Assessing TrueAllele® Genotype Identification on DNA Mixtures Containing up to Five Unknown Contributors

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ABSTRACT

Manual review of complex DNA evidence does not fully elicit all the data's identification information. Therefore, computer methods have been developed for mathematical interpretation of mixed and low-template DNA. The genotype modeling approach computationally separates out the contributors to a mixture, with uncertainty represented through probability. Comparison of a contributor genotype to another genotype, relative to a population, calculates a likelihood ratio (LR). Validating an interpretation method on a broad range of DNA mixtures having known composition can help predict an expected LR outcome in a particular case.

This randomized experimental design examined 40 DNA mixture items. The 4 mixture sets had 2, 3, 4 or 5 contributors, with each item specified as a random mixture weighting of randomly assigned known references. Both normal (1 ng) and low (200 pg) template amounts were studied, for a total of 8 groups (4 contributor numbers x 2 template amounts) each having 10 mixture items.

The mixture weight (MW) of each item's contributors had a predetermined design value, but was subject to laboratory variation. For each item, the TrueAllele system computed two MW estimates, one using all the known genotypes, and the other with all genotypes unknown. MW was also computed manually on the 2 contributor items. There was a strong association ($r^2 = 0.999$) between the three computed MWs for an item, and less ($r^2 = 0.907$) with the design value ($p < 10^{-12}$). The computed TrueAllele known-genotype MWs had the most precise values (average sd = 0.0195 log(LR) units), and were used in the remainder of the study.

Following a procedure used in a previous validation study [1], scatterplots were developed comparing a contributor's known DNA quantity (logarithm of MW x total DNA, x-axis) versus its identification information (log of LR, y-axis). This approach permitted examination of all the match results (all contributors of all items) within their groups across a single statistical analysis. The scatterplots of positive match results were roughly linear ($r^2 = 0.638$), showing expected log(LR) reductions for equal MWs and high DNA amounts. The average regression slope was 12.66 log(LR)/log(DNA) ($p < 10^{-40}$), so a ten-fold change in DNA amount yielded a trillion-fold change in LR.

Analysis of covariance (ANCOVA) of the eight groups showed different x-intercept values, but no significant difference in slope ($p = 0.348 > 0.05$). This slope invariance was observed across four different contributor numbers (2, 3, 4 and 5) and DNA template amounts (200 pg and 1 ng). This invariance indicates that TrueAllele's information response to DNA mixture data is relatively independent of contributor number or template amount. The ANCOVA outcome suggests that this genotype modeling method produces reliable match results, regardless of the DNA mixture composition.

The false exclusion rate (Type II error) was estimated as a function of MW. For normal DNA amounts, there were positive match results in 100% of comparisons ($0.10 \le MW \le 1.00$), 82% ($0.05 \le MW \le 0.10$), 40% ($0.01 \le MW \le 0.05$) and none below 0.01. With low-template DNA, positive match results were found in 100% of comparisons ($0.25 \le MW \le 1.00$), 91% ($0.10 \le MW \le 0.25$), 24% ($0.05 \le MW \le 0.10$) and none below 0.05. In addition to these sensitivity and specificity results, reproducibility was measured in all groups.

This validation study used randomly generated DNA mixtures (reflective of actual casework samples) of up to 5 contributors, with both high and low template amounts, to assess TrueAllele genotype modeling. The study found that the computer's MW values were reliable, and that match information changed with DNA quantity in a predictable way that did not significantly depend on contributor number or template amount. Type II error was determined as a function of MW. This in-depth experimental study and statistical analysis show the applicability and limitations of the TrueAllele method.

INFORMATION RESPONSE

How does DNA identification information change with DNA quantity?

The change is roughly log linear: a ten-fold change in quantity gives a trillion-fold change in LR.

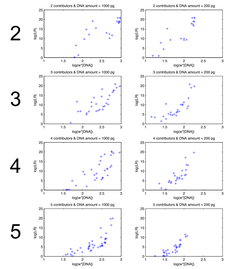

Figure 1. *DNA information vs. amount*. Scatterplots of TrueAllele-inferred log(LR) versus known DNA contributor amount, shown for different numbers of contributors (2, 3, 4 and 5 individuals) and DNA amounts (1 ng and 200 pg). Only match results having positive log(LR) are displayed.

INTERPRETATION INVARIANCE

Does TrueAllele interpretation vary much with the amount of DNA or the number of contributors?

No, the behavior stays about the same, as shown by an unchanging information-to-amount slope.

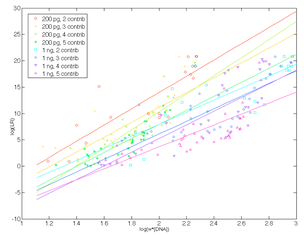

Figure 2. *Information change regression slopes*. Scatterplots of log(LR) vs. DNA amount are shown for eight different groups: 2, 3, 4 or 5 contributors, and either 1 ng or 200 pg of DNA. The scatterplots and regression lines are overlain to show their similar slope behavior.

CONTRIBUTOR SUFFICIENCY

If there are K contributors in a mixture, and TrueAllele assumes K or more contributors are present, what happens?

Once there are a sufficient number of assumed contributors, on average the LR stays the same or lower.

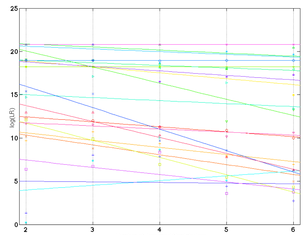

Figure 3. *Information with excess contributors (two person mixtures)*. In separate computer runs, TrueAllele assumed 2, 3, 4, 5 or 6 unknown contributors and inferred log(LR) match statistics. For each mixture component, the regression line and data points are shown under these five different contributor assumptions.

CONCLUSIONS

The computer interpretation of DNA evidence is a twenty-first century necessity. With ever-increasing numbers of STR loci, DNA mixtures having three or more contributors, low-level or degraded samples, and the potential for subjective examination bias, human analysts cannot be expected to fully process all the data. Such thorough and objective mathematical DNA mixture interpretation is the province of machines.

To be forensically useful, interpretation methods must be fully tested on realistic data. The history of mixture interpretation is strewn with unused software programs whose aspirations far exceeded their implementation (e.g., DNAMIX, I-3, LoComatioN, LSD, PENDULUM). Exceptionally, Cybergenetics TrueAllele Casework system has been repeatedly proven in extensive validation studies, both internal to laboratories and in peer-reviewed publications [2, 3].

This TrueAllele validation study used randomly generated DNA mixtures of known composition that were representative of actual casework. The samples contained up to five contributors, for both high and low template amounts. The study assessed the efficacy of the computer's genotype modeling, as quantified by LR.

The computer's mixture weight values were found to be reliable. The computed match information varied with DNA quantity in a predictable way that did not significantly depend on contributor number or template amount. Excess assumed contributors did not materially affect the conclusions.

The match statistic determination of inclusion and exclusion gave reproducible match values. The system was highly sensitive, preserving considerable identification information. It was also extremely specific, providing large exclusionary match statistics. Error rates were determined for false inclusions and exclusions. Inclusion accuracy was tabulated as a function of mixture weight.

This in-depth experimental study and statistical analysis establish the reliability of TrueAllele for the interpretation of DNA mixture evidence over a broad range of forensic casework conditions.

INCLUSION DISTRIBUTION

How well does TrueAllele include genotypes that have contributed to a low-template DNA mixture?

Very well, since the LR values usually include, and less often exclude, a true contributor.

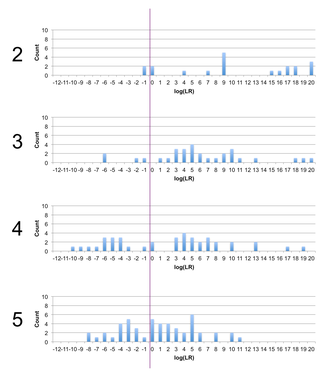

Figure 4. *Sensitivity (200 pg)*. Histograms of the log(LR) distribution for mixtures having 2, 3, 4 and 5 contributors. Average replicated log(LR) scores were used.

EXCLUSION DISTRIBUTION

How well does TrueAllele exclude genotypes that have not contributed to a low-template DNA mixture?

Very well, since the LR values almost always exclude, and very rarely include, a non-contributor.

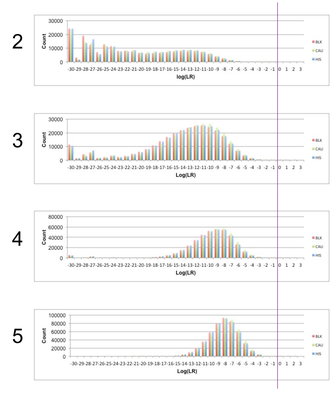

Figure 5. *Specificity (200 pg)*. The log(LR) specificity distribution for mixtures having 2, 3, 4 and 5 contributors. The LRs were computed relative to 10,000 randomly generated profiles across the FBI African-American (BLK, red), Caucasian (CAU, green) and Hispanic (HIS, blue) populations.

REPRODUCIBILITY COMPARISON

How well do repeated TrueAllele computer runs give the same match statistic with a low-template DNA mixture?

Very well, since replicate LR values for the same match comparison line up along the $LR_1 = LR_2$ line.

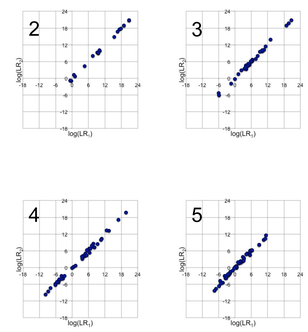

Figure 6. *Reproducibility (200 pg)*. Scatterplots of paired log(LR) values for duplicate computer runs on the same mixture sample. The mixtures had 2, 3, 4 and 5 contributors. Each point shows the first (LR_1) and second (LR_2) replicates.

REFERENCES

[1] Perlin MW, Sinelnikov A. An information gap in DNA evidence interpretation. PLoS ONE. 2009;4(12):e8327.

[2] Perlin MW, Legler MM, Spencer CE, Smith JL, Allan WP, Belrose JL, Duceman BW. Validating TrueAllele® DNA mixture interpretation. J Forensic Sci. 2011;56(6):1430-47.

[3] Perlin MW, Belrose JL, Duceman BW. New York State TrueAllele® Casework validation study. J Forensic Sci. 2013;58(6):1458-66.

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