ANSI/ASB Standard 018, First Edition 2020
Standard for Validation of Probabilistic Genotyping Systems

TrueAllele® Casework System

Cybergenetics, Pittsburgh, PA

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Introduction

This document describes how Cybergenetics TrueAllele® Casework system complies with the Standard for Validation of Probabilistic Genotyping Systems (ANSI/ASB Standard 018), as promulgated in the ANSI/ASB July 2020 document.

The document embeds the ANSI/ASB Standard 018 text, and gives a paragraph-by-paragraph description of system compliance. Separate appendices list the many TrueAllele validation studies that establish the system's reliability. There is also an appendix on the availability of the supporting documents referred to herein.

The ANSI/ASB Standard 018 document is downloadable from:

http://www.asbstandardsboard.org/wp-content/uploads/2020/07/018_Std_e1.pdf

Glossary
• *AAFS* is the American Academy of Forensic Sciences, an organization for forensic science professionals.
• *ANSI* is the American National Standards Institute, a standards organization that oversees standard conformity.
• *ASB* is the AAFS Standards Board, an organization that provides forensic standards.
• *Cybergenetics* is a Pittsburgh-based company founded in 1994 that specializes in computer interpretation of DNA evidence data.
• *Peer review* is an assessment scientific research by a journal that has two (or more) independent workers review a manuscript before accepting it for publication.
• *Probabilistic genotyping* is any method that interprets DNA data and produces more than one genotype, assigning probabilities to the possibilities.
• *SWGDAM* is the Scientific Working Group on DNA Analysis Methods, a standing committee that helps establish guidelines of interest to the FBI.
• *TrueAllele* Casework is a computer system that accurately and automatically interprets DNA evidence data, producing reliable match statistics.
• *Validation* is a testing procedure for establishing the reliability of a method.
• *Validation study* is a scientific study that documents validation testing.

Cybergenetics

Cybergenetics is a bio-information company that develops and uses TrueAllele Casework. Cybergenetics is not a traditional crime laboratory, but rather an independent forensic statistical consultant (IFSC). As an IFSC, Cybergenetics can review DNA data from a case and provide any party with the DNA information present in the data and the match statistics for any reference or evidence comparison. IFSCs are not are not crime labs. Thus, the standard requirements that apply to crime labs do not apply to IFSCs.

TrueAllele Casework

TrueAllele Casework is a probabilistic genotyping (PG) software that interprets DNA data. The system does not have user input analytical or statistical parameters that need to be set. Therefore, the computer does not rely upon historical data to calibrate or set parameters. The computer calculates the information from the data. In addition, these parameters do no vary from run to run. Moreover, there are no specific parameters used by TrueAllele that need to be tested outside of validation data sets.

Internal Validation

Crime laboratories use TrueAllele technology. Before implementing TrueAllele in casework, all TrueAllele labs perform an internal validation study. These laboratories also ensure that they comply with all standards relevant to their use of TrueAllele PG software. The labs document their internal validations, and these documents can be part of the lab's disclosure. Cybergenetics provides all validation studies (both developmental and internal) in every case where disclosure materials are needed.

The compliance described in the next section is applicable to all TrueAllele laboratories.

Standard for Validation of Probabilistic Genotyping Systems (ANSI/ASB Standard 018)

4. Requirements

4.1 The laboratory shall validate a probabilistic genotyping system prior to its use for casework samples in the laboratory.

The TrueAllele Casework system has been extensively validated on both laboratory and casework DNA samples, with over 40 studies completed. Eight of these validation studies have been published in peer-reviewed journals. Currently, TrueAllele validation studies have been completed on samples containing up to 10 unknown contributors with both high and low template samples tested across a range of conditions. Sensitivity, specificity, and reproducibility of the TrueAllele system have been thoroughly established, with other measures studied as well. Performance checks are done when software updates are made.

Appendix 1 (*TrueAllele Validation Summary*) lists all TrueAllele validation studies and describes the metrics tested in each validation study based on the 2015 SWGDAM Guidelines for Validation of Probabilistic Genotyping Systems.

4.1.1 Validations shall include both developmental and internal studies. Developmental validation may be conducted by the manufacturer/developer of the application or another laboratory/agency. Developmental validation shall not replace internal validation.

Appendix 1 (*TrueAllele Validation Summary*) lists all TrueAllele validation studies (both developmental and internal) and describes the metrics tested in each study based on the 2015 SWGDAM Guidelines for Validation of Probabilistic Genotyping Systems.

There are 8 TrueAllele developmental validation studies. Appendix 2 (*TrueAllele Developmental Validations*) lists these studies.

4.1.2 Developmental validation studies shall address the following: accuracy, sensitivity, specificity, and precision. These studies shall include case-type profiles of known composition that represent (in terms of number of contributors, mixture

ratios, and total DNA template quantities) the range of scenarios that would likely be encountered in casework. Studies shall not be limited to pristine DNA samples but shall also include compromised DNA samples (e.g., low template, degraded, and inhibited samples).

> These studies have been conducted. Appendix 1 (*TrueAllele Validation Summary*) describes the metrics tested in each developmental TrueAllele validation study based on the 2015 SWGDAM Guidelines for Validation of Probabilistic Genotyping Systems.

4.1.3 Internal validation studies shall address the following: accuracy, sensitivity, specificity, and precision. These studies shall include internally generated case-type profiles of known composition that represent (in terms of number of contributors, mixture ratios, and total DNA template quantities) the range of actual casework samples intended for analysis with the system at the laboratory. Studies shall not be limited to pristine DNA samples but shall also include compromised DNA samples (e.g., low template, degraded, and inhibited samples). The internal validation shall not exceed the scope of the conditions tested in the developmental validation. Case type profiles that fall outside the range of conditions explored in developmental validation shall require additional developmental validation studies. See Annex A.

> These studies have been conducted. Appendix 1 (*TrueAllele Validation Summary*) describes the metrics tested in each internal TrueAllele validation study based on the 2015 SWGDAM Guidelines for Validation of Probabilistic Genotyping Systems.

4.1.4 Internal validation studies shall include evaluating user input parameters that vary run to run. The effects of artifacts (e.g., stutter) and parameters that relate to the statistical algorithm (e.g., run time parameters for the software system that can vary from system to system) shall also be evaluated. The parameters may vary depending upon the approach or intended use of the software. Therefore, the specific parameters to be tested shall be determined by the laboratory.

> These studies have been conducted. Appendix 1 (*TrueAllele Validation Summary*) describes the metrics (including those for artifacts and run time

input) tested in each TrueAllele validation study based on the 2015
SWGDAM Guidelines for Validation of Probabilistic Genotyping Systems.

*4.1.5 Internal validation studies shall also include the evaluation of multiple
propositions for case type samples to aid in the development of propositions. Such
studies shall also consider the effect of overestimating and underestimating the
number of contributors.*

These studies have been conducted. Appendix 1 (*TrueAllele Validation
Summary*) describes the metrics (including those for propositions and
contributor number input) tested in each TrueAllele validation study based
on the 2015 SWGDAM Guidelines for Validation of Probabilistic
Genotyping Systems.

*4.1.6 For internal validation, the laboratory shall evaluate both the appropriate
sample types (i.e., number of contributors, mixture ratios, and template quantities)
and the number of samples within each type to demonstrate the potential limitations
and reliability of the software. The laboratory shall base this evaluation on the
intended application of the software.*

These studies have been conducted. Appendix 1 (*TrueAllele Validation
Summary*) describes the metrics tested in each TrueAllele validation study
based on the 2015 SWGDAM Guidelines for Validation of Probabilistic
Genotyping Systems.

*4.2 The underlying scientific principle(s) of the probabilistic genotyping model and
associative method and software including the mathematical basis and underlying
algorithms shall be published in peer-reviewed scientific journal(s).*

TrueAllele's underlying scientific principles, methods of analysis, mathematical
basis, underlying algorithms, and statistical formulae are described in various
peer-reviewed and other publications. The TrueAllele Methods: Statistical Model
document summarizes those methods and citations. Appendix 3 (TrueAllele
Peer-reviewed Papers) lists TrueAllele related peer-reviewed papers.

4.3 Quality assurance parameters, analytical procedures, and interpretation protocols shall be derived from internal validation studies. Developmental and manufacturer recommendations may be used in addition to internal validation studies but shall not replace internal validation.

Appendix 1 (*TrueAllele Validation Summary*) lists all TrueAllele validation studies and describes the metrics tested in each validation study based on the 2015 SWGDAM Guidelines for Validation of Probabilistic Genotyping Systems. These studies encompass the processes and procedures Cybergenetics follows when analyzing casework data. Cybergenetics TrueAllele workflow and interpretation guidelines are described in the *TrueAllele® Casework Process: Standard Operating Procedures* document.

4.4 Software modifications, changes to computing platform or changes to upstream analytical processes (i.e., amplification processes, detection platforms) that may impact the interpretation or reported result(s) shall be evaluated to determine whether a validation or performance check is required prior to implementation. Such modifications shall require a validation or performance check of the affected software component. If neither is conducted after a software modification, changes to computing platform or changes to upstream analytical processes, the laboratory shall document the justification (e.g., software update simply enhances visual output or displays, therefore no performance check was conducted). See Annex A.

When server code updates affect interpretation, validation is done before the new version is distributed and used in routine processing.

Additionally, when a new module is added, a performance check is done to test the new software. Once sufficient testing has been done, the software or server version is deployed for use in casework. This testing is documented, and any new software features are documented prior to release.

4.5 All validation and performance check studies conducted by the laboratory shall be documented and retained by the laboratory. See Annex A.

Appendix 1 (TrueAllele Validation Summary) lists all TrueAllele validation studies, including some performance check reports. All studies and reports are documented and available upon request.

4.6 The laboratory shall have a mechanism to record the software settings that are used each time an analysis is performed. See Annex A.

Each TrueAllele interpretation request stores the software setting information once it is uploaded to the TrueAllele server (e.g., contributor number, number of cycles, etc.). This information can be viewed in the Report module for each interpretation request. The *TrueAllele® VUIer™: Report Module* manual describes where the setting information can be found.

In addition, the analyst can save the interpretation requests in a *.req* file. This file saves the requests and settings for each session. The *TrueAllele® VUIer™: Request Module* manual has more information about this file type.

4.7 Prior to implementation, the laboratory shall verify the functionality of its defined software settings and parameters utilizing different data sets than what were originally used to establish those settings and parameters. See Annex A.

Sufficient testing is done on a variety of data sets before new software is distributed and used in routine processing. This testing is documented, and any new software features are documented prior to release.

5. Conformance

Documentation demonstrating conformance with the standards described in this document will be reviewed and approved by the laboratory's DNA technical leader (or equivalent) and will be made readily available in hard copy and/or electronic form for review.

The current document describes Cybergenetics TrueAllele Casework compliance with the Standard for Validation of Probabilistic Genotyping Systems (ANSI/ASB Standard 018).

Appendix 1: TrueAllele Validation Summary

Introduction

The TrueAllele Casework system has been thoroughly validated across a range of conditions. Cybergenetics and other groups have conducted over 40 validation studies. These studies have been presented either as peer-reviewed papers, or as written reports or presentations. Additional validation studies are currently being conducted.

This section contains a table describing the validation studies that fulfill the various developmental and internal validation guidelines presented in sections 3 and 4 of the 2015 SWGDAM Guidelines for Validation of Probabilistic Genotyping Systems. The table contains the SWGDAM *Guideline* number, a *Description* of the guideline, and a *Study* number that corresponds to the study fulfilling the guideline. These *Study* numbers correspond to both the *TrueAllele Validation Citations* section in this document as well as the study information contained in the *TrueAllele Validation Reports and Papers (ReadMe)* document. Many of these guidelines appear in other standards and guideline documents. Thus, this appendix can be used to show how TrueAllele complies with those standards and recommendations as well.

A Dropbox link to all of the papers and reports can be provided upon request. It should be noted that this table may not list every topic covered in a study but is representative of the major points covered in each study.

Note: SWGDAM guideline 4.1.12 (establishing in-house parameters) is not applicable to TrueAllele analysis.

TrueAllele Studies and SWGDAM Guidelines

Guideline	Description	Study
3.2.1, 4.1.13	Sensitivity	4, 5, 7, 8, 9, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 27, 28, 29, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43
3.2.1.1	Type I errors (False exclusions)	16, 21, 22, 23, 24, 27, 28, 32, 34, 36, 37, 39, 40, 42, 43
3.2.1.2	Sensitivity range of LR values expected for contributors	4, 5, 7, 8, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 27, 28, 31, 32, 33, 34, 35, 36, 37, 39, 40, 43
3.2.2, 4.1.13	Specificity	7, 8, 12, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 27, 28, 29, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 43
3.2.2.1	Type II errors (False inclusions)	16, 18, 19, 20, 21, 22, 23, 24, 27, 28, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 43
3.2.2.2	Specificity range of LR values expected for non-contributors	12, 15, 16, 18, 19, 20, 21, 22, 23, 24, 25, 27, 28, 31, 32, 33, 34, 35, 36, 37, 39, 40, 43
3.2.3, 4.1.13	Precision	2, 5, 7, 8, 9, 11, 12, 13, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 27, 28, 29, 31, 32, 33, 34, 35, 36, 37, 39, 40, 43
3.2.3.1	Range of LR values expected between multiple analyses (σ_w)	5, 7, 8, 13, 15, 16, 17, 19, 20, 21, 22, 23, 24, 25, 27, 28, 31, 32, 33, 34, 35, 36, 37, 39, 40, 43
3.2.3.2	Reducing the variability of LR variation (e.g., increasing MCMC iterations)	15, 16, 18, 28, 29, 31, 33, 34, 37, 39, 42
3.2.4, 3.2.4.1, 4.1.1	Case-type samples (reliable evaluation)	5, 6, 7, 9, 10, 13, 17, 19, 25, 27, 31, 33, 37, 38, 40, 43
3.2.5	Control samples	1, 9, 25
3.2.6	Accuracy	2, 4, 5, 6, 8, 9, 13, 15, 17, 19, 21, 24, 26, 27, 29, 31, 34, 35, 38, 39, 40, 43
3.2.6.1, 4.2	Comparison with manual review	1, 2, 4, 5, 6, 7, 8, 9, 10, 11, 13, 15, 17, 19, 25, 29, 31, 33, 35
3.2.6.2	Comparison of allele calling of raw data (.fsa) files	1, 17
4.1	Data from kits, instruments, and analysis software used in casework	1, 3, 4, 5, 7, 8, 9, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 43
4.1.1	Known contributor samples	4, 8, 9, 12, 14, 15, 16, 18, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43
4.1.2, 4.1.2.1	Hypothesis testing with contributors and non-contributors	4, 5, 9, 11, 12, 13, 17, 18, 19, 25, 26, 28, 29, 31, 32, 37, 38, 39, 40, 42, 43
4.1.3	Variable DNA typing conditions	9, 16, 18, 19, 22, 24, 28, 31, 32, 36, 37, 40, 43
4.1.4	Allelic peak height	3, 9, 16, 18, 19, 22, 24, 28, 30

4.1.5	Single-source samples	1, 5, 6, 8, 9, 12, 15, 25, 28, 29, 31, 35, 37, 38, 40, 43
4.1.6	Mixture samples	2, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43
4.1.6.1	Various contributor ratios	4, 7, 8, 9, 11, 12, 13, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 34, 35, 36, 37, 39, 40, 41, 42, 43
4.1.6.2	Various total DNA template quantities	4, 7, 8, 9, 11, 12, 15, 17, 18, 19, 20, 21, 27, 28, 32, 35, 36, 37, 40, 41, 43
4.1.6.3	Various numbers of contributors in samples	7, 10, 11, 12, 15, 16, 17, 18, 19, 21, 23, 24, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43
4.1.6.4	Over- and under- estimating of number of contributors input	8, 27, 28, 30, 32, 34, 39
4.1.6.5	Allele sharing among contributors	8, 11, 12, 18, 20, 26, 29, 38, 40
4.1.7	Partial profiles	5, 8, 9, 14, 15, 18, 28, 29, 35
4.1.7.1	Allele and locus drop-out	5, 8, 15, 18, 29, 34, 35, 39
4.1.7.2	DNA degradation	8, 12, 28, 29, 30, 32, 36, 37, 40, 43
4.1.7.3	Inhibition	30, 32, 36, 43
4.1.8	Allele drop-in	14
4.1.9	Forward and reverse stutter	1, 8, 13
4.1.10	Intra-locus peak height variation	1, 3, 29, 41
4.1.11	Inter-locus peak height variation (mixture weight modeling)	4, 5, 13, 14, 15, 17, 27, 41
4.1.14	Additional challenge testing (spikes, etc.)	1, 29
4.2.1	Determination if results produced are intuitive and consistent with expectations	1, 2, 4, 5, 6, 7, 8, 9, 10, 11, 13, 15, 17, 18, 19, 25, 29, 31, 33, 35
4.2.1.1	If included manually, also included with probabilistic genotyping	1, 2, 4, 5, 6, 7, 8, 9, 10, 13, 15, 17, 19, 25, 29, 31, 33, 35
4.2.1.2	Single-source concordance between manual and probabilistic genotyping methods	1, 5, 6, 8, 9, 15, 17, 25, 31, 35
4.2.1.3	Weightings given to individual genotypes decrease with increasing mixture complexity	5, 8, 11, 15, 16, 17, 18, 21, 22, 23, 24, 26, 27, 28, 31, 32, 33, 34, 35, 36, 37, 39, 42, 43

TrueAllele Validation Citations

This section lists the citations for all TrueAllele validation studies.

1. Kadash K, Kozlowski BE, Biega LA, Duceman BW. Validation study of the TrueAllele® automated data review system. *J Forensic Sci.* 2004;49(4):1-8.

2. Perlin MW. Scientific validation of mixture interpretation methods. *Promega's Seventeenth International Symposium on Human Identification*, 2006 Oct 10-12; Nashville, TN.

3. Cybergenetics. "TrueAllele® System 2 and Genotyper/Genescan Peak Heights and Orchid UK Data." *Cybergenetics (Pittsburgh, PA),* May 2007.

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

5. B.W. Duceman, M.W. Perlin, and J.L. Belrose. "New York State TrueAllele® Casework Developmental Validation." *New York State Police Forensic Investigation Center (Albany, NY), Cybergenetics (Pittsburgh, PA), and Northeast Regional Forensic Institute (Albany, NY),* February 2010.

6. Cybergenetics and Orchid Cellmark. "TrueAllele® Volume Crime Validation Study." *Cybergenetics (Pittsburgh, PA) and Orchid Cellmark (Abingdon, Oxfordshire, UK),* February 2010.

7. Cybergenetics. "NYSP TrueAllele® Validation." *Cybergenetics (Pittsburgh, PA),* May 2011.

8. M. Perlin, M. Legler, and J. Galdi. "Suffolk County TrueAllele® Validation." *Cybergenetics (Pittsburgh, PA) and Suffolk County Crime Laboratory (Hauppauge, NY),* May 2011.

9. NSW Review Team. "Phase 1 Evaluation Report of Cybergenetics TrueAllele® Expert System." *NSW Police Force (Lidcombe, New South Wales, Australia),* July 2011.

10. J. Sgueglia and K. Harrington. "Phase I: Internal Validation of TrueAllele Genetic Calculator as an Expert Assistant for Reads and Review of Data from Reported Sexual Assault Evidence." *Massachusetts State Police Forensic and Technology Center (Maynard, MA),* August 2011.

11. M.D. Coble and J.M. Butler. "Exploring the Capabilities of Mixture Interpretation Using True Allele Software." *National Institute for Standards and Technology (Gaithersburg, MD),* September 2011.

12. Cybergenetics. "Australia TrueAllele® Validation Report." *Cybergenetics (Pittsburgh, PA),* September 2011.

13. 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):1430-1447.

14. Ballantyne J, Hanson EK, Perlin MW. DNA mixture genotyping by probabilistic computer interpretation of binomially-sampled laser captured cell populations: Combining quantitative data for greater identification information. *Sci Justice.* 2013;53(2):103-114.

15. J. Caponera. "New York State Police Crime Laboratory System TrueAllele® Casework Validation Addendum." *New York State Police Forensic Investigation Center (Albany, NY),* June 2013.

16. M.W. Perlin, J. Hornyak, J. Caponera, and B. Duceman. "New York State TrueAllele® Validation on DNA Mixtures of Known Composition." *Cybergenetics (Pittsburgh, PA) and New York State Police Forensic Investigation Center (Albany, NY),* October 2013.

17. Perlin MW, Belrose JL, Duceman BW. New York State TrueAllele® Casework validation study. *J Forensic Sci.* 2013;58(6):1458-1466.

18. J. Caponera. "New York State Police Crime Laboratory System TrueAllele® Casework Validation Addendum." *New York State Police Forensic Investigation Center (Albany, NY),* December 2013.

19. Perlin MW, Dormer K, Hornyak J, Schiermeier-Wood L, Greenspoon S. TrueAllele® Casework on Virginia DNA mixture evidence: computer and manual interpretation in 72 reported criminal cases. *PLOS ONE.* 2014;9(3):e92837.

20. M.A. Clarke, J. Hornyak, W.P. Allan, and M.W. Perlin. "TrueAllele® Casework Separates DNA Mixtures that Share Alleles." *Cybergenetics (Pittsburgh, PA),* March 2014.

21. J. Hornyak, W.P. Allan, and M.W. Perlin. "TrueAllele® Casework Validation on PowerPlex® 21 Mixture Data." *Cybergenetics (Pittsburgh, PA),* March 2014.

22. J. Hornyak, W.P. Allan, and M.W. Perlin. "TrueAllele® Validation on Minifiler™ Mixture Data." *Cybergenetics (Pittsburgh, PA),* July 2014.

23. J. Hornyak, M. Bowkley, and M.W. Perlin. "TrueAllele® Validation on PowerPlex® 16 HS Mixture Data." *Cybergenetics (Pittsburgh, PA),* July 2014.

24. J. Hornyak, W.P. Allan, and M.W. Perlin. "TrueAllele® Validation on Identifiler® Plus Mixture Data." *Cybergenetics (Pittsburgh, PA),* August 2014.

25. G. Amick. "TrueAllele Validation." *Richland County Sheriff's Department (Columbia, SC),* March 2015.

26. K. Guest, L. Ludvico, L. Ferrara, and M. Perlin. "Development of Kinship Mixtures and Subsequent Analysis Using TrueAllele® Casework." *Master's Thesis*, *Duquesne University (Pittsburgh, PA),* April 2015.

27. Perlin MW, Hornyak J, Sugimoto G, Miller K. TrueAllele® genotype identification on DNA mixtures containing up to five unknown contributors. *J Forensic Sci*. 2015; 60(4):857-868.

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31. J. Donahue. "TrueAllele Casework Validation." *Beaufort County Sheriff's Office (Beaufort, SC),* January 2016.

32. J.M. Hornyak, E.M. Schmidt, and M.W. Perlin. "Georgia Bureau of Investigation Forensic Biology Unit TrueAllele® Validation." *Cybergenetics (Pittsburgh, PA) and Georgia Bureau of Investigation Forensic Biology Unit (Decatur, GA),* September 2016.

33. M.M. Legler, B.L. Harris, C.L. Booker, and M.W. Perlin. "Acadiana Criminalistics Laboratory TrueAllele® Casework Validation." *Cybergenetics (Pittsburgh, PA) and Acadiana Criminalistics Laboratory (New Iberia, LA),* October 2016.

34. D.W. Bauer, N. Butt, and M.W. Perlin. "Cuyahoga County TrueAllele® Validation Study." *Cybergenetics (Pittsburgh, PA) and Cuyahoga County Regional Forensic Science Laboratory (Cleveland, OH),* September 2016.

35. B.L Harris. "Acadiana Criminalistics Laboratory TrueAllele® Casework Validation Using Investigator® 24plex Kits & 2017 Server Upgrade Performance Check." Acadiana Criminalistics Laboratory (New Iberia, LA), May 2017.

36. E.M. Schmidt. "TrueAllele® GlobalFiler Performance Check." *Georgia Bureau of Investigation Forensic Biology Unit (Decatur, GA)*, August 2017.

37. J.M. Hornyak, C.L. Brown, and M.W. Perlin. "TrueAllele® Casework Validation of the PowerPlex® Fusion 6C STR Kit." Cybergenetics (Pittsburgh, PA) and Louisiana State Police Crime Laboratory (Baton Rouge, LA), July 2018.

38. G. Sugimoto. "Validation of the TrueAllele® Casework VUIer™ Kinship Application." Kern Regional Crime Laboratory (Bakersfield, CA), August 2019.

39. Bauer DW, Butt N, Hornyak JM, Perlin MW. Validating TrueAllele® interpretation of DNA mixtures containing up to ten unknown contributors. *J Forensic Sci*, 2020; 65(2):380-398.

40. B.A. Pujols, B.M. Browning, J.M. Bracamontes, M.M. Legler, D.W. Bauer, and M.W. Perlin. "TrueAllele® Casework Validation on Greenville County DNA Lab GlobalFiler™ Data." Cybergenetics (Pittsburgh, PA) and Greenville County Department of Public Safety Forensic DNA Laboratory (Greenville, SC), March, 2020.

41. S. Antillon. "Deconvolution of DNA mixtures using replicate sampling and TrueAllele® mixture interpretation [master's thesis]." George Mason University (Fairfax, VA), 2020.

42. H.S. Chaudhry. "Peeling away uncertainty: A probabilistic approach to DNA mixture deconvolution [master's thesis]." George Mason University (Fairfax, VA), 2020.

43. E.E. Mole, J.M. Bracamontes, I. Fleming, M.M. Legler, and M.W. Perlin. "Metro Nashville Police Department Crime Laboratory TrueAllele® Casework Validation on PowerPlex® Fusion 6C data." Cybergenetics (Pittsburgh, PA) and Metro Nashville Police Department Crime Laboratory (Nashville, TN), June 2023.

Appendix 2: TrueAllele Developmental Validations

This section lists the citations for TrueAllele developmental validation studies.

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):1430-1447.

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6. Perlin MW, Hornyak J, Sugimoto G, Miller K. TrueAllele® genotype identification on DNA mixtures containing up to five unknown contributors. *J Forensic Sci*. 2015; 60(4):857-868.

7. Greenspoon SA, Schiermeier-Wood L, Jenkins BA. Establishing the limits of TrueAllele® Casework: a validation study. *J Forensic Sci.* 2015;60(5):1263-1276.

8. Bauer DW, Butt N, Hornyak JM, Perlin MW. "Validating TrueAllele® interpretation of DNA mixtures containing up to ten unknown contributors." *J Forensic Sci*, 2020; 65(2):380-398.

Appendix 3: TrueAllele Peer-reviewed Papers

This section lists citations for TrueAllele-related peer-reviewed papers.

1. Perlin MW. Transforming conjunctive match into RETE: a call-graph caching approach, *International Journal of Software Engineering and Knowledge Engineering*, 1991;1(4):373:408.

2. Perlin MW, Burks MB, Hoop RC, Hoffman EP. Toward fully automated genotyping: allele assignment, pedigree construction, phase determination, and recombination detection in Duchenne muscular dystrophy. *Am J Hum Genet*. 1994;55(4):777-87.

3. 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.

4. Andrews C, Devlin B, Perlin M, Roeder K. Binning clones by hybridization with complex probes: statistical refinement of an inner product mapping method. *Genomics*, 1997;41(2):141-154.

5. Lancia G, Perlin M. Genotyping of pooled microsatellite markers by combinatorial optimization techniques. *Discrete Applied Math*. 1998;88(1-3):291-314.

6. Pálsson B, Pálsson F, Perlin M, Gubjartsson H, Stefánsson K, Gulcher J. Using quality measures to facilitate allele calling in high-throughput genotyping. *Genome Research*. 1999;9(10):1002-12.

7. Perlin MW, Szabady B. Linear mixture analysis: a mathematical approach to resolving mixed DNA samples. *J Forensic Sci*. 2001;46(6):1372-7.

8. Kadash K, Kozlowski BE, Biega LA, Duceman BW. Validation study of the TrueAllele® automated data review system. *J Forensic Sci.* 2004;49(4):1-8.

9. Hill SY, Shen S, Zezza N, Hoffman EK, Perlin M, Allan W. A genome wide search for alcoholism susceptibility genes. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics*. 2004;128B(1):102-13.

10. Perlin MW, Kadane JB, Cotton RW. Match likelihood ratio for uncertain genotypes. *Law, Probability and Risk*. 2009;8(3):289-302.

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

12. 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):1430-1447.

13. Ballantyne J, Hanson EK, Perlin MW. DNA mixture genotyping by probabilistic computer interpretation of binomially-sampled laser captured cell populations: Combining quantitative data for greater identification information. *Sci Justice.* 2013;53(2):103-114.

14. Perlin MW, Belrose JL, Duceman BW. New York State TrueAllele® Casework validation study. *J Forensic Sci.* 2013;58(6):1458-1466.

15. Perlin MW, Dormer K, Hornyak J, Schiermeier-Wood L, Greenspoon S. TrueAllele® Casework on Virginia DNA mixture evidence: computer and manual interpretation in 72 reported criminal cases. *PLOS ONE*. 2014;9(3):e92837.

16. Perlin MW. Inclusion probability for DNA mixtures is a subjective one-sided match statistic unrelated to identification information. *Journal of Pathology Informatics*, 6(1):59, 2015.

17. Perlin MW, Hornyak J, Sugimoto G, Miller K. TrueAllele® genotype identification on DNA mixtures containing up to five unknown contributors. *J Forensic Sci*. 2015; 60(4):857-868.

18. Greenspoon SA, Schiermeier-Wood L, Jenkins BA. Establishing the limits of TrueAllele® Casework: a validation study. *J Forensic Sci.* 2015;60(5):1263-1276.

19. Stokes NA, Stanciua CE, Brocatoa ER, Ehrhardta CR, Greenspoon SA. Simplification of complex DNA profiles using front end cell separation and probabilistic modeling. *Forensic Science International: Genetics*. 2018;36:205-212.

20. Perlin MW. Efficient construction of match strength distributions for uncertain multi-locus genotypes. *Heliyon*, 4(10):e00824, 2018.

21. Bauer DW, Butt N, Hornyak JM, Perlin MW. "Validating TrueAllele® interpretation of DNA mixtures containing up to ten unknown contributors." *J Forensic Sci*, 2020; 65(2):380-398.

Appendix 4: Other Reports and Supporting Documentation

Several supporting reports and other materials are mentioned throughout this document. These materials give additional support for TrueAllele's compliance with various guidelines and standards. A Dropbox link to these documents can be provided upon request.

TrueAllele reports

Perlin MW. Scientific validation of mixture interpretation methods. Promega's Seventeenth International Symposium on Human Identification, 2006; Nashville, TN.

Perlin MW. Explaining the likelihood ratio in DNA mixture interpretation. Promega's Twenty First International Symposium on Human Identification, 2010; San Antonio, TX.

Other supporting documents:
- *TrueAllele® Methods: Statistical Model*
- *TrueAllele® VUIer™* user manuals:
    - *Workflow Introduction*
    - *Getting Started*
    - *Analyze Module*
    - *Data Module*
    - *Request Module*
    - *Review Module*
    - *Report Module*
    - *Tools Module*
    - *Tutorial*
    - *Database Application Note*
    - *Specificity Application Note*
    - *Likelihood Ratio Calculation Application Note*
- Cybergenetics' *TrueAllele® Casework Process: Standard Operating Procedures*
- *TrueAllele® Server Quality Assurance Checklist*