ANSI/ASB Standard 020, First Edition 2018
Standard for Validation Studies of DNA Mixtures, and Development and Verification of a Laboratory's Mixture Interpretation Protocol

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 Studies of DNA Mixtures, and Development and Verification of a Laboratory's Mixture Interpretation Protocol (ANSI/ASB Standard 020), as promulgated in the ANSI/ASB September 2018 document.

The document embeds the ANSI/ASB Standard 020 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 020 document is downloadable from:
https://asb.aafs.org/wp-content/uploads/2018/09/020_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.

Standard for Validation Studies of DNA Mixtures, and Development and Verification of a Laboratory's Mixture Interpretation Protocol (ANSI/ASB Standard 020)

4. Requirements

4.1 Refer to Annex B, Requirements – Supporting Information, for additional normative information on the following requirements.

Annex B was reviewed and considered when responding to each standard listed in this document.

4.2 The laboratory shall perform DNA mixture studies as part of the internal validation to support interpretation protocols prior to their use for casework samples in the laboratory. The mixture studies shall include, at a minimum, mixed DNA samples that:

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 protocols and guidelines are described in the *TrueAllele® Casework Process: Standard Operating Procedures* document.

4.2.1 Are representative of those typically encountered and interpreted by the testing laboratory.

These studies have been conducted. 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.

4.2.2 Span the dynamic range of the detection platform.

These studies have been conducted. 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.

4.2.3 Include each number of contributors to be interpreted by the laboratory.

These studies have been conducted. 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.

4.2.4 Are constructed from extracted DNA samples of known origin (having known genotypes or sequences, etc.) combined: a) in varied input ratios based on the estimated DNA template amounts of the individual contributors; and b) with varied degrees of allele sharing.

These studies have been conducted. 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.

4.3 The data from the validation studies performed by the laboratory shall be the basis for the interpretation parameters and protocols developed by the laboratory and shall provide guidance for the types of mixed DNA profiles that will be interpreted by the laboratory. The studies shall:

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.

4.3.1 Support all of the interpretation methods and protocols used for DNA mixture analysis. The validation summary shall describe how the data from the validation studies performed led to the parameters used in the interpretation protocol.

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.

4.3.2 Aid in assessing and defining the limitations of the methodologies used for the range of samples to be tested and the interpretation of the data generated.

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.

4.3.3 Establish testing methodology and interpretation parameters for samples containing mixtures of DNA, including criteria for establishing the minimum and assumed number of contributors to a DNA mixture.

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 protocols and guidelines are described in the *TrueAllele® Casework Process: Standard Operating Procedures* document. Section 4.2 of that document describes the criteria for assessing the number of contributors in the DNA data.

4.4 The laboratory shall verify and document that the mixture interpretation protocols developed from the validation studies generate reliable and consistent interpretations and conclusions for the types of mixed DNA samples typically encountered by the laboratory.

TrueAllele's precision is described in various validation studies, where Cybergenetics procedures for operating TrueAllele Casework were used. 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.

4.4.1 Verification of the mixture protocols shall be performed on mixed DNA samples of known origin that are different from those in the initial validation studies used to establish the protocol.

Verification of the mixture interpretation protocol shall demonstrate that its use results in the correct inclusion of true contributors, exclusion of non-contributors, and the parameters considered in the interpretation protocols.

TrueAllele Casework has been tested on a variety of data sets, both documented as validation reports, and during software testing. 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.

4.4.2 Verification shall include a demonstration of consistency in the analysis and interpretation of mixed DNA data among analysts in the laboratory or laboratory system.

TrueAllele's precision is described in various validation studies, where Cybergenetics procedures for operating TrueAllele Casework were used. In addition, some precision studies were performed across laboratory systems showing the power of TrueAllele to operate reliably independent of computer location. 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.

4.4.3 Verification shall be performed on new, existing, and modified mixture interpretation protocols.

Sufficient testing of the TrueAllele Casework system is performed 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.

4.4.4 Verification of the protocol shall be performed by individuals in a blinded manner without knowledge of the expected results.

Sufficient testing of the TrueAllele Casework system is performed 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

Documented conformance to these requirements need to be: (1) approved by the laboratory's DNA Technical Leader or other appropriate personnel (2) communicated to all analysts during training, and (3) made readily available for review (e.g., by auditors or inspectors, stakeholders who use reports generated by the DNA mixture test protocols and parameters, etc.).

The current document describes Cybergenetics TrueAllele Casework compliance with the Standard for Validation Studies of DNA Mixtures, and Development and Verification of a Laboratory's Mixture Interpretation Protocol (ANSI/ASB Standard 020).

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.

28. J.M. Hornyak, T. Hebert, W.P. Allan, and M.W. Perlin. “Baltimore Police Department TrueAllele® Validation.” *Cybergenetics (Pittsburgh, PA) and Baltimore City Police Department Laboratory Section (Baltimore, MD),* August 2015.

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

30. S. Greenspoon, L. Schiermeier-Wood, and B. Jenkins. “Further Exploration of TrueAllele® Casework.” *Promega's Twenty Sixth International Symposium on Human Identification,* Grapevine, TX, October 2015.

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.

3. 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.

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

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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.

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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*