ANSI/ASB Standard 040, First Edition 2019
Standard for Forensic DNA Interpretation and Comparison Protocols

TrueAllele® Casework System

Cybergenetics, Pittsburgh, PA

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Introduction

This document describes how Cybergenetics TrueAllele® Casework system complies with the Standard for Forensic DNA Interpretation and Comparison Protocols (ANSI/ASB Standard 040), as promulgated in the ANSI/ASB September 2019 document.

The document embeds the ANSI/ASB Standard 040 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 040 document is downloadable from:
http://www.asbstandardsboard.org/wp-content/uploads/2019/10/Std_040_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 Forensic DNA Interpretation and Comparison Protocols (ANSI/ASB Standard 040)

4. Requirements

4.1 The laboratory interpretation protocols and comparison protocols, including criteria for drawing conclusions from comparisons between evidentiary data and reference (or other evidentiary) data, shall be based on, developed from, and supported by internal validation studies.

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 The laboratory shall maintain and follow documented DNA interpretation protocols that address the following.

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

4.2.1 Criteria for assessing the DNA data as originating from a single source or multiple sources.

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.

4.2.2 Criteria upon which assumptions may be made and the types of assumptions that may be used in data interpretation including, but not limited to, the number of contributors and the presence of assumed contributors.

Cybergenetics TrueAllele workflow, and interpretation protocols and guidelines are described in the *TrueAllele® Casework Process: Standard Operating Procedures* document. Sections 4, 5, 6, and 10 of that document describe the analyst input (e.g., contributor number, assumed known contributors, sampling time, etc.) when processing a sample using TrueAllele Casework.

4.2.3 Criteria for evaluating other considerations used in the interpretation of the data, such as the presence of major and minor contributors, the possibility of allele sharing, the relative mixture ratio for contributors, the possibility of inhibition or degradation for one or more contributors, the possibility of stochastic effects, and the presence of stutter.

Most of this standard is not applicable when using TrueAllele Casework. The computer uses all of the quantitative DNA data, models it, and separates out the DNA contributors. Sections 5 and 11 of the *TrueAllele® Casework Process: Standard Operating Procedures* document describe when to use the TrueAllele DNA degradation option.

4.2.4 The limitations of the interpretation methods used such as characterizing and defining the maximum number of contributors, and issues associated with low-level data, low-level contributors and potential contamination events.

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. Each study is documented, describing the reliability and limitations of the system on a wide variety of data sets.

4.2.5 Criteria for defining what are interpretable data versus data that cannot be interpreted.

This standard is not applicable when using TrueAllele Casework as the computer objectively uses all of the quantitative DNA data present in the sample during interpretation.

4.2.6 Criteria for defining data that are suitable for comparison versus data that are unsuitable for comparison.

This standard is not applicable when using TrueAllele Casework as the computer objectively uses all of the quantitative DNA data present in the sample during interpretation. Following the evidence data interpretation, any reference or evidence genotype can then be compared to calculate match statistics.

4.3 The laboratory shall have a documented policy requiring the interpretation of evidentiary data and documentation of any interpretation, including all assumptions used, prior to the comparison to any reference data.

Cybergenetics TrueAllele workflow, interpretation protocols and guidelines, and case documentation are described in the *TrueAllele® Casework Process: Standard Operating Procedures* document.

4.3.1 Interpretation of evidentiary data shall include documentation of the suitability of the single source or DNA mixture data for comparison.

This standard is not applicable when using TrueAllele Casework as the computer objectively uses all of the quantitative DNA data present in the sample during interpretation. Following the evidence data interpretation, any reference or evidence genotype can then be compared to calculate match statistics.

4.3.1.1 If the data or a subset of the data [e.g., major contributor(s)] are deemed suitable for comparison, the loci eligible for use in the comparison and in a subsequent statistical calculation(s) shall be documented in the case record.

When using TrueAllele Casework, all DNA loci are used both in the interpretation stage and when calculating DNA match statistics.

4.3.1.2 If the data or a subset of the data [e.g., minor contributor(s)] are deemed unsuitable for comparison, the qualitative reason(s) shall be documented in the case record.

This standard is not applicable when using TrueAllele Casework as the computer objectively uses all of the quantitative DNA data present in the sample during interpretation. Following the evidence data interpretation, any reference or evidence genotype can then be compared to calculate match statistics.

4.3.2 The subsequent interpretation of new evidentiary data shall be done by completing the interpretation and its documentation prior to comparison to any previously generated reference data.

This standard is not applicable when using TrueAllele Casework. The TrueAllele Casework system is inherently objective. The computer only looks at the evidence data during interpretation and does not know the comparison reference genotype. Reference genotype information is introduced only after the evidence data interpretation is complete to calculate match statistics.

4.3.3 When an assumption of an expected contributor is used for interpretation, the use of that assumption shall be documented in the case record along with the DNA data of the assumed contributor.

This information is documented in the case notes as well as the disclosure materials. These materials are described in the Cybergenetics TrueAllele the *TrueAllele® Casework Process: Standard Operating Procedures* document.

4.4 The laboratory shall maintain and follow documented protocols for drawing conclusions from the comparison of suitable evidentiary data derived from single source, mixed, and limited quality/quantity samples to reference (or other evidentiary) data.

Cybergenetics TrueAllele workflow, interpretation protocols and guidelines, and case documentation are described in the *TrueAllele® Casework Process: Standard Operating Procedures* document.

4.4.1 Laboratory protocols shall describe the criteria used for concluding that the source of the reference data is included, excluded, or inconclusive when compared to evidentiary data when those terms are used by the laboratory. If a comparison is deemed inconclusive, the reason(s) shall be documented in the case record.

This standard is not applicable when using TrueAllele Casework. Using TrueAllele, all separated evidence genotypes derived from the evidence data can be compared with all reference (or other evidence) genotypes to produce either an inclusionary or exclusionary DNA match statistic. This

match statistic shows the association between the evidence and reference (or other evidence) genotypes.

4.4.2 All re-evaluations of, and changes to, the original evidentiary data interpretation shall be thoroughly documented within the case record. The laboratory shall have protocols that address re- evaluation of evidentiary data after the comparison to reference (or other evidentiary) data has been performed.

This information is documented in the case notes as well as in the disclosure materials as described in the Cybergenetics TrueAllele the *TrueAllele® Casework Process: Standard Operating Procedures* document.

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.

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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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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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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.

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

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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*