Different TrueAllele® users, same DNA answer: a multi-center proficiency study

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Abstract

For two decades, interlaboratory studies (1, 2, 3) have highlighted considerable variation in DNA mixture interpretation outcomes. The same DNA evidence can produce widely different results – one laboratory may calculate a match statistic that connects a suspect to the mixture, another lab may exclude him, while a third can't reach any conclusions. This variability diminishes confidence in forensic DNA science.

Cybergenetics conducted a study of crime laboratories that use its TrueAllele® technology. A TrueAllele computer applies Bayesian inference and statistical search to derive genotypes from DNA mixture data. Each "probabilistic" genotype corresponds to one contributor to the mixture. These single-contributor genotypes are compared with reference profiles to calculate a likelihood ratio (LR) match statistic. The LR quantifies the statistical support for a person having left their DNA (or not) in the evidence.

Our study had two goals: assessing the TrueAllele proficiency of participating analysts, and examining the concordance of their reported results. Each TrueAllele laboratory sent us electronic data from one mixture item, along with a matching reference profile. The labs produced data using five different PCR kits and four different genetic analyzers. The DNA mixtures contained 3 to 5 contributors; 70% were four-contributor mixtures. The comparison person comprised 18% to 90% of the mixture.

We sent anonymized data from 10 mixtures to 32 analysts across 10 participating laboratories. Each TrueAllele analyst processed every item. Once an analyst had completed their TrueAllele processing, we sent them reference profiles for LR comparison. This two-stage data distribution assured objectivity – TrueAllele did not need or use reference information to interpret mixture data.

The lab analysts used TrueAllele comparisons to first determine which reference was associated with which mixture sample. They then calculated LR match statistics for the DNA associations. The analysts returned their match statistics to Cybergenetics, who collated their results and conducted ANOVA statistical tests. The ANOVA grouped the LR results by mixture item, laboratory, and analyst.

The study showed analyst proficiency in using TrueAllele – all were able to process DNA mixture data and produce match statistics. The ANOVA results demonstrated no statistical difference in LR outcomes between laboratories (p-value = 0.273 > 0.05), nor between analysts (p-value = 0.856 > 0.05).

The TrueAllele laboratories derived reliable results using STR data from other laboratories. No PCR kit or genetic analyzer calibration was needed, since TrueAllele learns this information directly from evidence data. It made no difference where the DNA data came from, nor what lab technology was employed to generate the data.

The study showed that TrueAllele results do not depend on where, when, who, how, why, or what DNA mixture data is generated and interpreted. The LR results are invariant across person and laboratory, DNA complexity and analysis procedure, motivation and bias, or time and space. The answers are the same regardless.

With TrueAllele mixture analysis, all laboratories and analysts get the same output LR answer from the same input DNA data. All qualified experts will report the same answer [4]. This cross-laboratory consistency improves on other approaches that showed high inter-laboratory reporting variation. Reporting concordant LR results increases confidence in forensic DNA science and human identification.

Materials

Mixture Contributors

DNA contributors	sample
three	2
four	7
five	1

The study included DNA mixture samples of 3, 4, and 5 contributors.

STR Kits

Kit	sample
Applied Biosystems™ GlobalFiler	4
Promega PowerPlex® 16	1
Promega PowerPlex® Fusion 5C	3
Promega PowerPlex® Fusion 6C	1
Qiagen Investigator® 24plex GO!	1

The study included data from 5 different PCR kits, across 3 different vendors.

Sequencers

Sequencer	sample
ABI310	1
ABI3130xl	2
ABI3500	5
ABI3500xl	2

The study included data from 4 different sequencer models.

Labs and Analysts

Laboratory	analyst
Lab01	9
Lab02	4
Lab03	2
Lab04	3
Lab05	2
Lab06	3
Lab07	2
Lab08	2
Lab09	3
Lab10	2
Total	32

The study included at least 2 trained TrueAllele analysts from each lab.

Methods

Multiple TrueAllele laboratories submitted the electronic data for a mixture sample created in their lab, using their sequencer and PCR kit. This mixture sample was either from an adjudicated case, or from validation data. The sample was to be representative of typical lab work. The lab also provided a matching reference profile.

In addition, analysts from the laboratories signed up to participate in the study. These analysts had some level of TrueAllele training.

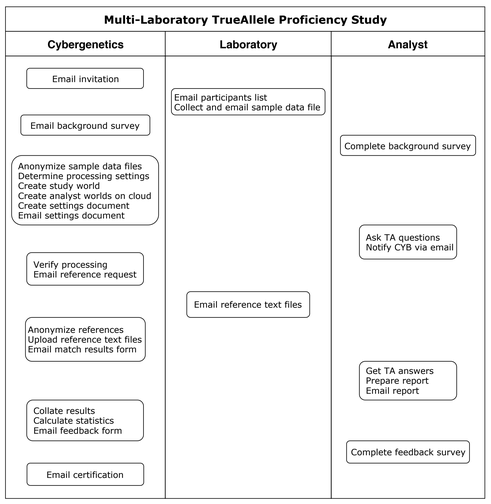
Once received, Cybergenetics anonymized the sample and reference names and files. Cybergenetics then provided each study participant with the electronic data for each of the 10 mixture data samples. Each analyst used TrueAllele to process each mixture sample in triplicate. One sample was from their laboratory, and 9 others were not. For consistency, the analysts were instructed on the number of contributors and the number of MCMC cycles to use for each sample.

TrueAllele mathematically separated the mixture data into separated genotypes for each contributor to the sample.

Once all the processing was completed, Cybergenetics sent out reference profiles for LR comparison. For objectivity, the analysts did not have references for mixture data processing (TrueAllele does not use comparison references when interpreting evidence data).

The analysts used TrueAllele to compare the separated genotypes with the reference profiles. Their task was twofold: to determine the corresponding reference, and to provide match statistics for that comparison.

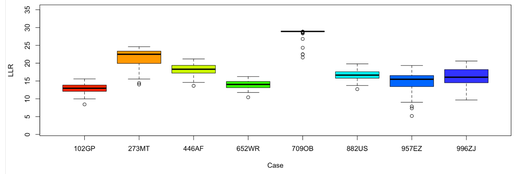
Since each sample was run in triplicate, each analyst returned a total of 30 match statistics.



Results

Cybergenetics collated the log(LR) results and performed summary and ANOVA statistical analyses on each sample. Summary statistics included average log(LR) for each sample. The ANOVA analyses were conducted separated for laboratories and analysts. F-statistics and p-values were examined to see how laboratory or analyst processing of the samples statistically affected the data's log(LR) values. Cybergenetics rendered box plots to visualize the assembled DNA identification information.

Sample log(LR) Values

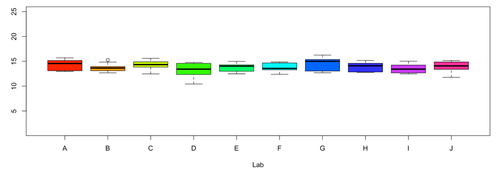


Box plot showing log(LR) values for 8 samples.

Between Laboratories

ANOVA results

- Degrees of Freedom: 9
- Sum of Squares: 3
- Mean Square: 4.2
- F-statistic: 1.231
- **p-value: 0.273 > 0.05**

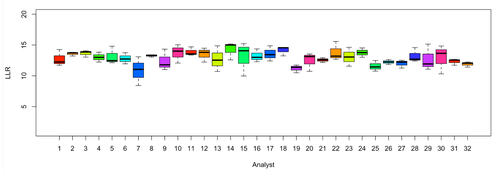


Box plot showing log(LR) values for 1 sample for the 10 different labs.

Between Analysts

ANOVA results

- Degrees of Freedom: 31
- Sum of Squares: 79
- Mean Square: 2.6
- F-statistic: 0.732
- **p-value: 0.856 > 0.05**



Box plot showing log(LR) values for 1 sample for the 32 participating analysts.

Conclusions

There are unfounded myths about the interpretation of complex DNA evidence. These myths arise from the use of older manual protocols and software programs that have limited capability. Such older methods depend on laboratory protocol steps and human data input decisions. Their limitations require conducting laboratory-specific calibrations prior to performing DNA interpretation.

However, high-dimensional Bayesian modeling of the STR experiment overcomes these artificial limitations. The mathematics accounts for data variation of PCR amplification and signal detection, hierarchically extending to individual locus experiments.

Detailed variation modeling permits the determination of accurate genotype probabilities at every locus for each separate contributor. Calibration is done dynamically on evidence data, not on historical laboratory runs.

Our hypothesis was that such powerful Bayesian computation is independent of laboratory and analyst. Akin to other fields of science, the genotype and LR results on DNA data should be invariant with respect to when, where, who and why the DNA interpretation was performed.

This hypothesis was tested using the Bayesian TrueAllele genotyping system. 32 trained DNA analysts at 10 different laboratories examined 8 representative complex mixture items from different labs.

The participating laboratories provided the evidence data. Each analyst used their own lab's TrueAllele system to interpret the DNA mixture data generated by the different laboratories. No was calibration needed or done. The analysts recorded their LR values.

Across eight cases, analysis of variance demonstrated no statistical difference between the LR information found by analysts or laboratories. The TrueAllele analysts showed mutual proficiency. The TrueAllele system was invariant across person, place and time.

We conclude that any trained TrueAllele analyst at any TrueAllele site can run their TrueAllele system on complex DNA mixture data produced by any accredited DNA laboratory to obtain reliable LR match results.

References

[1] Butler, Kline, Coble. "NIST interlaboratory studies involving DNA mixtures (MIX05 and MIX13): Variation observed and lessons learned." Forensic Sci Int Genet, 2018 Nov:37:81-94

[2] Buckleton, Bright, Cheng, Budowle, Coble. "NIST interlaboratory studies involving DNA mixtures (MIX13): A modern analysis." Forensic Sci Int Genet, 2018 Nov:37:172-179

[3] Brinkac, Richetelli, Davoren, Bever, Hicklin. "DNAmix 2021: Laboratory policies, procedures, and casework scenarios summary and dataset." Data Brief, 2023 Apr 14:48:109150.

[4] Bauer, D.W., Butt, N., Hornyak, J.M., and Perlin, M.W. Validating TrueAllele® interpretation of DNA mixtures containing up to ten unknown contributors. Journal of Forensic Sciences, 65(2):380-398, 2020.