the 2 profiles, *when the starting concentration of template DNA is low?*
- In 3 person mixtures, what is the limit at which TrueAllele can detect and resolve accurately the *three profiles*?
- In 3 person mixtures, what is the limit at which TrueAllele can detect and resolve accurately the three profiles, when one of the components is noticeably *degraded*?

Validation methods

This proposal centers on the scientific validation of the TrueAllele Casework DNA interpretation system (3). The statistical approach uses DNA match information as the key metric (4), since that is the single measure of association used by law enforcement and the courts (5).

Efficacy

The outcome of any genotype inference from evidence data is a probability distribution over allele pair values at each locus. With quantitative computer-based methods (6, 7) such as the TrueAllele system (8), the probabilities arise from Bayesian inference. With qualitative binary methods such as CPI or CLR, a genotype list of length N is formed that contains reportable allele pairs, each one assigned a probability of $1/N$ (5). A LR compares this evidence genotype to a suspect genotype, relative to a population genotype, through their probability distributions to obtain match information (5). Thus the LR provides a universal mechanism for comparing match information between mixture genotypes inferred by different methods, relative to the same suspect and population (9).

The log(LR) is a standard measure of information (10, 11). All currently reported match statistics (e.g., TrueAllele, kinship, CLR, CPI) can all be viewed as LRs (5). Therefore, we can

compare the relative efficacy of two mixture interpretation methods by examining the difference in their $\log_{10}$(LR) scores. For a set of cases, we can also look at the mean value of these information differences. Statistical significance between these differences can be measured using a t-test.

In this project we will compare differences in identification information between quantitative and qualitative mixture interpretation methods (3). When the victim genotype is known and used, the difference is $\log_{10}$(TrueAllele) – $\log_{10}$(CLR). When the victim is not available for genotype inference, this information difference is $\log_{10}$(TrueAllele) – $\log_{10}$(CPI).

Through these measures of efficacy, the validation study can verify that the TrueAllele system extracts at least much information as current manual review methods. Moreover, the efficacy measures can quantify the extent of additional information that the computer is able to derive from the data.

Reproducibility

An important aspect of scientific reliability is a method's reproducibility (12). The reproducibility of a set of measurements is conventionally reported as the standard deviation of these numbers (13). Any mixture interpretation method applied to some DNA data will infer a genotype, which yields a single information $\log_{10}$(LR) measurement when compared with a suspect and population. Independent interpretations using the same method on the same DNA mixture data, relative to the same suspect and population, produce a set of $\log_{10}$(LR) values. From this set of information measurements, we can assess the method's reproducibility by computing a standard deviation.

To sharpen the reproducibility estimate of a mixture interpretation method, we use more cases. The "within-case" standard deviation σ_w (14) describes the method's reproducibility over a population of mixture cases (4). We can compute σ_w as the root mean square deviation of replicated $\log_{10}$(LR) information scores, relative to the mean value within each case (14), as shown.

$$\sigma_w^2 = \frac{\sum_{i=1}^{I} \sum_{j=1}^{J_i} \left(s_{ij} - \overline{s}_i \right)^2}{\sum_{i=1}^{I} J_i}$$

Here, I is the number of cases, J_i is the number of independent interpretations of the i^{th} case, s_{ij} is the $\log_{10}$(LR) score of the j^{th} interpretation of the i^{th} case, and $\overline{s}_i$ is the mean score of the s_{ij} values within the i^{th} case.

Through these measures of reproducibility, the validation study will quantify the reliability of the TrueAllele system under different casework situations. This quantification will be done by assessing reproducibility on subgroups of DNA items of differing sample complexity (number of contributors, mixture weight, DNA amount, DNA degradation, etc.).

Cybergenetics: Automating DNA interpretation

Cybergenetics is the world leader in computer interpretation of DNA evidence. The American Pittsburgh-based company was founded in 1994. Cybergenetics specializes in innovating computer solutions to DNA identification problems using probability modeling of biochemical processes. The flagship product is TrueAllele Casework, an automated computer system that mines DNA evidence data to extract maximal identification information in minimal time.

Cybergenetics has used TrueAllele technology to complete many time-critical high-profile DNA interpretation projects. These efforts include eliminating the UK Forensic Science Service millennial backlog of 350,000 DNA database samples, working with the US Army identification lab to prepare DNA profiles for the 2004 Olympic Games, and re-analyzing World Trade Center disaster DNA data for the New York City Office of the Chief Medical Examiner. Cybergenetics has developed scientific validation techniques for assessing the efficacy and reproducibility of DNA interpretation methods based on DNA match information. Cybergenetics was also the first group to successfully introduce the objective computer-based statistical interpretation of DNA evidence into an American courtroom.

Dr. Mark W. Perlin is the senior scientist at Cybergenetics who will be responsible for conducting the validation project. His responsibilities include planning the project, supervising the Cybergenetics team, spearheading the validation effort, coordinating the information technology components, conducting user training, preparing reports and disseminating the results of the study. Dr. Perlin developed the mathematics underlying the TrueAllele technology, directed its software development, designed the training curriculum, innovated the validation methodology, has published and presented the underlying interpretation methods, and has testified about TrueAllele methods in court. He holds a PhD in Mathematics, a medical MD degree and a PhD in Computer Science.

Validation Results

The study has seven validation axes that were explored by Cybergenetics and the New South Wales Police. The TrueAllele requests were processed in duplicate in order to assess reproducibility. The LRs were recorded at a 1% theta value, to account for coancestry.

Two contributor mixtures - Validation mixtures were analyzed and interpreted in the TrueAllele® system assuming a possible number of contributors. Mixtures that appeared to have two contributors were run as "two unknown" genotype requests.

Three contributor mixtures - Validation mixtures were analyzed and interpreted in the TrueAllele® system assuming a possible number of contributors. Mixtures that appeared to have three contributors were run as "three unknown" genotype requests.

Three contributor mixtures with one degraded contributor – After obtaining results from the three contributor mixtures, Cybergenetics analyzed the mixture data again to determine if any sets appeared to contain degraded DNA. Questions were posed to the TrueAllele system, running fifteen samples with the degraded feature turned on.

Joint amplifications – Fifteen duplicate amplifications of validation samples were run (a) individually and (b) jointly using amplifications from the same sample. The amplification requests had an "a" appended onto the request name.

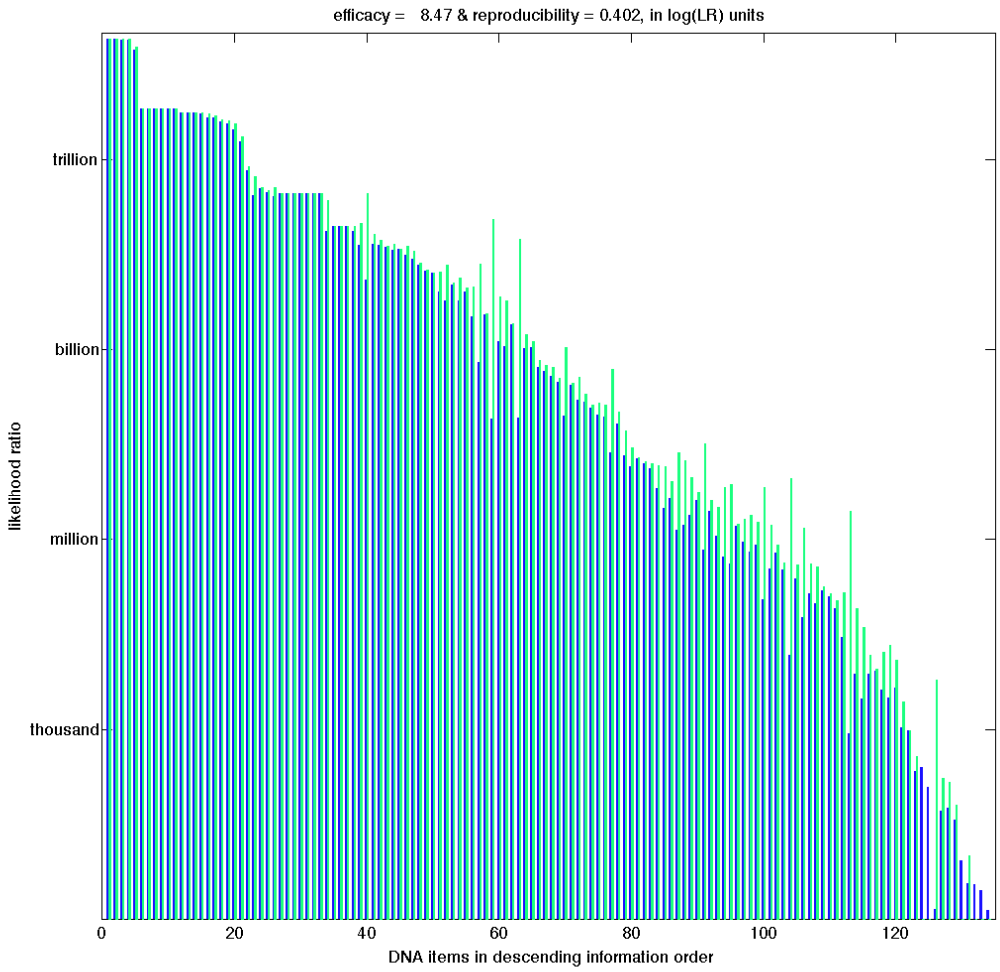
Joint items – Thirteen sets of two unknown joint requests were run in TrueAllele. The goal was to determine whether joint interpretation of two different evidence items extracted more identification information than separate interpretations of the same evidence items. Items were chosen that (a) were comprised of the same contributors, and (b) had dissimilar mixture weights (i.e. a 90:10 mixture was joined with a 50:50).

Using a known reference – Twelve requests that had either a very minor contributor or were 50:50 mixtures were run using a designated known "victim". The question was whether using a victim reference in TrueAllele would extract more identification information from a previously less informative contributor match.

Relatives – Eight relatives of the mixture contributors were analyzed as reference samples, and compared to the validation mixture samples. Human mixture interpretation uses thresholds to determine lists of alleles, so shared alleles with relatives can give partial matches. The question was whether computer separation into underlying genotypes exhibited similar cross-matching based on allele sharing.

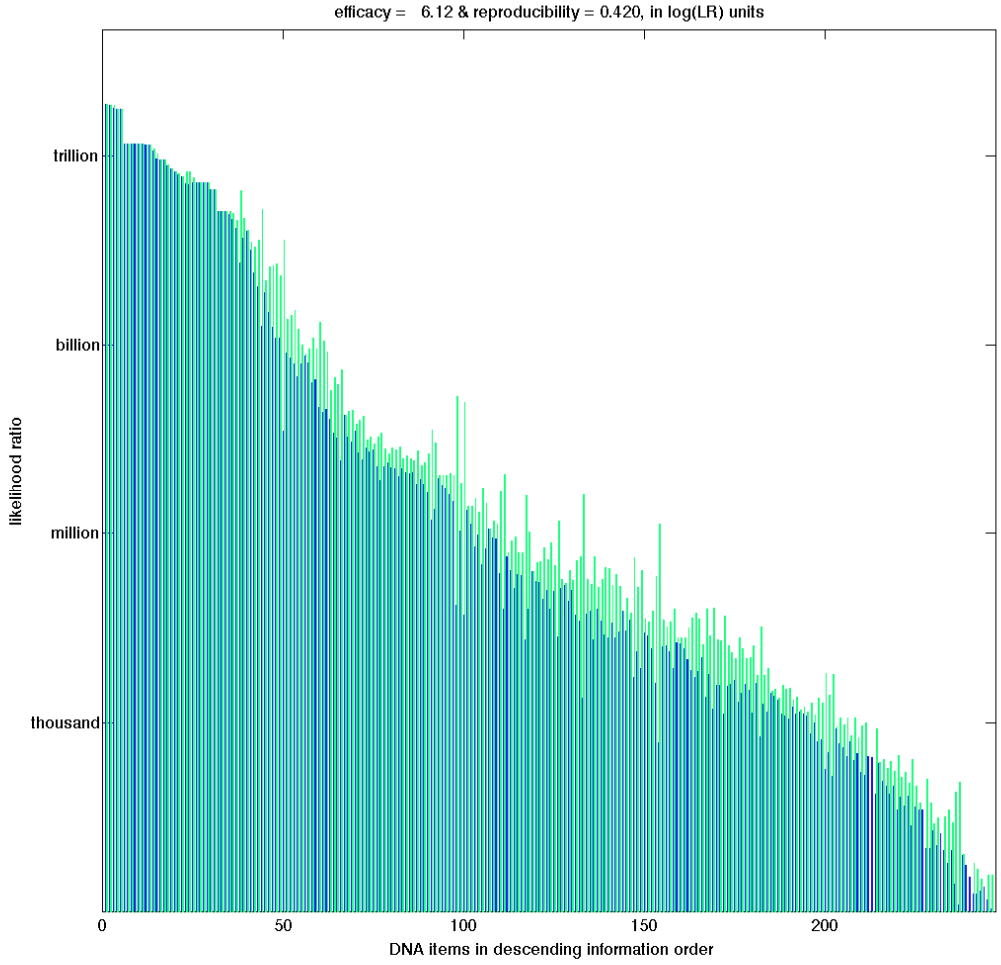
Two contributor mixtures

We analyzed and interpreted apparent two person mixtures in the TrueAllele system in duplicate as "two unknown" genotype requests. In additive log(LR) units, the information efficacy was 8.47, and the reproducibility was 0.402. In multiplicative LR units, these numbers correspond to factors of 292 million (efficacy) and 2.53 (reproducibility).



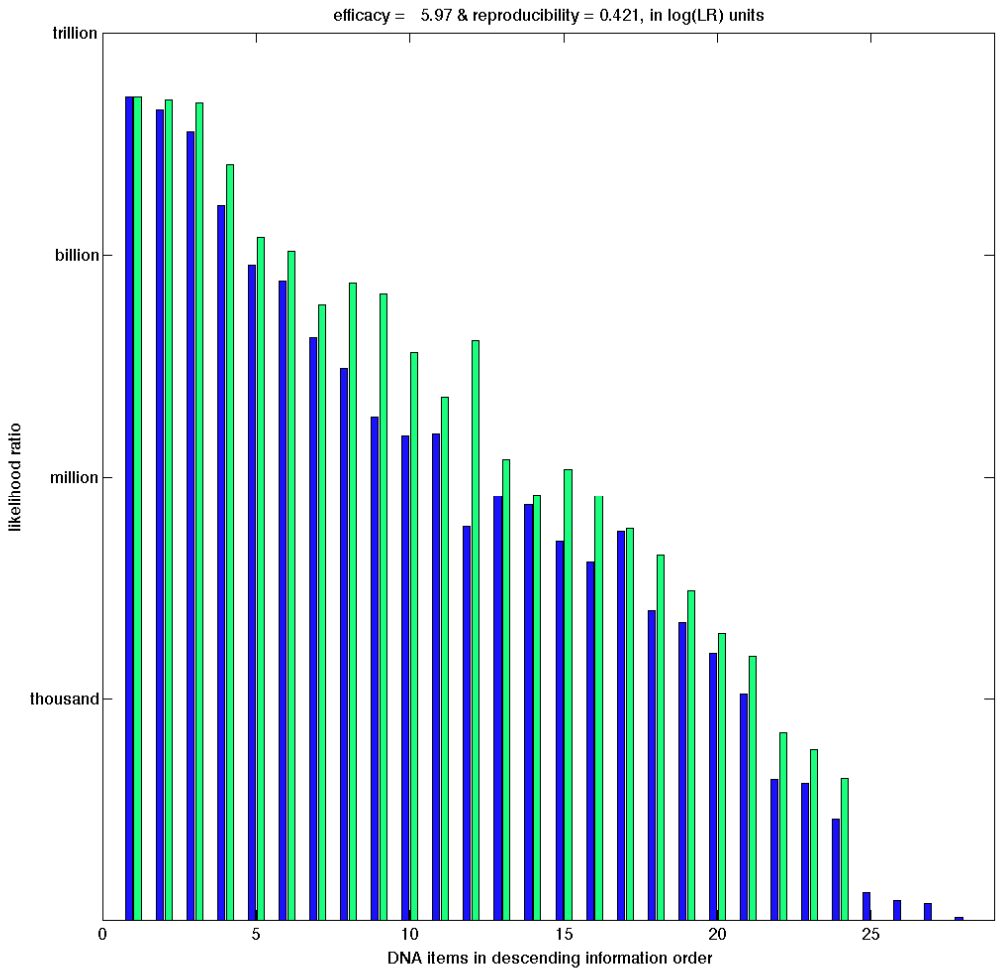
Three contributor mixtures

We analyzed and interpreted apparent three person mixtures in the TrueAllele system in duplicate as "three unknown" genotype requests. In additive log(LR) units, the information efficacy was 6.12, and the reproducibility was 0.420. In multiplicative LR units, these numbers correspond to factors of 1.31 million (efficacy) and 2.63 (reproducibility).



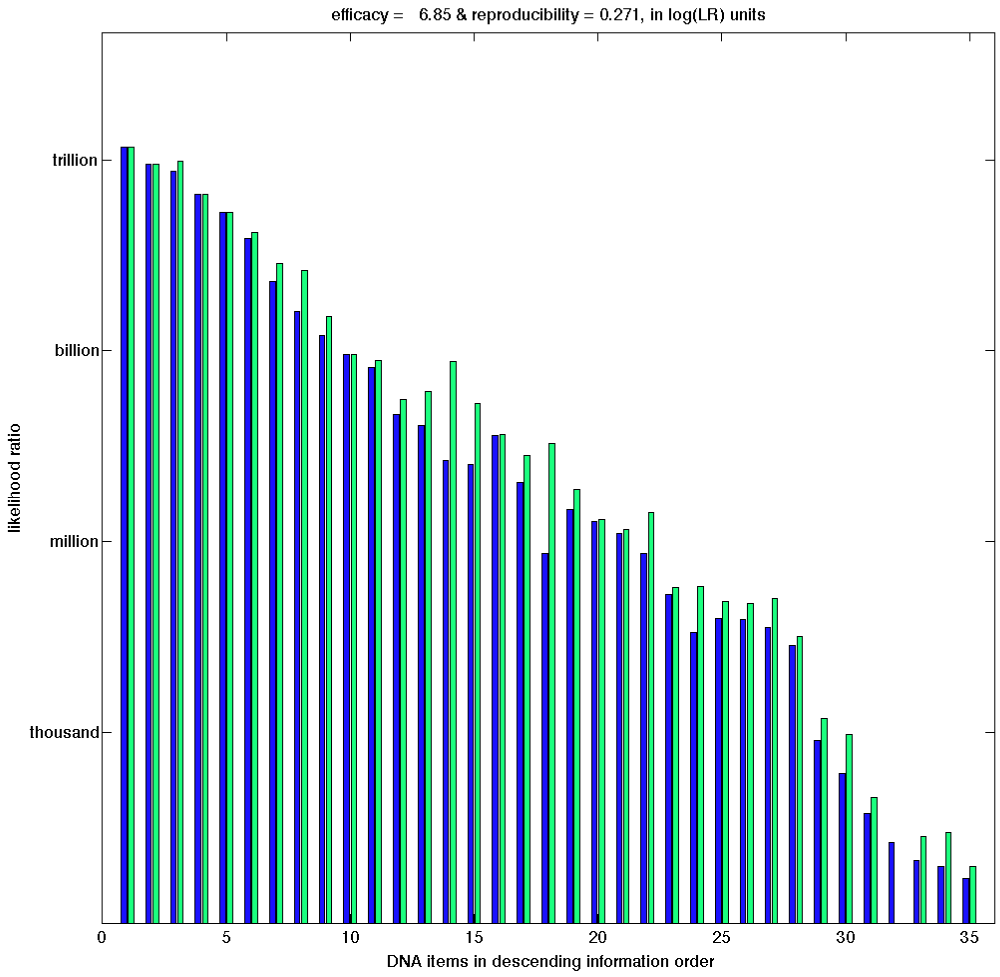
Three contributor mixtures with one degraded contributor

We analyzed the three contributor mixture data a second time on items that appeared to contain degraded DNA. We had the TrueAllele system rerun the fifteen items twice more, this time with the degraded feature turned on. In additive log(LR) units, the information efficacy on this degraded subset was 5.97, and the reproducibility was 0.421. In multiplicative LR units, these numbers correspond to factors of 934 thousand (efficacy) and 2.64 (reproducibility).



Joint amplifications

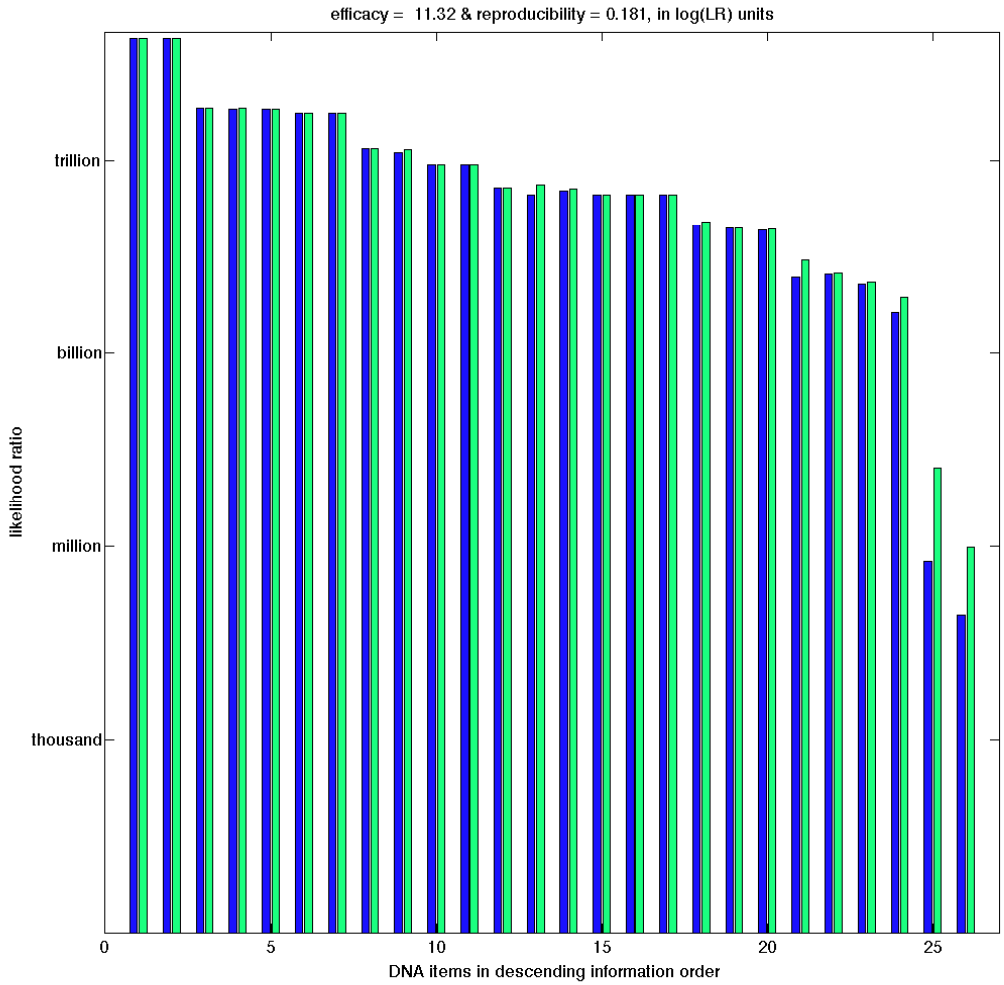
Fifteen duplicate amplifications of validation samples were run (a) individually and (b) jointly using amplifications from the same sample. In additive log(LR) units, the information efficacy was 6.85, and the reproducibility was 0.271. In multiplicative LR units, these numbers correspond to factors of 7 million (efficacy) and 1.86 (reproducibility).



Joint items

Thirteen two unknown requests were run in TrueAllele on multiple mixture items. The goal was to determine whether joint computer interpretation of two different evidence items could extract more identification information than separate interpretations of the same items. We chose items that had (a) the same contributors, but (b) dissimilar mixture weights (for example, combining a 90:10 mixture with a 50:50 mixture).

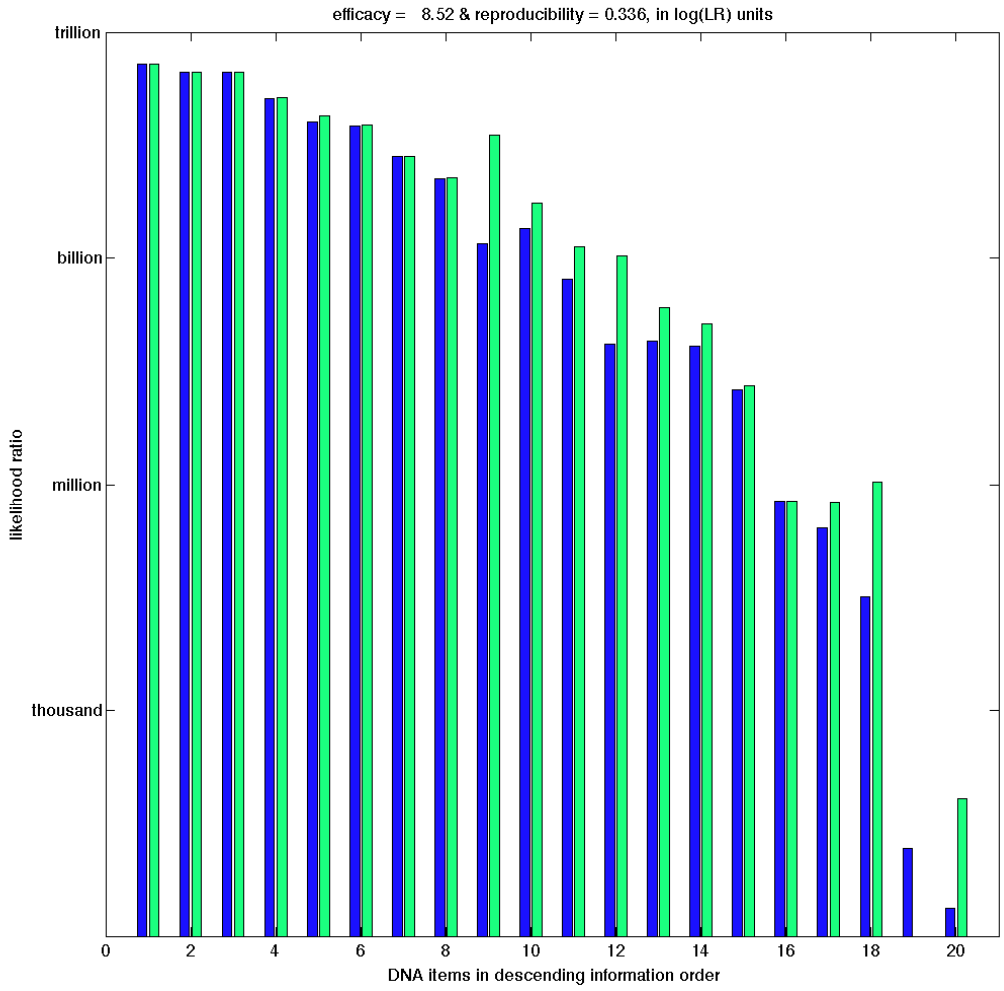
The replicated LR results showed that combining evidence items can increase both information yield and reproducibility. In additive log(LR) units, the information efficacy was 11.32, and the reproducibility was 0.181. In multiplicative LR units, these numbers correspond to factors of 207 billion (efficacy) and 1.52 (reproducibility).



Using a known reference

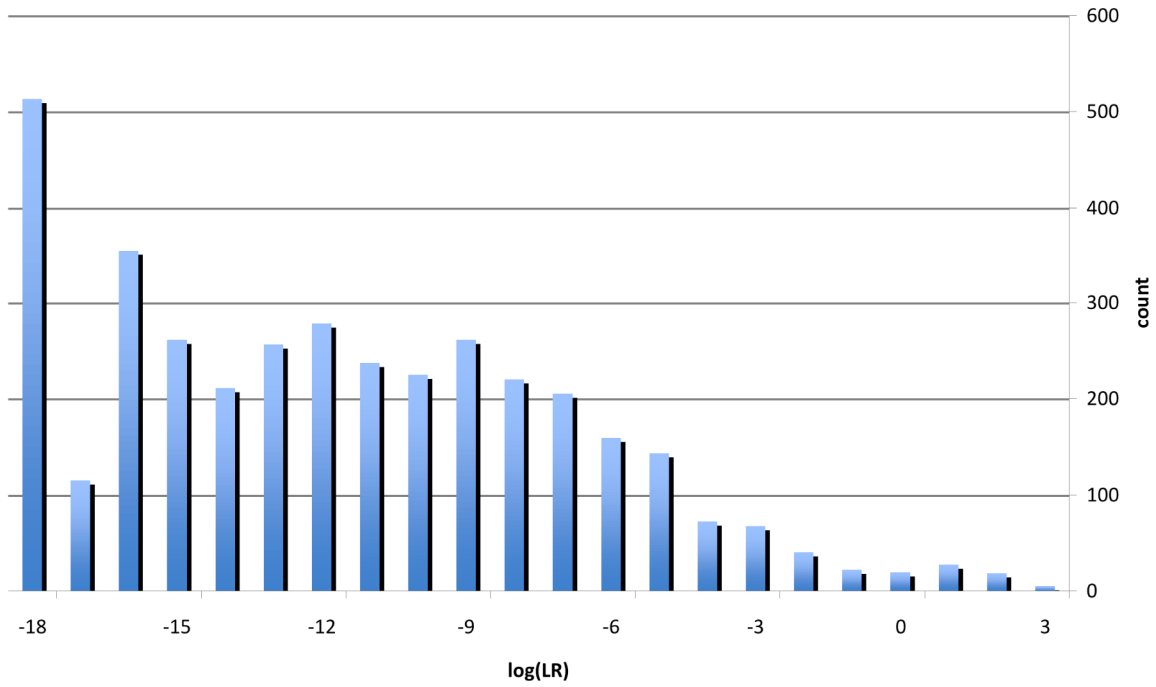
There were twelve requests that had either a very minor contributor or were a 50:50 mixture. We reran these items in TrueAllele using a known "victim" reference.

These computer experiments demonstrated that TrueAllele can extract more identification information from an evidence item when using a victim reference. In additive log(LR) units, the information efficacy was 8.52, and the reproducibility was 0.336. In multiplicative LR units, these numbers correspond to factors of 335 million (efficacy) and 2.17 (reproducibility).



Relatives

To assess cross matching between related individuals, relatives of the mixture contributors were analyzed as reference samples, and then compared with the validation mixture samples. We observed that when there is allele sharing with a relative, there can be a small degree of positive log(LR) that is inconclusive for a match. Otherwise, the log(LR) values tend to be overwhelmingly negative, indicating that TrueAllele has high specificity and does not produce false matches.



Appendix

Validation Plan

INITIAL SCHEMA FOR TRUEALLELE VALIDATION

A. AIMS

a. The aim of this study is to test the ability of TrueAllele to deduce accurately the genotypes of the various persons that have contributed DNA to a set of laboratory-generated profiles.

   In particular, the questions that we seek answers to are:

   i. What are the lower limits of amplified DNA that TrueAllele will detect and type correctly (from single-source material)?

   ii. In 2-person mixtures of varying ratios, what is the limit at which TrueAllele can detect and resolve accurately the 2 profiles?

   iii. In 3 person mixtures, what is the limit at which TrueAllele can detect and resolve accurately the 3 profiles?

   Note that it is unnecessary for the study to replicate all steps of the STR procedure – we are not trying to assess DNA recovery techniques, amplification efficiencies, electrophoretic resolutions or casework scenarios (although labs will certainly wish to assess TrueAllele with some case work examples).

   Rather the intent of the study is to assess the abilities and limitations of TrueAllele in determining, resolving and assigning DNA profiles, including as assessment of its "limits of resolution".

   Most of the assessment experiments can be performed with a set of standard human DNA preparations, singly or in various combinations. It is therefore proposed that five DNA extracts be prepared from volunteers to serve as the template test panel. Most test samples will be either mixtures of post-PCR products (normalised to provide precise ratios), or mixtures of templates prior to amplification (which deliver less precise final product, but do allow for stochastic effects to be assessed).

B.1. WHAT ARE THE LOWER LIMITS OF *PCR PRODUCT* THAT TRUEALLELE WILL DETECT AND TYPE CORRECTLY FROM SINGLE-SOURCE MATERIAL?

i. PCR amplification of 5 selected single DNA profiles of known genotype – standard template amount of (e.g.) 2 nanogram.

ii. Construct dilution series of the PCR mix. Suggest:
Undiluted, 1:4; 1:8; 1:16; 1:64;
iii. Resolve un-diluted and the dilutions using the lab's standard conditions - ~1 μL PCR product per load)

This would be a total of FIVE amplifications, and twenty-five 3130 separations.

B.2. WHAT ARE THE LOWER LIMITS OF *TEMPLATE DNA* THAT TA WILL DETECT AND TYPE CORRECTLY FROM SINGLE-SOURCE MATERIAL?

i. Construct dilution series (suggest undiluted 1:4; 1:8 1:16; 1:64;) of template – from 2ng per reaction down of each of 5 templates (i.e. from 2 ng per amplification down to ~ 30pg per amplification)
ii. Amplify & resolve each dilution.

This would be a total of TWENTY amplifications, and twenty-five 3130 separations.

C.1. IN 2-PERSON MIXTURES OF VARYING RATIOS OF *PCR PRODUCT*, WHAT IS THE LIMIT AT WHICH TRUEALLELE CAN DETECT AND RESOLVE ACCURATELY THE 2 PROFILES?

1. PCR amplification of 5 selected single DNA profiles of known genotype, selected to include some that are distinct form each other, some which have significant sharing of alleles. (multiple amplification of each will be necessary → then pool single-source samples to give a constant mix). (Call them profiles A, B, C, D & E)

2. Quantitate & normalise resulting PCR product (via 310 or 3130 run). (normalisation by for example total peak areas of all loci)

3. Compile Matrix of ratios of 2-source samples: - i.e. this is mixing of PCR products – no necessity to repeat PCRs for each mixture.

Source (major):	**A**	**B**	**C**	**D**	**E**
Source (minor):					
A	---	1:1	1:2	1:5	1:10
B	1:10	---	1:1	1:2	1:5
C	1:5	1:10	---	1:1	1:2
D	1:2	1:5	1:10	----	1:1
E	1:1	1:2	1:5	1:10	---

4. This is a total of 20 samples.

C.2. IN 2-PERSON MIXTURES OF VARYING RATIOS OF *TEMPLATE,* WHAT IS THE LIMIT AT WHICH TRUEALLELE CAN DETECT AND RESOLVE ACCURATELY THE 2 PROFILES?

1. Prepare template mixes at the following ratios, where the major template component is 1 ng, and the minor component is a proportion of this, down to 100pg

2. Template mixes are distributed to labs for amplification and resolution.

Template Source (major):	**A**	**B**	**C**	**D**	**E**
Template Source (minor):					
A	---	1:1	1:2	1:5	1:10
B	1:10	---	1:1	1:2	1:5
C	1:5	1:10	---	1:1	1:2
D	1:2	1:5	1:10	----	1:1
E	1:1	1:2	1:5	1:10	---

C.3. IN 2-PERSON MIXTURES OF VARYING RATIOS OF TEMPLATE, WHAT IS THE LIMIT AT WHICH TRUE ALLELE CAN DETECT AND RESOLVE ACCURATELY THE 2 PROFILES, *WHEN THE STARTING CONCENTRATION OF TEMPLATE IS LOW?*

1. Prepare template mixes at the following ratios, where the major template component is 0.3 ng (300 pg), and the minor component is a proportion of this, down to 30pg

2. Template mixes are distributed to labs for amplification and resolution.

Template Source (major):	**A**	**B**	**C**	**D**
Template Source (minor):				
A	---	1:1	1:2	1:5
B	1:5	---	1:1	1:2
C	1:2	1:5	---	1:1
D	1:1	1:2	1:5	----

D.1. IN 3 PERSON MIXTURES, WHAT IS THE LIMIT AT WHICH TRUE ALLELE CAN DETECT AND RESOLVE ACCURATELY THE 3 PROFILES?

1. For 3 person mixes template compositions will consist of the following amounts (nanograms) of each template:

Nanograms of samples	Sample X	Sample Y	Sample Z
Test 1	1	1	1
2	1	0.5	0.5
3	1	0.2	0.2
4	1	0.1	0.1
5	0.5	1	0.5
6	0.5	0.5	1
7	0.5	0.2	0.1
8	0.5	0.1	0.2
9	0.2	1	0.2
10	0.2	0.5	0.1
11	0.2	0.2	1
12	0.2	0.1	0.5
13	0.1	1	0.1
14	0.1	0.5	0.2
15	0.1	0.2	0.5
16	0.1	0.1	1

2. This is an orthogonal array which mathematically covers all possibilities of ratios of 3 x DNAs in 16 samples, and can be analysed statistically as to accuracy.

3. This will be done by pooling the templates and amplifying all 3 together then running on 3130 – in triplicate – a total of 48 samples.

D.2. IN 3 PERSON MIXTURES, WHAT IS THE LIMIT AT WHICH TRUEALLELE CAN DETECT AND RESOLVE ACCURATELY THE 3 PROFILES, WHEN ONE OF THE COMPONENTS IS NOTICEABLY DEGRADED?

1. For 3 person mixes template compositions will consist of the following amounts (nanograms) of each template:
2. Sample Z' will be degraded by environmental exposure (e.g. sonication, temperature &/or limited DNAse I treatment) sufficient to reveal "significant" degradation on agarose gel)

Nanograms of samples	Sample X	Sample Y	Sample Z'
Test 1	0.5	0.5	0.5
2	0.5	0.25	0.25
3	0.5	0.1	0.1
4	0.5	0.05	0.05

5	0.25	0.5	0.25
6	0.25	0.25	0.5
7	0.25	0.1	0.05
8	0.25	0.05	0.1
9	0.1	0.5	0.1
10	0.1	0.25	0.05
11	0.1	0.1	0.5
12	0.1	0.05	0.25
13	0.05	0.5	0.05
14	0.05	0.25	0.1
15	0.05	0.1	0.25
16	0.05	0.05	0.5

3. This is an orthogonal array which mathematically covers all possibilities of ratios of 3 x DNAs in 16 samples, and can be analysed statistically as to accuracy.

4. This would be done by pooling the templates and amplifying all 3 together then running on 3130

Bibliography

1. Perlin MW, Lancia G, Ng S-K. Toward fully automated genotyping: genotyping microsatellite markers by deconvolution. Am J Hum Genet. 1995;57(5):1199-210.
2. Perlin MW, Szabady B. Linear mixture analysis: a mathematical approach to resolving mixed DNA samples. J Forensic Sci. 2001;46(6):1372-7.
3. Perlin MW, Sinelnikov A. An information gap in DNA evidence interpretation. PLoS ONE. 2009;4(12):e8327.
4. Perlin MW. Scientific validation of mixture interpretation methods. Promega's Seventeenth International Symposium on Human Identification, 2006 Oct 10-12; Nashville, TN. 2006.
5. Perlin MW, Kadane JB, Cotton RW. Match likelihood ratio for uncertain genotypes. Law, Probability and Risk. 2009;8(3):289-302.
6. Cowell RG, Lauritzen SL, Mortera J. Probabilistic modelling for DNA mixture analysis. Forensic Sci Int Genet Suppl. 2008;1(1):640-2.
7. Curran J. A MCMC method for resolving two person mixtures. Sci Justice. 2008;48(4):168-77.
8. Perlin MW. Simple reporting of complex DNA evidence: automated computer interpretation. Promega's Fourteenth International Symposium on Human Identification, 2003; Phoenix, AZ. 2003.
9. Buckleton JS, Triggs CM, Walsh SJ, editors. Forensic DNA Evidence Interpretation. Boca Raton, FL: CRC Press, 2004.
10. Good IJ. Probability and the Weighing of Evidence. London: Griffin, 1950.
11. Savage LJ. The Foundations of Statistics. New York: John Wiley & Sons, 1954.
12. Faigman DL, Kaye DH, Saks MJ, Sanders J. Science in the Law: Standards, Statistics, and Research Issues: West Group, 2002.
13. Snedecor GW, Cochran WG. Statistical Methods. Eighth ed. Ames: Iowa State University, 1989.
14. Scheffé H. The Analysis of Variance. New York: John Wiley & Sons, 1959.