# American Academy of Forensic Sciences

## VIRTUAL WORKSHOP W19

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2021 AAFS VIRTUAL MEETING

# MVPs of Forensic DNA: Examining the Most Valuable Publications in the Field


*Chair*

**John M. Butler, PhD**

NIST FORENSIC SCIENCE

*RESEARCH. STANDARDS. FOUNDATIONS.*

*Co-Chair*

**Robin W. Cotton, PhD**

BOSTON UNIVERSITY

**Mechthild K. Prinz, PhD**

JOHN JAY COLLEGE OF CRIMINAL JUSTICE

**Charlotte J. Word, PhD**

# Reference Lists Compared

| | | MVPs Feb 2021 | OSAC 10-26-20 | SWGDAM July 2020 |
|---|---|---|---|---|
| | **Informative Textbooks on Forensic DNA** | **17** | **16** | **5 + 2** |
| | | | | 6 websites |
| A | Plain Language Guides to Forensic DNA Analysis | 4 | 3 | -- |
| B | Serology and Body Fluid Identification | 24 | 15 + 2 | -- |
| C | Collection and Storage of Biological Material | 25 | 19 | -- |
| D | DNA Extraction/Purification, Differential Extraction | 18 | 14 | 1 |
| E | DNA Quantitation, Degraded DNA | 10 | 9 + 1 | 1 |
| F | PCR Amplification, Inhibition, and Artifacts | 13 | 10 | 3 |
| G | Capillary Electrophoresis Separation and Detection | 12 | 12 | 6 |
| H | Assessing Sample Suitability and Complexity, Low-Template DNA | 7 | 8 | -- |
| I | Estimating the Number of Contributors | 12 | 12 | -- |
| J | Data Interpretation, Mixture Deconvolution, Interlaboratory Studies | 12 | 12 | 2 + 4 |
| K | Interpretation: Binary Approaches (CPI, RMP, LR) | 11 | 9 | 3 |
| L | Interpretation: Probabilistic Genotyping Software (Discrete, Continuous) | 44 | 41 | 7 + 11 |
| M | Report Writing and Technical Review | 8 | 8 | -- |
| N | Court Testimony, Communication of Results, Juror Comprehension | 22 | 21 | 3 |
| O | Autosomal STR Markers and Kits | 29 | 27 | 4 |
| P | Mitochondrial DNA Testing | 11 | 10 + 1 | 3 + 32 |
| Q | Y-Chromosome and X-Chromosome Testing | 17 | 11 | 4 + 6 |
| R | DNA Databases and Investigative Genetic Genealogy | 14 | 14 | -- |
| S | Statistical Analysis | 11 | 9 | 3 + 2 |
| T | Population Genetics | 11 | 10 | 1 |
| U | DNA Phenotyping (Ancestry, Appearance, Age) | 24 | 20 | -- |
| V | New Technologies (Rapid DNA, Massively Parallel Sequencing) | 35 | 31 | -- |
| W | DNA Transfer and Activity Level Reporting | 57 | 54 | -- |
| X | Non-Human DNA Testing | 15 | 15 | -- |
| Y | Method Validation, Quality Control, and Human Factors | 23 | 23 | 1 + 5 |
| Z | General Forensic Science Topics | 11 | 11 | 1 |
| | Historical | | | 19 |
| *Underlined numbers designate those articles only found in that list* | TOTAL | **497** | **448** | **135** |

# Informative Textbooks on Forensic DNA (17)

*The following informative textbooks are listed by publication date in ascending order with the most recent ones listed last. This list is not comprehensive (e.g., earlier editions of some of these textbooks not included).*

1. National Research Council (1996) *The Evaluation of Forensic DNA Evidence*. National Academy Press: Washington, D.C.

2. Evett, I.W. and Weir, B.S. (1998) *Interpreting DNA Evidence: Statistical Genetics for Forensic Scientists.* Sinauer Associates: Sunderland MA.

3. Inman, K. and Rudin, N. (2001) *Principles and Practice of Criminalistics: The Profession of Forensic Science*. CRC Press: Boca Raton.

4. Fung, W.K. and Hu, Y.-Q. (2008) *Statistical DNA Forensics: Theory, Methods and Computation*. Wiley: Chichester, UK.

5. Butler, J.M. (2010) *Fundamentals of Forensic DNA Typing*. Elsevier Academic Press: San Diego.

6. Goodwin, W., Linacre, A., Hadi, S. (2011) *An Introduction to Forensic Genetics Second Edition*. Wiley: Chichester, UK.

7. Butler, J.M. (2012) *Advanced Topics in Forensic DNA Typing: Methodology*. Elsevier Academic Press: San Diego.

8. Shewale, J.G. and Liu, R.H. (Editors) (2013) *Forensic DNA Analysis: Current Practices and Emerging Technologies.* CRC Press: Boca Raton.

9. Gill, P. (2014) *Misleading DNA Evidence: Reasons for Miscarriages of Justice*. Elsevier Academic Press: San Diego.

10. Butler, J.M. (2015) *Advanced Topics in Forensic DNA Typing: Interpretation*. Elsevier Academic Press: San Diego.

11. Balding, D. J. and Steele, C. D. (2015). *Weight-of-evidence for Forensic DNA Profiles Second Edition*. Wiley: Chichester, UK.

12. Buckleton, J.S., Bright, J.-A., Taylor, D. (Editors) (2016) *Forensic DNA Evidence Interpretation Second Edition*. CRC Press: Boca Raton.

13. Robertson, B., Vignaux, G.A., Berger, C.E.H. (2016) *Interpreting Evidence: Evaluating Forensic Science in the Courtroom Second Edition*. Wiley: Chichester, UK.

14. Jamieson, A. and Bader, S. (Editors) (2016) *A Guide to Forensic DNA Profiling*. Wiley: Chichester, UK.

15. Amorim, A. and Budowle, B. (Editors) (2017) *Handbook of Forensic Genetics: Biodiversity and Heredity in Civil and Criminal Investigation*. World Scientific Publishing: London.

16. Bright, J.-A. and Coble, M. (2020) *Forensic DNA Profiling: A Practical Guide to Assigning Likelihood Ratios*. CRC Press: Boca Raton.

17. Gill, P., Bleka, Ø., Hansson, O., Benschop, C., Haned, H. (2020) *Forensic Practitioner's Guide to the Interpretation of Complex DNA Profiles*. Elsevier Academic Press: San Diego.

# Informative Forensic DNA Reviews and Research Studies (A to Z) (480)

*Below 26 categories are defined covering topics of interest in forensic DNA analysis and interpretation (listed arbitrarily from A to Z). Neither the categories nor this reference list are intended to be exhaustive. Suggestions for additional, appropriate references and categories are welcome. A #1 article (in bold font) was subjectively selected in each category and then followed by reference citations defined by date in ascending order with the most recent publications at the end of each category. This letter and number system (e.g., A1, B3, F7) provides a simple method to locate specific articles and enables opportunities for expansion as the literature grows. Although some articles could logically appear under multiple categories, no duplicate listings were used. Recommended references from the SWGDAM 2020 Training Guidelines have been included as well.*

## A. Plain Language Guides to Forensic DNA Analysis

1. **Sense about Science (2017) *Making Sense of Forensic Genetics*. A 40-page plain language guide available at https://senseaboutscience.org/activities/making-sense-of-forensic-genetics/.**

2. Jobling, M.A. and Gill, P. (2004) Encoded evidence: DNA in forensic analysis. *Nature Reviews: Genetics* 5(10): 739-751.

3. The Royal Society (2017) *Forensic DNA Analysis: A Primer for Courts*. A 60-page plain language guide available at https://royalsociety.org/-/media/about-us/programmes/science-and-law/royal-society-forensic-dna-analysis-primer-for-courts.pdf.

4. Press, R. (2019) *DNA Mixtures: A Forensic Science Explainer*. Available at https://www.nist.gov/featured-stories/dna-mixtures-forensic-science-explainer. (see also *Forensic Science Review* 31: 87-91 available at http://forensicsciencereview.com/Abstract/31(2)-(R&C)%20Full%20text.pdf)

## B. Serology and Body Fluid Identification

1. **Gaensslen, R.E. (1983) *Sourcebook in Forensic Serology, Immunology, and Biochemistry*. U.S. Department of Justice, National Institute of Justice: Washington, D.C.**

2. Cox, M. (1991) A study of the sensitivity and specificity of four presumptive tests for blood. *Journal of Forensic Sciences* 36(5): 1503-1511.

3. Hochmeister, M.N., Budowle, B., Rudin, O., Gehrig, C., Borer, U., Thali, M., Dirnhofer, R. (1999) Evaluation of prostate-specific antigen (PSA) membrane test assays for the forensic identification of seminal fluid. *Journal of Forensic Sciences* 44(5): 1057-1060.

4. Kobus, H.J., Silenieks, E., Scharnberg, J. (2002) Improving the effectiveness of fluorescence for the detection of semen stains on fabrics. *Journal of Forensic Sciences* 47(4): 819-823.

5. Tobe, S.S., Watson, N., Daéid, N.N. (2007) Evaluation of six presumptive tests for blood, their specificity, sensitivity, and effect on high molecular-weight DNA. *Journal of Forensic Sciences* 52: 102-109.

6. Schweers BA, Old J, Boonlayangoor PW, Reich KA. (2008) Developmental validation of a novel lateral flow strip test for rapid identification of human blood (Rapid Stain Identification--Blood). *Forensic Science International: Genetics* 2(3): 243-247.

7. Desroches, A.N., Buckle, J.L., Fourney, R.M. (2009) Forensic biology evidence screening: past and present. *Canadian Society of Forensic Science Journal* 42(2): 101-120.

8. Old, J.B., Schweers, B.A., Boonlayangoor, P.W., Reich, K.A. (2009) Developmental validation of RSID-saliva: a lateral flow immunochromatographic strip test for the forensic detection of saliva. *Journal of Forensic Sciences* 54(4): 866-873.

9. Virkler, K. and Lednev, I.K. (2009) Analysis of body fluids for forensic purposes: from laboratory testing to non-destructive rapid confirmatory identification at a crime scene. *Forensic Science International* 188: 1-17.

10. Harteveld, J., Lindenbergh, A. and Sijen, T. (2013) RNA cell typing and DNA profiling of mixed samples: can cell types and donors be associated? *Science & Justice* 53: 261-269.

11. Redhead, P. and Brown, M.K. (2013) The acid phosphatase test two minute cut-off: an insufficient time to detect some semen stains. *Science & Justice* 53(2): 187-191.

12. Sijen, T. (2015) Molecular approaches for forensic cell type identification: On mRNA, miRNA, DNA methylation and microbial markers. *Forensic Science International: Genetics* 18: 21-32.

13. Cotton, R.W. and Fisher, M.B. (2015) Review: Properties of sperm and seminal fluid, informed by research on reproduction and contraception. *Forensic Science International: Genetics* 18: 66-77.

14. Zapata, F., Fernández de la Ossa, M.Á., García-Ruiz, C. (2015) Emerging spectrometric techniques for the forensic analysis of body fluids. *TrAC - Trends in Analytical Chemistry* 64: 53-63.

15. Harbison, S. and Fleming, R.I. (2016) Forensic body fluid identification: state of the art. *Research and Reports in Forensic Medical Science* 6: 11-23.

16. Vidaki, A., Giangasparo, F., Syndercombe Court, D. (2016) Discovery of potential DNA methylation markers for forensic tissue identification using bisulphite pyrosequencing. *Electrophoresis* 37(21): 2767-2779.

17. Silva, D.S.B.S., Antunes, J., Balamurugan, K., Duncan, G., Alho, C.S., McCord, B. (2016) Developmental validation studies of epigenetic DNA methylation markers for the detection of blood, semen and saliva samples. *Forensic Science International: Genetics* 23: 55-63.

18. Wornes, D.J., Speers, S.J., Murakami, J.A. (2018) The evaluation and validation of Phadebas® paper as a presumptive screening tool for saliva on forensic exhibits. *Forensic Science International* 288: 81-88.

19. Dørum, G., Ingold, S., Hanson, E., Ballantyne, J., Snipen, L., Haas, C. (2018) Predicting the origin of stains from next generation sequencing mRNA data. *Forensic Science International: Genetics* 34: 37-48.

20. Ingold, S., Dørum, G., Hanson, E., Berti, A., Branicki, W., Brito, P., Elsmore, P., Gettings, K.B., Giangasparo, F., Gross, T.E., Hansen, S., Hanssen, E.N., Kampmann, M.L., Kayser, M., Laurent, F.X., Morling, N., Mosquera-Miguel, A., Parson, W., Phillips, C., Porto, M.J., Pośpiech, E., Roeder, A.D., Schneider, P.M., Schulze, J.K., Steffen, C.R., Syndercombe-Court, D., Trautmann, M., van den Berge, M., van der Gaag, K.J., Vannier, J., Verdoliva, V., Vidaki, A., Xavier, C., Ballantyne, J., Haas, C. (2018) Body fluid identification using a targeted mRNA massively parallel sequencing approach - results of a EUROFORGEN/EDNAP collaborative exercise. *Forensic Science International: Genetics* 34: 105-115.

21. Albani, P.P. and Fleming, R. (2019) Developmental validation of an enhanced mRNA-based multiplex system for body fluid and cell type identification. *Science & Justice* 59(3): 217-227.

22. Kulstein, G., Pably, P., Fürst, A., Wiegand, P., Hadrys, T. (2019) "The acid test"-validation of the ParaDNA® Body Fluid ID Test for routine forensic casework. *International Journal of Legal Medicine* 133(3): 751-757.

23. Noël, S., Lagacé, K., Raymond, S., Granger, D., Loyer, M., Bourgoin, S., Jolicoeur, C., Séguin, D.(2019) Repeatedly washed semen stains: Optimal screening and sampling strategies for DNA analysis. *Forensic Science International: Genetics* 38: 9-14.

24. Ingold, S., Dørum, G., Hanson, E., Ballard, D., Berti, A., Gettings, K.B., Giangasparo, F., Kampmann, M.L., Laurent, F.X., Morling, N., Parson, W., Steffen, C.R., Ulus, A., van den Berge, M., van der Gaag, K.J., Verdoliva, V., Xavier, C., Ballantyne, J., Haas, C. (2020) Body fluid identification and assignment to donors using a targeted mRNA massively parallel sequencing approach - results of a second EUROFORGEN/EDNAP collaborative exercise. *Forensic Science International: Genetics* 45: 102208.

## C. Collection and Storage of Biological Material

1. **Mapes, A.A., Kloosterman, A.D., van Marion, V., de Poot, C.J. (2016) Knowledge on DNA success rates to optimize the DNA analysis process: from crime scene to laboratory. *Journal of Forensic Sciences* 61(4): 1055-1061.**

2. Bär, W., Kratzer, A., Mächler, M., Schmid, W. (1988) Postmortem stability of DNA. *Forensic Science International* 39(1): 59-70.

3. Sweet, D., Lorente, M., Lorente, J.A., Valenzuela, A., Villanueva, E. (1997) An improved method to recover saliva from human skin: the double swab technique. *Journal of Forensic Sciences* 42(2): 320-322.

4. Lee, H.C. and Ladd, C. (2001) Preservation and collection of biological evidence. *Croatian Medical Journal* 42(3): 225-228.

5. Kline, M.C., Duewer, D.L., Redman, J.W., Butler, J.M., Boyer, D.A. (2002) Polymerase chain reaction amplification of DNA from aged blood stains: quantitative evaluation of the "suitability for purpose" of four filter papers as archival media. *Analytical Chemistry* 74(8): 1863-1869.

6. Bond, J.W. and Hammond, C. (2008) The value of DNA material recovered from crime scenes. *Journal of Forensic Sciences* 53(4): 797-801.

7. Vandewoestyne, M. and Deforce, D. (2010) Laser capture microdissection in forensic research: a review. *International Journal of Legal Medicine* 124(6): 513-521.

8. van Oorschot, R.A.H. (2012) Assessing DNA profiling success rates: need for more and better collection of relevant data. *Forensic Science Policy and Management* 3: 37-41.

9. Goray, M., van Oorschot, R.A., Mitchell, J.R. (2012) DNA transfer within forensic exhibit packaging: potential for DNA loss and relocation. *Forensic Science International: Genetics* 6(2): 158-166.

10. Technical Working Group on Biological Evidence Preservation (2013) *The Biological Evidence Preservation Handbook: Best Practices for Evidence Handlers*. National Institute of Standards and Technology and National Institute of Justice Available at https://doi.org/10.6028/NIST.IR.7928.

11. Allen-Hall, A. and McNevin, D. (2013) Non-cryogenic forensic tissue preservation in the field: a review. *Australian Journal of Forensic Sciences* 45(4): 450-460.

12. Higgins, D. and Austin, J.J. (2013) Teeth as a source of DNA for forensic identification of human remains: A review. *Science & Justice* 53(4): 433-441.

13. Adamowicz, M.S., Stasulli, D.M., Sobestanovich, E.M., Bille, T.W. (2014) Evaluation of methods to improve the extraction and recovery of DNA from cotton swabs for forensic analysis. *PLoS ONE* 9(12): e116351.

14. Verdon, T.J., Mitchell, R.J. and van Oorschot, R.A. (2014) Swabs as DNA collection devices for sampling different biological materials from different substrates. *Journal of Forensic Sciences* 59(4): 1080-1089.

15. Verdon, T.J., Mitchell, R.J. and van Oorschot, R.A. (2014) Evaluation of tapelifting as a collection method for touch DNA. *Forensic Science International: Genetics* 8(1): 179-186.

16. Verdon, T.J., Mitchell, R.J., van Oorschot, R.A. (2015) Preliminary investigation of differential tapelifting for sampling forensically relevant layered deposits. *Legal Medicine* 17(6): 553-559.

17. Baechler, S. (2016) Study of criteria influencing the success rate of DNA swabs in operational conditions: A contribution to an evidence-based approach to crime scene investigation and triage. *Forensic Science International: Genetics* 20: 130-139.

18. Pickrahn, I., Kreindl, G., Müller, E., Dunkelmann, B., Zahrer, W., Cemper-Kiesslich, J., Neuhuber, F. (2017) Contamination incidents in the pre-analytical phase of forensic DNA analysis in Austria—Statistics of 17 years. *Forensic Science International: Genetics* 31: 12-18.

19. Hess, S. and Haas, C. (2017) Recovery of trace DNA on clothing: A comparison of mini-tape lifting and three other forensic evidence collection techniques. *Journal of Forensic Sciences* 62(1): 187-191.

20. Kanokwongnuwut, P., Kirkbride, K.P., Linacre, A. (2018) Detection of latent DNA. *Forensic Science International: Genetics* 37: 95-101.

21. Sujatha, G., Muruganandhan, J., Priya, V.V., Srinivasan, M.R. (2019) Determination of reliability and practicality of saliva as a genetic source in forensic investigation by analyzing DNA yield and success rates: A systematic review. *Journal of Oral and Maxillofacial Surgery, Medicine, and Pathology* 31(3): 218-227.

22. Prasad, E., Hitchcock, C., Raymond, J., Cole, A., Barash, M., Gunn, P., McNevin, D., van Oorschot, R.A. (2020) DNA recovery from unfired and fired cartridge cases: A comparison of swabbing, tape lifting, vacuum filtration, and direct PCR. *Forensic Science International* 317: 110507.

23. Hedman, J., Jansson, L., Akel, Y., Wallmark, N., Gutierrez Liljestrand, R., Forsberg, C., Ansell, R. (2020) The double-swab technique versus single swabs for human DNA recovery from various surfaces. *Forensic Science International: Genetics* 46: 102253.

24. Sherier, A.J., Kieser, R.E., Novroski, N.M.M., Wendt, F.R., King, J.L., Woerner, A.E., Ambers, A., Garofano, P., Budowle, B. (2020) Copan microFLOQ® Direct Swab collection of bloodstains, saliva, and semen on cotton cloth. *International Journal of Legal Medicine* 134(1): 45-54.

25. McLamb, J.M., Adams, L.D., Kavlick, M.F. (2020) Comparison of the M-Vac® wet-vacuum-based collection method to a wet-swabbing method for DNA recovery on diluted bloodstained substrates. *Journal of Forensic Sciences* 65(6): 1828-1834.

## D. DNA Extraction/Purification, Differential Extraction

**1. Gill, P., Jeffreys, A.J., Werrett, D.J. (1985) Forensic application of DNA 'fingerprints'. *Nature* 318: 577-579.**

2. Walsh, P.S., Metzger, D.A., Higuchi, R. (1991) Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *Biotechniques* 10(4): 506-513.

3. Comey, C.T., Koons, B.W., Presley, K.W., Smerick, J.B., Sobieralski, C.A., Stanley, D.M., Baechtel, F.S. (1994) DNA extraction strategies for amplified fragment length polymorphism analysis. *Journal of Forensic Sciences* 39(5): 1254-1269.

4. Rådström, P., Knutsson, R., Wolffs, P., Lövenklev, M., Löfström, C. (2004). Pre-PCR processing: Strategies to generate PCR-compatible samples. *Molecular Biotechnology* 26(2): 133-146.

5. Nagy, M., Otremba, P., Krüger, C., Bergner-Greiner, S., Anders, P., Henske, B., Prinz, M., Roewer, L. (2005) Optimization and validation of a fully automated silica-coated magnetic beads purification technology in forensics. *Forensic Science International* 152(1): 13-22.

6. Anslinger, K., Bayer, B., Rolf, B., Keil, W., Eisenmenger, W. (2005) Application of the BioRobot EZ1 in a forensic laboratory. *Legal Medicine* 7(3): 164-168.

7. Montpetit, S.A., Fitch, I.T., O'Donnell, P.T. (2005) A simple automated instrument for DNA extraction in forensic casework. *Journal of Forensic Sciences* 50(3): 1-9.

8. Castella, V., Dimo-Simonin, N., Brandt-Casadevall, C., Mangin, P. (2006) Forensic evaluation of the QIAshredder/QIAamp DNA extraction procedure. *Forensic Science International* 156(1): 70-73.

9. Loreille, O.M., Diegoli, T.M., Irwin, J.A., Coble, M.D., Parsons, T.J. (2007) High efficiency DNA extraction from bone by total demineralization. *Forensic Science International: Genetics* 1(2): 191-195.

10. Brevnov, M.G., Pawar, H.S., Mundt, J., Calandro, L.M., Furtado, M.R., Shewale, J.G. (2009) Developmental validation of the PrepFiler Forensic DNA Extraction Kit for extraction of genomic DNA from biological samples. *Journal of Forensic Sciences* 54(3): 599-607.

11. Stray, J.E., Liu, J.Y., Brevnov, M.G., Shewale, J.G. (2010) Extraction of DNA from forensic biological samples for genotyping. *Forensic Science Review* 22(2): 159-175.

12. Stray, J.E. and Shewale, J.G. (2010) Extraction of DNA from human remains. *Forensic Science Review* 22(2): 177-185.

13. Frégeau, C.J., Lett, C.M., Fourney, R.M. (2010) Validation of a DNA IQ™-based extraction method for TECAN robotic liquid handling workstations for processing casework. *Forensic Science International: Genetics* 4(5): 292-304.

14. Schneider, H., Sommerer, T., Rand, S., Wiegand, P. (2011) Hot flakes in cold cases. *International Journal of Legal Medicine* 125: 543-548.

15. Farash, K., Hanson, E.K., Ballantyne, J. (2018) Single source DNA profile recovery from single cells isolated from skin and fabric from touch DNA mixtures in mock physical assaults. *Science & Justice* 58: 191-199.

16. Samie, L., Champod, C., Glutz, V., Garcia, M., Castella, V., Taroni F. (2019) The efficiency of DNA extraction kit and the efficiency of recovery techniques to release DNA using flow cytometry. *Science & Justice* 59(4): 405-410.

17. Romsos, E.L. and Vallone, P.M. (2019) Estimation of extraction efficiency by droplet digital PCR. *Forensic Science International: Genetics Supplement Series* 7: 515-517.

18. Chapman, B.R., Blackwell, S.J., Müller, L.H. (2020) Forensic techniques for the isolation of spermatozoa from sexual assault samples - A review. *Forensic Science Review* 32(2): 105-116.

## E. DNA Quantitation, Degraded DNA

**1. Grgicak, C.M., Urban, Z.M., Cotton, R.W. (2010) Investigation of reproducibility and error associated with qPCR methods using Quantifiler® Duo DNA quantification kit. *Journal of Forensic Sciences* 55(5):1331-1339.**

2. Lindahl, T. (1993) Instability and decay of the primary structure of DNA. *Nature* 362: 709-715.

3. Butler, J.M., Shen, Y., McCord, B.R. (2003) The development of reduced size STR amplicons as tools for analysis of degraded DNA. *Journal of Forensic Sciences* 48(5) 1054-1064.

4. Green, R.L., Roinestad, I.C., Boland, C., Hennessy, L.K. (2005) Developmental validation of the Quantifiler real-time PCR kits for the quantification of human nuclear DNA samples. *Journal of Forensic Sciences* 50(4): 809-825.

5. Barbisin, M., Fang, R., O'Shea, C.E., Calandro, L.M., Furtado, M.R., Shewale, J.G. (2009) Developmental validation of the Quantifiler Duo DNA Quantification kit for simultaneous quantification of total human and human male DNA and detection of PCR inhibitors in biological samples. *Journal of Forensic Sciences* 54(2): 305-319.

6. Alaeddini, R., Walsh, S.J., Abbas, A. (2010) Forensic implications of genetic analyses from degraded DNA--a review. *Forensic Science International: Genetics* 4(3):148-157.

7. Lee, S.B., McCord, B., Buel, E. (2014) Advances in forensic DNA quantification: a review. *Electrophoresis* 35: 3044-3052.

8. Vieira-Silva, C., Afonso-Costa, H., Ribeiro, T., Porto, M.J., Dias, M., Amorim, A. (2015) Quantifiler® Trio DNA validation and usefulness in casework samples. *Forensic Science International: Genetics Supplement Series* 5: e246-e247.

9. Holt, A., Olson, S., Marfori, M., Yong Ning Oh, D. (2015) A DNA-based screening assay to streamline sexual assault sample processing. *American Laboratory* 47(6): 42-44.

10. Holt, A., Wootton, S.C., Mulero, J.J., Brzoska, P.M., Langit, E., Green, R.L. (2016) Developmental validation of the Quantifiler(®) HP and Trio Kits for human DNA quantification in forensic samples. *Forensic Science International: Genetics* 21: 145-157.

## F. PCR Amplification, Inhibition, and Artifacts

**1. Walsh, P.S., Erlich, H.A. and Higuchi, R. (1992) Preferential PCR amplification of alleles: mechanisms and solutions. *PCR Methods & Applications* 1(4): 241-250.**

2. Saiki, R.K., Gelfand, D.H., Stoffel, S., Scharf, S.J., Higuchi, R., Horn, G.T., Mullis, K.B., Erlich, H.A. (1988) Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* 239: 487-491.

3. Clark, J.M. (1988). Novel non-templated nucleotide addition reactions catalyzed by procaryotic and eucaryotic DNA polymerases. *Nucleic Acids Research* 16(20): 9677-9686.

4. Bloch, W. (1991) A biochemical perspective of the polymerase chain reaction. *Biochemistry* 30: 2735-2747.

5. Reynolds, R., Sensabaugh, G., Blake, E. (1991) Analysis of genetic markers in forensic DNA samples using the polymerase chain reaction. *Analytical Chemistry* 63(1): 2-15.

6. Walsh, P.S., Fildes, N.J., Reynolds, R. (1996) Sequence analysis and characterization of stutter products at the tetranucleotide repeat locus vWA. *Nucleic Acids Research* 24(14): 2807-2812.

7. Leclair, B., Sgueglia, J.B., Wojtowicz, P.C., Juston, A.C., Frégeau, C.J,, Fourney, R.M. (2003) STR DNA typing: increased sensitivity and efficient sample consumption using reduced PCR reaction volumes. *Journal of Forensic Sciences* 48(5): 1001-1013.

8. Alaeddini, R. (2012) Forensic implications of PCR inhibition—A review. *Forensic Science International: Genetics* 6(3): 297-305.

9. Brookes, C., Bright, J.-A., Harbison, S., Buckleton, J. (2012) Characterizing stutter in forensic STR multiplexes. *Forensic Science International: Genetics* 6(1): 58-63.

10. Kumar, P., Gupta, R., Singh, R., Jasuja, O.P. (2015) Effects of latent fingerprint development reagents on subsequent forensic DNA typing: a review. *Journal of Forensic and Legal Medicine* 32:64-69.

11. Cavanaugh, S.E. and Bathrick, A.S. (2018) Direct PCR amplification of forensic touch and other challenging DNA samples: A review. *Forensic Science International: Genetics* 32: 40-49.

12. Sidstedt, M., Hedman, J., Romsos, E.L., Waitara, L., Wadsö, L., Steffen, C.R., Vallone, P.M., Rådström, P. (2018) Inhibition mechanisms of hemoglobin, immunoglobulin G, and whole blood in digital and real-time PCR. *Analytical and Bioanalytical Chemistry* 410(10): 2569-2583.

13. Martin, B. and Linacre, A. (2020) Direct PCR: A review of use and limitations. *Science & Justice* 60: 303-310.

## G. Capillary Electrophoresis Separation and Detection

**1. Butler, J.M., Buel, E., Crivellente, F., McCord, B.R. (2004) Forensic DNA typing by capillary electrophoresis using the ABI Prism 310 and 3100 Genetic Analyzers for STR analysis. *Electrophoresis* 25: 1397-1412.**

2. Guttman, A. and Cooke, N. (1991) Effect of temperature on the separation of DNA restriction fragments in capillary gel electrophoresis. *Journal of Chromatography* 559: 285-294.

3. Issaq, H.J., Chan, K.C., Muschik, G.M. (1997) The effect of column length, applied voltage, gel type, and concentration on the capillary electrophoresis separation of DNA fragments and polymerase chain reaction products. *Electrophoresis* 18(7): 1153-1158.

4. Lazaruk, K., Walsh, P.S., Oaks, F., Gilbert, D., Rosenblum, B.B., Menchen, S., Scheibler, D., Wenz, H.M., Holt, C., Wallin, J. (1998) Genotyping of forensic short tandem repeat (STR) systems based on sizing precision in a capillary electrophoresis instrument. *Electrophoresis* 19(1): 86-93.

5. Mansfield, E.S., Robertson, J.M., Vainer, M., Isenberg, A.R., Frazier, R.R., Ferguson, K., Chow, S., Harris, D.W., Barker, D.L., Gill, P.D., Budowle, B., McCord, B.R. (1998) Analysis of multiplexed short tandem repeat (STR) systems using capillary array electrophoresis. *Electrophoresis* 19(1): 101-107.

6. Heller, C. (2001) Principles of DNA separation with capillary electrophoresis. *Electrophoresis* 22(4): 629-643.

7. Moretti, T.R., Baumstark, A.L., Defenbaugh, D.A., Keys, K.M., Smerick, J.B. and Budowle, B. (2001) Validation of short tandem repeats (STRs) for forensic usage: performance testing of fluorescent multiplex STR systems and analysis of authentic and simulated forensic samples. *Journal of Forensic Sciences* 46(3): 647-660.

8. Moretti, T.R., Baumstark, A.L., Defenbaugh, D.A., Keys, K.M., Brown, A.L. and Budowle, B. (2001) Validation of STR typing by capillary electrophoresis. *Journal of Forensic Sciences* 46(3): 661-676.

9. Sgueglia, J.B., Geiger, S., Davis, J. (2003) Precision studies using the ABI Prism 3100 Genetic Analyzer for forensic DNA analysis. *Analytical and Bioanalytical Chemistry* 376(8): 1247-1254.

10. Gilder, J.R., Doom, T.E., Inman, K. and Krane, D.E. (2007) Run-specific limits of detection and quantitation for STR-based DNA testing. *Journal of Forensic Sciences* 52(1): 97-101.

11. Rakay, C.A., Bregu, J. and Grgicak, C.M. (2012) Maximizing allele detection: Effects of analytical threshold and DNA levels on rates of allele and locus drop-out. *Forensic Science International: Genetics* 6(6): 723-728.

12. Adelman, J.D., Zhao, A., Eberst, D.S. and Marciano, M.A. (2019) Automated detection and removal of capillary electrophoresis artifacts due to spectral overlap. *Electrophoresis* 40(14): 1753-1761.

## H. Assessing Sample Suitability and Complexity, Low-Template DNA

1. **Gill, P., Whitaker, J., Flaxman, C., Brown, N., Buckleton, J. (2000) An investigation of the rigor of interpretation rules for STRs derived from less than 100 pg of DNA. *Forensic Science International* 112(1): 17-40.**

2. Taberlet, P., Griffin, S., Goossens, B., Questiau, S., Manceau, V., Escaravage, N., Waits, L.P. Bouvet, J. (1996) Reliable genotyping of samples with very low DNA quantities using PCR. *Nucleic Acids Research* 24(16): 3189-3194.

3. Findlay, I., Taylor, A., Quirke, P., Frazier, R., Urquhart, A. (1997) DNA fingerprinting from single cells. *Nature* 389: 555-556.

4. Gill, P. (2001) Application of low copy number DNA profiling. *Croatian Medical Journal* 42(3): 229-232.

5. Balding, D.J. and Buckleton, J. (2009) Interpreting low template DNA profiles. *Forensic Science International: Genetics* 4(1): 1-10.

6. Butler, J.M. and Hill, C.R. (2010) Scientific issues with analysis of low amounts of DNA. *Profiles in DNA (Promega)*, 13(1). Available at https://www.promega.com/resources/profiles-in-dna/2010/scientific-issues-with-analysis-of-low-amounts-of-dna/.

7. Benschop, C.C., van der Beek, C.P., Meiland, H.C., van Gorp, A.G., Westen, A.A. and Sijen, T. (2011) Low template STR typing: effect of replicate number and consensus method on genotyping reliability and DNA database search results. *Forensic Science International: Genetics* 5(4): 316-328.

## I. Estimating the Number of Contributors

1. **Buckleton, J.S., Curran, J.M. and Gill, P. (2007) Towards understanding the effect of uncertainty in the number of contributors to DNA stains. *Forensic Science International: Genetics* 1(1): 20-28.**

2. Egeland, T., Dalen, I. and Mostad, P.F. (2003) Estimating the number of contributors to a DNA profile. *International Journal of Legal Medicine* 117(5): 271-275.

3. Evett, I.W. and Pope, S. (2014) Is it to the advantage of a defendant to infer a greater number of contributors to a questioned sample than is necessary to explain the observed DNA profile? *Science & Justice* 54(5): 373-374.

4. Benschop, C.C., Haned, H., Jeurissen, L., Gill, P.D. and Sijen, T. (2015) The effect of varying the number of contributors on likelihood ratios for complex DNA mixtures. *Forensic Science International: Genetics* 19: 92-99.

5. Coble, M.D., Bright, J.A., Buckleton, J.S. and Curran, J.M. (2015) Uncertainty in the number of contributors in the proposed new CODIS set. *Forensic Science International: Genetics* 19: 207-211.

6. Swaminathan, H., Grgicak, C.M., Medard, M. and Lun, D.S. (2015) NOCIt: a computational method to infer the number of contributors to DNA samples analyzed by STR genotyping. *Forensic Science International: Genetics* 16: 172-180.

7. Alfonse, L.E., Tejada, G., Swaminathan, H., Lun, D.S. and Grgicak, C.M. (2017) Inferring the number of contributors to complex DNA mixtures using three methods: exploring the limits of low-template DNA interpretation. *Journal of Forensic Sciences* 62(2): 308-316.

8. Marciano, M.A. and Adelman, J.D. (2017) PACE: Probabilistic Assessment for Contributor Estimation- A machine learning-based assessment of the number of contributors in DNA mixtures. *Forensic Science International: Genetics* 27: 82-91.

9. Norsworthy, S., Lun, D.S., Grgicak, C.M. (2018) Determining the number of contributors to DNA mixtures in the low-template regime: Exploring the impacts of sampling and detection effects. *Legal Medicine* 32: 1-8.

10. Dembinski, G.M., Sobieralski, C., Picard C.J. (2018) Estimation of the number of contributors of theoretical mixture profiles based on allele counting: Does increasing the number of loci increase success rate of estimates? *Forensic Science International: Genetics* 33: 24-32.

11. Young, B.A., Gettings, K.B., McCord, B., Vallone, P.M. (2019) Estimating number of contributors in massively parallel sequencing data of STR loci. *Forensic Science International: Genetics* 38: 15-22.

12. Marciano, M.A. and Adelman, J.D. (2019) Developmental validation of PACE™: Automated artifact identification and contributor estimation for use with GlobalFiler™ and PowerPlex® Fusion 6C generated data. *Forensic Science International: Genetics* 43: 102140.

## J. Data Interpretation, Mixture Deconvolution, Interlaboratory Studies

**1. Gill, P., Sparkes, R. and Kimpton, C. (1997) Development of guidelines to designate alleles using an STR multiplex system. *Forensic Science International* 89(3): 185-197.**

2. Gill, P., Sparkes, R., Pinchin, R., Clayton, T., Whitaker, J., Buckleton, J. (1998) Interpreting simple STR mixtures using allele peak areas. *Forensic Science International* 91(1): 41-53.

3. Clayton, T.M., Whitaker, J.P., Sparkes, R. and Gill, P. (1998) Analysis and interpretation of mixed forensic stains using DNA STR profiling. *Forensic Science International* 91(1): 55-70.

4. Crouse, C.A., Rogers, S., Amiott, E., Gibson, S., Masibay, A. (1999) Analysis and interpretation of short tandem repeat microvariants and three-banded allele patterns using multiple allele detection systems. *Journal of Forensic Sciences* 44(1): 87-94. Erratum in: J Forensic Sci 1999 May;44(3):667.

5. Duewer, D.L., Kline, M.C., Redman, J.W., Newall, P.J. and Reeder, D.J. (2001) NIST Mixed Stain Studies #1 and #2: interlaboratory comparison of DNA quantification practice and short tandem repeat multiplex performance with multiple-source samples. *Journal of Forensic Sciences* 46: 1199-1210.

6. Duewer, D.L., Kline, M.C., Redman, J.W. and Butler, J.M. (2004) NIST Mixed Stain Study #3: signal intensity balance in commercial short tandem repeat multiplexes. *Analytical Chemistry* 76: 6928-6934.

7. Prieto, L., Haned, H., Mosquera, A., Crespillo, M., Aleman, M., Aler, M., Alvarez, F., Baeza-Richer, C., Dominguez, A., Doutremepuich, C., Farfán, M.J., Fenger-Grøn, M., García-Ganivet, J.M., González-Moya, E., Hombreiro, L., Lareu, M.V., Martínez-Jarreta, B., Merigioli, S., Milans Del Bosch, P., Morling, N., Muñoz-Nieto, M., Ortega-González, E., Pedrosa, S., Pérez, R., Solís, C., Yurrebaso, I. and Gill, P. (2014) Euroforgen-NoE collaborative exercise on LRmix to demonstrate standardization of the interpretation of complex DNA profiles. *Forensic Science International: Genetics* 9: 47-54.

8. Benschop, C.C., Connolly, E., Ansell, R. and Kokshoorn, B. (2017) Results of an inter and intra laboratory exercise on the assessment of complex autosomal DNA profiles. *Science & Justice* 57(1): 21-27.

9. Butler, J.M., Kline, M.C. and Coble, M.D. (2018) NIST interlaboratory studies involving DNA mixtures (MIX05 and MIX13): variation observed and lessons learned. *Forensic Science International: Genetics* 37: 81-94.

10. Lynch, P.C. and Cotton, R.W. (2018) Determination of the possible number of genotypes which can contribute to DNA mixtures: non-computer assisted deconvolution should not be attempted for greater than two person mixtures. *Forensic Science International: Genetics* 37: 235-240.

11. Tao, R., Wang, S., Zhang, J., Zhang, J., Yang, Z., Sheng, X., Hou, Y., Zhang, S., Li, C. (2018) Separation/extraction, detection, and interpretation of DNA mixtures in forensic science (review). *International Journal of Legal Medicine* 132(5): 1247-1261.

12. Sanz-Piña, E., Santurtún, A., Zarrabeitia, M.T. (2019) Forensic implications of the presence of chimerism after hematopoietic stem cell transplantation. *Forensic Science International* 302: 109862.

## K. Interpretation: Binary Approaches (CPI, RMP, LR)

**1. Gill, P., Brenner, C.H., Buckleton, J.S., Carracedo, A., Krawczak, M., Mayr, W.R., Morling, N., Prinz, M., Schneider, P.M. and Weir, B.S. (2006) DNA Commission of the International Society of Forensic Genetics: Recommendations on the interpretation of mixtures. *Forensic Science International* 160: 90-101.**

2. Schneider, P.M., Gill, P., Carracedo, A. (2006) Editorial on the recommendations of the DNA commission of the ISFG on the interpretation of mixtures. *Forensic Science International* 160: 89.

3. Buckleton, J. and Triggs, C. (2006) Is the 2p rule always conservative? *Forensic Science International* 159: 206-209.

4. Morling, N., Bastisch, I., Gill, P. and Schneider, P.M. (2007) Interpretation of DNA mixtures--European consensus on principles. *Forensic Science International: Genetics* 1: 291-292.

5. Buckleton, J. and Curran, J. (2008) A discussion of the merits of random man not excluded and likelihood ratios. *Forensic Science International: Genetics* 2(4): 343-348.

6. Schneider, P.M., Fimmers, R., Keil, W., Molsberger, G., Patzelt, D., Pflug, W., Rothämel, T., Schmitter, H., Schneider, H. and Brinkmann, B. (2009) The German Stain Commission: recommendations for the interpretation of mixed stains. *International Journal of Legal Medicine* 123(1): 1-5. [Originally published in German in *Rechtsmedizin* (2006) 16: 401-404].

7. Budowle, B., Onorato, A.J., Callaghan, T.F., Della, M.A., Gross, A.M., Guerrieri, R.A., Luttman, J.C., McClure, D.L. (2009) Mixture interpretation: defining the relevant features for guidelines for the assessment of mixed DNA profiles in forensic casework. *Journal of Forensic Sciences* 54(4): 810-821.

8. Bright, J.-A., Turkington, J., Buckleton, J. (2010) Examination of the variability in mixed DNA profile parameters for the Identifiler™ multiplex. *Forensic Science International: Genetics* 4(2): 111-114.

9. Bille, T., Bright, J.-A., Buckleton, J. (2013) Application of random match probability calculations to mixed STR profiles. *Journal of Forensic Sciences* 58(2): 474-485.

10. Bieber, F.R., Buckleton, J.S., Budowle, B., Butler, J.M., Coble, M.D. (2016) Evaluation of forensic DNA mixture evidence: protocol for evaluation, interpretation, and statistical calculations using the combined probability of inclusion. *BMC Genetics* 17(1):125.

11. Marsden, C.D., Rudin, N., Inman, K., Lohmueller, K.E. (2016) An assessment of the information content of likelihood ratios derived from complex mixtures. *Forensic Science International: Genetics* 22: 64-72.

## L. Interpretation: Probabilistic Genotyping Software (Discrete, Continuous)


1. **Coble, M.D. and Bright, J.-A. (2019) Probabilistic genotyping software: An overview. *Forensic Science International: Genetics* 38: 219-224.**

2. Evett, I.W. (1983) What is the probability that this blood came from that person? A meaningful question? *Journal of the Forensic Science Society (Science & Justice)* 23:35-39.

3. Gill, P., Kirkham, A., Curran, J. (2007) LoComatioN: A software tool for the analysis of low copy number DNA profiles. *Forensic Science International.* 166:128-138.

4. Cowell, R.G. (2009) Validation of an STR peak area model. *Forensic Science International: Genetics* 3(3): 193-199.

5. Perlin, M.W. and Sinelnikov, A. (2009) An information gap in DNA evidence interpretation. *PLoS ONE* 4(12): e8327.

6. Tvedebrink, T., Eriksen, P.S., Mogensen, H.S., Morling, N. (2010) Evaluating the weight of evidence by using quantitative short tandem repeat data in DNA mixtures. *Applied Statistics* 59(5): 855-874.

7. Cowell, R.G., Lauritzen, S.L., Mortera, J. (2011) Probabilistic expert systems for handling artifacts in complex DNA mixtures. *Forensic Science International: Genetics* 5(3): 202-209.

8. Perlin, M.W., Legler, M.M., Spencer, C.E., Smith, J.L., Allan, W.P., Belrose, J.L., Duceman, B.W. (2011) Validating TrueAllele® DNA mixture interpretation. *Journal of Forensic Sciences* 56(6): 1430-1447.

9. Tvedebrink, T., Eriksen, P.S., Mogensen, H.S., Morling, N. (2012) Statistical model for degraded DNA samples and adjusted probabilities for allelic drop-out. *Forensic Science International: Genetics* 6: 97-101.

10. Kelly, H., Bright, J.-A., Curran, J.M., Buckleton, J. (2012) Modelling heterozygote balance in forensic DNA profiles. *Forensic Science International: Genetics* 6: 729-734.

11. Gill, P., Gusmao, L., Haned, H., Mayr, W.R., Morling, N., Parson, W., Prieto, L., Prinz, M., Schneider, H., Schneider, P.M., Weir, B.S. (2012) DNA Commission of the International Society of Forensic Genetics: Recommendations on the evaluation of STR typing results that may include drop-out and/or drop-in using probabilistic methods. *Forensic Science International: Genetics* 6(6): 679-688.

12. Gill, P. and Haned, H. (2013) A new methodological framework to interpret complex DNA profiles using likelihood ratios. *Forensic Science International: Genetics* 7(2): 251-263.

13. Bright, J.-A., Curran, J.M., Buckleton, J.S. (2013) Investigation into the performance of different models for predicting stutter. *Forensic Science International: Genetics* 7(4): 422-427.

14. Taylor, D., Bright, J.-A., Buckleton, J. (2013) The interpretation of single source and mixed DNA profiles. *Forensic Science International: Genetics* 7(5): 516-528.

15. Puch-Solis, R., Rodgers, L., Mazumder, A., Pope, S., Evett, I., Curran, J., Balding, D. (2013) Evaluating forensic DNA profiles using peak heights, allowing for multiple donors, allelic dropout and stutters. *Forensic Science International: Genetics* 7(5): 555-563.

16. Ramos, D. and Gonzalez-Rodriguez, J. (2013) Reliable support: measuring calibration of likelihood ratios. *Forensic Science International* 230: 156-169.

17. Kelly, H., Bright, J.A., Buckleton, J.S., Curran, J.M. (2014) A comparison of statistical models for the analysis of complex forensic DNA profiles. *Science & Justice* 54(1): 66-70.

18. Taylor, D., Bright, J.-A., Buckleton, J. (2014) The 'factor of two' issue in mixed DNA profiles. *Journal of Theoretical Biology* 363: 300-306.

19. Taylor, D., Bright, J.-A., Buckleton, J. (2014) Interpreting forensic DNA profiling evidence without specifying the number of contributors. *Forensic Science International: Genetics* 13: 269-280.

20. Taylor, D., Bright, J.-A., Buckleton, J. (2014) Considering relatives when assessing the evidential strength of mixed DNA profiles. *Forensic Science International: Genetics* 13: 259-263.

21. Bright, J.-A., Taylor, D., Curran, J.M., Buckleton, J. (2014) Searching mixed DNA profiles directly against profile databases. *Forensic Science International: Genetics* 9: 102-110.

22. Bille, T.W., Weitz, S.M., Coble, M.D., Buckleton, J., Bright, J.-A. (2014) Comparison of the performance of different models for the interpretation of low level mixed DNA profiles. *Electrophoresis* 35: 3125-3133.

23. Steele, C.D., Greenhalgh, M., Balding, D.J. (2014) Verifying likelihoods for low template DNA profiles using multiple replicates. *Forensic Science International: Genetics* 13: 82-89.

24. Steele, C.D. and Balding, D.J. (2014) Statistical evaluation of forensic DNA profile evidence. *Annual Review of Statistics and its Application* 1: 361-384.

25. Bright, J.-A., Evett, I.W., Taylor, D., Curran, J.M., Buckleton, J. (2015) A series of recommended tests when validating probabilistic DNA profile interpretation software. *Forensic Science International: Genetics* 14: 125-131.

26. Gill, P., Haned, H., Bleka, O., Hansson, O., Dørum, G. and Egeland, T. (2015) Genotyping and interpretation of STR-DNA: Low-template, mixtures and database matches-Twenty years of research and development. *Forensic Science International: Genetics* 18: 100-117.

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

28. Greenspoon, S.A., Schiermeier-Wood, L., Jenkins, B.C. (2015) Establishing the limits of TrueAllele® Casework: A validation study. *Journal of Forensic Sciences* 60(5): 1263-1276.

29. Bleka, Ø., Benschop, C.C., Storvik, G. and Gill, P. (2016) A comparative study of qualitative and quantitative models used to interpret complex STR DNA profiles. *Forensic Science International: Genetics* 25: 85-96.

30. Bright, J.-A., Taylor, D., McGovern, C., Cooper, S., Russell, L., Abarno, D., Buckleton, J. (2016) Developmental validation of STRmix, expert software for the interpretation of forensic DNA profiles. *Forensic Science International: Genetics* 23: 226-239.

31. Steele, C.D., Greenhalgh, M. and Balding, D.J. (2016) Evaluation of low-template DNA profiles using peak heights. *Statistical Applications in Genetics and Molecular Biology* 15(5): 431-445.

32. Haned, H., Gill, P., Lohmueller, K., Inman, K., Rudin, N. (2016) Validation of probabilistic genotyping software for use in forensic DNA casework: Definitions and illustrations. *Science & Justice* 56(2): 104-108.

33. Moretti, T.R., Just, R.S., Kehl, S.C., Willis, L.E., Buckleton, J.S., Bright, J.A., Taylor, D.A. and Onorato, A.J. (2017) Internal validation of STRmix for the interpretation of single source and mixed DNA profiles. *Forensic Science International: Genetics* 29: 126-144.

34. Bright, J.-A., Taylor, D., Gittelson, S., Buckleton, J. (2017) The paradigm shift in DNA profile interpretation. *Forensic Science International: Genetics* 31: e24-e32.

35. Meuwly, D., Ramos, D., Haraksim, R. (2017) A guideline for the validation of likelihood ratio methods used for forensic evidence evaluation. *Forensic Science International* 276: 142-153.

36. Bright, J.-A., Richards, R., Kruijver, M., Kelly, H., McGovern, C., Magee, A., McWhorter, A., Ciecko, A., Peck, B., Baumgartner, C., Buettner, C., McWilliams, S., McKenna, C., Gallacher, C., Mallinder, B., Wright, D., Johnson, D., Catella, D., Lien, E., O'Connor, C., Duncan, G., Bundy, J., Echard, J., Lowe, J., Stewart, J., Corrado, K., Gentile, S., Kaplan, M., Hassler, M., McDonald, N., Hulme, P., Oefelein, R.H., Montpetit, S., Strong, M., Noel, S., Malsom, S., Myers, S., Welti, S., Moretti, T., McMahon, T., Grill, T., Kalafut, T., Greer-Ritzheimer, M.M., Beamer, V., Taylor, D.A., Buckleton, J.S. (2018) Internal validation of STRmix™– a multi laboratory response to PCAST. *Forensic Science International: Genetics* 34: 11-24.

37. Slooten, K. (2018) The information gain from peak height data in DNA mixtures. *Forensic Science International: Genetics* 36: 119-123.

38. Swaminathan, H., Qureshi, M.O., Grgicak, C.M., Duffy, K. and Lun, D.S. (2018) Four model variants within a continuous forensic DNA mixture interpretation framework: effects on evidential inference and reporting. *PLoS ONE* 13(11): e0207599.

39. Benschop, C.C.G., Nijveld, A., Duijs, F.E. and Sijen, T. (2019) An assessment of the performance of the probabilistic genotyping software EuroForMix: Trends in likelihood ratios and analysis of Type I & II errors. *Forensic Science International: Genetics* 42: 31-38.

40. Bright, J.-A., Cheng, K., Kerr, Z., McGovern, C., Kelly, H., Moretti, T.R., Smith, M.A., Bieber, F.R., Budowle, B., Coble, M.D., Alghafri, R., Allen, P.S., Barber, A., Beamer, V., Buettner, C., Russell, M., Gehrig, C., Hicks, T., Charak, J., Cheong-Wing, K., Ciecko, A., Davis, C.T., Donley, M., Pedersen, N., Gartside, B., Granger, D., Greer-Ritzheimer, M., Reisinger, E., Kennedy, J., Grammer, E., Kaplan, M., Hansen, D., Larsen, H.J., Laureano, A., Li, C., Lien, E., Lindberg, E., Kelly, C., Mallinder, B., Malsom, S., Yacovone-Margetts, A., McWhorter, A., Prajapati, S.M., Powell, T., Shutler, G., Stevenson, K., Stonehouse, A.R., Smith, L., Murakami, J., Halsing, E., Wright, D., Clark, L., Taylor, D.A., Buckleton. J. (2019) STRmix™ collaborative exercise on DNA mixture interpretation. *Forensic Science International: Genetics* 40:1-8.

41. You, Y. and Balding, D. (2019) A comparison of software for the evaluation of complex DNA profiles. *Forensic Science International: Genetics* 40: 114-119.

42. Lin, M.H., Bright, J.-A, Pugh, S.N., Buckleton, J.S. (2020) The interpretation of mixed DNA profiles from a mother, father, and child trio. *Forensic Science International: Genetics* 44: 102175.

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

44. Schuerman, C., Kalafut, T., Buchanan, C., Sutton, J., Bright, J.-A. (2020) Using the nondonor distribution to improve communication and inform decision making for low LRs from minor contributors in mixed DNA profiles. *Journal of Forensic Sciences* 65(4): 1072-1084.

## M. Report Writing and Technical Review

**1. Association of Forensic Science Providers (2009) Standards for the formulation of evaluative forensic science expert opinion. *Science & Justice* 49: 161-164.**

2. Cook, R., Evett, I.W., Jackson, G., Jones, P.J. and Lambert, J.A. (1998) A model for case assessment and interpretation. *Science & Justice* 38(3): 151-156.

3. Cook, R., Evett, I.W., Jackson, G., Jones, P.J. and Lambert, J.A. (1998) A hierarchy of propositions: deciding which level to address in casework. *Science & Justice* 38(4): 231-239.

4. Evett, I.W., Jackson, G., Lambert, J.A. (2000) More on the hierarchy of propositions: exploring the distinction between explanations and propositions. *Science & Justice* 40(1): 3-10.

5. Evett, I.W., Jackson, G., Lambert, J.A., McCrossan, S. (2000) The impact of the principles of evidence interpretation on the structure and content of statements. *Science & Justice* 40(4): 233-239.

6. Doak, S. and Assimakopoulos, D. (2007) How do forensic scientists learn to become competent in casework reporting in practice: A theoretical and empirical approach. *Forensic Science International* 167: 201-206.

7. Ballantyne, K.N., Edmond, G. and Found, B. (2017) Peer review in forensic science. *Forensic Science International* 277: 66-76.

8. Barrio, P.A., Crespillo, M., Luque, J.A., Aler, M., Baeza-Richer, C., Baldassarri, L., Carnevali, E., Coufalova, P., Flores, I., García, O. and García, M.A. (2018) GHEP-ISFG collaborative exercise on mixture profiles (GHEP-MIX06). Reporting conclusions: results and evaluation. *Forensic Science International: Genetics* 35: 156-163.

## N. Court Testimony, Communication of Results, Juror Comprehension Studies

**1. Eldridge, H. (2019) Juror comprehension of forensic expert testimony: a literature review and gap analysis. *Forensic Science International: Synergy* 1: 24-34.**

2. Thompson, W.C. and Schumann, E.L. (1987) Interpretation of statistical evidence in criminal trials: the prosecutor's fallacy and the defense attorney's fallacy. *Law and Human Behavior* 11(3): 167-187.

3. Evett, I.W. (1995) Avoiding the transposed conditional. *Science & Justice* 35(2): 127-131.

4. Evett, I.W., Gill, P.D., Jackson, G., Whitaker, J. and Champod, C. (2002) Interpreting small quantities of DNA: the hierarchy of propositions and the use of Bayesian networks. *Journal of Forensic Sciences* 47(3): 520-530.

5. Jackson, G., Jones, S., Booth, G., Champod, C., Evett, I.W. (2006) The nature of forensic science opinion--a possible framework to guide thinking and practice in investigations and in court proceedings. *Science & Justice* 46(1): 33-44.

6. Gill, P., Brown, R.M., Fairley, M., Lee, L., Smyth, M., Simpson, N., Irwin, B., Dunlop, J., Greenhalgh, M., Way, K., Westacott, E.J., Ferguson, S.J., Ford, L.V., Clayton, T. and Guiness, J. (2008) National recommendations of the Technical UK DNA working group on mixture interpretation for the NDNAD and for court going purposes. *Forensic Science International: Genetics* 2(1): 76-82.

7. Smith, L.L., Bull, R. and Holliday, R. (2011) Understanding juror perceptions of forensic evidence: investigating the impact of case context on perceptions of forensic evidence strength. *Journal of Forensic Sciences* 56(2): 409-414.

8. de Keijser, J. and Elffers, H. (2012) Understanding of forensic expert reports by judges, defense lawyers and forensic professionals. *Psychology, Crime & Law* 18(2): 191-207.

9. Howes, L.M., Kirkbride, K.P., Kelty, S.F., Julian, R., Kemp, N. (2013) Forensic scientists' conclusions: how readable are they for non-scientist report-users? *Forensic Science International* 231: 102-112.

10. Taroni, F., Biedermann, A., Vuille, J., and Morling, N. (2013). Whose DNA is this? How relevant a question? (a note for forensic scientists). *Forensic Science International: Genetics* 7: 467-470.

11. Howes, L.M. (2015) The communication of forensic science in the criminal justice system: A review of theory and proposed directions for research. *Science & Justice* 55(2): 145-154.

12. Ritchie, J. (2015) Probabilistic DNA evidence: the layperson's interpretation. *Australian Journal of Forensic Sciences* 47(4): 440-449.

13. Marquis, R., Biedermann, A., Cadola, L., Champod, C., Gueissaz, L., Massonnet, G., Mazzella, W.D., Taroni, F., Hicks, T. (2016) Discussion on how to implement a verbal scale in a forensic laboratory: Benefits, pitfalls and suggestions to avoid misunderstandings. *Science & Justice* 56(5): 364-370.

14. de Keijser, J.W., Malsch, M., Luining, E.T., Weulen Kranenbarg, M. and Lenssen, D.J.H.M. (2016) Differential reporting of mixed DNA profiles and its impact on jurists' evaluation of evidence. An international analysis. *Forensic Science International: Genetics* 23: 71-82.

15. Evett, I., Pope, S. and Puch-Solis, R. (2016) Providing scientific guidance on DNA to the judiciary. *Science & Justice* 56(4): 278-281.

16. Gittelson, S., Kalafut, T., Myers, S., Taylor, D., Hicks, T., Taroni, F., Evett, I.W., Bright, J.A. and Buckleton, J. (2016) A practical guide for the formulation of propositions in the Bayesian approach to DNA evidence interpretation in an adversarial environment. *Journal of Forensic Sciences* 61(1): 186-195.

17. Gill, P., Hicks, T., Butler, J.M., Connolly, E., Gusmão, L., Kokshoorn, B., Morling, N., van Oorschot, R.A.H., Parson, W., Prinz, M., Schneider, P.M., Sijen, T. and Taylor, D. (2018) DNA Commission of the International Society for Forensic Genetics: Assessing the value of forensic biological evidence – guidelines highlighting the importance of propositions. Part I: Evaluations of DNA profiling comparisons given (sub-) source propositions. *Forensic Science International: Genetics* 36: 189-202.

18. Machado, H. and Silva, S. (2019) What influences public views on forensic DNA testing in the criminal field? A scoping review of qualitative evidence. *Human Genomics* 13:23.

19. Gill, P. (2019) Interpretation continues to be the main weakness in criminal justice systems: developing roles of the expert witness and court. *WIREs Forensic Sci.* 1:e1321. Available at https://doi.org/10.1002/wfs2.1321.

20. Gill, P. (2019) DNA evidence and miscarriages of justice. *Forensic Science International* 294: e1-e3.

21. Jackson, G. and Biedermann, A. (2019) "Source" or "activity": What is the level of issue in a criminal trial? *Significance* 16(2): 36-39.

22. Gill, P., Hicks, T., Butler, J.M., Connolly, E., Gusmão, L., Kokshoorn, B., Morling, N., van Oorschot, R.A.H., Parson, W., Prinz, M., Schneider, P.M., Sijen, T. and Taylor, D. (2020) DNA Commission of the International Society for Forensic Genetics: Assessing the value of forensic biological evidence - guidelines highlighting the importance of propositions. Part II: Evaluation of biological traces considering activity level propositions. *Forensic Science International: Genetics* 44: 102186.

## O. Autosomal STR Markers and Kits

1. **Butler, J.M. (2006) Genetics and genomics of core STR loci used in human identity testing. *Journal of Forensic Sciences* 51(2): 253-265.**

2. Edwards, A., Civitello, A., Hammond, H.A., Caskey, C.T. (1991) DNA typing and genetic mapping with trimeric and tetrameric tandem repeats. *American Journal of Human Genetics* 49(4): 746-756.

3. Frégeau, C.J. and Fourney, R.M. (1993) DNA typing with fluorescently tagged short tandem repeats: a sensitive and accurate approach to human identification. *BioTechniques* 15(1): 100-119.

4. Hammond, H.A., Jin, L., Zhong, Y., Caskey, C.T., Chakraborty, R. (1994) Evaluation of 13 short tandem repeat loci for use in personal identification applications. *American Journal of Human Genetics* 55(1): 175-189.

5. Wallin, J.M., Buoncristiani, M.R., Lazaruk, K.D., Fildes, N., Holt, C.L., Walsh, P.S. (1998) TWGDAM validation of the AmpFISTR™ Blue PCR amplification kit for forensic casework analysis. *Journal of Forensic Sciences* 43(4): 854-870.

6. Gill, P., Sparkes, R., Fereday, L., Werrett, D.J. (2000) Report of the European Network of Forensic Science Institutes (ENSFI): formulation and testing of principles to evaluate STR multiplexes. *Forensic Science International* 108(1): 1-29.

7. Cotton, E.A., Allsop, R.F., Guest, J.L., Frazier, R.R.E., Koumi, P., Callow, I.P., Seager, A. Sparkes, R.L. (2000) Validation of the AMPFlSTR® SGM Plus™ system for use in forensic casework. *Forensic Science International* 112: 151-161.

8. Ruitberg, C.M., Reeder, D.J., Butler, J.M. (2001) STRBase: a short tandem repeat DNA database for the human identity testing community. *Nucleic Acids Research* 29: 320-322.

9. Frank, W.E., Llewellyn, B.E., Fish, P.A., Riech, A.K., Marcacci, T.L., Gandor, D.W., Parker, D., Carter, R.R., Thibault, S.M. (2001) Validation of the AmpFlSTR Profiler Plus PCR amplification kit for use in forensic casework. *Journal of Forensic Sciences* 46(3): 642-646.

10. LaFountain, M.J., Schwartz, M.B., Svete, P.A., Walkinshaw, M.A., Buel, E. (2001) TWGDAM validation of the AmpFlSTR Profiler Plus and AmpFlSTR COfiler STR multiplex systems using capillary electrophoresis. *Journal of Forensic Sciences* 46(5): 1191-1198.

11. Krenke, B.E., Tereba, A., Anderson, S.J., Buel, E., Culhane, S., Finis, C.J., Tomsey, C.S., Zachetti, J.M., Masibay, A., Rabbach, D.R., Amiott, E.A. (2002) Validation of a 16-locus fluorescent multiplex system. *Journal of Forensic Sciences* 47(4): 773-785.

12. Collins, P.J., Hennessy, L.K., Leibelt, C.S., Roby, R.K., Reeder, D.J., Foxall, P.A. (2004) Developmental validation of a single-tube amplification of the 13 CODIS STR loci, D2S1338, D19S433, and amelogenin: the AmpFlSTR Identifiler PCR Amplification Kit. *Journal of Forensic Sciences* 49(6): 1265-1277.

13. Gill, P., Fereday, L., Morling, N., Schneider, P.M. (2006) The evolution of DNA databases—recommendations for new European STR loci. *Forensic Science International* 156: 242-244.

14. Mulero, J.J., Chang, C.W., Lagace, R.E., Wang, D.Y., Bas, J.L., McMahon, T.P., Hennessy, L.K. (2008). Development and validation of the AmpFℓSTR® MiniFiler™ PCR Amplification Kit: a miniSTR multiplex for the analysis of degraded and/or PCR inhibited DNA. *Journal of Forensic Sciences* 53(4): 838-852.

15. Luce, C., Montpetit, S., Gangitano, D., O'Donnell, P. (2009) Validation of the AMPFlSTR MiniFiler PCR amplification kit for use in forensic casework*. *Journal of Forensic Sciences* 54(5): 1046-1054.

16. Wang, D.Y., Chang, C.W., Lagacé, R.E., Calandro, L.M., Hennessy, L.K. (2012) Developmental validation of the AmpFℓSTR® Identifiler® Plus PCR Amplification Kit: an established multiplex assay with improved performance. *Journal of Forensic Sciences* 57(2): 453-465.

17. Butler, J.M. and Hill, C.R. (2012) Biology and genetics of new autosomal STR loci useful for forensic DNA analysis. *Forensic Science Review* 24(1): 15-26.

18. Green, R.L., Lagacé, R.E., Oldroyd, N.J., Hennessy, L.K., Mulero, J.J. (2013) Developmental validation of the AmpFℓSTR® NGM SElect™ PCR Amplification Kit: A next-generation STR multiplex with the SE33 locus. *Forensic Science International: Genetics* 7(1): 41-51.

19. Hares, D.R. (2015) Selection and implementation of expanded CODIS core loci in the United States. *Forensic Science International: Genetics* 17: 33-34.

20. Gettings, K.B., Aponte, R.A., Vallone, P.M., Butler, J.M. (2015) STR allele sequence variation: current knowledge and future issues. *Forensic Science International: Genetics* 18: 118-130.

21. Ensenberger, M.G., Lenz, K.A., Matthies, L.K., Hadinoto, G.M., Schienman, J.E., Przech, A.J., Morganti, M.W., Renstrom, D.T., Baker, V.M., Gawrys, K.M., Hoogendoorn, M., Steffen, C.R., Martín, P., Alonso, A., Olson, H.R., Sprecher, C.J., Storts, D.R. (2016) Developmental validation of the PowerPlex(®) Fusion 6C System. *Forensic Science International: Genetics* 21: 134-144.

22. Kraemer, M., Prochnow, A., Bussmann, M., Scherer, M., Peist, R., Steffen, C. (2017) Developmental validation of QIAGEN Investigator® 24plex QS Kit and Investigator® 24plex GO! Kit: Two 6-dye multiplex assays for the extended CODIS core loci. *Forensic Science International: Genetics* 29: 9-20.

23. Gettings, K.B., Borsuk, L.A., Ballard, D., Bodner, M., Budowle, B., Devesse, L., King, J., Parson, W., Phillips, C., Vallone, P.M. (2017) STRSeq: A catalog of sequence diversity at human identification Short Tandem Repeat loci. *Forensic Science International: Genetics* 31: 111-117.

24. Ludeman, M.J., Zhong, C., Mulero, J.J., Lagacé, R.E., Hennessy, L.K., Short, M.L., Wang, D.Y. (2018) Developmental validation of GlobalFiler™ PCR amplification kit: a 6-dye multiplex assay designed for amplification of casework samples. *International Journal of Legal Medicine* 132(6): 1555-1573.

25. Gettings, K.B., Borsuk, L.A., Steffen, C.R., Kiesler, K.M., Vallone, P.M. (2018) Sequence-based U.S. population data for 27 autosomal STR loci. *Forensic Science International: Genetics* 37: 106-115.

26. Novroski, N.M.M., Woerner, A.E., Budowle, B. (2018) Potential highly polymorphic short tandem repeat markers for enhanced forensic identity testing. *Forensic Science International: Genetics* 37:162-171.

27. Novroski, N.M.M., Wendt, F.R., Woerner, A.E., Bus, M.M., Coble, M., Budowle, B. (2019) Expanding beyond the current core STR loci: An exploration of 73 STR markers with increased diversity for enhanced DNA mixture deconvolution. *Forensic Science International: Genetics* 38: 121-129.

28. Devesse, L., Davenport, L., Borsuk, L., Gettings, K., Mason-Buck, G., Vallone, P.M., Syndercombe Court, D., Ballard, D. (2020) Classification of STR allelic variation using massively parallel sequencing and assessment of flanking region power. *Forensic Science International: Genetics* 48: 102356.

29. Wyner, N., Barash, M., McNevin, D. (2020) Forensic autosomal short tandem repeats and their potential association with phenotype. *Frontiers in Genetics* 11: 884. doi: 10.3389/fgene.2020.00884.

## P. Mitochondrial DNA Testing

**1. Parson, W., Gusmão, L., Hares, D.R., Irwin, J.A., Mayr, W.R., Morling, N., Pokorak, E., Prinz, M., Salas, A., Schneider, P.M., Parsons, T.J. (2014) DNA Commission of the International Society for Forensic Genetics: revised and extended guidelines for mitochondrial DNA typing. *Forensic Science International: Genetics* 13: 134-142.**

2. Wilson, M.R., DiZinno, J.A., Polanskey, D., Replogle, J., Budowle, B. (1995) Validation of mitochondrial DNA sequencing for forensic casework analysis. *International Journal of Legal Medicine* 108(2): 68-74.

3. Budowle, B., Allard, M.W., Wilson, M.R., Chakraborty, R. (2003) Forensics and mitochondrial DNA: Applications, debates, and foundations. *Annual Review of Genomics and Human Genetics* 4: 119-141.

4. Melton, T. (2004) Mitochondrial DNA heteroplasmy. *Forensic Science Review* 16: 2-19.

5. van der Gaag, K.J., Hoogenboom, J., Sijen, T. (2017) Validation and implementation of MPS mtDNA control region analysis for forensic casework: Determination of C-stretch lengths by the FDSTools noise correction feature. *Forensic Science International: Genetics Supplement Series*. 6: e558-eE559.

6. Peck, M.A., Sturk-Andreaggi, K., Thomas, J.T., Oliver, R.S., Barritt-Ross, S., Marshall, C. (2018) Developmental validation of a Nextera XT mitogenome Illumina MiSeq sequencing method for high-quality samples. *Forensic Science International: Genetics* 34: 25-36.

7. Holland, M.M., Makova, K.D., McElhoe, J.A. (2018) Deep-coverage MPS analysis of heteroplasmic variants within the mtGenome allows for frequent differentiation of maternal relatives. *Genes (Basel)* 9(3): 124.

8. Huber, N., Parson, W., Dur, A. (2018) Next generation database search algorithm for forensic mitogenome analyses. *Forensic Science International: Genetics* 37: 204-214.

9. Pereira, V., Longobardi, A., Borsting, C. (2018) Sequencing of mitochondrial genomes using the Precision ID mtDNA Whole Genome Panel. *Electrophoresis* 39(21): 2766-2775.

10. Amorim, A., Fernandes, T., Taveira, N. (2019) Mitochondrial DNA in human identification: a review. *PeerJ* 7: e7314.

11. Brandhagen, M.D., Just, R.S., Irwin, J.A. (2020) Validation of NGS for mitochondrial DNA casework at the FBI Laboratory. *Forensic Science International: Genetics* 44: 102151.

## Q. Y-Chromosome and X-Chromosome Testing

1. **Roewer, L., Andersen, M.M., Ballantyne, J., Butler, J.M., Caliebe, A., Corach, D., D'Amato, M.E., Gusmão, L., Hou, Y., de Knijff, P., Parson, W., Prinz, M., Schneider, P.M., Taylor, D., Vennemann, M., Willuweit, S. (2020) DNA commission of the International Society of Forensic Genetics (ISFG): Recommendations on the interpretation of Y-STR results in forensic analysis. *Forensic Science International: Genetics* 48: 102308.**

2. Gill, P., Brenner, C., Brinkmann, B., Budowle, B., Carracedo, A., Jobling, M.A., de Knijff, P., Kayser, M., Krawczak, M., Mayr, W.R., Morling, N., Olaisen, B., Pascali, V., Prinz, M., Roewer, L., Schneider, P.M., Sajantila, A., Tyler-Smith, C. (2001) DNA Commission of the International Society of Forensic Genetics: recommendations on forensic analysis using Y-chromosome STRs. *Forensic Science International* 124(1): 5-10.

3. Butler, J.M. (2003) Recent developments in Y-short tandem repeat and Y-single nucleotide polymorphism analysis. *Forensic Science Review* 15: 91-111.

4. Jobling, M.A. and Tyler-Smith, C. (2003) The human Y chromosome: an evolutionary marker comes of age. *Nature Reviews: Genetics* 4(8): 598-612.

5. Gusmão, L., Butler, J.M., Carracedo, A., Gill, P., Kayser, M., Mayr, W.R., Morling, N., Prinz, M., Roewer, L., Tyler-Smith, C., Schneider, P.M. (2006) DNA Commission of the International Society of Forensic Genetics (ISFG): an update of the recommendations on the use of Y-STRs in forensic analysis. *Forensic Science International* 157(2-3): 187-197.

6. Mulero, J.J., Chang, C.W., Calandro, L.M., Green, R.L., Li, Y., Johnson, C.L., Hennessy, L.K. (2006) Development and validation of the AmpFlSTR Yfiler PCR amplification kit: a male specific, single amplification 17 Y-STR multiplex system. *Journal of Forensic Sciences* 51(1): 64-75.

7. Ballantyne, K.N., Goedbloed, M., Fang, R., Schaap, O., Lao, O., Wollstein, A., Choi, Y., van Duijn, K., Vermeulen, M., Brauer, S., Decorte, R., Poetsch, M., von Wurmb-Schwark, N., de Knijff, P., Labuda, D., Vézina, H., Knoblauch, H., Lessig, R., Roewer, L., Ploski, R., Dobosz, T., Henke, L., Henke, J., Furtado, M.R., Kayser, M. (2010) Mutability of Y-chromosomal microsatellites: rates, characteristics, molecular bases, and forensic implications. *American Journal of Human Genetics* 87(3): 341-353.

8. Thompson, J.M., Ewing, M.M., Frank, W.E., Pogemiller, J.J., Nolde, C.A., Koehler, D.J., Shaffer, A.M., Rabbach, D.R., Fulmer, P.M., Sprecher, C.J., Storts, D.R. (2013) Developmental validation of the PowerPlex® Y23 System: a

single multiplex Y-STR analysis system for casework and database samples. *Forensic Science International: Genetics* 7(2): 240-50.

9. Ballantyne, K.N., Ralf, A., Aboukhalid, R., Achakzai, N.M., Anjos, M.J., Ayub, Q., Balažic, J., Ballantyne, J., Ballard, D.J., Berger, B., Bobillo, C., Bouabdellah, M., Burri, H., Capal, T., Caratti, S., Cárdenas, J., Cartault, F., Carvalho, E.F., Carvalho, M., Cheng, B., Coble, M.D., Comas, D., Corach, D., D'Amato, M.E., Davison, S., de Knijff, P., De Ungria, M.C., Decorte, R., Dobosz, T., Dupuy, B.M., Elmrghni, S., Gliwiński, M., Gomes, S.C., Grol, L., Haas, C., Hanson, E., Henke, J., Henke, L., Herrera-Rodríguez, F., Hill, C.R., Holmlund, G., Honda, K., Immel, U.D., Inokuchi, S., Jobling, M.A., Kaddura, M., Kim, J.S., Kim, S.H., Kim, W., King, T.E., Klausriegler, E., Kling, D., Kovačević, L., Kovatsi, L., Krajewski, P., Kravchenko, S., Larmuseau, M.H., Lee, E.Y., Lessig, R., Livshits, L.A., Marjanović, D., Minarik, M., Mizuno, N., Moreira, H., Morling, N., Mukherjee, M., Munier, P., Nagaraju, J., Neuhuber, F., Nie, S., Nilasitsataporn, P., Nishi, T., Oh, H.H., Olofsson, J., Onofri, V., Palo, J.U., Pamjav, H., Parson, W., Petlach, M., Phillips, C., Ploski, R., Prasad, S.P., Primorac, D., Purnomo, G.A., Purps, J., Rangel-Villalobos, H., Rębała, K., Rerkamnuaychoke, B., Gonzalez, D.R., Robino, C., Roewer, L., Rosa, A., Sajantila, A., Sala, A., Salvador, J.M., Sanz, P., Schmitt, C., Sharma, A.K., Silva, D.A., Shin, K.J., Sijen, T., Sirker, M., Siváková, D., Skaro, V., Solano-Matamoros, C., Souto, L., Stenzl, V., Sudoyo, H., Syndercombe-Court, D., Tagliabracci, A., Taylor, D., Tillmar, A., Tsybovsky, I.S., Tyler-Smith, C,, van der Gaag, K.J., Vanek, D., Völgyi, A., Ward, D., Willemse, P., Yap, E.P., Yong, R.Y., Pajnič, I.Z., Kayser, M. (2014) Toward male individualization with rapidly mutating y-chromosomal short tandem repeats. *Human Mutation* 35(8): 1021-1032.

10. Diegoli, T.M. (2015) Forensic typing of short tandem repeat markers on the X and Y chromosomes. *Forensic Science International: Genetics* 18: 140-151.

11. Barbaro, A. and Cormaci, P. (2015) Validation of PowerPlex® Y23 System (Promega) using reduced reaction volume. *Forensic Science International: Genetics Supplement Series* 5: e373-e374.

12. Gopinath, S., Zhong, C., Nguyen, V., Ge, J., Lagacé, R.E., Short, M.L., Mulero, J.J. (2016) Developmental validation of the Yfiler(®) Plus PCR Amplification Kit: An enhanced Y-STR multiplex for casework and database applications. *Forensic Science International: Genetics* 24: 164-175.

13. Tillmar, A.O., Kling, D., Butler, J.M.**,** Parson, W., Prinz, M., Schneider, P.M., Egeland, T., Gusmão, L. (2017) DNA Commission of the International Society for Forensic Genetics (ISFG): Guidelines on the use of X-STRs in kinship analysis. *Forensic Science International: Genetics* 29: 269-275.

14. Kayser, M. (2017) Forensic use of Y-chromosome DNA: a general overview. *Human Genetics* 136: 621-635.

15. Jobling, M.A. and Tyler-Smith, C. (2017) Human Y-chromosome variation in the genome-sequencing era. *Nature Reviews: Genetics* 18(8): 485-497.

16. Caliebe, A. and Krawczak, M. (2018) Match probabilities for Y-chromosomal profiles: a paradigm shift. *Forensic Science International: Genetics* 37: 200-203.

17. Gomes, I., Pinto, N., Antão-Sousa, S., Gomes, V., Gusmão, L., Amorim, A. (2020). Twenty years later: A comprehensive review of the X chromosome use in forensic genetics. *Frontiers in Genetics* 11: 926.

## R. DNA Databases and Investigative Genetic Genealogy

**1. Struyf, P., De Moor, S., Vandeviver, C., Renard, B., van der Beken, T. (2019) The effectiveness of DNA databases in relation to their purpose and content: A systematic review. *Forensic Science International* 301: 371-381.**

2. Werrett, D.J. (1997) The national DNA database. *Forensic Science International* 88: 33-42.

3. Bieber, F.R., Brenner, C.H., Lazer, D. (2006) Finding criminals through DNA of their relatives. *Science* 312: 1315-1316.

4. Curran, J.M. and Buckleton, J.S. (2008) Effectiveness of familial searches. *Science & Justice* 48(4): 164-167.

5. Guerrini, C.J., Robinson, J.O., Petersen, D., McGuire, A.L. (2018) Should police have access to genetic genealogy databases? Capturing the Golden State Killer and other criminals using a controversial new forensic technique. *PLoS Biology* 16(10): e2006906.

6. Murphy, E. (2018) Law and policy oversight of familial searches in recreational genealogy databases. *Forensic Science International* 292: e5-e9.

7. Phillips, C. (2018) The Golden State Killer investigation and the nascent field of forensic genealogy. *Forensic Science International: Genetics* 36: 186-188.

8. Ram, N., Guerrini, C.J., McGuire, A.L. (2018) Genealogy databases and the future of criminal investigation. *Science* 360: 1078-1079.

9. Greytak, E.M., Kaye, D.H., Budowle, B., Moore, C., Armentrout, S.L. (2018) Privacy and genetic genealogy data. *Science* 361:857.

10. Samuel, G., Howard, H.C., Cornel, M., van El, C., Hall, A., Forzano, F., Prainsack, B. (2018) A response to the forensic genetics policy initiative's report "Establishing Best Practice for Forensic DNA Databases." *Forensic Science International: Genetics* 36: e19-e21.

11. Syndercombe Court, D. (2018) Forensic genealogy: Some serious concerns. *Forensic Science International: Genetics* 36: 203-204.

12. Greytak, E.M., Moore, C., Armentrout, S.L. (2019) Genetic genealogy for cold case and active investigations. *Forensic Science International* 299: 103-113.

13. Kennett, D. (2019) Using genetic genealogy databases in missing persons cases and to develop suspect leads in violent crimes. *Forensic Science International* 301: 107-117.

14. Thomson, J., Clayton, T., Cleary, J., Gleeson, M., Kennett, D., Leonard, M., Rutherford, D. (2020) An empirical investigation into the effectiveness of genetic genealogy to identify individuals in the UK. *Forensic Science International: Genetics* 46: 102263.

## S. Statistical Analysis

**1. Curran, J.M. (2013) Is forensic science the last bastion of resistance against statistics? *Science & Justice* 53: 251-252.**

2. Clopper, C.J., Pearson, E.S. (1934). The use of confidence or fiducial limits illustrated in the case of the binomial. *Biometrika 26*(4): 404-413.

3. DNA Advisory Board (2000) Statistical and population genetics issues affecting the evaluation of the frequency of occurrence of DNA profiles calculated from pertinent population database (s). *Forensic Science Communications*, *2*(3), 1-8. Available at https://archives.fbi.gov/archives/about-us/lab/forensic-science-communications/fsc/july2000/dnastat.htm.

4. Foreman, L.A. and Evett, I.W. (2001). Statistical analyses to support forensic interpretation for a new ten-locus STR profiling system. *International Journal of Legal Medicine* 114(3): 147-155.

5. Foreman, L.A., Champod, C., Evett, I.W., Lambert, J.A., Pope, S. (2003) Interpreting DNA evidence: A review. *International Statistical Review* 71(3): 473-495.

6. Curran, J.M. (2005). An introduction to Bayesian credible intervals for sampling error in DNA profiles. *Law, Probability and Risk* 4(1-2): 115-126.

7. Aitken, C., Roberts, P. and Jackson, G. (2010) Practitioner Guide No. 1. *Fundamentals of Probability and Statistical Evidence in Criminal Proceedings: Guidance for Judges, Lawyers, Forensic Scientists and Expert Witnesses*. Royal Statistical Society's Working Group on Statistics and the Law. Available at https://www.maths.ed.ac.uk/~cgga/Guide-1-WEB.pdf.

8. Biedermann, A. and Taroni, F. (2012) Bayesian networks for evaluating forensic DNA profiling evidence: A review and guide to literature. *Forensic Science International: Genetics* 6(2): 147-157.

9. Puch-Solis, R., Roberts, P., Pope, S. and Aitken, C. (2012) Practitioner Guide No. 2. *Assessing the Probative Value of DNA Evidence: Guidance for Judges, Lawyers, Forensic Scientists and Expert Witnesses*. Royal Statistical Society's Working Group on Statistics and the Law. Available at https://www.maths.ed.ac.uk/~cgga/Guide-2-WEB.pdf.

10. Roberts, P. and Aiken, C. (2014) Practitioner Guide No. 3. *The Logic of Forensic Proof: Inferential Reasoning in Criminal Evidence and Forensic Science: Guidance for Judges, Lawyers, Forensic Scientists and Expert Witnesses*. Royal Statistical Society's Working Group on Statistics and the Law. Available at https://rss.org.uk/RSS/media/File-library/Publications/rss-inferential-reasoning-criminal-evidence-forensic-science.pdf.

11. Jackson, G., Aitken, C., Roberts, P. (2014) Practitioner Guide No. 4. *Case Assessment and Interpretation of Expert Evidence: Guidance for Judges, Lawyers, Forensic Scientists and Expert Witnesses*. Royal Statistical Society's Working Group on Statistics and the Law. Available at https://rss.org.uk/RSS/media/File-library/Publications/rss-case-assessment-interpretation-expert-evidence.pdf.

## T. Population Genetics

1. **Balding, D.J. and Nichols, R.A. (1994) DNA profile match probability calculation: how to allow for population stratification, relatedness, database selection and single bands. *Forensic Science International* 64: 125-140.**

2. Hardy, G.H. (1908) Mendelian proportions in a mixed population. *Science* 28: 49-50.

3. Stern, C. (1943) The Hardy-Weinberg law. *Science* 97: 137-138.

4. Weir, B.S. and Cockerham, C.C. (1984) Estimating F-statistics for the analysis of population structure. *Evolution* 38(6): 1358-1370.

5. Chakraborty, R. (1992) Sample size requirements for addressing the population genetic issues of forensic use of DNA typing. *Human Biology* 64(2): 141-159.

6. Guo, S.W. and Thompson, E.A. (1992) Performing the exact test of Hardy-Weinberg proportion for multiple alleles. *Biometrics* 48(2): 361-372.

7. Weir, B.S. (1994) The effects of inbreeding on forensic calculations. *Annual Review of Genetics* 28: 597-621.

8. Buckleton, J.S., Curran, J.M., Walsh, S.J. (2006) How reliable is the sub-population model in DNA testimony? *Forensic Science International* 157(2-3): 144-148.

9. Curran, J.M., Walsh, S.J., Buckleton, J. (2007) Empirical testing of estimated DNA frequencies. *Forensic Science International: Genetics* 1: 267-272.

10. Steele, C.D., Syndercombe-Court, D., Balding, D.J. (2014) Worldwide F(ST) estimates relative to five continental-scale populations. *Annals of Human Genetics* 78(6): 468-477.

11. Buckleton, J., Curran, J., Goudet, J., Taylor, D., Thiery, A., Weir, B.S. (2016) Population-specific FST values for forensic STR markers: A worldwide survey. *Forensic Science International: Genetics* 23: 91-100.

## U. DNA Phenotyping (Ancestry, Appearance, Age)

**1. Kayser, M. (2015) Forensic DNA Phenotyping: Predicting human appearance from crime scene material for investigative purposes. *Forensic Science International: Genetics* 18: 33-48.**

2. Kayser, M. and Schneider, P.M. (2009) DNA-based prediction of human externally visible characteristics in forensics: motivations, scientific challenges, and ethical considerations. *Forensic Science International: Genetics* 3(3): 154-161.

3. Walsh, S., Liu, F., Wollstein, A., Kovatsi, L., Ralf, A., Kosiniak-Kamysz, A., Branicki, W., Kayser, M. (2013) The HIrisPlex system for simultaneous prediction of hair and eye colour from DNA. *Forensic Science International: Genetics* 7(1): 98-115.

4. Phillips, C. (2015) Forensic genetic analysis of bio-geographical ancestry. *Forensic Science International: Genetics* 18: 49-65.

5. Freire-Aradas, A., Phillips, C., Lareu, M.V. (2017) Forensic individual age estimation with DNA: From initial approaches to methylation tests. *Forensic Science Review* 29(2): 121-144.

6. Chaitanya, L., Breslin, K., Zuñiga, S., Wirken, L., Pośpiech, E., Kukla-Bartoszek, M., Sijen, T., Knijff, P., Liu, F., Branicki, W., Kayser, M., Walsh, S. (2018) The HIrisPlex-S system for eye, hair and skin colour prediction from DNA: Introduction and forensic developmental validation. *Forensic Science International: Genetics* 35: 123-135.

7. Vidaki, A. and Kayser, M. (2017) From forensic epigenetics to forensic epigenomics: broadening DNA investigative intelligence. *Genome Biology* 18(1): 238.

8. Vidaki, A. and Kayser, M. (2018) Recent progress, methods and perspectives in forensic epigenetics. *Forensic Science International: Genetics* 37: 180-195.

9. Parson, W. (2018) Age estimation with DNA: from forensic DNA fingerprinting to forensic (epi)genomics: a mini-review. Gerontology 64(4): 326-332.

10. Naue, J., Hoefsloot, H.C.J., Kloosterman, A.D., Verschure, P.J. (2018) Forensic DNA methylation profiling from minimal traces: How low can we go? *Forensic Science International: Genetics* 33: 17-23.

11. Scudder, N., McNevin, D., Kelty, S.F., Walsh, S.J., Robertson, J. (2018) Forensic DNA phenotyping: Developing a model privacy impact assessment. *Forensic Science International: Genetics* 34: 222-230.

12. Pośpiech, E., Chen, Y., Kukla-Bartoszek, M., Breslin, K., Aliferi, A., Andersen, J.D., Ballard, D., Chaitanya, L., Freire-Aradas, A., van der Gaag, K.J., Girón-Santamaría, L., Gross, T.E., Gysi, M., Huber, G., Mosquera-Miguel, A., Muralidharan, C., Skowron, M., Carracedo, Á., Haas, C., Morling, N., Parson, W., Phillips, C., Schneider, P.M., Sijen, T., Syndercombe-Court, D., Vennemann, M., Wu, S., Xu, S., Jin, L., Wang, S., Zhu, G., Martin, N.G., Medland, S.E., Branicki, W., Walsh, S., Liu, F., Kayser, M. (EUROFORGEN-NoE Consortium) (2018) Towards broadening Forensic DNA Phenotyping beyond pigmentation: Improving the prediction of head hair shape from DNA. *Forensic Science International: Genetics* 37: 241-251.

13. Wienroth, M. (2018) Governing anticipatory technology practices: forensic DNA phenotyping and the forensic genetics community in Europe. *New Genetics and Society* 37(2): 137-152.

14. Samuel, G. and Prainsack, B. (2019) Forensic DNA phenotyping in Europe: views "on the ground" from those who have a professional stake in the technology. *New Genetics and Society* 38(2): 119-141.

15. Schneider, P. M., Prainsack, B., & Kayser, M. (2019) The use of forensic DNA phenotyping in predicting appearance and biogeographic ancestry. *Deutsches Arzteblatt International* 116: 873–880.

16. Katsara, M.-A, and Nothnagel, M. (2019) True colors: A literature review on spatial distribution of eye and hair pigmentation. *Forensic Science International: Genetics* 39: 109-118.

17. Xiong, Z., Dankova, G., Howe, L.J., Lee, M.K., Hysi, P.G., de Jong, M.A., Zhu, G., Adhikari, K., Li, D., Li, Y., Pan, B., Feingold, E., Marazita, M.L., Shaffer, J.R., McAloney, K., Xu, S.H., Jin, L., Wang, S., de Vrij, F.M., Lendemeijer, B., Richmond, S., Zhurov, A., Lewis, S., Sharp, G.C., Paternoster, L., Thompson, H., Gonzalez-Jose, R., Bortolini, M.C., Canizales-Quinteros, S., Gallo, C., Poletti, G., Bedoya, G., Rothhammer, F., Uitterlinden, A.G., Ikram, M.A., Wolvius, E., Kushner, S.A., Nijsten, T.E., Palstra, R.T., Boehringer, S., Medland, S.E., Tang, K., Ruiz-Linares, A., Martin, N.G., Spector, T.D., Stergiakouli, E., Weinberg, S.M., Liu, F., Kayser, M. (2019) Novel genetic loci affecting facial shape variation in humans. *eLIFE* 8: e49898.

18. Peng, F., Zhu, G., Hysi, P.G., Eller, R.J., Chen, Y., Li, Y., Hamer, M.A., Zeng, C., Hopkins, R.L., Jacobus, C.L., Wallace, P.L., Uitterlinden, A.G., Ikram, M.A., Nijsten, T., Duffy, D.L., Medland, S.E., Spector, T.D., Walsh, S., Martin, N.G., Liu, F., Kayser, M. (2019) Genome-wide association studies identify multiple genetic loci influencing eyebrow color variation in Europeans. *Journal of Investigative Dermatology* 139(7): 1601-1605.

19. Liu, F., Zhong, K., Jing, X., Uitterlinden, A.G., Hendriks, A.E.J., Drop, S.L.S., Kayser, M. (2019) Update on the predictability of tall stature from DNA markers in Europeans. *Forensic Science International: Genetics* 42: 8-13.

20. Kukla-Bartoszeka, M., Pośpiech, E., Woźniak, A., Boroń, M., Karłowska-Pik, J., Teisseyre, P., Zubańska, M., Bronikowska, A., Grzybowski, T., Płoski, R., Spólnicka, M., Branicki, W. (2019) DNA-based predictive models for the presence of freckles. *Forensic Science International: Genetics* 42: 252-259.

21. Freire-Aradas, A., Pośpiech, E., Aliferi, A., Giron-Santamarie, L., Mosquera-Miguel, A., Pisarek, A., Ambroa-Conde, A., Phillips, C., Casares De Cal, M., Gomez-Tato, A., Spolnicka, M., Wozniak, M., Alvarez-Dios, J., Ballard, D., Syndercombe-Court, D., Branicki, W., Carracedo, Á., Lareu, M.V. (2020) A comparison of forensic age prediction models using data from four DNA methylation technologies. *Frontiers in Genetics* 11: 932. https://doi.org/10.3389/fgene.2020.00932.

22. Heidegger, A., Xavier, C., Niederstätter, H., de la Puente, M., Pośpiech, E., Pisarek, A., Kayser, M., Branicki, W., Parson, W. (VISAGE Consortium) (2020) Development and optimization of the VISAGE basic prototype tool for forensic age estimation. *Forensic Science International: Genetics* 48: 102322.

23. Palencia-Madrid, L., Xavier, C., de la Puente, M., Hohoff, C., Phillips, C., Kayser, M., Parson, W. (2020) Evaluation of the VISAGE basic tool for appearance and ancestry prediction using PowerSeq chemistry on the MiSeq FGx System. *Genes (Basel)* 11(6): 708.

24. Xavier, C., de la Puente, M., Mosquera-Miguel, A., Freire-Aradas, A., Kalamara, V., Vidaki, A., Gross, T.E., Revoir, A., Pośpiech, E., Kartasińska, E., Spólnicka, M., Branicki, W., Ames, C.E., Schneider, P.M., Hohoff, C., Kayser, M., Phillips, C., Parson, W. (VISAGE Consortium) (2020) Development and validation of the VISAGE AmpliSeq basic tool to predict appearance and ancestry from DNA. *Forensic Science International: Genetics* 48: 102336.

## V. New Technologies (Rapid DNA, Massively Parallel Sequencing)

1. **Butler, J.M. (2015) The future of forensic DNA analysis. *Philosophical Transactions of the Royal Society of London Series B, Biological Sciences* 370: 20140252.**

2. Gill, P. (2001) An assessment of the utility of single nucleotide polymorphisms (SNPs) for forensic purposes. *International Journal of Legal Medicine* 114: 204-210.

3. Gill, P., Werrett, D.J., Budowle, B., Guerrieri, R. (2004) An assessment of whether SNPs will replace STRs in national DNA databases--joint considerations of the DNA working group of the European Network of Forensic Science Institutes (ENFSI) and the Scientific Working Group on DNA Analysis Methods (SWGDAM). *Science & Justice* 44(1): 51-53.

4. Butler, J.M., Coble, M.D., Vallone, P.M. (2007) STRs vs SNPs: thoughts on the future of forensic DNA testing. *Forensic Science Medicine and Pathology* 3: 200-205.

5. Blackman, S., Dawnay, N., Ball, G., Stafford-Allen, B., Tribble, N., Rendell, P., Neary, K., Hanson, E.K., Ballantyne, J., Kallifatidis, B., Mendel, J., Mills, D.K., Wells, S. (2015) Developmental validation of the ParaDNA(®) Intelligence System-A novel approach to DNA profiling. *Forensic Science International: Genetics* 17: 137-148.

6. Date-Chong, M., Hudlow, W.R., Buoncristiani, M.R. (2016) Evaluation of the RapidHIT 200 and RapidHIT GlobalFiler® Express kit for fully automated STR genotyping. *Forensic Science International: Genetics* 23: 1-8.

7. Parson, W., Ballard, D., Budowle, B., Butler, J.M., Gettings, K.B., Gill, P., Gusmão, L., Hares, D.R., Irwin, J.A., King, J.L., Knijff, P., Morling, N., Prinz, M., Schneider, P.M., Neste, C.V., Willuweit, S., Phillips, C. (2016) Massively parallel sequencing of forensic STRs: Considerations of the DNA commission of the International Society for Forensic Genetics (ISFG) on minimal nomenclature requirements. *Forensic Science International: Genetics* 22: 54-63.

8. van der Gaag, K.J., de Leeuw, R.H., Hoogenboom, J., Patel, J., Storts, D.R., Laros, J.F.J., de Knijff, P. (2016) Massively parallel sequencing of short tandem repeats-Population data and mixture analysis results for the PowerSeq™ system. *Forensic Science International: Genetics* 24: 86-96.

9. Bruijns, B., van Asten, A., Tiggelaar, R., Gardeniers, H. (2016) Microfluidic devices for forensic DNA analysis: a review. *Biosensors (Basel)* 6(3): 41.

10. Della Manna, A., Nye, J.V., Carney, C., Hammons, J.S., Mann, M., Al Shamali, F., Vallone, P.M., Romsos, E.L., Marne, B.A., Tan, E., Turingan, R.S., Hogan, C., Selden, R.F., French, J.L. (2016) Developmental validation of the DNAscan™ Rapid DNA Analysis™ instrument and expert system for reference sample processing. *Forensic Science International: Genetics* 25: 145-156.

11. Mapes, A.A., Kloosterman, A.D., de Poot, C.J., van Marion, V. (2016) Objective data on DNA success rates can aid the selection process of crime samples for analysis by rapid mobile DNA technologies. *Forensic Science International* 264: 28-33.

12. Moreno, L.I., Brown, A.L., Callaghan, T.F. (2017) Internal validation of the DNAscan/ANDE Rapid DNA Analysis platform and its associated PowerPlex® 16 high content DNA biochip cassette for use as an expert system with reference buccal swabs. *Forensic Science International: Genetics* 29: 100-108.

13. Wiley, R., Sage, K., LaRue, B., Budowle, B. (2017) Internal validation of the RapidHIT® ID system. *Forensic Science International: Genetics* 31: 180-188.

14. Mehta, B., Daniel, R., Phillips, C., McNevin, D. (2017) Forensically relevant SNaPshot® assays for human DNA SNP analysis: a review. *International Journal of Legal Medicine* 131(1): 21-37.

15. Jäger, A.C., Alvarez, M.L., Davis, C.P., Guzmán, E., Han, Y., Way, L., Walichiewicz, P., Silva, D., Pham, N., Caves, G., Bruand, J., Schlesinger, F., Pond, S.J.K., Varlaro, J., Stephens, K.M., Holt, C.L. (2017) Developmental validation of the MiSeq FGx Forensic Genomics System for targeted next generation sequencing in forensic DNA casework and database laboratories. *Forensic Science International: Genetics* 28: 52-70.

16. Sharma, V., Chow, H.Y., Siegel, D., Wurmbach, E. (2017) Qualitative and quantitative assessment of Illumina's forensic STR and SNP kits on MiSeq FGx. *PLoS ONE* 12(11): e0187932.

17. Phillips, C., Gettings, K.B., King, J.L., Ballard, D., Bodner, M., Borsuk, L., Parson, W. (2018) "The devil's in the detail": Release of an expanded, enhanced and dynamically revised forensic STR sequence guide. *Forensic Science International: Genetics* 34: 162-169.

18. Alonso, A., Barrio, P.A., Müller, P., Köcher, S., Berger, B., Martin, P., Bodner, M., Willuweit, S., Parson, W., Roewer, L., Budowle, B. (2018) Current state-of-art of STR sequencing in forensic genetics. *Electrophoresis* 39(21): 2655-2668.

19. Bruijns, B., Tiggelaar, R., Gardeniers, H. (2018) Massively parallel sequencing techniques for forensics: a review. *Electrophoresis* 39(21): 2642-2654.

20. Morrison, J., Watts, G., Hobbs, G., Dawnay, N. (2018) Field-based detection of biological samples for forensic analysis: Established techniques, novel tools, and future innovations. *Forensic Science International* 285: 147-160.

21. Liu, Y.-Y. and Harbison, S. (2018) A review of bioinformatic methods for forensic DNA analyses. *Forensic Science International: Genetics* 33: 117-128.

22. Buscaino, J., Barican, A., Farrales, L., Goldman, B., Klevenberg, J., Kuhn, M., Lin, F., Nguyen, P., Salceda, S., Schueren, R., Smith, C., Troup, C., Tsou, D., Vangbo, M., King, D. (2018) Evaluation of a rapid DNA process with the RapidHIT® ID system using a specialized cartridge for extracted and quantified human DNA. *Forensic Science International: Genetics* 34: 116-127.

23. Shackleton, D., Pagram, J., Ives, L., Vanhinsbergh, D. (2018) Development and validation of the RapidHIT™ 200 utilising NGMSElect™ Express for the processing of buccal swabs. *Forensic Science International* 289: 244-252.

24. Shackleton, D., Gray, N., Ives, L., Malsom, S., Vanhinsbergh, D. (2019) Development of RapidHIT® ID using NGM SElect™ Express chemistry for the processing of reference samples within the UK criminal justice system. *Forensic Science International* 295: 179-188.

25. Shackleton, D., Pagram, J., Andrews, N., Malsom, S., Ives, L., Vanhinsbergh, D. (2019) Development of enhanced sensitivity protocols on the RapidHIT™ 200 with a view to processing casework material. *Science & Justice* 59: 411-417.

26. Wu, J., Li, J.L., Wang, M.L., Li, J.P., Zhao, Z.C., Wang, Q., Yang, S.D., Xiong, X., Yang, J.L., Deng, Y.J. (2019) Evaluation of the MiSeq FGx system for use in forensic casework. *International Journal of Legal Medicine* 133(3): 689-697.

27. de Knijff, P. (2019) From next generation sequencing to now generation sequencing in forensics. *Forensic Science International: Genetics* 38: 175-180.

28. Oldoni, F. and Podini, D. (2019) Forensic molecular biomarkers for mixture analysis. *Forensic Science International: Genetics* 41: 107-119.

29. Bennett, L., Oldoni, F., Long, K., Cisana, S., Madella, K., Wootton, S., Chang, J., Hasegawa, R., Lagacé, R., Kidd, K., Podini, D. (2019) Mixture deconvolution by massively parallel sequencing of microhaplotypes. *International Journal of Legal Medicine* 133*:* 719-729.

30. Morgan, R., Illidge, S., Wilson-Wilde, L. (2019) Assessment of the potential investigative value of a decentralized rapid DNA workflow for reference DNA samples. *Forensic Science International* 294: 140-149.

31. Mapes, A.A., Stoel, R.D., de Poot, C.J., Vergeer, P., Huyck, M. (2019) Decision support for using mobile Rapid DNA analysis at the crime scene. *Science & Justice* 59: 29-45.

32. Carney, C., Whitney, S., Vaidyanathan, J., Persick, R., Noel, F., Vallone, P.M., Romsos, E.L., Tan, E., Grover, R., Turingan, R.S., French, J.L., Selden, R.F. (2019) Developmental validation of the ANDE™ rapid DNA system with FlexPlex™ assay for arrestee and reference buccal swab processing and database searching. *Forensic Science International: Genetics* 40: 120-130.

33. Romsos, E.L., French, J.L., Smith, M., Figarelli, V., Harran, F., Vandegrift, G., Moreno, L.I., Callaghan, T.F., Brocato, J., Vaidyanathan, J., Pedroso, J.C., Amy, A., Stoiloff, S., Morillo, V.H., Czetyrko, K., Johnson, E.D., de Tagyos, J., Murray, A., Vallone, P.M. (2020) Results of the 2018 Rapid DNA Maturity Assessment. *Journal of Forensic Sciences* 65(3): 953-959.

34. Ballard, D., Winkler-Galicki, J., Wesoly, J. (2020) Massive parallel sequencing in forensics : advantages, issues, technicalities, and prospects. *International Journal of Legal Medicine* 134: 1292-1303.

35. Bleka, Ø., Just, R., Le, J., Gill, P. (2020) An examination of STR nomenclatures, filters and models for MPS mixture interpretation. *Forensic Science International: Genetics* 48: 102319.

## W. DNA Transfer and Activity Level Reporting

**1. van Oorschot, R.A.H., Szkuta, B., Meakin, G.E., Kookshoorn, B., Goray, M. (2019) DNA transfer in forensic science: a review. *Forensic Science International: Genetics* 38: 140-166.**

2. van Oorschot, R.A. and Jones, M.K. (1997) DNA fingerprints from fingerprints. *Nature* 387: 767.

3. Lowe, A., Murray, C., Whitaker, J., Tully, G. and Gill, P. (2002) The propensity of individuals to deposit DNA and secondary transfer of low level DNA from individuals to inert surfaces. *Forensic Science International* 129: 25-34.

4. Wickenheiser, R.A. (2002) Trace DNA: a review, discussion of theory, and application of the transfer of trace quantities of DNA through skin contact. *Journal of Forensic Sciences* 47(3): 442-450.

5. Port, N.J., Bowyer, V.L., Graham, E.A.M., Batuwangala, M.S., Rutty, G.N. (2006) How long does it take a static speaking individual to contaminate the immediate environment? *Forensic Science, Medicine, and Pathology* 2(3): 157-163.

6. Phipps, M. and Petricevic, S. (2007) The tendency of individuals to transfer DNA to handled items. *Forensic Science International* 168(2-3): 162-168.

7. Malsom, S., Flanagan, N., McAlister, C., Dixon, L. (2009) The prevalence of mixed DNA profiles in fingernail samples taken from couples who co-habit using autosomal and Y-STRs. *Forensic Science International: Genetics* 3(2): 57-62.

8. Goray, M., Mitchell, R.J., van Oorschot, R.A.H. (2010) Investigation of secondary DNA transfer of skin cells under controlled test conditions. *Legal Medicine* 12(3): 117-120.

9. Goray, M., Eken, E., Mitchell, R. J., van Oorschot, R.A.H. (2010) Secondary DNA transfer of biological substances under varying test conditions. *Forensic Science International: Genetics* 4(2): 62-67.

10. Daly, D.J., Murphy, C., McDermott, S.D. (2012) The transfer of touch DNA from hands to glass, fabric and wood. *Forensic Science International: Genetics* 6(1): 41-46.

11. Meakin, G. and Jamieson, A. (2013) DNA transfer: review and implications for casework. *Forensic Science International: Genetics* 7: 434-443.

12. Ballantyne, K.N., Poy, A.L. and van Oorschot, R.A.H. (2013) Environmental DNA monitoring: beware of the transition to more sensitive typing methodologies. *Australian Journal of Forensic Sciences* 45(3): 323-340.

13. Verdon, T.J., Mitchell, R.J. and van Oorschot, R.A. (2013) The influence of substrate on DNA transfer and extraction efficiency. *Forensic Science International: Genetics* 7(1): 167-175.

14. Jackson, G. (2013) The impact of commercialization on the evaluation of DNA evidence. *Frontiers in Genetics* 4: 227.

15. McKenna, L. (2013) Understanding DNA results within the case context: importance of the alternative proposition. *Frontiers in Genetics* 4: 242.

16. Champod, C. (2013) DNA transfer: informed judgment or mere guesswork? *Frontiers in Genetics* 4: 300.

17. van Oorschot, R.A.H., Glavich, G., and Mitchell, R.J. (2014) Persistence of DNA deposited by the original user on objects after subsequent use by a second person. *Forensic Science International: Genetics* 8(1): 219-225.

18. Cale, C.M., Earll, M.E., Latham, K.E., Bush, G.L. (2015) Could secondary DNA transfer falsely place someone at the scene of a crime? *Journal of Forensic Sciences* 61(1): 196-203.

19. Lehmann, V.J.., Mitchell, R.J., Ballantyne, K.N., van Oorschot, R.A.H. (2015) Following the transfer of DNA: How does the presence of background DNA affect the transfer and detection of a target source of DNA? *Forensic Science International: Genetics.* 19: 68-75.

20. Fonneløp, A.E., Egeland, T., Gill, P. (2015) Secondary and subsequent DNA transfer during criminal investigation. *Forensic Science International: Genetics* 17: 155-162.

21. Kamphausen, T., Fandel, S.B., Gutmann, J.S., Bajanowski, T., Poetsch, M. (2015) Everything clean? Transfer of DNA traces between textiles in the washtub. *International Journal of Legal Medicine* 129(4): 709-714.

22. ENFSI (2015) ENFSI Guideline for Evaluative Reporting in Forensic Science; available at http://enfsi.eu/wp-content/uploads/2016/09/m1_guideline.pdf.

23. Goray, M. and van Oorschot, RA. (2015) The complexities of DNA transfer during a social setting. *Legal Medicine* 17(2): 82-91.

24. Lapointe, M., Rogic, A., Bourgoin, S., Jolicoeur, C. and Séguin, D. (2015) Leading-edge forensic DNA analyses and the necessity of including crime scene investigators, police officers and technicians in a DNA elimination database. *Forensic Science International: Genetics* 19: 50-55.

25. Taylor, D., Abarno, D., Rowe, E., Rask-Nielsen, L. (2016) Observations of DNA transfer within an operational Forensic Biology Laboratory. *Forensic Science International: Genetics* 23: 33-49.

26. Jones, S., Scott, K., Lewis, J., Davidson, G., Allard, J. E., Lowrie, C., McBride, B.M., McKenna, L., Teppett, G., Rogers, C., Clayson, N., Baird, A. (2016) DNA transfer through nonintimate social contact. *Science & Justice* 56(2): 90-95.

27. Noël, S., Lagacé, K., Rogic, A., Granger, D., Bourgoin, S., Jolicoeur, C., Séguin, D. (2016) DNA transfer during laundering may yield complete genetic profiles. *Forensic Science International: Genetics* 23: 240-247.

28. Breathnach, M., Williams, L., McKenna, L., Moore, E. (2016) Probability of detection of DNA deposited by habitual wearer and/or the second individual who touched the garment. *Forensic Science International: Genetics* 20: 53-60.

29. Helmus, J., Bajanowski, T., Poetsch, M. (2016) DNA transfer-a never ending story: a study on scenarios involving a second person as carrier. *International Journal of Legal Medicine* 130(1): 121-125.

30. Oldoni, F., Castella, V., Hall, D. (2016) Shedding light on the relative DNA contribution of two persons handling the same object. *Forensic Science International: Genetics* 24: 148-157.

31. Goray, M., Fowler, S., Szkuta, B., van Oorschot, R.A.H. (2016) Shedder status – an analysis of self and non-self DNA in multiple handprints deposited by the same individuals over time. *Forensic Science International: Genetics* 23: 190-196.

32. Biedermann, A. and Hicks, T. (2016) The importance of critically examining the level of propositions when evaluating forensic DNA results. *Frontiers in Genetics* 7: 8.

33. Biedermann, A., Champod, C., Jackson, G., Gill, P., Taylor, D., Butler, J., Morling, N., Hicks, T., Vuille, J., Taroni, F. (2016) Evaluation of forensic DNA traces when propositions of interest relate to activities: analysis and discussion of recurrent concerns. *Frontiers in Genetics* 7: 215.

34. Taylor, D., Biedermann, A., Samie, L., Pun, K.M., Hicks, T. and Champod, C. (2017) Helping to distinguish primary from secondary transfer events for trace DNA. *Forensic Science International: Genetics* 28: 155-177.

35. Steensma, K., Ansell, R., Clarisse, L., Connolly, E., Kloosterman, A.D., McKenna, L.G., van Oorschot, R.A.H., Szkuta, B. and Kokshoorn, B. (2017) An inter-laboratory comparison study on transfer, persistence and recovery of DNA from cable ties. *Forensic Science International: Genetics* 31: 95-104.

36. Meakin, G.E., Butcher, E.V., van Oorschot, R.A.H., Morgan, R.M. (2017) Trace DNA evidence dynamics: An investigation into the deposition and persistence of directly- and indirectly-transferred DNA on regularly-used knives. *Forensic Science International: Genetics* 29: 38-47.

37. Pfeifer, C.M. and Wiegand, P. (2017) Persistence of touch DNA on burglary-related tools. *International Journal of Legal Medicine* 131(4): 941-953.

38. Kokshoorn, B., Blankers, B.J., de Zoete, J., Berger, C.E.H. (2017) Activity level DNA evidence evaluation: On propositions addressing the actor or the activity. *Forensic Science International* 278: 115-124.

39. Stella, C.J., Mitchell, R.J., van Oorschot, R.A.H. (2017) Hand activities during robberies—Relevance to consideration of DNA transfer and detection. *Forensic Science International: Genetics Supplement Series* 6: e3-e5.

40. Szkuta, B., Ballantyne, K.N., van Oorschot, R.A.H. (2017) Transfer and persistence of DNA on the hands and the influence of activities performed. *Forensic Science International: Genetics* 28: 10-20.

41. Kanokwongnuwut, P., Martin, B., Kirkbride, K.P., Linacre, A. (2018) Shedding light on shedders. *Forensic Science International: Genetics* 36: 20-25.

42. Szkuta, B., Ballantyne, K.N., Kokshoorn, B., and van Oorschot, R.A.H. (2018) Transfer and persistence of non-self DNA on hands over time: Using empirical data to evaluate DNA evidence given activity level propositions. *Forensic Science International: Genetics* 33: 84-97.

43. Taylor, D., Kokshoorn, B. and Biedermann, A. (2018) Evaluation of forensic genetics findings given activity level propositions: A review. *Forensic Science International: Genetics* 36: 34-49.

44. Taylor, D., Biedermann, A., Hicks, T. and Champod, C. (2018) A template for constructing Bayesian networks in forensic biology cases when considering activity level propositions. *Forensic Science International: Genetics* 33: 136-146.

45. Kokshoorn, B., Aarts, L.H.J., Ansell, R., Connolly, E., Drotz, W., Kloosterman, A.D., McKenna, L.G., Szkuta, B., van Oorschot, R.A.H. (2018) Sharing data on DNA transfer, persistence, prevalence and recovery: Arguments for harmonization and standardization. *Forensic Science International: Genetics* 37: 260-269.

46. Voskoboinik, L., Amiel, M., Reshef, A., Gafny, R., Barash, M. (2018) Laundry in a washing machine as a mediator of secondary and tertiary DNA transfer. *International Journal of Legal Medicine* 132(2): 373-378.

47. Goray, M., Pirie, E., van Oorschot, R. A. (2019) DNA transfer: DNA acquired by gloves during casework examinations. *Forensic Science International: Genetics* 38: 167-174.

48. Burrill, J., Daniel, B., Frascione, N. (2019) A review of trace "touch DNA" deposits: Variability factors and an exploration of cellular composition. *Forensic Science International: Genetics* 39:8-18.

49. Gosch, A. and Courts, C. (2019) On DNA transfer: the lack and difficulty of systematic research and how to do it better. *Forensic Science International: Genetics* 40: 24-36.

50. Rolo, M., Sampaio, L., Balsa, F., Bento, A.M., Gouveia, N., Serra, A., Brito, P., Lopes, V., Sao-Bento, M., Bogas, V., Cunha, P., Porto, M. J., Carneiro de Sousa, M.J. (2019) Assessment of individual shedder status and background DNA on objects: Direct or indirect transfer? *Forensic Science International Genetics Supplement Series* 7(1): 622-623.

51. Romero-García, C., Rosell-Herrera, R., Revilla, C.J., Baeza-Richer, C., Gomes, C., Palomo-Díez, S., Arroyo-Pardo, E., López-Parra, A.M. (2019) Effect of the activity in secondary transfer of DNA profiles. *Forensic Science International: Genetics Supplement Series* 7(1): 578-579.

52. Szkuta, B., Ansell, R., Boiso, L., Connolly, E., Kloosterman, A.D., Kokshoorn, B., McKenna, L.G., Steensma, K. and van Oorschot, R.A.H. (2019) Assessment of the transfer, persistence, prevalence and recovery of DNA traces from clothing: An inter-laboratory study on worn upper garments. *Forensic Science International: Genetics* 42: 56-68.

53. Taylor, D., Samie, L., Champod, C. (2019) Using Bayesian networks to track DNA movement through complex transfer scenarios. *Forensic Science International: Genetics* 42: 69-80.

54. Burrill, J., Daniel, B., Frascione, N. (2020) Illuminating touch deposits through cellular characterization of hand rinses and body fluids with nucleic acid fluorescence. *Forensic Science International: Genetics* 46: 102269.

55. Gosch, A., Euteneuer, J., Preuss-Wossner, J., Courts, C. (2020) DNA transfer to firearms in alternative realistic handling scenarios. *Forensic Science International: Genetics* 48: 102355.

56. Samie, L., Taroni, F., Champod, C. (2020) Estimating the quantity of transferred DNA in primary and secondary transfers. *Science & Justice* 60(2): 128-135.

57. Samie, L., Champod, C., Taylor, D., Taroni, F. (2020) The use of Bayesian Networks and simulation methods to identify the variables impacting the value of evidence assessed under activity level propositions in stabbing cases. *Forensic Science International: Genetics* 48: 102334.

## X. Non-Human DNA Testing

**1. Linacre, A., Gusmão, L., Hecht, W., Hellmann, A.P., Mayr, W.R., Parson, W., Prinz, M., Schneider, P.M., Morling, N. (2011) ISFG: recommendations regarding the use of non-human (animal) DNA in forensic genetic investigations. *Forensic Science International: Genetics* 5(5): 501-505.**

2. Budowle, B., Schutzer, S.E., Einseln, A., Kelley, L.C., Walsh, A.C., Smith, J.A., Marrone, B.L., Robertson, J., Campos, J. (2003) Building microbial forensics as a response to bioterrorism. *Science* 301: 1852-1853.

3. Budowle, B., Garofano, P., Hellman, A., Ketchum, M., Kanthaswamy, S., Parson, W., van Haeringen, W., Fain, S., Broad, T. (2005) Recommendations for animal DNA forensic and identity testing. *International Journal of Legal Medicine* 119(5): 295-302.

4. Budowle, B., Murch, R., Chakraborty, R. (2005) Microbial forensics: the next forensic challenge. *International Journal of Legal Medicine* 119(6): 317-330.

5. Menotti-Raymond, M.A., David, V.A., Wachter, L.A., Butler, J.M., O'Brien, S.J. (2005) An STR forensic typing system for genetic individualization of domestic cat (*Felis catus*) samples. *Journal of Forensic Sciences* 50(5): 1061-1070.

6. Ogden, R. (2010) Forensic science, genetics and wildlife biology: getting the right mix for a wildlife DNA forensics lab. *Forensic Science Medicine and Pathology* 6(3): 172-179.

7. Alacs, E.A., Georges, A., FitzSimmons, N.N., Robertson, J. (2010) DNA detective: a review of molecular approaches to wildlife forensics. *Forensic Science Medicine and Pathology* 6(3): 180-194.

8. Linacre, A. and Tobe, S.S. (2011) An overview to the investigative approach to species testing in wildlife forensic science. *Investigative Genetics* 2(1): 2. doi: 10.1186/2041-2223-2-2.

9. Iyengar, A. (2014) Forensic DNA analysis for animal protection and biodiversity conservation: A review. *Journal for Nature Conservation* 22(3): 195-205.

10. Iyengar, A. and Hadi, S. (2014) Use of non-human DNA analysis in forensic science: a mini review. *Medicine, Science, and the Law* 54(1): 41-50.

11. Ogden, R. and Linacre, A. (2015) Wildlife forensic science: A review of genetic geographic origin assignment. *Forensic Science International: Genetics* 18: 152-159.

12. Arenas, M., Pereira, F., Oliveira, M., Pinto, N., Lopes, A.M., Gomes, V., Carracedo, A., Amorim, A. (2017) Forensic genetics and genomics: Much more than just a human affair. *PLoS Genetics* 13(9): e1006960.

13. Oliveira, M. and Amorim, A. (2018) Microbial forensics: new breakthroughs and future prospects. *Applied Microbiology and Biotechnology* 102(24): 10377-10391.

14. Metcalf, J.L. (2019) Estimating the postmortem interval using microbes: Knowledge gaps and a path to technology adoption. *Forensic Science International: Genetics* 38: 211-218.

15. Berger, B., Heinrich, J., Niederstätter, H., Hecht, W., Morf, N., Hellmann, A., Rohleder, U., Schleenbecker, U., Berger, C., Parson, W (The CaDNAP Group) (2019) Forensic characterization and statistical considerations of the CaDNAP 13-STR panel in 1,184 domestic dogs from Germany, Austria, and Switzerland. *Forensic Science International: Genetics* 42: 90-98.

## Y. Method Validation, Quality Control, and Human Factors

**1. Kloosterman, A., Sjerps, M., & Quak, A. (2014) Error rates in forensic DNA analysis: Definition, numbers, impact and communication. *Forensic Science International: Genetics* 12: 77-85.**

2. Budowle, B., Bottrell, M.C., Bunch, S.G., Fram, R., Harrison, D., Meagher, S., Oien, C.T., Peterson, P.E., Seiger, D.P., Smith, M.B., Smrz, M.A., Soltis, G.L., Stacey, R.B. (2009) A perspective on errors, bias, and interpretation in the forensic sciences and direction for continuing advancement. *Journal of Forensic Sciences* 54(4): 798-809.

3. Buckleton, J. (2009) Validation issues around DNA typing of low level DNA. *Forensic Science International: Genetics* 3(4): 255-260.

4. Thompson, W.C. (2009) Painting the target around the matching profile: the Texas sharpshooter fallacy in forensic DNA interpretation. *Law, Probability and Risk* 8(3): 257-276.

5. Dror, I.E and Hampikian G. (2011) Subjectivity and bias in forensic DNA mixture interpretation. *Science and Justice* 51: 204-208.

6. Thompson, W.C. (2011) What role should investigative facts play in the evaluation of scientific evidence? *Australian Journal of Forensic Sciences* 43(2-3): 123-134.

7. Dror, I. E. (2012). Cognitive forensics and experimental research about bias in forensic casework. *Science & Justice* 52(2): 128-130.

8. Kassin, S.M., Dror, I.E., Kukucka, J. (2013) The forensic confirmation bias: Problems, perspectives, and proposed solutions. *Journal of Applied Research in Memory and Cognition* 2(1): 42-52.

9. ENFSI (2014) Guidelines for the single laboratory validation of instrumental and human based methods in forensic science. Available at http://enfsi.eu/wp-content/uploads/2017/06/Guidance-QCC-VAL-002.pdf.

10. Hansson, O., Gill, P., Egeland, T. (2014) STR-validator: an open source platform for validation and process control. *Forensic Science International: Genetics* 13: 154-166.

11. Dror, I.E. (2015) Cognitive neuroscience in forensic science: understanding and utilizing the human element. *Philosophical Transactions of the Royal Society of London Series B, Biological Sciences* 370: 20140255.

12. Bodner, M., Bastisch, I., Butler, J.M., Fimmers, R., Gill, P., Gusmão, L., Morling, N., Phillips, C., Prinz, M., Schneider, P.M., Parson, W. (2016) Recommendations of the DNA Commission of the International Society for Forensic Genetics (ISFG) on quality control of autosomal Short Tandem Repeat allele frequency databasing (STRidER). *Forensic Science International: Genetics* 24:97-102.

13. Peters, K.C., Swaminathan, H., Sheehan, J., Duffy, K.R., Lun, D.S., Grgicak, C.M. (2017) Production of high-fidelity electropherograms results in improved and consistent DNA interpretation: Standardizing the forensic validation process. *Forensic Science International: Genetics* 31: 160-170.

14. Jeanguenat, A.M., Budowle, B., Dror, I.E. (2017) Strengthening forensic DNA decision making through a better understanding of the influence of cognitive bias. *Science & Justice* 57(6): 415-420.

15. Alfonse, L.E., Garrett, A.D., Lun, D.S., Duffy, K.R. and Grgicak, C.M. (2018) A large-scale dataset of single and mixed-source short tandem repeat profiles to inform human identification strategies: PROVEDIt. *Forensic Science International: Genetics* 32: 62-70.

16. Jeanguenat, A.M. and Dror, I.E. (2018) Human factors effecting forensic decision making: workplace stress and well-being. *Journal of Forensic Sciences* 63(1): 258-261.

17. Basset, P. and Castella, V. (2018) Lessons from a study of DNA contaminations from police services and forensic laboratories in Switzerland. *Forensic Science International: Genetics* 33: 147-154.

18. Basset, P. and Castella, V. (2019) Positive impact of DNA contamination minimization procedures taken within the laboratory. *Forensic Science International: Genetics* 38: 232-235.

19. Wickenheiser, R. and Farrell, L. (2020) Collaborative versus traditional method validation approach: Discussion and business case. *Forensic Science International: Synergy* 2: 230-237.

20. Bodner, M. and Parson, W. (2020) The STRidER report on two years of quality control of autosomal STR population datasets. *Genes (Basel)* 11(8): 901.

21. Dror, I.E. (2020) Cognitive and human factors in expert decision making: six fallacies and the eight sources of bias. *Analytical Chemistry* 92(12): 7998-8004.

22. Dror, I.E. (2020) The error in "error rate": why error rates are so needed, yet so elusive. *Journal of Forensic Sciences* 65(4): 1034-1039.

23. FBI (2020) Quality Assurance Standards for Forensic DNA Testing Laboratories. Available at https://www.fbi.gov/file-repository/quality-assurance-standards-for-forensic-dna-testing-laboratories.pdf/view.

## Z. General Forensic Science Topics

1. **National Academy of Sciences, Committee on the Conduct of Science (1989) On being a scientist. *Proceedings of the National Academy of Sciences of the United States of America* 86(23): 9053-9074.**

2. National Research Council, Committee on Identifying the Needs of the Forensic Science Community (2009) *Strengthening Forensic Science in the United States: A Path Forward*. The National Academies Press: Washington DC.

3. Mnookin, J.L., Cole, S.A., Dror, I.E., Fisher, B.A.J., Houck, M.M., Inman, K., Kaye, D.H., Koehler, J.J., Langenburg, G., Risinger, D.M., Rudin, N., Siegel, J., Stoney, D.A. (2010). The need for a research culture in the forensic sciences. *UCLA Law Review* 58: 725-779.

4. Evett, I.W. (2015) The logical foundations of forensic science: Towards reliable knowledge. *Philosophical Transactions of the Royal Society of London Series B, Biological Sciences* 370: 20140263.

5. Butler, J.M. (2015) U.S. initiatives to strengthen forensic science & international standards in forensic DNA. *Forensic Science International: Genetics* 18: 4-20. [open access]

6. Edmond, G., Found, B., Martire, K., Ballantyne, K., Hamer, D., Searston, R., Thompson, M., Cunliffe, E., Kemp, R., San Roque, M., Tangen, J., Dioso-Villa, R., Ligertwood, A., Hibbert, D., White, D., Ribeiro, G., Porter, G., Towler, A. and Roberts, A. (2016) Model forensic science. *Australian Journal of Forensic Science* 48(5): 496-537.

7. President's Council of Advisors on Science and Technology (PCAST) (2016) Report to the President - Forensic Science in Criminal Courts: Ensuring Scientific Validity of Feature-Comparison Methods. Issued September 2016 with an addendum in January 2017. Available at https://obamawhitehouse.archives.gov/sites/default/files/microsites/ostp/PCAST/pcast_forensic_science_report_final.pdf and https://obamawhitehouse.archives.gov/sites/default/files/microsites/ostp/PCAST/pcast_forensics_addendum_finalv2.pdf.

8. Butler, J.M. (2017) Recent activities in the United States involving the National Commission on Forensic Science and the Organization of Scientific Area Committees for Forensic Science. *Australian Journal of Forensic Sciences* 49(5): 526-540 [open access]

9. National Commission on Forensic Science (2017) *Reflecting Back – Looking Toward the Future.* Available at https://www.justice.gov/archives/ncfs/page/file/959356/download.

10. Evett, I.W., Berger, C.E.H., Buckleton, J.S., Champod, C., Jackson, G. (2017) Finding the way forward for forensic science in the US-A commentary on the PCAST report. *Forensic Science International* 278: 16-23.

11. Edmond, G., Towler, A., Growns, B., Ribeiro, G., Found, B., White, D., Ballantyne, K., Searston, R.A., Thompson, M.B., Tangen, J.M., Kemp, R.I. and Martire, K. (2017) Thinking forensics: cognitive science for forensic practitioners. *Science & Justice* 57: 144-154.

American Academy of Forensic Sciences
VIRTUAL WORKSHOP W19 (MVPs of Forensic DNA)
February 16, 2021

2021 AAFS VIRTUAL MEETING

# MVPs of Forensic DNA:
## Examining the Most Valuable Publications in the Field

Chair
John M. Butler
NIST FORENSIC SCIENCE RESEARCH. STANDARDS. FOUNDATIONS.

Co-Chair
Robin W. Cotton
BOSTON UNIVERSITY

Mechthild K. Prinz
JOHN JAY

Charlotte J. Word

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## Workshop Purposes

1. **Review principles and practices** underlying DNA analysis and interpretation
2. **Examine core foundational literature** supporting these practices
3. **Provide information to strengthen training programs** for DNA analysts

***We plan to review key publications covering forensic DNA analysis and interpretation***

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## The Ultimate Goal

**Creation of a defined body of knowledge** covering historical and foundational literature ***that qualified DNA analysts should know and understand***

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American Academy of Forensic Sciences
VIRTUAL WORKSHOP W19 (MVPs of Forensic DNA)
February 16, 2021

2021 AAFS VIRTUAL MEETING

# Introduction to Workshop and Criteria for Developing a Literature List

**John M. Butler, PhD**
*National Institute of Standards and Technology*

NIST FORENSIC SCIENCE
RESEARCH. STANDARDS. FOUNDATIONS.

**Module 1**

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## Presentation Outline

- Motivations for doing this workshop
  - OSAC task group request (Sept 2020)
  - Foundational review on DNA mixture interpretation (2017-present)
  - New SWGDAM Training Guidelines (July 2020)
  - INTERPOL DNA Review (2016-2019)
- Why these presenters
  - Robin (ISHI 2000 talk), Mecki (ISFG), Charlotte (reviews for FSIG & JFS)
- Workshop topics and schedule
- Some thoughts on how to read an article
- Creation of our literature list
  - How categories are organized
  - List is "informative" rather than "foundational"

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## Motivation for Doing This MVPs (Best Papers) Workshop

1. In the past year, I was asked to review potential reference lists for an **OSAC task group** and training guidelines for the ENFSI DNA Working Group
   - Desire to help forensic DNA analysts and technical leaders strengthen their training
   - Training requirements exist, but often specific helpful sources are not identified
2. Examination of the literature while conducting a **NIST scientific foundation review** covering DNA mixture interpretation
   - Increased familiarity with what is currently available in the literature
   - Desire to improve information available for review when seeking to assess the degree of reliability of probabilistic genotyping software systems as defense challenges and admissibility hearings have increased in recent years
3. Analysis of the new **July 2020 SWGDAM Training Guidelines**
   - Recognizing the value of a standard body of knowledge to assist in training
   - **Can we define what are the best sources to learn from and why?**

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## What Has Inspired Me Personally Over the Years

1. An ISHI 2000 Presentation by Robin Cotton
   - **From the Scientist's Point of View: What Constitutes General Acceptance?**
     - See https://promega.media/-/media/files/resources/conference-proceedings/ishi-11/oral-presentations/cotton.pdf?la=en
   - Her sections and reference lists cover by category the loci, features of the PCR and forensic applications, and electrophoresis and fluorescent detection
   - ***This was the first effort I had seen to try and document from the literature why we know specific things in our field***
2. Writing my textbooks on *Forensic DNA Typing* (2001, 2005, 2010, 2012, 2015) and trying to find the best citations to reference for each topic
3. PCAST Report and the Reference List Gathered
   - There are 294 references listed (but my analysis found only ~75 relevant to DNA mixture interpretation and no helpful subcategories); see https://obamawhitehouse.archives.gov/sites/default/files/microsites/ostp/PCAST/pcast_forensics_references.pdf

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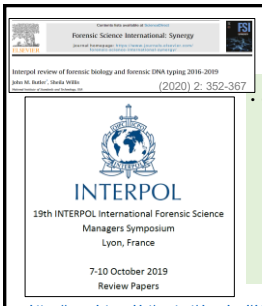
Forensic Science International: Synergy

Interpol review of forensic biology and forensic DNA typing 2016-2019

(2020) 2: 352-367

INTERPOL

19th INTERPOL International Forensic Science Managers Symposium
Lyon, France

7-10 October 2019
Review Papers

## My Experience with the Recent INTERPOL DNA Review

I was asked to report on publications from 2016 to 2019

- Category selection and article selection:
  1. Core Loci Expansion
  2. Rapid Analysis of STR Markers
  3. Investigative Genetic Genealogy
  4. Next-Generation Sequencing
  5. DNA Mixture Interpretation and Probabilistic Genotyping Software
  6. DNA Transfer and Activity Level Evaluations
  7. Forensic Biology and Body Fluid Identification
  8. DNA Phenotyping
  9. Privacy and Ethical Issues
  10. Guidance Documents (SWGDAM, OSAC, ASB, ENFSI, UK FS Regulator)
  11. Contamination Avoidance and DNA Success Rates
  12. Recent Special Issues and Review Articles of Note

https://www.interpol.int/content/download/14458/file/Interpol%20Review%20Papers%202019.pdf

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## Background and Qualification of Presenters

- **John M. Butler, PhD**:
  - Author of five textbooks (2001, 2005, 2010, 2012, 2015) and >180 research articles
  - Conducted dozens of workshops and served as an editor for the top journal
- **Robin W. Cotton, PhD**:
  - Professor at Boston University
  - Former Cellmark Laboratory Director
- **Mechthild K. "Mecki" Prinz, PhD**:
  - Professor at John Jay College
  - Former NYC OCME Forensic Biology Laboratory Director
- **Charlotte J. Word, PhD**:
  - Consultant, OSAC, ASB, regular reviewer for multiple journals
  - Former Cellmark Laboratory Director

**Collectively we have >120 years of experience in forensic DNA and have taught and written extensively on the subject**

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## Planned Workshop Schedule (1)

| | Time (Central) | Topic *MVPs = Most Valuable Publications* | Presenter(s) |
|---|---|---|---|
| 1 | 1:00pm (15 minutes) | **Introduction to Workshop** and Criteria for **Developing a Literature List** | John Butler |
| 2 | 1:15pm (15 minutes) | The **Value of a Knowledge Base** for Educating Students and Practitioners | Robin Cotton |
| 3 | 1:30pm (15 minutes) | A Review of **Training Standards** | Charlotte Word |
| 4 | 1:45pm (15 minutes) | MVPs on **DNA Collection, Extraction, and Quantitation** | Robin Cotton |
| 5 | 2:00pm (15 minutes) | MVPs on **PCR, STRs, and CE** | John Butler |
| 6 | 2:15pm (15 minutes) | MVPs on **Population Genetics and Statistical Analysis** | Robin Cotton |
| | 2:30pm (15 minutes) | **Question and Answers** (live Zoom meeting) | All |

2:45pm to 2:55pm 10-minute BREAK

**Please put questions in the chat box** while watching our pre-recorded presentations and we will answer them over the chat or through a live Zoom session at the end

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## Planned Workshop Schedule (2)

| | Time (Central) | Topic *MVPs = Most Valuable Publications* | Presenter(s) |
|---|---|---|---|
| 7 | 2:55pm (15 minutes) | MVPs on **Binary Approaches to Mixture Interpretation** | Mecki Prinz |
| 8 | 3:10pm (15 minutes) | MVPs on **Probabilistic Genotyping Systems** | John Butler |
| 9 | 3:25pm (15 minutes) | MVPs on **DNA Transfer and Activity Level Propositions** | John Butler |
| 10 | 3:40pm (15 minutes) | MVPs on **Lineage Markers** | Robin Cotton |
| 11 | 3:55pm (15 minutes) | MVPs on **Phenotyping and New Technologies** | Mecki Prinz |
| 12 | 4:10pm (15 minutes) | MVPs on **Method Validation, Quality Control, and Human Factors** | John Butler |
| 13 | 4:25pm (15 minutes) | Wrap-up and Workshop Conclusion | John Butler |
| | 4:40pm (20 minutes) | **Question and Answers** (live Zoom meeting) | All |

**Please put questions in the chat box** while watching our pre-recorded presentations and we will answer them over the chat or through a live Zoom session at the end

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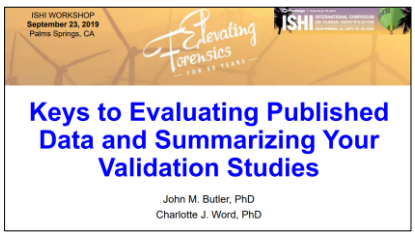
## How to Read an Article
### Discussed in this ISHI 2019 Workshop




https://strbase.nist.gov/pub_pres/ISHI2019workshop-EvaluatingPublishedData.pdf

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## Different Types of Articles

- **Original research articles**
- **Review articles**
- Short communications (termed "technical notes" in *JFS*)
- Book reviews
- Case studies (termed "case reports" in *JFS*)
- Opinion or commentary
- Letters to the Editor
  - typically correcting or commenting on a previous publication
- With *FSI Genetics*: Forensic population genetics (original paper, short communication, or correspondence)

**Different journals can have different categories and/or required structures for manuscript submission**

https://www.elsevier.com/journals/forensic-science-international-genetics/1872-4973/guide-for-authors

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## The "IMRAD" Format to Scientific Articles

- **I**ntroduction – what question is being studied?
- **M**ethods (& Materials) – how study was performed?
- **R**esults – what were the findings in the study?
- **A**nd
- **D**iscussion – what do these findings mean?

- **The first scientific journals appeared in 1665** but early articles were descriptive in nature
- The IMRAD approach began to be used in the mid-20th century to focus articles and to make indexing and reviewing easier
- **IMRAD was formally defined in 1979** by the American National Standards Institute (ANSI Z39.16-1979) "American National Standard for the Preparation of Scientific Papers for Written or Oral Presentation"

From Day, R.A. (1998). *How to Write & Publish a Scientific Paper, 5th edition*. Oryx Press: Phoenix, Arizona.

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## How to Read a Scientific Article

- Skim the article first
  - Start with title and abstract (may consider authors as well)
  - Scan tables, figures and figure captions

John Butler's perspective and not a formal standard!

- Examine results and conclusions
  - **Do the data presented support the statements made?**
- Do not worry about trying to comprehend the entire article at first
  - Most articles will be skimmed rather than read from start to finish
  - **Many articles are never read in detail**
- **Highlight key points and make notes on the paper** itself so you can go back to them later to refresh your memory

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## Reference List Provided with Slide Handouts
### *497 References Across 26 Topics (A-to-Z)*

**Informative Forensic DNA Reviews and Research Studies (A-to-Z)**

***In our reference list, 26 categories are defined covering topics of interest in forensic DNA analysis and interpretation (listed arbitrarily from A to Z).***

*Neither the categories nor this reference list are intended to be exhaustive. Suggestions for additional, appropriate references and categories are welcome.*

*A #1 article (in bold font) was subjectively selected in each category and then followed by reference citations defined by date in ascending order with the most recent publications at the end of each category. This letter and number system (e.g., A1, B3, F7) provides a simple method to locate specific articles and enables opportunities for expansion as the literature grows. Although some articles could logically appear under multiple categories, no duplicate listings were used. Recommended references from the SWGDAM 2020 Training Guidelines have been included as well.*

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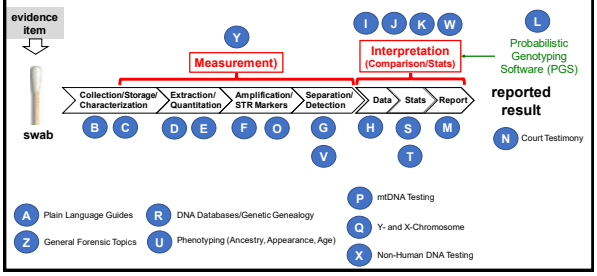
## Reference List Provided with Slide Handouts
### *497 References Across 26 Topics (A-to-Z)*
(480) Articles + (17) Informative Textbooks

**A (4)** Plain Language Guides to Forensic DNA Analysis
**B (24)** Serology and Body Fluid Identification
**C (25)** Collection and Storage of Biological Material — Module 4
**D (18)** DNA Extraction/Purification, Differential Extraction
**E (10)** DNA Quantitation, Degraded DNA
**F (13)** PCR Amplification, Inhibition, and Artifacts — Module 5
**G (12)** Capillary Electrophoresis Separation and Detection
**H (7)** Assessing Sample Suitability and Complexity, Low-Template DNA
**I (12)** Estimating the Number of Contributors
**J (12)** Data Interpretation, Mixture Deconvolution, Interlaboratory Studies
**K (11)** Interpretation: Binary Approaches (CPI, RMP, LR) — Module 7
**L (44)** Interpretation: Probabilistic Genotyping Software (Discrete, Continuous)
**M (8)** Report Writing and Technical Review — Module 8
**N (22)** Court Testimony, Communication of Results, Juror Comprehension Studies
**O (29)** Autosomal STR Markers and Kits — Module 5
**P (11)** Mitochondrial DNA Testing — Module 10
**Q (17)** Y-Chromosome and X-Chromosome Testing
**R (14)** DNA Databases and Investigative Genetic Genealogy
**S (11)** Statistical Analysis — Module 6
**T (11)** Population Genetics
**U (24)** DNA Phenotyping (Ancestry, Appearance, Age) — Module 11
**V (35)** New Technologies (Rapid DNA, Massively Parallel Sequencing)
**W (57)** DNA Transfer and Activity Level Reporting
**X (15)** Non-Human DNA Testing — Module 9
**Y (23)** Method Validation, Quality Control, Human Factors
**Z (11)** General Forensic Science Topics — Module 12

Module 2 Value of a Knowledge Base  Module 3 Training Standards  Module 13 Wrap-Up

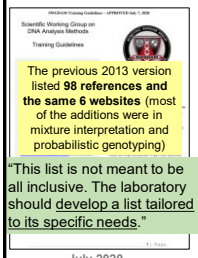
17

## Mapping Our A-to-Z Reference Categories onto the DNA Testing Process




18

## New SWGDAM Training Guidelines (July 2020)




The previous 2013 version listed **98 references and the same 6 websites** (most of the additions were in mixture interpretation and probabilistic genotyping)

"This list is not meant to be all inclusive. The laboratory should develop a list tailored to its specific needs."

**Recommended References** (**129** + 6 websites)

*The following resources may be helpful to the trainer in defining the breadth and scope of the materials for the trainee's reading. This list is not meant to be all inclusive. The laboratory should develop a list tailored to its specific needs.*

1. General Forensic DNA and Autosomal STRs (**42**)
2. Mixture Interpretation/Population Genetics/ Probabilistic Genotyping/Statistics (**40**)
3. Mitochondrial DNA (**37**)
   - General Mitochondrial DNA Information (**6**)
   - Heteroplasmy (**15**)
   - Maternal Inheritance (**1**)
   - Population Studies (**1**)
4. Y STRs (**10**)
5. Informational Websites (**6**)

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## Origins of Our Literature List

- On September 10, 2020, Phil Danielson (University of Denver), representing a team of seven OSAC members compiling foundational literature, reached out to me and shared their list for my input (it had 105 references + possible additions) :
  - **5 "foundational" textbooks**,
  - **41 "foundational" reviews** (subtopics: field of forensic sciences in general, serology, collection and storage of biological material, epigenetics, DNA quantification, PCR process, trace/touch type DNA, advanced and emerging DNA profiling technologies, mitochondrial DNA haplotyping, DNA profile interpretation, presenting forensic DNA in the courtroom, and non-human DNA analysis)
  - **59 salient research studies** (subtopics: serology, human factors, DNA extraction/purification, DNA quantification, DNA profiling and validation, mtDNA haplotyping, probabilistic genotyping, presenting DNA in the courtroom, and validation software)

*I examined these references along with those in the SWGDAM 2020 Training Guidelines, created a more comprehensive set of categories (from A-to-Z), added many new references, created uniform reference formatting, and changed the titles to "informative textbooks" and "informative forensic DNA reviews and research studies" – this updated information was returned to Phil Danielson* ***on September 24, 2020***

20

## Additional Input to Our MVP Reference List

- **Discussion with fellow presenters as presentations developed**
  - Mecki Prinz, Robin Cotton, Charlotte Word
- Examination of updated OSAC 10-26-2020 list
  - Included additional PGS, DNA transfer, and non-human DNA articles
- Feedback from Other Practitioners and Educators
  - **Amy Brodeur** (Boston University) – serology & body fluid ID, collection & storage
  - **Teresa Cheromcha** (Colorado Bureau of Investigation) – DNA transfer

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## Plan for Examining MVPs in This Workshop

1. Discuss important principles involved with the category topic (e.g., DNA extraction or PCR amplification)
2. In each examined category, briefly review the number and types of articles in our reference list and number of times cited in Google Scholar
3. Focus on one or a few specific articles and the findings reported
4. Summarize and review key takeaways

22

## Acknowledgments and Disclaimers

*Thank you to SWGDAM and Phil Danielson with the OSAC Literature Task Group for their starting materials in developing these MVPs (most valuable publications)*

**Points of view are those of each presenter** and do not necessarily represent the official position or policies of the National Institute of Standards and Technology.

**Identification does not imply endorsement**
Certain commercial entities are identified in order to specify experimental procedures as completely as possible. In no case does such identification imply a recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that any of the entities identified are necessarily the best available for the purpose.

23

## Thank you for your attention!

**John M. Butler**
john.butler@nist.gov

**Robin W. Cotton**
rwcotton@bu.edu

**Mechthild "Mecki" Prinz**
mprinz@jjay.cuny.edu

**Charlotte J. Word**
cjword@comcast.net

**Please put questions in the chat box** while watching our pre-recorded presentations and we will answer them over the chat or through a live Zoom session at the end – you can also email any of us…

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American Academy of Forensic Sciences
VIRTUAL WORKSHOP W19 (MVPs of Forensic DNA)
February 16, 2021

2021 AAFS VIRTUAL MEETING

# Value of a Knowledge Base for Educating Students and Practitioners

**Robin W. Cotton, PhD**
*Boston University Biomedical Forensic Sciences*

BOSTON UNIVERSITY

**Module 2**

1

## Presentation Outline

- What is the value to you and your laboratory of keeping current by reading the scientific literature?

1. There are requirements to read the literature.
2. Expectations of Students vs Analysts
3. The literature provides the basis of protocol development (in the past and now)
4. Change happens and is part of the scientific process
5. Comments from students who are now in the lab
6. Access to the scientific literature may not be easy
7. Reading takes time

2

## *Requirement for Reading the Literature*

from the FBI DNA Quality Assurance Standards (2020)

**STANDARD 16.1** The laboratory shall have and follow a program to ensure technical qualifications are maintained through **participation in continuing education.**

**16.1.1** …analyst(s)…shall stay abreast of topics relevant to the field of forensic DNA analysis **by attending seminars…in relevant subject areas for a minimum of eight (8) cumulative hours each calendar year.**

**16.1.2** The laboratory shall have and follow a program approved by the technical leader for the annual review of scientific literature that documents the analysts' **ongoing reading of scientific literature.**

**16.1.2.1** The laboratory shall maintain or have physical or electronic access to a collection of current books, reviewed journals, or other literature applicable to DNA analysis.

**Current QAS (2020)** – available on FBI website (approved January 11, 2018): https://www.fbi.gov/file-repository/quality-assurance-standards-for-forensic-dna-testing-laboratories.pdf/view

3

## Student *vs* Analyst

- Students initially learn from textbooks; literature is added in. Goal is to understand the basic principles that underpin forensic DNA analysis.
  - Refer to "THE List"
- In lab classes students have limited "hands on" exposure to the techniques used in DNA analysis
- The literature basis needed for students is exceeded by "THE List" assembled for this workshop.
- Students doing a thesis project for an MS degree are reading more deeply in one or two areas related to their project and..
- Are getting extensive exposure to some DNA analysis procedures.
- They will begin to appreciate "THE List".

4

## Student *vs* Analyst

- The Analyst's learning process begins where the student's learning process ended and, in the best circumstances, continues throughout the Analyst's career
- Analyst training begins with additional reading and hands-on practice.
  - Will gradually be responsible for many procedures
  - Will be responsible for making protocol choices to maximize the chance of obtaining results (one reason to further appreciate "THE List"
- Post training, an analyst will make independent decisions regarding methods and data interpretation.
- Post training an analyst will present data in court.
  - **NOTE**…"THE List " is looking useful.

5

- Academic training in:
  - Biochemistry & molecular biology & cell biology
    - DNA and protein structure
    - Enzymes
      - Nucleases, polymerases, proteases
    - Cell structure
      - Body fluid cell types and their special characteristics
      - Including: epithelial cells, sperm cells and other cell types
  - Genetics
    - Mendelian inheritance, Chromosome structure (Autosomes, X & Y), Polymorphic loci,
  - Population genetics.
    - Allele, genotype and DNA profile frequencies, Haplotype inheritance
  - Statistics and Probability
    - Measured DNA profile metrics, stutter, PH, PHR, mixture proportion probability of drop-out,
    - Analytical and other thresholds
    - Methods for DNA profile evaluation, Likelihood ratio formulation and calculation, probabilistic genotyping methods and reporting

**In DNA analysis: where does our basic knowledge and our protocols come from?**

6

Analyst training provides - Information on the use of simple and complex instrumentation & software used in the laboratory.

- Common Equipment Includes:
  - ph meter
  - Bio-hazzard hood and PPE
  - Centrifuges, pipettes and other small equipment
  - DNA extraction sample handling robot and associated software
  - qPCR instrument and associated software
  - Capillary electrophoresis instrument and associated software
  - GeneMapper or similar software
  - STRmix, TrueAllele, DNA Mixture Solution or similar software

7

For the DNA analyst the documents below are added to the list of journal articles that becomes the "literature" basis for procedures and analysis:

- Current journal articles related to laboratory procedures
- Laboratory protocols
- Equipment and software manuals
- Safety procedures
- QA/QC procedures
- Information about sample contamination
- Other training information

8

## What makes you a forensic "*scientist*"

**Definition of "scientist":**

An **expert** who **studies** or **works** in one of the **sciences**

https://dictionary.cambridge.org/us/dictionary/english/scientist

A person learned in **science** and especially natural science **:** a **scientific** investigator

https://www.merriam-webster.com/dictionary/scientist

Some hallmarks of a scientist:

Curiosity

Continuous learning

Seeing the need for change as scientific processes and understanding changes

National Academy of Sciences, Committee on the Conduct of Science (1989) On being a scientist. *PNAS of the United States of America* 86(23): 9053-9074.

9

When did DNA testing begin in the US?

1987-1988

Are we doing the same thing we did in 1988

NO

If you do not read and remain interested in the scientific literature, will you get left behind?

YES

10

Therefore, informed change is critical.

11

**In talking to students who have recently become DNA analysts:**

- They understand the importance of protocols.
- Discussions underscored the **different requirement's** for reading during training in different laboratories.
  - Some training uses mainly textbooks for required reading
  - Other incorporate more journal articles
- New trainees are using, for as long as possible, their University library facilities.
- They are surprised by the lack of access to scientific journals.
  - **Some have paid, on their own, through AAFS**
  - Some are not members of AAFS

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## Challenges the Forensic DNA Community Faces with Continuing Education

- **QAS requirement for continuing education are only a start**
  - Minimum of eight (8) hours per year for seminars and **one** (1) or more articles to read will not cover much ground
  - **How does anyone know if you learned anything since there is no assessment of what was learned?**
  - **For example, which articles are essential for you to understand and will expand your expertise in DNA mixture interpretation?**
- **Rapid and continuous evolution of the field**
  - New STR kits, new CE instruments, new software, new potential approaches for analysis (e.g., NGS) and interpretation (e.g., probabilistic genotyping software)
  - **There are lots of articles to chose from based on interest or need…**
- **Numerous articles are being published each year**
  - **Which articles should you choose to study?**

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## Open access to scientific information:

- Via authors' permission (i.e., authors' payment)
- Open access journals
  - PLOS One; https://journals.plos.org/plosone/
  - Peer J; https://peerj.com/
- Other open access journals or articles are "noted" when looking at the results of a search:
  - Can be identified using PubMed; https://www.ncbi.nlm.nih.gov/pmc/
  - Google Scholar

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## Opportunities to Get Electronic Access to Journals

ISFG membership includes free access to the print and online editions of Forensic Science International: Genetics. Please log in to read and download articles via the section reserved for members. ISFG members have also access to the workshop presentations and lectures of invited speakers at the most recent ISFG congresses.

FSI GENETICS

https://www.aafs.org/

ISFG International Society for Forensic Genetics

https://www.isfg.org/
**60 euros (~$72.50/year)**

ELSEVIER

**#1 Journal on Forensic DNA**


**Elsevier Forensics Package ($133/year)** includes electronic access to AAFS members (**$165/year**):

- *Forensic Science International*
- *Forensic Science International: Genetics*
- *Journal of Forensic and Legal Medicine and Legal Medicine*
- *Legal Medicine*
- *Science & Justice*

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American Academy of Forensic Sciences
VIRTUAL WORKSHOP W19 (MVPs of Forensic DNA)
February 16, 2021

2021 AAFS VIRTUAL MEETING

# A Review of Training Standards

**Charlotte J. Word, PhD**
*Independent Consultant*

**Module 3**

1

## Presentation Outline

- Existing Standards for Training for Forensic DNA Testing
- Brief Overview of OSAC and ASB Process
- Training Standards Being Drafted and Developed

OSAC Organization of Scientific Area Committees for Forensic Science

ASB AAFS Standards Board

2

Quality Assurance Standards Documents

## Existing Standards

https://www.swgdam.org/publications

**2020 Quality Assurance Standards**

| | |
|---|---|
| The Quality Assurance Standards for DNA Databasing Laboratories, Effective July 1, 2020 | 6. TRAINING 6.1-6.11; ~2 pages<br>16. Professional Development 16.1-16.2; 1 page |
| The Quality Assurance Standards for Forensic DNA Testing Laboratories, Effective July 1, 2020 | 6. TRAINING 6.1-6.13; ~2.5 pages<br>16. Professional Development 16.1-16.2; 1 page |
| The FBI Quality Assurance Standards Audit for DNA Databasing Laboratories, Effective July 1, 2020 | |
| The FBI Quality Assurance Standards Audit for Forensic DNA Testing Laboratories, Effective July 1, 2020 | |
| The Guidance Document for the FBI Quality Assurance Standards for Forensic DNA Testing and DNA Databasing Laboratories, Effective 07/01/2020 | 6. TRAINING 6.1-6.13; ~6 pages<br>16. Professional Development 16.1-16.2; ~2 pages |

3

SWGDAM Training Guidelines – APPROVED July 7, 2020

Scientific Working Group on
DNA Analysis Methods

Training Guidelines

https://www.swgdam.org/

4

https://www.iso.org/standard/66912.html

ICS › 03 › 03.120 › 03.120.20

## ISO/IEC 17025:2017

**General requirements for the competence of testing and calibration laboratories**

- A new section has been added introducing the concept of **risk-based thinking** and describes the commonalities with the new version of ISO 9001:2015, *Quality management systems – Requirements.*

"Training" only used 3 times

5

Designation: E2917 – 19a

https://www.astm.org/Standards/E2917.htm

**Standard Practice for Forensic Science Practitioner Training, Continuing Education, and Professional Development Programs**[1]

This standard is issued under the fixed designation E2917; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

INTRODUCTION

Some material in this practice is based on the Technical Working Group for Education and Training in Forensic Science, National Institute of Justice (TWGED, NIJ), Special Report, *Education and Training in Forensic Science: A Guide for Forensic Science Laboratories, Educational Institutions, and Students* (1).[2]

Revisions being considered as new annexes for Crime Scene Investigation & Seized Drugs

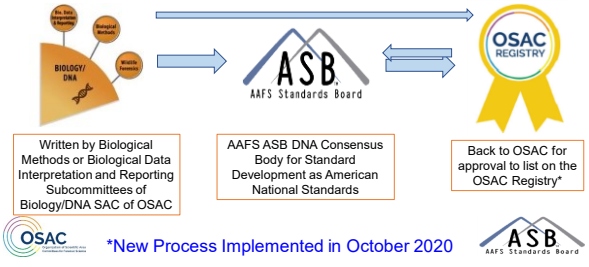
6

## General Requirements

- Documented training program
- Education and training in procedures for all steps of process, evaluation of data, reporting, testimony
- Practical exercises
- Demonstrate skills and knowledge
- Oral communication skills with mock trial
- Competency
- Modifications to training – based on previous vs. re-training
- Documentation of training with record retention

7

## Training Standards History




Written by Biological Methods or Biological Data Interpretation and Reporting Subcommittees of Biology/DNA SAC of OSAC

AAFS ASB DNA Consensus Body for Standard Development as American National Standards

Back to OSAC for approval to list on the OSAC Registry*

OSAC

*New Process Implemented in October 2020

ASB AAFS Standards Board

8

## ANSI/ASB Published Standards for Forensic Training

ASB AAFS Standards Board

5 published; more coming!

**ANSI/ASB Standard 022, *Standard for Forensic DNA Analysis Training Programs*, First Edition, 2019**
This standard provides the general requirements for a forensic DNA laboratory's training program in DNA analysis including data interpretation.

**ANSI/ASB Standard 023, *Standard for Training in Forensic DNA Isolation and Purification Methods*, First Edition, 2020**
This document provides requirements to ensure proper training in the methods of DNA isolation and purification used within the trainee's forensic DNA laboratory.

**ANSI/ASB Standard 110, *Standards for Training in Forensic Serological Methods*, First Edition, 2020**
This standard provides the general requirements for a forensic serology training program to evaluate body fluids, stains, or residues related to forensic investigations. This standard does not address training in forensic DNA analysis procedures.

**ANSI/ASB Standard 115, *Standard for Training in Forensic Short Tandem Repeat Typing Methods using Amplification, DNA Separation, and Allele Detection*, First Edition, 2020**
This standard provides the requirements of a forensic DNA laboratory's training program in forensic Short Tandem Repeat typing methods using amplification, DNA separation and allele detection.

**ANSI/ASB Standard 116, *Standard for Training in Forensic DNA Quantification Methods*, First Edition, 2020**
This standard provides the requirements for a forensic DNA laboratory's training program in DNA quantification.

http://www.asbstandardsboard.org/published-documents/dna-published-documents/

9

**OSAC Registry**

- Organizational Structure +
- Governing Documents
- OSAC Registry –
  - Standards Under Consideration
  - Standards Open for Comment
  - Public Documents
- OSAC Registry Implementation

https://www.nist.gov/osac/osac-registry

10

**ASB Published Standard & on OSAC Registry**

[ANSI/ASB Standard 022 Standard for Forensic DNA Analysis Training Programs, First Edition, 2019]

**This standard provides the general requirements for a forensic DNA laboratory's training program in DNA analysis including data interpretation.**

An "umbrella" normative standard required for meeting the requirements in the other training standards.

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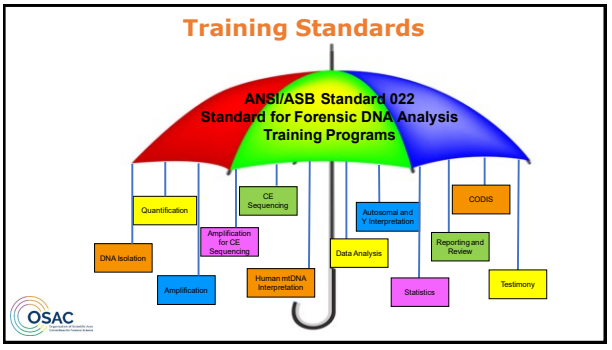
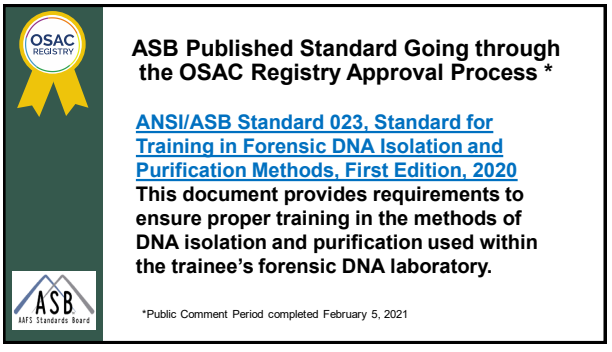
**ANSI/ASB Standard 022 Standard for Forensic DNA Analysis Training Programs, First Edition, 2019**

- Personnel
  - All laboratory personnel shall have a successfully completed training prior to…
  - Training coordinator; Trainee with experience; New methods
- Training Program
  - Content of the Training Program – General; Quality; Safety; Lectures & Exercises in all steps; Bias; Ethics; Limitations
  - Documentation; Administration; Revisions; New methods; Re-training
- Competency Testing
  - General
  - Required – oral, written
  - Administration; Assessment; Re-testing
  - Documentation

12


Training Standards
ANSI/ASB Standard 022
Standard for Forensic DNA Analysis
Training Programs
Quantification
CE Sequencing
CODIS
Autosomal and Y Interpretation
Amplification for CE Sequencing
Reporting and Review
DNA Isolation
Data Analysis
Human mtDNA Interpretation
Statistics
Testimony
Amplification
OSAC

13


OSAC REGISTRY
ASB
AAFS Standards Board
ASB Published Standard Going through the OSAC Registry Approval Process *
ANSI/ASB Standard 023, Standard for Training in Forensic DNA Isolation and Purification Methods, First Edition, 2020
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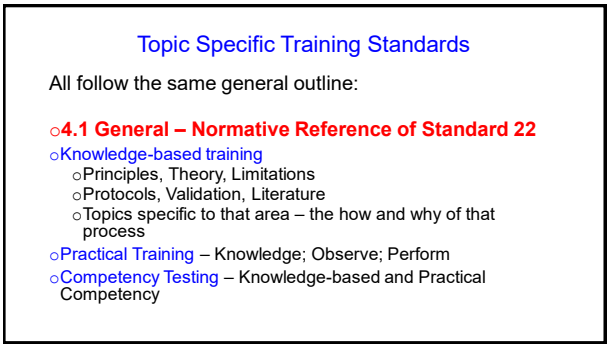
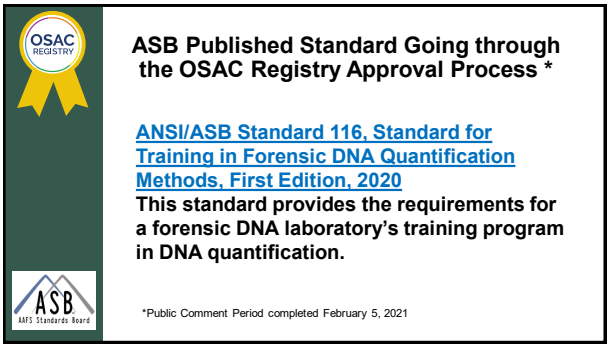
*Public Comment Period completed February 5, 2021

14


Topic Specific Training Standards
All follow the same general outline:
4.1 General – Normative Reference of Standard 22
Knowledge-based training
Principles, Theory, Limitations
Protocols, Validation, Literature
Topics specific to that area – the how and why of that process
Practical Training – Knowledge; Observe; Perform
Competency Testing – Knowledge-based and Practical Competency

15


OSAC REGISTRY
ASB Published Standard Going through the OSAC Registry Approval Process *
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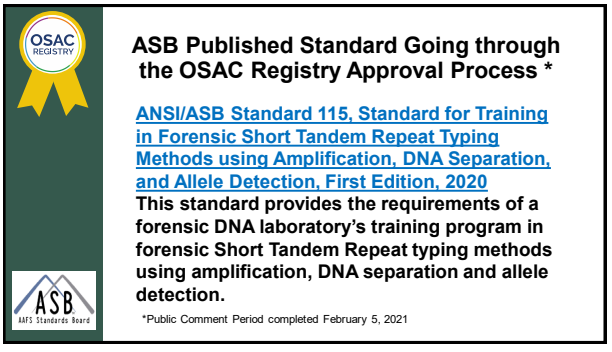
*Public Comment Period completed February 5, 2021
ASB
AAFS Standards Board

16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ANSI/ASB Standard 115, Standard for Training in Forensic Short Tandem Repeat Typing Methods using Amplification, DNA Separation, and Allele Detection, First Edition, 2020
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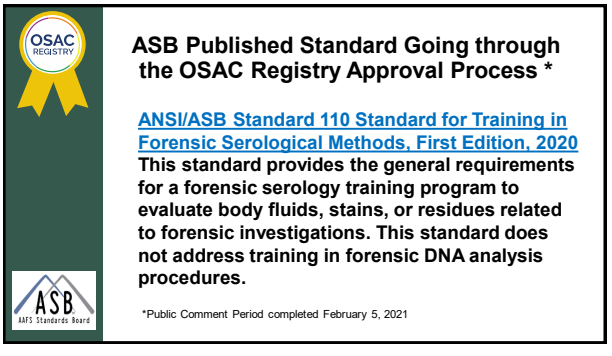
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*Public Comment Period completed February 5, 2021
ASB
AAFS Standards Board

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## Training Standards In Progress

- *Standard 91 – Standard for Training of Analysis of Forensic STR Data*
- *Standard 78 – Standard for Training of Forensic Autosomal and Y STR Data Interpretation*
- *Standard 81 – Standard for Training in the Use of Statistics in Interpretation of Forensic DNA Evidence*
- *Standard 80 – Standard for Training of Forensic DNA Reporting and Review*
- *Standard 154 – Standard for Training of Courtroom Testimony for Forensic DNA Analysis*
- *Standard 79 – Standard for Training of CODIS*
- *Standard 140 – Mitochondrial DNA Analysis, Interpretation, Statistical Evaluation, and Reporting*
- *Standard 130 – Standard for Training in Forensic DNA Amplification Methods for Capillary Electrophoresis Sequencing*
- *Standard 131 – Standard for Training in Forensic DNA Sequencing Using Capillary Electrophoresis Sequencing*

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## Biology/DNA Standards and Best Practices Developed by OSAC & ASB Webinar Series

Promega

#1 - Publication of New Standards and Best Practices – The Process – July 15, 2020
Presenters: John Paul Jones, Robyn Ragsdale and Teresa Ambrosius

#2 - Mixture Interpretation Validation, and Protocol Development and Verification – August 1, 2020
Presenters: Charlotte Word and Joanna Johnson

**#3 - Training Standards Overview** – September 9, 2020
**Presenters: Kim Murga and Beth Ordeman**

#4 – Validation of Probabilistic Genotyping Systems – January 20, 2021
Presenters: Brian Higgins and Joel Sutton

OSAC **Available On Demand at: www.promega.com/webinars** ASB AAFS Standards Board

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## How To Participate

1) Help draft documents – apply to join
- OSAC Human Forensic Biology Subcommittee (2020)
  - https://www.nist.gov/topics/organization-scientific-area-committees-forensic-science/apply-join-osac
- ASB DNA Consensus Body
  - http://www.asbstandardsboard.org/asb-standards/

2) Review and submit comments on document

Download document and comment template
- ASB Standard Development
  - http://www.asbstandardsboard.org/notice-of-standard-development-and-coordination/
- OSAC Registry
  - https://www.nist.gov/topics/organization-scientific-area-committees-forensic-science/standards-open-comment

3) Implement Standards in your laboratory

ASB AAFS Standards Board

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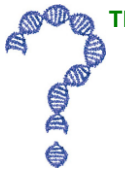

**Thank you for your attention!**

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john.butler@nist.gov

**Robin W. Cotton**
rwcotton@bu.edu

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mprinz@jjay.cuny.edu

**Charlotte J. Word**
cjword@comcast.net

Thank You to:
OSAC
ASB
Commenters
Promega

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American Academy of Forensic Sciences
VIRTUAL WORKSHOP W19 (MVPs of Forensic DNA)
February 16, 2021

2021 AAFS VIRTUAL MEETING

*MVPs = Most Valuable Publications*

# MVPs on DNA Collection, Extraction, and Quantitation

**Robin W. Cotton, PhD**
*Boston University Biomedical Forensic Sciences*

BOSTON UNIVERSITY

**Module 4**

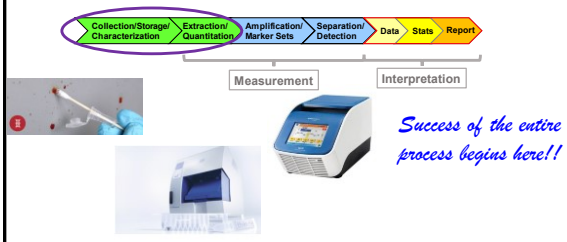
1

## Presentation Outline

- Principles used in DNA collection, DNA extraction and DNA quantification
- MVP's on DNA Collection
  - #1 article and why
- MVP's on DNA Extraction
  - #1 article and why
- MVP's on DNA Quantitation
  - #1 article and why
- Summary and other thoughts…

2

## Steps in Forensic DNA Testing




*Success of the entire process begins here!!*

3

# Most Valuable Publications of Forensic DNA

(J.M. Butler, R.W. Cotton, M.K. Prinz, C.J. Word)

16 February 2021

## Critical Principles in DNA Collection and Extraction and Quantitation

- Collection (Section C)
  1. The efficiency of collection will depend on the deposition substrate and the collection device substrate
  2. Contamination occurs as early as the evidence collection step
  3. Validation of collection methods is complicated by the number of variables
- Extraction (Section D)
  1. The efficiency of DNA Recovery varies with extraction methods.
  2. Efficiency is measured relative to total possible DNA, comparison of methods does not measure efficiency
  3. Reduction of co-purification of inhibitors varies with extraction methods
  4. Differential Extraction relies on the di-sulfide bonds which are within and between protamines I and II and package the DNA in the sperm nucleus
- Quantitation (Section E)
  1. Reproducibility is related to the standard curve
  2. Kits are robust and can also assay for degradation and inhibition

4

## Collection and Storage of Biological Material-page 1

| Journal Article | Source | Number of Citations |
|---|---|---|
| Prasad, E., et.al. (2020) DNA recovery from unfired and fired cartridge cases: A comparison of swabbing, tape lifting, vacuum filtration, and direct PCR. | FSI | not available |
| Technical Working Group on Biological Evidence Preservation (2013) The Biological Evidence Preservation Handbook: Best Practices for Evidence Handlers. | NIST and NIJ | not available |
| Sweet, D., et.al. (1997) An improved method to recover saliva from human skin: the double swab technique. | JFS | 322 |
| Sar, W., et.al. (1988) Postmortem stability of DNA. | FSI | 251 |
| Higgins, D. and Austin, J.J. (2013) Teeth as a source of DNA for forensic identification of human remains: A review. | Sci & Justice | 129 |
| Lee, H.C. and Ladd, C. (2001) Preservation and collection of biological evidence. | CMJ | 96 |
| Bond, J.W. and Hammond, C. (2008) The value of DNA material recovered from crime scenes. | JFS | 84 |
| Verdon, T.J., et.al. (2014) Evaluation of tapelifting as a collection method for touch DNA. | FSI Genetics | 83 |
| Sandewoestyne, M. and Deforce, D. (2010) Laser capture microdissection in forensic research: a review. | Int. J of Legal Med. | 76 |
| Verdon, T.J., et al.(2014) Swabs as DNA collection devices for sampling different biological materials from different substrates. JFS | JFS | 73 |
| Goray, M., et.al. (2012) DNA transfer within forensic exhibit packaging: potential for DNA loss and relocation. | FSI Genetics | 66 |

5

## Collection and Storage of Biological Material-page 2

| Journal Article | Source | Number of Citations |
|---|---|---|
| Kline, M.C., et.al. (2002) Polymerase chain reaction amplification of DNA from aged blood stains: quantitative evaluation of the "suitability for purpose" of four filter papers as archival media. | Anal. Chem. | 59 |
| Adamowicz, M.S., Stasulli, D.M., et.al. (2014) Evaluation of methods to improve the extraction and recovery of DNA from cotton swabs for forensic analysis. | PLoS ONE | 57 |
| Mapes, A.A., et.al.(2016) Knowledge on DNA success rates to optimize the DNA analysis process: from crime scene to laboratory. | JFS | 33 |
| Hess, S. and Haas, C. (2017) Recovery of trace DNA on clothing: A comparison of mini-tape lifting and three other forensic evidence collection techniques. | JFS | 28 |
| Kanokwongnuwut, P., et.al. (2018) Detection of latent DNA. | FSI Genetics | 26 |
| Pickrahn, I., et.al. (2017) Contamination incidents in the pre-analytical phase of forensic DNA analysis in Austria—Statistics of 17 years. | Legal Med. | 22 |
| Verdon, T.J., et.al. (2015) Preliminary investigation of differential tapelifting for sampling forensically relevant layered deposits. | Legal Med. | 13 |
| Baechler, S. (2016) Study of criteria influencing the success rate of DNA swabs in operational conditions: A contribution to an evidence-based approach to crime scene investigation and triage. | FSI Genetics | 12 |
| Allen-Hall, A. and McNevin, D. (2013) Non-cryogenic forensic tissue preservation in the field: a review. | Aust. J of For. Sci. | 9 |
| Sujatha, G., et.al. (2019) Determination of reliability and practicality of saliva as a genetic source in forensic investigation by analyzing DNA yield and success rates: A systematic review. | J Oral ...& Path. | 5 |
| Hedman, J., et.al. (2020) The double-swab technique versus single swabs for human DNA recovery from various surfaces. | FSI Genetics | 4 |
| van Oorschot, R.A.H. (2012) Assessing DNA profiling success rates: need for more and better collection of relevant data. | For Policy & Management | 4 |
| Sherier, A.J., Kieser, R.E., et.al. (2020) Copan microFLOQ® Direct Swab collection of bloodstains, saliva, and semen on cotton cloth. | Int. J of Legal Med. | 3 |
| McLamb, J.M., Adams, L.D., et.al. (2020) Comparison of the M-Vac® wet-vacuum-based collection method to a wet-swabbing method for DNA recovery on diluted bloodstained substrates. | JFS | 1 |

#1

#2

6

## Collection and Storage of Biological Material
**Topic Categories**-C-25 articles)

- DNA degradation
- C2
- Contamination upon sampling
- C9 transfer when packaging evidence, C18
- Standard practices
- C10-from TWG on Bio.Evid. Samples
- Sampling methods
- C3- the double swab technique
- C5, C7, C11, C14, C15, C16, C19, 23, C24, C25
- Specific sample type
- C12, C20, C21, C22
- Success rates
- C1, C8,
- C13 defines issues with cell release from swabs
- C17

7

## #1 MVP on Collection & Storage of Biological Material

JOURNAL OF FORENSIC SCIENCES

TECHNICAL NOTE

CRIMINALISTICS

#1

Knowledge on DNA Success Rates to Optimize the DNA Analysis Process: From Crime Scene to Laboratory*

***2020 Publication***

***33 Citations***
*(Jan 16, 2020)*

- **Why is this article valuable?**
- **This article provides a method to analyze laboratory DNA results and use this data to predict success rates and eliminate wasting resources on evidence that is unlikely to produce results.**

8

## Runner Up: Collection & Storage of Biological Material

Forensic Science International: Genetics

#2

The double-swab technique versus single swabs for human DNA recovery from various surfaces

***2020 Publication***

- **Why is this article valuable?**
- **The paper takes a close look at published 1997 procedure that is in common use and re-investigates with updated experiments**

9

**DNA Extraction/Purification, Differential Extraction**

| Journal Article | Source | Number of Citations |
|---|---|---|
| Romsos, E.L. and Vallone, P.M. (2019) Estimation of extraction efficiency by droplet digital PCR. | FSI Genetics | not available |
| Chapman, B.R., Blackwell, S.J., Müller, L.H. (2020) Forensic techniques for the isolation of spermatozoa from sexual assault samples - A review. Forensic Science Review 32(2): 105-116. | FS Review | not available |
| Walsh, P.S., Metzger, D.A., et.al. (1991) Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. | Biotechniques | 6839 |
| #1 Gill, P., Jeffreys, A.J., et.al. (1985) Forensic application of DNA 'fingerprints'. | Nature | 1331 |
| Comey, C.T., Koons, B.W., et.al. (1994) DNA extraction strategies for amplified fragment length polymorphism analysis. | JFS | 282 |
| Loreille, O.M., Diegoli, T.M., et.al. (2007) High efficiency DNA extraction from bone by total demineralization. | FSI Genetics | 282 |
| Montpetit, S.A., Fitch, I.T., et.al. (2005) A simple automated instrument for DNA extraction in forensic casework. | JFS | 126 |
| Nagy, M., Otremba, P., et.al. (2005) Optimization and validation of a fully automated silica-coated magnetic beads purification technology in forensics. | FSI | 118 |
| Brennov, M.G., Pawar, H.S., et.al. (2009) Developmental validation of the PrepFiler Forensic DNA Extraction Kit for extraction of genomic DNA from biological samples. | JFS | 89 |
| Castella, V., Dimo-Simonin, N., et.al. (2006) Forensic evaluation of the QIAshredder/QIAamp DNA extraction procedure. | FSI | 78 |
| Anslinger, K., Bayer, B., et.al. (2005) Application of the BioRobot EZ1 in a forensic laboratory. | Legal Med. | 59 |
| Frégeau, C.J., Lett, C.M., et.al. (2010) Validation of a DNA IQ™-based extraction method for TECAN robotic liquid handling workstations for processing casework. | FSI Genetics | 39 |
| Schneider, H., Sommerer, T., et.al. (2011) Hot flakes in cold cases. | Int. J of Legal Med. | 27 |
| Stray, J.E., Liu, J.Y., et.al. (2010) Extraction of DNA from forensic biological samples for genotyping. | FS Review | 15 |
| Stray, J.E. and Shewale, J.G. (2010) Extraction of DNA from human remains. Forensic Science Review 22(2): 177-185. | FS Review | 9 |
| Rådström, P., Knutsson, R., et.al. (2004). Pre-PCR processing: Strategies to generate PCR-compatible samples. | Molec. Biotech. | 8 |
| Farash, K., Hanson, E.K., et.al. (2018) Single source DNA profile recovery from single cells isolated from skin and fabric from touch DNA mixtures in mock physical assaults. | Sci & Justice | 7 |
| Samie, L., Champod, C., et.al. (2019) The efficiency of DNA extraction kit and the efficiency of recovery techniques to release DNA using flow cytometry. | Sci & Justice | 5 |

10

## DNA Extraction/Purification, Differential Extraction

**Topic Categories;** Section D-18 articles

- Differential extraction
  - D1 and D18
- Extraction methods
  - D2, D3, D5, D8, D9, D10
- Measuring extraction efficiency
  - D16 and D17
- Specific sample type
  - D12 and D15
- Robotics
  - D6, D7, D13

11

## #1 MVP on DNA Extraction/Purification, Differential Extraction

*Nature 1985, v318: 577-579*

#1 **Forensic application of DNA 'fingerprints'**

*1985 Publication*

***Cited 1331 times***
*(Jan 16, 2020)*

Peter Gill*, Alec J. Jeffreys† & David J. Werrett*

* Central Research Establishment, Home Office Forensic Science Service, Aldermaston, Reading, Berkshire RG7 4PN, UK
† Department of Genetics, University of Leicester, University Road, Leicester LE1 7RH, UK

- **Why is this article valuable?**
- **This is the original differential extraction paper. Current procedures still use this basic method with few modifications for sexual assault evidence samples.**

12

## Runners up: DNA Extraction/Purification, Differential Extraction

Samie, L., Champod, C., et.al. (2019) The efficiency of DNA extraction kit and the efficiency of recovery techniques to release DNA using flow cytometry. Science & Justice 59(4):405-410

Romsos, E.L. and Vallone, P.M. (2019) Estimation of extraction efficiency by droplet digital PCR. Forensic Science Int. Genetics Suppl. Series7: 515-517

**Why are these articles important?**

**Both of these papers examine the actual efficiency of DNA extraction. That is, they use known amounts of starting cells and measure the amount of DNA obtained post extraction.**

13

**DNA Quantitation, Degraded DNA**

| *Journal Article* | *Source* | *Number of Citations* |
|---|---|---|
| Holt, A., Olson, S., et.al. (2015) A DNA-based screening assay to streamline sexual assault sample processing. | Amer. Lab. | not available |
| Lindahl, T. (1993) Instability and decay of the primary structure of DNA. | Nature | 6030 |
| Butler, J.M., Shen, Y., et.al. (2003) The development of reduced size STR amplicons as tools for analysis of degraded DNA. | JFS | 630 |
| Alaeddini, R., Walsh, S.J., et.al. (2010) Forensic implications of genetic analyses from degraded DNA--a review. | FSI Genetics | 264 |
| Green, R.L., Roinestad, I.C., et.al. (2005) Developmental validation of the Quantifiler real-time PCR kits for the quantification of human nuclear DNA samples. | JFS | 150 |
| Barbisin, M., Fang, R., et.al. (2009) Developmental validation of the Quantifiler Duo DNA Quantification kit for simultaneous quantification of total human and human male DNA and detection of PCR inhibitors in biological samples. | JFS | 86 |
| Grgicak, C.M., Urban, Z.M., et.al. (2010) Investigation of reproducibility and error associated with qPCR methods using Quantifiler® Duo DNA quantification kit. | JFS | 67 |
| Holt, A., Wootton, S.C., et.al. (2016) Developmental validation of the Quantifiler® HP and Trio Kits for human DNA quantification in forensic samples. | FSI Genetics | 48 |
| Lee, S.B., McCord, B., et.al. (2014) Advances in forensic DNA quantification: a review. | Electrophoresis | 38 |
| Vieira-Silva, C., Afonso-Costa, H., et.al. (2015) Quantifiler® Trio DNA validation and usefulness in casework samples. | FSI Genetics | 5 |

#1

14

## DNA Quantitation, Degraded DNA

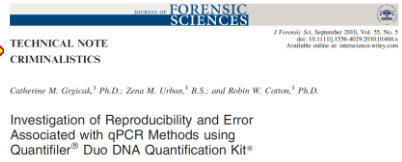
**Topic Categories-**Section E-10 articles)

- qPCR
  - E1
- DNA degradation
  - E2, E6
- Mini STR loci
  - E3
- qPCR Kits
  - E4, E5, E8, E9, E10,
- qPCR Reproducibility
  - E7
- Robotics
  - D6, D7, D13

15

## #1 MVP on DNA Quantitation & Degraded DNA

#1


JOURNAL OF FORENSIC SCIENCES

TECHNICAL NOTE
CRIMINALISTICS

Catherine M. Grgicak,[1] Ph.D.; Zena M. Urban,[1] B.S.; and Robin W. Cotton,[1] Ph.D.

Investigation of Reproducibility and Error Associated with qPCR Methods using Quantifiler® Duo DNA Quantification Kit*

*2010 Publication*

***Cited 67 times***
*(Jan 16, 2020)*

- Why is this article valuable?
- This article demonstrates that use of a new standard curve for each run increases the variation in quant results.
- Use of a "virtual" standard curve based on multiple standard curves provides increased reproducibility (i.e., reduces variation) and saves reagent costs

16

## Thoughts…

- The procedures used for, and the research done, on collection of DNA evidence samples and the use and characterization of DNA extraction procedures represent the best potential to increase overall DNA profile success rates.
- More DNA means better profiles and fewer low template profiles.

17

## Thank you for your attention!

**John M. Butler**
john.butler@nist.gov

**Robin W. Cotton**
rwcotton@bu.edu

**Mechthild "Mecki" Prinz**
mprinz@jjay.cuny.edu

**Charlotte J. Word**
cjword@comcast.net

**Please put questions in the chat box** while watching our pre-recorded presentations and we will answer them over the chat or through a live Zoom session at the end – you can also email any of us…

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American Academy of Forensic Sciences
VIRTUAL WORKSHOP W19 (MVPs of Forensic DNA)
February 16, 2021

2021 AAFS VIRTUAL MEETING

*MVPs = Most Valuable Publications*

# MVPs on PCR, STRs, and CE

**John M. Butler, PhD**
*National Institute of Standards and Technology*

NIST FORENSIC SCIENCE
RESEARCH. STANDARDS. FOUNDATIONS.

**Module 5**

1

## Acknowledgments and Disclaimer

*Thank you to SWGDAM and Phil Danielson with the OSAC Literature Task Group for their starting materials in developing these MVPs (most valuable publications)*

**Points of view are mine** and do not necessarily represent the official position or policies of the National Institute of Standards and Technology.

Certain commercial entities are identified in order to specify experimental procedures as completely as possible. In no case does such identification imply a recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that any of the entities identified are necessarily the best available for the purpose.

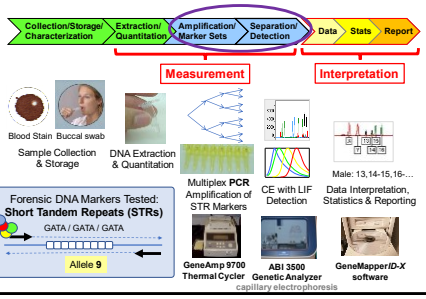
2

## Presentation Outline

- Principles involved with DNA Measurements (PCR, STRs, and CE)
- MVPs on PCR
  - Number and types of publications in this category
  - #1 article and why
- MVPs on STRs
  - Number and types of publications in this category
  - #1 article and why
- MVPs on CE
  - Number and types of publications in this category
  - #1 article and why
- Summary and Key Takeaways

3

## Steps in Forensic DNA Testing




4

## Basic Principles with PCR, STRs, and CE

1. The polymerase chain reaction (PCR) is a widely-used molecular biology technique that **amplifies available DNA material into measurable amounts**.
2. During the PCR process, each amplified molecule can be **labeled with a fluorescent tag for detection** purposes. Tags with different colors can be used.
3. Forensic DNA testing examines **selected short tandem repeat (STR) regions** in the human genome, which possess multiple alleles and vary in length.
4. Commercial **kits provide PCR reagents** to copy, label, and measure multiple STR markers simultaneously. Commonly used STR kits examine 24 markers.
5. Capillary electrophoresis (CE) is a widely-used analytical chemistry technique that **separates DNA molecules by length** and enables PCR-amplified STR alleles to be analyzed by size and fluorescent dye color.
6. CE **results are displayed in an electropherogram** (EPG).

5

## Some Further Principles with PCR, STRs, and CE

7. When **low amounts of DNA** are amplified, the PCR results **may not exactly represent the original DNA sample**, including the relative quantities of each allele and genotype.
8. When amplifying STR alleles, **the PCR process introduces artifacts**, including stutter products, that complicate interpretation of the resulting DNA profile.
9. Relative fluorescence unit (RFU) **variance (uncertainty) of a DNA profile peak height is inversely proportional to the peak height**.
10. Peak **positions more accurately reflect allele calls** than peak heights represent relative allele amounts in EPGs.

6

## PCR Amplification, Inhibition, and Artifacts
(Category F – 13 articles)

| Google Scholar Number of citations (4 Jan 2021) | |
|---|---|
| **386** | 1. **Walsh, P.S., Erlich, H.A. and Higuchi, R. (1992) Preferential PCR amplification of alleles: mechanisms and solutions. *PCR Methods & Applications* 1(4): 241-250.** |
| 22,498 | 2. Saiki, R.K., Gelfand, D.H., Stoffel, S., Scharf, S.J., Higuchi, R., Horn, G.T., Mullis, K.B., Erlich, H.A. (1988) Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* 239: 487-491. |
| 1248 | 3. Clark, J.M. (1988). Novel non-templated nucleotide addition reactions catalyzed by procaryotic and eucaryotic DNA polymerases. *Nucleic Acids Research* 16(20): 9677-9686. |
| 184 | 4. Bloch, W. (1991) A biochemical perspective of the polymerase chain reaction. *Biochemistry* 30: 2735-2747. |
| 139 | 5. Reynolds, R., Sensabaugh, G., Blake, E. (1991) Analysis of genetic markers in forensic DNA samples using the polymerase chain reaction. *Analytical Chemistry* 63(1): 2-15. |
| 443 | 6. Walsh, P.S., Fildes, N.J., Reynolds, R. (1996) Sequence analysis and characterization of stutter products at the tetranucleotide repeat locus vWA. *Nucleic Acids Research* 24(14): 2807-2812. |
| 67 | 7. Leclair, B., Sgueglia, J.B., Wojtowicz, P.C., Juston, A.C., Frégeau, C.J., Fourney, R.M. (2003) STR DNA typing: increased sensitivity and efficient sample consumption using reduced PCR reaction volumes. *Journal of Forensic Sciences* 48(5): 1001-1013. |
| 222 | 8. Alaeddini, R. (2012) Forensic implications of PCR inhibition—A review. *Forensic Science International: Genetics* 6(3): 297-305. |
| 122 | 9. Brookes, C., Bright, J.-A., Harbison, S., Buckleton, J. (2012) Characterizing stutter in forensic STR multiplexes. *Forensic Science International: Genetics* 6(1): 58-63. |
| 29 | 10. Kumar, P., Gupta, R., Singh, R., Jasuja, O.P. (2015) Effects of latent fingerprint development reagents on subsequent forensic DNA typing: a review. *Journal of Forensic and Legal Medicine* 32:64-69. |
| 72 | 11. Cavanaugh, S.E. and Bathrick, A.S. (2018) Direct PCR amplification of forensic touch and other challenging DNA samples: A review. *Forensic Science International: Genetics* 32: 40-49. |
| 52 | 12. Sidstedt, M., Hedman, J., Romsos, E.L., Waitara, L., Wadsö, L., Steffen, C.R., Vallone, P.M., Rådström, P. (2018) Inhibition mechanisms of hemoglobin, immunoglobulin G, and whole blood in digital and real-time PCR. *Analytical and Bioanalytical Chemistry* 410(10): 2569-2583. |
| 0 (too new) | 13. Martin, B. and Linacre, A. (2020) Direct PCR: A review of use and limitations. *Science & Justice* 60: 303-310. |

**Location Published**

*FSI Genetics* (3)
*Nucleic Acids Res.* (2)
*PCR Method Appl.* (1)
*Science* (1)
*Biochemistry* (1)
*Anal. Chem.* (1)
*Anal. Bioanal. Chem.* (1)
*J. Forensic Sci.* (1)
*J. Forensic Legal Med.* (1)
*Sci. Justice* (1)

7

## PCR Amplification, Inhibition, and Artifacts
(Category F – 13 articles)

- **PCR biochemistry:**
  - Thermostable polymerase (F2)
  - Components and conditions (F4)
  - Early review on forensic use (F5)
- **Amplification adjustments**:
  - Reduced PCR volumes (F7)
  - Direct PCR (F11, F13)
  - Stochastic effects with low DNA amounts and potential solutions (F1)

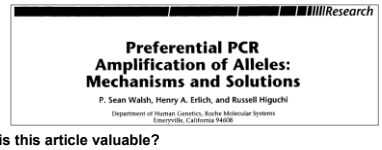
#1

- **Inhibition:**
  - Review (F8)
  - Effects of latent fingerprint development reagents (F10)
  - Mechanisms with blood (F12)
- **Artifacts:**
  - Non-nucleotide addition (F3)
  - Stutter of STR alleles (F6, F9)

8

## #1 MVP on PCR

F1. **Walsh, P.S., Erlich, H.A. and Higuchi, R. (1992) Preferential PCR amplification of alleles: mechanisms and solutions. *PCR Methods & Applications* 1(4): 241-250.**




Google Scholar
**Cited 386 times**
(4 Jan 2021)

**Why is this article valuable?**

- **Discusses stochastic effects with low levels of DNA** ("the possibility of an unequal sampling of the two alleles of a heterozygote…when only a few DNA molecules are used to initiate PCR")
- **Introduces the first concept of a stochastic threshold** ("adjusting the cycle number such that approximately 20 or more copies of target DNA [~125 pg] are required to give a typing result for that PCR system")

9

## Autosomal STR Markers and Kits

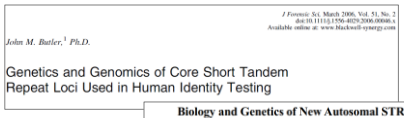
(Category O – 29 articles)

- **Early work:**
  - Caskey et al. (O2, O3) and Canadian (O4)
  - ENFSI testing principles (O6)
- **Validation of STR kits**:
  - AmpFlSTR Blue (O5)
  - GlobalFiler (O24)
  - Identifiler (O12), Identifiler Plus (O16)
  - Investigator 24plex QS and GO! (O22)
  - MiniFiler (O14, O15)
  - NGM SElect (O18)
  - Profiler Plus (O9)
  - Profiler Plus & COfiler (O10)
  - PowerPlex 16 (O11)
  - PowerPlex Fusion 6C (O21)
  - SGM Plus (O7)
- **Description of new STR loci:** #1
  - Core loci details (O1, O17)
  - European expansion (O13)
  - U.S. expansion (O19)
  - Beyond the current core (O26, O27)
  - STRBase (O8)
  - Any disease associations? (O29)
- **Sequence information:**
  - STR allele variation (O20, O28)
  - U.S. population data for 27 loci (O25)
  - STRseq (O23)

10

## #1 MVP(s) on STRs

**O1. Butler, J.M. (2006) Genetics and genomics of core STR loci used in human identity testing.** *Journal of Forensic Sciences* 51(2): 253-265.



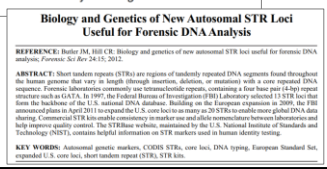
*J Forensic Sci*, March 2006, Vol. 51, No. 2
doi:10.1111/j.1556-4029.2006.00046.x
Available online at: www.blackwell-synergy.com

*John M. Butler,[1] Ph.D.*

Genetics and Genomics of Core Short Tandem Repeat Loci Used in Human Identity Testing

Google Scholar
**Cited 757 times**
(4 Jan 2021)

**O17.** Butler, J.M. and Hill, C.R. (2012) Biology and genetics of new autosomal STR loci useful for forensic DNA analysis. *Forensic Science Review* 24(1): 15-26.



**Biology and Genetics of New Autosomal STR Loci Useful for Forensic DNA Analysis**

**REFERENCE:** Butler JM, Hill CR: Biology and genetics of new autosomal STR loci useful for forensic DNA analysis, *Forensic Sci Rev* 24:15; 2012.

**ABSTRACT:** Short tandem repeats (STRs) are regions of tandemly repeated DNA segments found throughout the human genome that vary in length (through insertion, deletion, or mutation) with a core repeated DNA sequence. Forensic laboratories commonly use tetrameric/tetrade repeats, containing a four base pair (4-bp) repeat structure such as GATA. In 1997, the Federal Bureau of Investigation (FBI) Laboratory selected 13 STR loci that form the backbone of the U.S. national DNA database. Building on the European expansion in 2009, the FBI announced plans in April 2011 to expand the U.S. core loci to as many as 20 STRs to enable more global DNA data sharing. Commercial STR kits enable consistency in marker use and allelic nomenclature between laboratories and help improve quality control. The STRBase website, maintained by the U.S. National Institute of Standards and Technology (NIST), contains helpful information on STR markers used in human identity testing.

**KEY WORDS:** Autosomal genetic markers, CODIS STRs, core loci, DNA typing, European Standard Set, expanded U.S. core loci, short tandem repeat (STR), STR kits.

Google Scholar
**Cited 131 times**
(4 Jan 2021)

11

## Autosomal STR Markers and Kits

(**Category O** – 29 articles) – Part 1

| Google Scholar Number of citations (4 Jan 2021) | | Location Published |
|---|---|---|
| 757 | **1. Butler, J.M. (2006) Genetics and genomics of core STR loci used in human identity testing. *Journal of Forensic Sciences* 51(2): 253-265.** | |
| 1744 | 2. Edwards, A., Civitello, A., Hammond, H.A., Caskey, C.T. (1991) DNA typing and genetic mapping with trimeric and tetrameric tandem repeats. *American Journal of Human Genetics* 49(4): 746-756. | |
| 315 | 3. Frégeau, C.J. and Fourney, R.M. (1993) DNA typing with fluorescently tagged short tandem repeats: a sensitive and accurate approach to human identification. *BioTechniques* 15(1): 100-119. | |
| 612 | 4. Hammond, H.A., Jin, L., Zhong, Y., Caskey, C.T., Chakraborty, R. (1994) Evaluation of 13 short tandem repeat loci for use in personal identification applications. *American Journal of Human Genetics* 55(1): 175-189. | |
| 122 | 5. Wallin, J.M., Buoncristiani, M.R., Lazaruk, K.D., Fildes, N., Holt, C.L., Walsh, P.S. (1998) TWGDAM validation of the AmpFlSTR™ Blue PCR amplification kit for forensic casework analysis. *Journal of Forensic Sciences* 43(4): 854-870. | |
| 69 | 6. Gill, P., Sparkes, R., Fereday, L., Werrett, D.J. (2000) Report of the European Network of Forensic Science Institutes (ENSFI): formulation and testing of principles to evaluate STR multiplexes. *Forensic Science International* 108(1): 1-29. | |
| 217 | 7. Cotton, E.A., Allsop, R.F., Guest, J.L., Frazier, R.R.E., Koumi, P., Callow, I.P., Seager, A. Sparkes, R.L. (2000) Validation of the AMPFlSTR® SGM Plus™ system for use in forensic casework. *Forensic Science International* 112: 151-161. | |
| 358 | 8. Ruitberg, C.M., Reeder, D.J., Butler, J.M. (2001) STRBase: a short tandem repeat DNA database for the human identity testing community. *Nucleic Acids Research* 29: 320-322. | |
| 48 | 9. Frank, W.E., Llewellyn, B.E., Fish, P.A., Riech, A.K., Marcacci, T.L., Gandor, D.W., Parker, D., Carter, R.R., Thibault, S.M. (2001) Validation of the AmpFlSTR Profiler Plus PCR amplification kit for use in forensic casework. *Journal of Forensic Sciences* 46(3): 642-646. | |
| 72 | 10. LaFountain, M.J., Schwartz, M.B., Svete, P.A., Walkinshaw, M.A., Buel, E. (2001) TWGDAM validation of the AmpFlSTR Profiler Plus and AmpFlSTR COfiler STR multiplex systems using capillary electrophoresis. *Journal of Forensic Sciences* 46(5): 1191-1198. | |

**Location Published**

*FSI Genetics* (10)
*J. Forensic Sci.* (9)
*Forensic Sci. Int.* (3)
*Am. J. Hum. Genet.* (2)
*BioTechniques* (1)
*Forensic Sci. Rev.* (1)
*Front. Genet.* (1)
*Int. J. Legal Med.* (1)
*Nucleic Acids Res.* (1)

12

## Autosomal STR Markers and Kits

(**Category O** – 29 articles) – Part 2

| Google Scholar Number of citations (4 Jan 2021) | | Location Published |
|---|---|---|
| 413 | 11. Krenke, B.E., Tereba, A., Anderson, S.J., Buel, E., Culhane, S., Finis, C.J., Tomsey, C.S., Zachetti, J.M., Masibay, A., Rabbach, D.R., Amiott, E.A. (2002) Validation of a 16-locus fluorescent multiplex system. *Journal of Forensic Sciences* 47(4): 773-785. | |
| 277 | 12. Collins, P.J., Hennessy, L.K., Leibelt, C.S., Roby, R.K., Reeder, D.J., Foxall, P.A. (2004) Developmental validation of a single-tube amplification of the 13 CODIS STR loci, D2S1338, D19S433, and amelogenin: the AmpFlSTR Identifiler PCR Amplification Kit. *Journal of Forensic Sciences* 49(6): 1265-1277. | |
| 255 | 13. Gill, P., Fereday, L., Morling, N., Schneider, P.M. (2006) The evolution of DNA databases—recommendations for new European STR loci. *Forensic Science International* 156: 242-244. | |
| 163 | 14. Mulero, J.J., Chang, C.W., Lagace, R.E., Wang, D.Y., Bas, J.L., McMahon, T.P., Hennessy, L.K. (2008). Development and validation of the AmpFlSTR® MiniFiler™ PCR Amplification Kit: a miniSTR multiplex for the analysis of degraded and/or PCR inhibited DNA. *Journal of Forensic Sciences* 53(4): 838-852. | |
| 28 | 15. Luce, C., Montpetit, S., Gangitano, D., O'Donnell, P. (2009) Validation of the AMPFISTR MiniFiler PCR amplification kit for use in forensic casework". *Journal of Forensic Sciences* 54(5): 1046-1054. | |
| 125 | 16. Wang, D.Y., Chang, C.W., Lagacé, R.E., Calandro, L.M., Hennessy, L.K. (2012) Developmental validation of the AmpFlSTR® Identifiler® Plus PCR Amplification Kit: an established multiplex assay with improved performance. *Journal of Forensic Sciences* 57(2): 453-465. | |
| 131 | 17. Butler, J.M. and Hill, C.R. (2012) Biology and genetics of new autosomal STR loci useful for forensic DNA analysis. *Forensic Science Review* 24(1): 15-26. | |
| 77 | 18. Green, R.L., Lagacé, R.E., Oldroyd, N.J., Hennessy, L.K., Mulero, J.J. (2013) Developmental validation of the AmpFlSTR® NGM SElect™ PCR Amplification Kit: A next-generation STR multiplex with the SE33 locus. *Forensic Science International: Genetics* 7(1): 41-51. | |
| 143 | 19. Hares, D.R. (2015) Selection and implementation of expanded CODIS core loci in the United States. *Forensic Science International: Genetics* 17: 33-34. | |
| 124 | 20. Gettings, K.B., Aponte, R.A., Vallone, P.M., Butler, J.M. (2015) STR allele sequence variation: current knowledge and future issues. *Forensic Science International: Genetics* 18: 118-130. | |

*FSI Genetics* (10)
*J. Forensic Sci.* (9)
*Forensic Sci. Int.* (3)
*Am. J. Hum. Genet.* (2)
*BioTechniques* (1)
*Forensic Sci. Rev.* (1)
*Front. Genet.* (1)
*Int. J. Legal Med.* (1)
*Nucleic Acids Res.* (1)

13

## Autosomal STR Markers and Kits

(**Category O** – 29 articles) – Part 3

| Google Scholar Number of citations (4 Jan 2021) | | Location Published |
|---|---|---|
| 60 | 21. Ensenberger, M.G., Lenz, K.A., Matthies, L.K., Hadinoto, G.M., Schienman, J.E., Przech, A.J., Morganti, M.W., Renstrom, D.T., Baker, V.M., Gawrys, K.M., Hoogendoorn, M., Steffen, C.R., Martin, P., Alonso, A., Olson, H.R., Sprecher, C.J., Storts, D.R. (2016) Developmental validation of the PowerPlex(®) Fusion 6C System. *Forensic Science International: Genetics* 21: 134-144. | |
| 37 | 22. Kraemer, M., Prochnow, A., Bussmann, M., Scherer, M., Peist, R., Steffen, C. (2017) Developmental validation of QIAGEN Investigator® 24plex QS Kit and Investigator® 24plex GO! Kit: Two 6-dye multiplex assays for the extended CODIS core loci. *Forensic Science International: Genetics* 29: 9-20. | |
| 41 | 23. Gettings, K.B., Borsuk, L.A., Ballard, D., Bodner, M., Budowle, B., Devesse, L., King, J., Parson, W., Phillips, C., Vallone, P.M. (2017) STRSeq: A catalog of sequence diversity at human identification Short Tandem Repeat loci. *Forensic Science International: Genetics* 31: 111-117. | |
| 44 | 24. Ludeman, M.J., Zhong, C., Mulero, J.J., Lagacé, R.E., Hennessy, L.K., Short, M.L., Wang, D.Y. (2018) Developmental validation of GlobalFiler™ PCR amplification kit: a 6-dye multiplex assay designed for amplification of casework samples. *International Journal of Legal Medicine* 132(6): 1555-1573. | |
| 35 | 25. Gettings, K.B., Borsuk, L.A., Steffen, C.R., Kiesler, K.M., Vallone, P.M. (2018) Sequence-based U.S. population data for 27 autosomal STR loci. *Forensic Science International: Genetics* 37: 106-115. | |
| 10 | 26. Novroski, N.M.M., Woerner, A.E., Budowle, B. (2018) Potential highly polymorphic short tandem repeat markers for enhanced forensic identity testing. *Forensic Science International: Genetics* 37:162-171. | |
| 8 | 27. Novroski, N.M.M., Wendt, F.R., Woerner, A.E., Bus, M.M., Coble, M., Budowle, B. (2019) Expanding beyond the current core STR loci: An exploration of 73 STR markers with increased diversity for enhanced DNA mixture deconvolution. *Forensic Science International: Genetics* 38: 121-129. | |
| 0 (too new) | 28. Devesse, L., Davenport, L., Borsuk, L., Gettings, K., Mason-Buck, G., Vallone, P. M., Syndercombe Court, D., Ballard, D. (2020) Classification of STR allelic variation using massively parallel sequencing and assessment of flanking region power. *Forensic Science International: Genetics* 48: 102356. | |
| 1 | 29. Wyner, N., Barash, M., McNevin, D. (2020) Forensic autosomal short tandem repeats and their potential association with phenotype. *Frontiers in Genetics* 11: 884. doi: 10.3389/fgene.2020.00884 | |

*FSI Genetics* (10)
*J. Forensic Sci.* (9)
*Forensic Sci. Int.* (3)
*Am. J. Hum. Genet.* (2)
*BioTechniques* (1)
*Forensic Sci. Rev.* (1)
*Front. Genet.* (1)
*Int. J. Legal Med.* (1)
*Nucleic Acids Res.* (1)

14

## #1 MVP(s) on STRs

**O1. Butler, J.M. (2006) Genetics and genomics of core STR loci used in human identity testing. *Journal of Forensic Sciences* 51(2): 253-265.**

- **Provides genomic information and characterization of original core STR loci** → updated and refined with newer articles, such as Gettings et al. (see O20, O23, O25) and Devesse et al. (see O28)

Table 3 – Genomic locations of core STR loci

| Locus (UniSTS) | GenBank Accession (Allele Repeat #) | Chromosomal Location | Physical Position (July 2003, NCBI Build 34) | Physical Position (May 2004, NCBI Build 35) |
|---|---|---|---|---|
| TPOX (240638) | M68651 (11) | 2p25.3 thyroid peroxidase, 10th intron | Chr 2: 1,436 Mb | Chr 2: 1,472 Mb |
| D2S1338 (385004) | AC010136 (20) | 2q35 | Chr 2: 219,082 Mb | Chr 2: 218,703 Mb |
| D3S1358 (1402226) | AC098539 (16) | 3p21.31 | Chr 3: 45,543 Mb | Chr 3: 45,557 Mb |
| FGA (240635) | M64982 (21) | 4q31.3 alfenioegen, 3rd intron | Chr 4: 156,086 Mb | Chr 4: 155,866 Mb |
| D5S818 (545780) | AC000112 (11) | 5q23.2 | Chr 5: 123,187 Mb | Chr 5: 123,139 Mb |
| CSF1PO (176169) | X14720 (12) | 5q33.1 c-fms proto-oncogene, 6th intron | Chr 5: 149,464 Mb | Chr 5: 149,436 Mb |
| SE33 (ACTBP2) (none reported) | V00481 (26.2) | 6q14 β-actin related pseudogene | Chr 6: 88,982 Mb | Chr 6: 89,043 Mb |
| D7S820 (745093) | AC004848 (13) | 7q21.11 | Chr 7: 83,401 Mb | Chr 7: 83,433 Mb |
| D8S1179 (163698) | AF216671 (13) | 8q24.13 | Chr 8: 125,663 Mb | Chr 8: 125,976 Mb |
| TH01 (248639) | D00269 (9) | 11p15.5 tyrosine hydroxylase, 1st intron | Chr 11: 2.156 Mb | Chr11: 2.149 Mb |
| VWA (240640) | M25858 (18) | 12p13.33 von Willebrand Factor, 40th intron | Chr 12: 19,826 Mb | Chr 12: 5.963 Mb |
| D13S317 (7734) | AL353628 (11) | 13q31.1 | Chr 13: 80,520 Mb | Chr 13: 81,620 Mb |
| Penta E (none reported) | AC027004 (5) | 15q26.2 | Chr 15: 95,116 Mb | Chr 15: 95,173 Mb |
| D16S539 (425590) | AC024591 (11) | 16q24.1 | Chr 16: 86,168 Mb | Chr 16: 84,944 Mb |
| D18S51 (444489) | AP001534 (18) | 18q21.33 | Chr 18: 59,090 Mb | Chr 18: 59,180 Mb |
| D19S433 (133588) | AC008507 (16) | 19q12 | Chr 19: 35,109 Mb | Chr 19: 35,109 Mb |
| D21S11 (240842) | AP000433 (29) | 21q21.1 | Chr 21: 19,476 Mb | Chr 21: 16,476 Mb |
| Penta D (none reported) | AP001752 (13) | 21q22.3 | Chr 21: 43,912 Mb | Chr 21: 43,880 Mb |

15

## Recent Information on Number of Alleles Per STR Locus

**O28.** Devesse, L., Davenport, L., Borsuk, L., Gettings, K., Mason-Buck, G., Vallone, P.M., Syndercombe Court, D., Ballard, D. (2020) Classification of STR allelic variation using massively parallel sequencing and assessment of flanking region power. *Forensic Science International: Genetics* 48: 102356

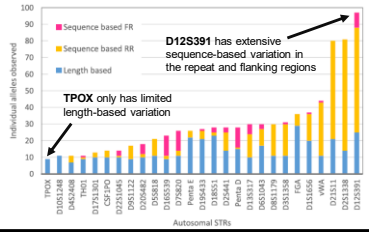


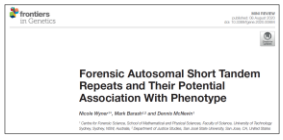

Fig. 2. Increase in the number of individual alleles observed in terms of repeat region (RR) and flanking region (FR) variants across all population groups (1007 individuals). Repeat region variation includes any changes that affect the repeat region, irrespective of traditional repeat motif designation (specifically in the cases of D5S818 and D13S317).

16

## Forensic STR Alleles Are Not Associated with Disease

**O29.** Wyner, N., Barash, M., McNevin, D. (2020) Forensic autosomal short tandem repeats and their potential association with phenotype. *Frontiers in Genetics* 11: 884.

- It is a widely accepted notion that genetic markers used in forensic applications are not predictive of phenotype.
- An extensive search of the literature returned 107 articles associating a forensic STR with a trait.
- TH01 had the greatest number of associations with 27 traits reportedly linked to 40 different genotypes. Five of the articles associated TH01 with schizophrenia. None of the associations found were independently causative or predictive of disease.
- **At present, there has been no demonstration of forensic STR variants directly causing or predicting disease.**




Conclusion: "While the results of this study did indicate a large number of phenotypic traits associated with forensic STRs, **none were found to be independently causative or predictive of disease**."

17

## Capillary Electrophoresis Separation and Detection

(**Category G** – 12 articles)

| *Google Scholar* Number of citations (4 Jan 2021) | |
|---|---|
| **299** | **1. Butler, J.M., Buel, E., Crivellente, F., McCord, B.R. (2004) Forensic DNA typing by capillary electrophoresis using the ABI Prism 310 and 3100 Genetic Analyzers for STR analysis. *Electrophoresis* 25: 1397-1412.** |
| 80 | 2. Guttman, A. and Cooke, N. (1991) Effect of temperature on the separation of DNA restriction fragments in capillary gel electrophoresis. *Journal of Chromatography* 559: 285-294. |
| 34 | 3. Issaq, H.J., Chan, K.C., Muschik, G.M. (1997) The effect of column length, applied voltage, gel type, and concentration on the capillary electrophoresis separation of DNA fragments and polymerase chain reaction products. *Electrophoresis* 18(7): 1153-1158. |
| 165 | 4. Lazaruk, K., Walsh, P.S., Oaks, F., Gilbert, D., Rosenblum, B.B., Menchen, S., Scheibler, D., Wenz, H.M., Holt, C., Wallin, J. (1998) Genotyping of forensic short tandem repeat (STR) systems based on sizing precision in a capillary electrophoresis instrument. *Electrophoresis* 19(1): 86-93. |
| 61 | 5. Mansfield, E.S., Robertson, J.M., Vainer, M., Isenberg, A.R., Frazier, R.R., Ferguson, K., Chow, S., Harris, D.W., Barker, D.L., Gill, P.D., Budowle, B., McCord, B.R. (1998) Analysis of multiplexed short tandem repeat (STR) systems using capillary array electrophoresis. *Electrophoresis* 19(1): 101-107. |
| 263 | 6. Heller, C. (2001) Principles of DNA separation with capillary electrophoresis. *Electrophoresis* 22(4): 629-643. |
| 264 | 7. Moretti, T.R., Baumstark, A.L., Defenbaugh, D.A., Keys, K.M., Smerick, J.B. and Budowle, B. (2001) Validation of short tandem repeats (STRs) for forensic usage: performance testing of fluorescent multiplex STR systems and analysis of authentic and simulated forensic samples. *Journal of Forensic Sciences* 46(3): 647-660. |
| 96 | 8. Moretti, T.R., Baumstark, A.L., Defenbaugh, D.A., Keys, K.M., Brown, A.L. and Budowle, B. (2001) Validation of STR typing by capillary electrophoresis. *Journal of Forensic Sciences* 46(3): 661-676. |
| 39 | 9. Sgueglia, J.B., Geiger, S., Davis, J. (2003) Precision studies using the ABI Prism 3100 Genetic Analyzer for forensic DNA analysis. *Analytical and Bioanalytical Chemistry* 376(8): 1247-1254. |
| 66 | 10. Gilder, J.R., Doom, T.E., Inman, K. and Krane, D.E. (2007) Run-specific limits of detection and quantitation for STR-based DNA testing. *Journal of Forensic Sciences* 52(1): 97-101. |
| 44 | 11. Rakay, C.A., Bregu, J. and Grgicak, C.M. (2012) Maximizing allele detection: Effects of analytical threshold and DNA levels on rates of allele and locus drop-out. *Forensic Science International: Genetics* 6(6): 723-728. |
| 2 | 12. Adelman, J.D., Zhao, A., Eberst, D.S. and Marciano, M.A. (2019) Automated detection and removal of capillary electrophoresis artifacts due to spectral overlap. *Electrophoresis* 40(14): 1753-1761. |

**Location Published**

- *Electrophoresis* (6)
- *J. Forensic Sci.* (3)
- *FSI Genetics* (1)
- *J. Chromatogr.* (1)
- *Anal. Bioanal. Chem.* (1)

18

## Capillary Electrophoresis* Separation and Detection (Category G – 12 articles)

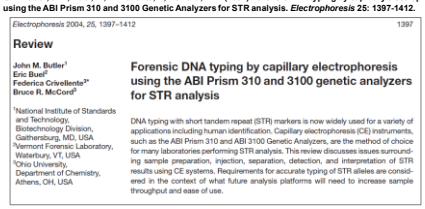
- **Principles and parameters:** #1
  - Injection, separation, detection (G1)
  - Separation (G6)
  - Temperature effects (G2)
  - Column length, voltage, polymer (G3)
- **Early instruments**:
  - ABI 310 Genetic Analyzer (G4)
  - Prototype 48-capillary array (G5)
- **Validation efforts:**
  - FBI validation of ABI 310 (G7, G8)
  - Precision studies with ABI 3100 (G9)
- **Setting analytical thresholds**:
  - Run-specific (G10)
  - Allele and locus drop-out rates (G11)
- **Spectral calibration**:
  - Automated detection (G12)

**STR alleles can also be separated by gel electrophoresis or detected by next-generation sequencing methods*

19

## #1 MVP on CE

**G1. Butler, J.M., Buel, E., Crivellente, F., McCord, B.R. (2004) Forensic DNA typing by capillary electrophoresis using the ABI Prism 310 and 3100 Genetic Analyzers for STR analysis. *Electrophoresis* 25: 1397-1412.**



*Electrophoresis* 2004, *25*, 1397–1412 1397

Review

John M. Butler[1]
Eric Buel[2]
Federica Crivellente[3]*
Bruce R. McCord[3]

[1]National Institute of Standards and Technology, Biotechnology Division, Gaithersburg, MD, USA
[2]Vermont Forensic Laboratory, Waterbury, VT, USA
[3]Ohio University, Department of Chemistry, Athens, OH, USA

**Forensic DNA typing by capillary electrophoresis using the ABI Prism 310 and 3100 genetic analyzers for STR analysis**

DNA typing with short tandem repeat (STR) markers is now widely used for a variety of applications including human identification. Capillary electrophoresis (CE) instruments, such as the ABI Prism 310 and ABI 3100 Genetic Analyzers, are the method of choice for many laboratories performing STR analysis. This review discusses issues surrounding sample preparation, injection, separation, detection, and interpretation of STR results using CE systems. Requirements for accurate typing of STR alleles are considered in the context of what future analysis platforms will need to increase sample throughput and ease of use.

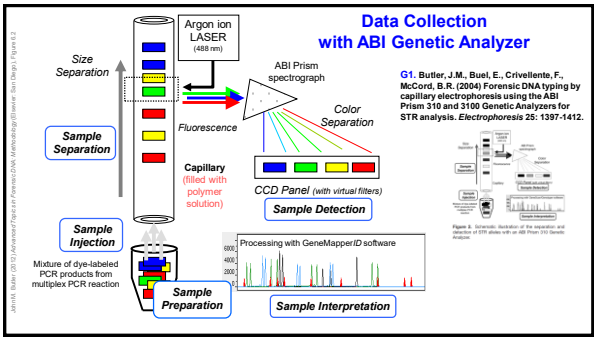
*Google Scholar*
**Cited 299 times**
(4 Jan 2021)

**Why is this article valuable?**

- Sample preparation, injection, separation, and detection principles are examined and explained

20

## Data Collection with ABI Genetic Analyzer




**G1. Butler, J.M., Buel, E., Crivellente, F., McCord, B.R. (2004) Forensic DNA typing by capillary electrophoresis using the ABI Prism 310 and 3100 Genetic Analyzers for STR analysis. *Electrophoresis* 25: 1397-1412.**



21

## Summary and Key Takeaways

- The polymerase chain reaction (PCR) enables sensitive DNA detection; stochastic effects occur when amplifying low quantities of DNA
- Core sets of short tandem repeat (STR) markers are used in forensic DNA testing; these markers have been extensively characterized and possess no known association with any genetic diseases
- STR alleles can vary in their overall length (number of repeat units), with their internal sequence of repeats, and in the flanking region; some STR markers vary more than others as reported in recent sequencing efforts
- Capillary electrophoresis (CE) remains the primary method for STR typing in use today, although research efforts show benefits of STR allele sequencing to extract more information from samples

22

## Thank you for your attention!

John M. Butler
john.butler@nist.gov

Robin W. Cotton
rwcotton@bu.edu

Mechthild "Mecki" Prinz
mprinz@jjay.cuny.edu

Charlotte J. Word
cjword@comcast.net

**Please put questions in the chat box** while watching our pre-recorded presentations and we will answer them over the chat or through a live Zoom session at the end – you can also email any of us…

23

American Academy of Forensic Sciences
VIRTUAL WORKSHOP W19 (MVPs of Forensic DNA)
February 16, 2021

2021 AAFS VIRTUAL MEETING

*MVPs = Most Valuable Publications*

# MVPs on Population Genetics and Statistical Analysis

**Robin W. Cotton, PhD**
*Boston University Biomedical Forensic Sciences*

BOSTON UNIVERSITY

**Module 6**

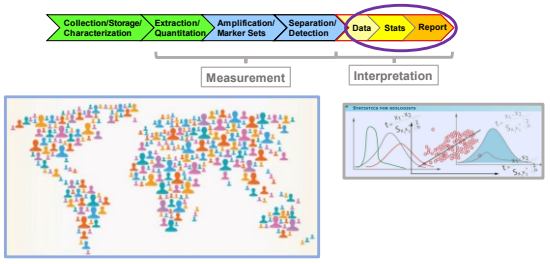
1

## Presentation Outline

- Principles of Population Genetics and Statistical Analysis which impact the analysis and interpretation of DNA profiles
- MVP's on Statistical Analysis (Section S)
  - #1 article and why
- MVP's on Population Genetics (Section T)
  - #1 article and why
- Summary and other thoughts

2

## Steps in Forensic DNA Testing




3

## Critical Principles in Population Genetics and Statistical Analysis

- Population Genetics
  1. Hardy Weinberg Equilibrium and the required assumptions
  2. Allele frequency and genotype frequency calculations
  3. Requirements for use of the product rule (Linkage Equilibrium)

- Statistical Analysis
  1. Training and working knowledge of basic statistics is needed for all analysts
  2. Understanding of normal and other distributions
  3. Hypothesis testing and confidence intervals
  4. Laws of probability and use of LR calculations

4

## Population Genetics

| Journal Article | Source | Number of Citations |
|---|---|---|
| Weir, B.S. and Cockerham, C.C. (1984) Estimating F-statistics for the analysis of population structure. | Evolution | 18415 |
| Guo, S.W. and Thompson, E.A. (1992) Performing the exact test of Hardy-Weinberg proportion for multiple alleles. | Biometrics | 5911 |
| Hardy, G.H. (1908) Mendelian proportions in a mixed population. | Science | 1555 |
| Balding, D.J. and Nichols, R.A. (1994) DNA profile match probability calculation: how to allow for population stratification, relatedness, database selection and single bands. | FSI | 484 |
| Stern, C. (1943) The Hardy-Weinberg law. | Science | 270 |
| Chakraborty, R. (1992) Sample size requirements for addressing the population genetic issues of forensic use of DNA typing. | Human Biology | 150 |
| Weir, B.S. (1994) The effects of inbreeding on forensic calculations. | Annual Rev. of Genetics | 78 |
| Buckleton, J., Curran, J., et.al. (2016) Population-specific FST values for forensic STR markers: A worldwide survey. | FSI Genetics | 48 |
| Buckleton, J.S., Curran, J.M., et.al. (2006) How reliable is the sub-population model in DNA testimony? | FSI | 26 |
| Curran, J.M., Walsh, S.J., et.al. (2007) Empirical testing of estimated DNA frequencies. | FSI Genetics | 23 |
| Steele, C.D., Syndercombe-Court, D., et.al. (2014) Worldwide F(ST) estimates relative to five continental-scale populations. | Annals of Human Genetics | 20 |

#1

5

## Population Genetics

**Topic Categories;** Section **T**-11 articles

- Original Theory
- T2 (1908)
- T3(1943)

- Population structure
- T4(1994)
- T7(1994)
- T10(2014)
- T11(2016)

- Population samples and allele frequencies
- T1(1994)
- T5(1992)
- T6(1992)
- T8(2006)
- T9(2007)

6

## #1 MVP on Population Genetics

Forensic Science International
Volume 64, Issues 2–3, February 1994, Pages 125-140

DNA profile match probability calculation: how to allow for population stratification, relatedness, database selection and single bands

David J. Balding A, Richard A. Nichols

*1994 Publication*

*Cited 484 times*
*(Jan 16, 2020)*

- **Why is this article valuable?**
- **Foundational article, calls 1992 NRC suggested calculation method "ad-hoc & overly conservative". WHICH IT WAS**
- **This article proposes the use of LR, as well as adjustments to allele frequencies that are "sound" from population genetic view.**

7

## Statistical Analysis

| *Journal Article* | *Source* | *Number of Citations* |
|---|---|---|
| Clopper, C.J., Pearson, E.S. (1934). The use of confidence or fiducial limits illustrated in the case of the binomial. | Biometrika | 3844 |
| Biedermann, A. and Taroni, F. (2012) Bayesian networks for evaluating forensic DNA profiling evidence: A review and guide to literature. | FSI Genetics | 73 |
| Foreman, L.A. and Evett, I.W. (2001). Statistical analyses to support forensic interpretation for a new ten-locus STR profiling system. | Int. J of Legal Med. | 72 |
| Curran, J.M. (2005). An introduction to Bayesian credible intervals for sampling error in DNA profiles. | Law, Prob., and Risk | 68 |
| Aitken, C., Roberts, P., et.al. (2010) Practitioner Guide No. 1. Fundamentals of Probability and Statistical Evidence in Criminal Proceedings: Guidance for Judges, Lawyers, Forensic Scientists and Expert Witnesses. | Royal Stat. Society's Working Group... | 58 |
| Foreman, L.A., Champod, et.al. (2003) Interpreting DNA evidence: A review. | Inter. Stat. Review | 50 |
| DNA Advisory Board (2000) Statistical and population genetics issues affecting the evaluation of the frequency of occurrence of DNA profiles calculated from pertinent population database(s). | FS Communications | 34 |
| Roberts, P. and Aiken, C. (2014) Practitioner Guide No. 3. The Logic of Forensic Proof: Inferential Reasoning in Criminal Evidence and Forensic Science: Guidance for Judges, Lawyers, Forensic Scientists and Expert Witnesses. | Royal Stat. Society's Working Group... | 17 |
| **Curran, J.M. (2013) Is forensic science the last bastion of resistance against statistics?** | **Science & Justice** | **13** |
| Jackson, G., Aitken, C., et.al. (2014) Practitioner Guide No. 4. Case Assessment and Interpretation of Expert Evidence: Guidance for Judges, Lawyers, Forensic Scientists and Expert Witnesses. | Royal Stat. Society's Working Group... | 12 |
| Puch-Solis, R., Roberts, P., et.al. (2012) Practitioner Guide No. 2. Assessing the Probative Value of DNA Evidence: Guidance for Judges, Lawyers, Forensic Scientists and Expert Witnesses. | Royal Stat. Society's Working Group... | 9 |

#1

8

## Statistical Analysis
**Topic Categories;** S-(11 articles)

- Challenge to Forensic Science:
- S1 (2013)

- Review articles from Royal Statistical Society Working Group on Statistics & the Law
- S7 (2010)
- S9 (2012)
- S10 (2014)
- S11 (2014)

- Statistical Methods/Bayesian
- S2 (1934)
- S6 (2005)
- S8 (2012)

- DNA profile frequencies
- S3 (2000)
- S4 (2001)
- S5 (2003)

9

## #1 MVP on Statistical Analysis


Science and Justice 53 (2013) 251–252

Contents lists available at SciVerse ScienceDirect

Science and Justice

journal homepage: www.elsevier.com/locate/scijus

Editorial

#1 Is forensic science the last bastion of resistance against statistics?

*2013 Publication*
*13 Citations*
*Jan 16, 2020*

- **Why is this article valuable?**
- **Author asks: Why is there so much resistance to change?**
- **"Statistical interpretation is a vital part of a modern forensic scientist's toolbox. It is incumbent upon us, as a community, to make sure that we have the best tool set available and that everyone knows how to use it."**

10

## Other Thoughts:

- Textbooks in the first section have good reviews of statistics and population genetics as noted in their titles.
- Courses in probability more advanced courses (past one semester) in statistics will prove valuable in your work and your understanding of results.
- There are numerous papers in Sections E, G, I, L and Y that provide examples of the applications of statistics to understanding and analysis of DNA results.

11

## Thank you for your attention!

**John M. Butler**
john.butler@nist.gov

**Robin W. Cotton**
rwcotton@bu.edu

**Mechthild "Mecki" Prinz**
mprinz@jjay.cuny.edu

**Charlotte J. Word**
cjword@comcast.net

**Please put questions in the chat box** while watching our pre-recorded presentations and we will answer them over the chat or through a live Zoom session at the end – you can also email any of us…

12

American Academy of Forensic Sciences
VIRTUAL WORKSHOP W19 (MVPs of Forensic DNA)
February 16, 2021

2021 AAFS VIRTUAL MEETING

*MVPs = Most Valuable Publications*

# MVPs on Binary Approaches to Mixture Interpretation

**Mechthild K. "Mecki" Prinz, PhD**
*John Jay College of Criminal Justice*

**Module 7**

1

## Presentation Outline

- Basic Principles of Binary Mixture Interpretation
- MVPs on Binary Approaches (CPI, RMP, LR)
  - Number and types of publications in this category
- Discussion of three MVP papers
  - #1 article - 2006 ISFG Recommendations on Mixture Interpretation
  - 1998 Article on Mixture Deconvolution (borrowed from section J)
  - 2016 Guidance on CPI
- Summary and Current Status

2

## Basic Principles of Binary Mixture Interpretation

- Binary interpretation is based on the presence and absence of allele peaks
- This approach still requires **peak height** considerations to:
  - Apply analytical and stochastic **thresholds**
  - Decide which **type of mixture** is present
- Mixture types can be
  - **Distinguishable mixture** with a major and a minor component
  - **Indistinguishable mixture** without an obvious major component but high peak heights and no evidence of stochastic effects
  - **Indistinguishable mixture** with evidence of stochastic effects -possibility of drop out
- Mixture type decides next steps

3

## Interpretation: Binary Approaches (CPI, RMP, LR)
(Category K – 11 articles)

| Google Scholar Number of citations (12 Jan 2021) | |
|---|---|
| 332 | 1. **Gill, P., Brenner, C.H., Buckleton, J.S., Carracedo, A., Krawczak, M., Mayr, W.R., Morling, N., Prinz, M., Schneider, P.M. and Weir, B.S. (2006) DNA Commission of the International Society of Forensic Genetics: Recommendations on the interpretation of mixtures.** ***Forensic Science International*** **160: 90-101.** |
| 11 | 2. Schneider, P.M., Gill, P., Carracedo, A. (2006) Editorial on the recommendations of the DNA commission of the ISFG on the interpretation of mixtures. *Forensic Science International* 160: 89. |
| 55 | 3. Buckleton, J. and Triggs, C. (2006) Is the 2p rule always conservative? *Forensic Science International* 159: 206-209. |
| 25 | 4. Morling, N., Bastisch, I., Gill, P. and Schneider, P.M. (2007) Interpretation of DNA mixtures--European consensus on principles. *Forensic Science International: Genetics* 1: 291-292. |
| 64 | 5. Buckleton, J. and Curran, J. (2008) A discussion of the merits of random man not excluded and likelihood ratios. *Forensic Science International: Genetics* 2(4): 343-348. |
| 56 | 6. Schneider, P.M., Finmers, R., Keil, W., Molsberger, G., Petzelt, D., Pflug, W., Rothämel, T., Schmitter, H., Schneider, H. and Brinkmann, B. (2009) The German Stain Commission: recommendations for the interpretation of mixed stains. *International Journal of Legal Medicine* 123(1): 1-5. |
| 153 | 7. Budowle, B., Onorato, A.J., Callaghan, T.F., Della, M.A., Gross, A.M., Guerrieri, R.A., Luttman, J.C., McClure, D.L. (2009) Mixture interpretation: defining the relevant features for guidelines for the assessment of mixed DNA profiles in forensic casework. *Journal of Forensic Sciences* 54(4): 810-821. |
| 67 | 8. Bright, J.-A., Turkington, J., Buckleton, J. (2010) Examination of the variability in mixed DNA profile parameters for the Identifiler™ multiplex. *Forensic Science International: Genetics* 4(2): 111-114. |
| 29 | 9. Bille, T., Bright, J.-A., Buckleton, J. (2013) Application of random match probability calculations to mixed STR profiles. *Journal of Forensic Sciences* 58(2): 474-485. |
| 55 | 10. Bieber, F.R., Buckleton, J.S., Budowle, B., Butler, J.M., Coble, M.D. (2016) Evaluation of forensic DNA mixture evidence: protocol for evaluation, interpretation, and statistical calculations using the combined probability of inclusion. *BMC Genetics* 17(1):125. |
| 11 | 11. Marsden, C.D., Rudin, N., Inman, K., Lohmueller, K.E. (2016) An assessment of the information content of likelihood ratios derived from complex mixtures. *Forensic Science International: Genetics* 22: 64-72. |

**Location Published**

*FSI Genetics (4)*
*For Sci Int (3)*
*J For Sci (2)*
*Int J Leg Med (1)*
*BMC Genetics (1)*

4

## Interpretation: Binary Approaches (CPI, RMP, LR)
(Category K – 11 articles)

#1

- **Recommendations:**
  - ISFG (K1) with editorial (K2)
  - ENFSI (K4)
  - German Stain Commission (K6)
  - FBI Laboratory (K7)
  - Use of CPI (K10)
- **Defining parameters:**
  - Identifiler Mixtures (K8)

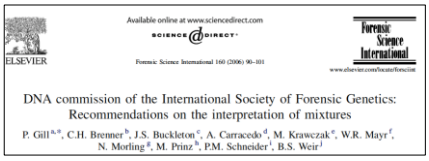
*More on variability of mixture parameters and effectiveness of different approaches in Mixture Deconvolution (J) and Probabilistic Genotyping Systems (L)*

- **Mathematical Evaluation:**
  - 2p rule (K3)
  - RMNE vs LR (K5)
- **Practical evaluation:**
  - RMP and RMNE (K9)
  - LR (K11)

5

## MVP Binary Approaches

**K1 Gill, P., Brenner, C.H., Buckleton, J.S., Carracedo, A., Krawczak, M., Mayr, W.R., Morling, N., Prinz, M., Schneider, P.M. and Weir, B.S. (2006) DNA Commission of the International Society of Forensic Genetics: Recommendations on the interpretation of mixtures.** ***Forensic Science International*** **160: 90-101.**


Available online at www.sciencedirect.com
SCIENCE DIRECT
Forensic Science International 160 (2006) 90–101
Forensic Science International
www.elsevier.com/locate/forsciint
ELSEVIER
DNA commission of the International Society of Forensic Genetics: Recommendations on the interpretation of mixtures
P. Gill [a,*], C.H. Brenner [b], J.S. Buckleton [c], A. Carracedo [d], M. Krawczak [e], W.R. Mayr [f], N. Morling [g], M. Prinz [h], P.M. Schneider [i], B.S. Weir [j]

Google Scholar
**Cited 332 times**
(12 Jan 2021)

**Why is this article valuable?**

- Includes critical evaluation of CPI (or RMNE) calculation
- Provides recommendations for mixture interpretation
- Addresses stutter, drop-out, degradation

6

## MVP Binary Approaches

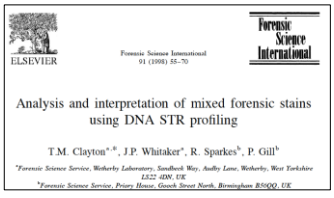
K1 **Gill, P., Brenner, C.H., Buckleton, J.S., Carracedo, A., Krawczak, M., Mayr, W.R., Morling, N., Prinz, M., Schneider, P.M. and Weir, B.S. (2006) DNA Commission of the International Society of Forensic Genetics: Recommendations on the interpretation of mixtures.** ***Forensic Science International*** **160: 90-101.**

> • *Recommendation* 1: The likelihood ratio is the preferred approach to mixture interpretation. The RMNE approach is restricted to DNA profiles where the profiles are unambiguous. If the DNA crime stain profile is low level and some minor alleles are the same size as stutters of major alleles, and/or if drop-out is possible, then the RMNE method may not be conservative.

- **Rec 2** and **3** discuss use of LR in court and recommend approach for unrestricted LR calculation.
- **Rec 4** and **5** endorse mixture deconvolution and competing LR propositions.
- **Rec 6** requires the consideration of minor alleles masked by stutter.
- **Rec 7** and **8** explain when to consider drop-out and when to make minor alleles not suitable for comparison.
- **Rec 9** issues caution on using thresholds and mixture parameters for LCN.

7

## Important Article on Mixture Deconvolution


ELSEVIER

Forensic Science International 91 (1998) 55–70

Forensic Science International

Analysis and interpretation of mixed forensic stains using DNA STR profiling

T.M. Clayton[a,*], J.P. Whitaker[a], R. Sparkes[b], P. Gill[b]

[a]Forensic Science Service, Wetherby Laboratory, Sandbeck Way, Audby Lane, Wetherby, West Yorkshire LS22 4DN, UK

[b]Forensic Science Service, Priory House, Gooch Street North, Birmingham B50QQ, UK

Article J3 on MVP list

Google Scholar
**Cited 321 times**
(12 Jan 2021)

**Reference recommended in Gill et al. 2006 – K1**
**Important interpretation step – goal DNA database profile**

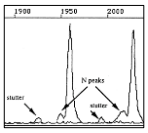
8

## Important Article on Mixture Deconvolution

J3. **Clayton, T.M., Whitaker, J.P., Sparkes, R. and Gill, P. (1998) Analysis and interpretation of mixed forensic stains using DNA STR profiling.** ***Forensic Science International*** **91(1): 55-70.**

**Steps towards mixture interpretation:**

- Recognize artefacts to identify true alleles
- Determine number of contributors
- Determine mixture ratio
- Formulate genotype combinations, if possible discount some combinations based on peak heights and ratio
- Compare to references




9

## MVP Binary Approaches

**Indistinguishable mixtures:**

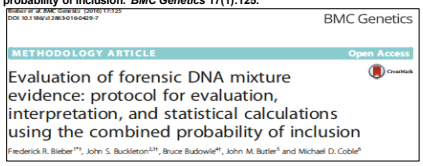
**Most widely used mixture statistic for many years**
**RMNE or CPI**

- Variety of protocols on
  - how to apply stochastic thresholds
  - deal stutter and possible allele sharing
- Wide variation in which loci were disqualified, not only between different SOP's but also within a laboratory.

10

## MVP Binary Approaches

**K10. Bieber, F.R., Buckleton, J.S., Budowle, B., Butler, J.M., Coble, M.D. (2016) Evaluation of forensic DNA mixture evidence: protocol for evaluation, interpretation, and statistical calculations using the combined probability of inclusion. *BMC Genetics* 17(1):125.**


BMC Genetics

METHODOLOGY ARTICLE Open Access

Evaluation of forensic DNA mixture evidence: protocol for evaluation, interpretation, and statistical calculations using the combined probability of inclusion

Frederick R. Bieber[*†], John S. Buckleton[2,3†], Bruce Budowle[4†], John M. Butler[5] and Michael D. Coble[6]

*Google Scholar*
**Cited 55 times**
(12 Jan 2021)

**Why is this article valuable?**

- Includes discussion of merits and limitations of CPI calculation
- Provides **very specific** rules and guidance for locus-by-locus decisions required for CPI calculation

11

## MVP Binary Approaches

**K10. Bieber, F.R., Buckleton, J.S., Budowle, B., Butler, J.M., Coble, M.D. (2016) Evaluation of forensic DNA mixture evidence: protocol for evaluation, interpretation, and statistical calculations using the combined probability of inclusion. *BMC Genetics* 17(1):125.**

To formalize the interpretation the overriding principle (P) for use of loci in CPI calculations is:

$P_1$: Any locus that has a reasonable probability of allele drop-out should be disqualified from use in calculation of the CPI statistic.

**Recommendations R1- R8 with Guidance**

- Covers how to apply stochastic thresholds and consider potential allele masking and additive effects prior to disqualifying a locus.
- Covers exceptions, e.g. for interpreting only major alleles.
- Emphasizes use of all loci and peak height considerations for exculpatory purposes.

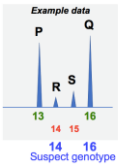
12

## Value of Peak Height Consideration

Manually including or excluding a person of interest prior to calculating statistical weight still requires consideration of **peak height** and the **determination of number of contributors**.

Also true for CPI.

Main disadvantage of binary systems is loss of information for distinguishable mixtures.




**Unrestricted**
genotype combinations

| | |
|---|---|
| PQ & RS | RS & PQ |
| PR & SQ | SQ & PR |
| PS & RQ | RQ & PS |

**Restricted**
genotype combinations

| | |
|---|---|
| PQ & RS | RS & PQ |

Image courtesy of John Butler (2015) Advanced Topics in DNA Typing: Interpretation

13

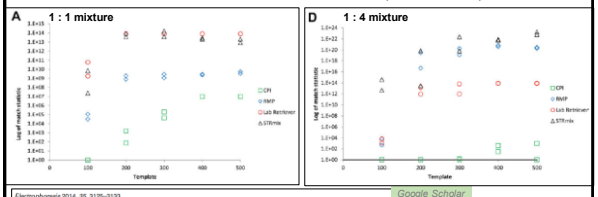
## Use MVP list to find related Articles

1. Need to understand effects of individual mixture characteristics on statistical evaluation
   - Applicability of each approach.
   - Differences in statistical weight for the same mixtures.
2. One article comparing software programs in that respect is K11 – Marsden et al. 2016.
3. Other relevant papers evaluating binary mixture interpretation can be found in section L.

14

## Impact of Peak Height Information on Statistical Weight

**Article borrowed from Section L** (L22 on MVP list)




*Electrophoresis* 2014, 35, 3125–3133

Todd W. Bille[1]
Steven M. Weitz[1]
Michael D. Coble[2]
John Buckleton[3]
Jo-Anne Bright[3]

[1]Bureau of Alcohol, Tobacco, Firearms and Explosives

Research Article

**Comparison of the performance of different models for the interpretation of low level mixed DNA profiles**

*Google Scholar*
**Cited 32 times**
(08 Jan 2021)

15

## Summary and Current Status

1. Binary mixture interpretation works BUT
   - Does not make use of valuable information.
   - Manual approach is difficult to standardize.
   - Interpretation protocol becomes very complicated especially for large multiplexes, complex mixtures, and low template amounts.

2. Continuous interpretation approaches with probabilistic genotyping and likelihood ratio assigning software are recommended.

16

## Thank you for your attention!

**John M. Butler**
john.butler@nist.gov

**Robin W. Cotton**
rwcotton@bu.edu

**Mechthild "Mecki" Prinz**
mprinz@jjay.cuny.edu

**Charlotte J. Word**
cjword@comcast.net

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17

American Academy of Forensic Sciences
VIRTUAL WORKSHOP W19 (MVPs of Forensic DNA)
February 16, 2021

2021 AAFS VIRTUAL MEETING

*MVPs = Most Valuable Publications*

# MVPs on Probabilistic Genotyping Systems

**John M. Butler, PhD**
*National Institute of Standards and Technology*

NIJ FORENSIC SCIENCE
RESEARCH. STANDARDS. FOUNDATIONS.

**Module 8**

1

## Acknowledgments and Disclaimer

*Thank you to SWGDAM and Phil Danielson with the OSAC Literature Task Group for their starting materials in developing these MVPs (most valuable publications)*

**Points of view are mine** and do not necessarily represent the official position or policies of the National Institute of Standards and Technology.

Certain commercial entities are identified in order to specify experimental procedures as completely as possible. In no case does such identification imply a recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that any of the entities identified are necessarily the best available for the purpose.

2

## Presentation Outline

- Principles involved with Probabilistic Genotyping Software (PGS) Systems
- MVPs of PGS
  - #1 article and why
  - Number and types of publications in this category
- Summary and Key Takeaways

3

## Some Important Principles

1. **High sensitivity DNA testing** can result in complex DNA mixtures, especially from touch evidence
2. Complex DNA mixtures with 3 or more contributors often involve low level DNA **where STR allele dropout may occur**; allele stacking and stutter artifacts also complicate interpretation
3. With previous binary approaches to mixture interpretation, "inconclusive" may be the only option available to analysts
4. Probabilistic genotyping **uses computer simulations** to infer the likelihood of possible genotype combinations for mixture contributors

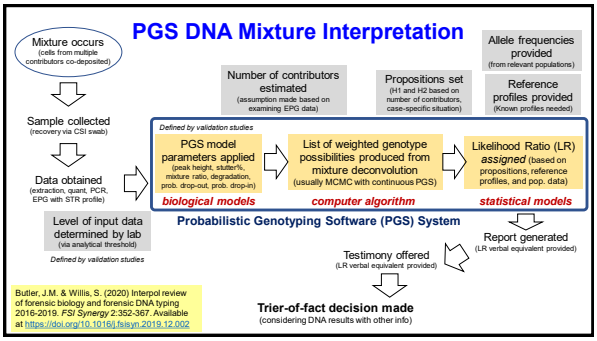
4

## Probabilistic Genotyping Software (PGS)

- Consists of two primary approaches:
  1. discrete (evaluates alleles with a probability of dropout) – e.g., FST, Lab Retriever
  2. continuous (utilizes alleles and their peak heights, etc.) – e.g., STRmix, TrueAllele
- **Uses statistical modeling** informed by biological data (in the case of continuous approaches), statistical theory, computer algorithms and/or probability distributions
- Infers potential genotypes and/or **calculates likelihood ratios (LRs)**
  - Requires user inputs and propositions (e.g., estimated number of contributors)
- **Multiple software programs and models exist**
  - Some are open-source, and some are commercial (proprietary code)

5

## PGS DNA Mixture Interpretation




6

## Interpretation: Probabilistic Genotyping Software (Discrete, Continuous)

(Category L – 44 articles)

- **Reviews:**
  - DNA Commission on allele drop-out/in (L11)
  - PGS overview and history (L1)
  - Comparison of statistical models (L17)
  - Historical: 20 years of R&D (L26)
  - Paradigm shift (L34)
  - Statistical evaluation of forensic evidence (L24)
- **Validation**:
  - Definitions and recommended tests (L25, L32)
  - EuroForMix studies (L29, L39)
  - TrueAllele studies (L5, L8, L27, L28, L43)
  - STRmix theory and studies (L14, L18, L19, L20, L21, L30, L33, L36, L42, L44)
  - STRmix collaborative exercise (L40)
  - Comparing model performance (L22, L29, L38, L41)
- **Continuous Models:**
  - Early work (L3, L4, L6, L7, L9, L10)
  - Modeling stutter (L13, L15)
  - Low template profiles (L23, L31)
  - Information gain from peak heights (L37)
- **Likelihood Ratios:**
  - Framework for addressing questions (L2)
  - Exploring nondonor distributions (L12, L44)
  - Calibration and method validation (L16, L35)

7

## #1 MVP(s) on PGS

**L1. Coble, M.D. and Bright, J.-A. (2019) Probabilistic genotyping software: An overview. *Forensic Science International: Genetics* 38: 219-224.**


Forensic Science International: Genetics 38 (2019) 219–224

Contents lists available at ScienceDirect

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsigen

ELSEVIER

Probabilistic genotyping software: An overview

Michael D. Coble[a,*], Jo-Anne Bright[b]

[a] Center for Human Identification, Department of Microbiology, Immunology, and Genetics, University of North Texas Health Science Center, 3500 Camp Bowie Blvd., Fort Worth, TX 76107, USA
[b] Institute of Environmental Science and Research Limited, Private Bag 92021, Auckland, 1142 New Zealand

*Google Scholar*
**Cited 40 times**
(8 Jan 2021)

- **Why is this article valuable?**
  - Provides a historical perspective and overview on the movement from binary methods of interpretation to probabilistic methods of interpretation

8

## Interpretation: Probabilistic Genotyping Software

(Category L – 44 articles) – Part 1

| *Google Scholar* Number of citations (8 Jan 2021) | | |
|---|---|---|
| 40 | 1. | **Coble, M.D. and Bright, J.-A. (2019) Probabilistic genotyping software: An overview. *Forensic Science International: Genetics* 38: 219-224.** |
| 92 | 2. | Evett, I.W. (1983) What is the probability that this blood came from that person? A meaningful question? *Journal of the Forensic Science Society (Science & Justice)* 23:35-39. |
| 122 | 3. | Gill, P., Kirkham, A., Curran, J. (2007) LoComatioN: A software tool for the analysis of low copy number DNA profiles. *Forensic Science International*. 166:128-138. |
| 23 | 4. | Cowell, R.G. (2009) Validation of an STR peak area model. *Forensic Science International: Genetics* 3(3): 193-199. |
| 81 | 5. | Perlin, M.W. and Sinelnikov, A. (2009) An information gap in DNA evidence interpretation. *PLoS ONE* 4(12): e8327. |
| 37 | 6. | Tvedebrink, T., Eriksen, P.S., Mogensen, H.S., Morling, N. (2010) Evaluating the weight of evidence by using quantitative short tandem repeat data in DNA mixtures. *Applied Statistics* 59(5): 855-874. |
| 61 | 7. | Cowell, R.G., Lauritzen, S.L., Mortera, J. (2011) Probabilistic expert systems for handling artifacts in complex DNA mixtures. *Forensic Science International: Genetics* 5(3): 202-209. |
| 191 | 8. | Perlin, M.W., Legler, M.M., Spencer, C.E., Smith, J.L., Allan, W.P., Belrose, J.L., Duceman, B.W. (2011) Validating TrueAllele® DNA mixture interpretation. *Journal of Forensic Sciences* 56(6): 1430-1447. |
| 57 | 9. | Tvedebrink, T., Eriksen, P.S., Mogensen, H.S., Morling, N. (2012) Statistical model for degraded DNA samples and adjusted probabilities for allelic drop-out. *Forensic Science International: Genetics* 6: 97-101. |
| 32 | 10. | Kelly, H., Bright, J.-A., Curran, J.M., Buckleton, J. (2012) Modelling heterozygote balance in forensic DNA profiles. *Forensic Science International: Genetics* 6: 729-734. |

**Location Published**

*FSI Genetics* (26)
*J. Forensic Sci.* (5)
*Forensic Sci. Int.* (3)
*Sci. Justice* (3)
*PLoS ONE* (2)
*Appl. Stat.* (1)
*Electrophoresis* (1)
*J. Theor. Biol.* (1)
*Ann. Rev. Stats. Appl.* (1)
*Stat. Appl. Genet. Mol. Biol.* (1)

9

## Interpretation: Probabilistic Genotyping Software
(Category L – 44 articles) – Part 2

| Google Scholar Number of citations (8 Jan 2021) | | |
|---|---|---|
| 107 | 11. | Gill, P., Gusmao, L., Haned, H., Mayr, W.R., Morling, N., Parson, W., Prieto, L., Prinz, M., Schneider, H., Schneider, P.M., Weir, B.S. (2012) DNA Commission of the International Society of Forensic Genetics: Recommendations on the evaluation of STR typing results that may include drop-out and/or drop-in using probabilistic methods. *Forensic Science International: Genetics* 6(6): 679-688. |
| 112 | 12. | Gill, P. and Haned, H. (2013) A new methodological framework to interpret complex DNA profiles using likelihood ratios. *Forensic Science International: Genetics* 7(2): 251-263. |
| 28 | 13. | Bright, J.-A., Curran, J.M., Buckleton, J.S. (2013) Investigation into the performance of different models for predicting stutter. *Forensic Science International: Genetics* 7(4): 422-427. |
| 178 | 14. | Taylor, D., Bright, J.-A., Buckleton, J. (2013) The interpretation of single source and mixed DNA profiles. *Forensic Science International: Genetics* 7(5): 516-528. |
| 75 | 15. | Puch-Solis, R., Rodgers, L., Mazumder, A., Pope, S., Evett, I., Curran, J., Balding, D. (2013) Evaluating forensic DNA profiles using peak heights, allowing for multiple donors, allelic dropout and stutters. *Forensic Science International: Genetics* 7(5): 555-563. |
| 78 | 16. | Ramos, D. and Gonzalez-Rodriguez, J. (2013) Reliable support: measuring calibration of likelihood ratios. *Forensic Science International* 230: 156-169. |
| 48 | 17. | Kelly, H., Bright, J.A., Buckleton, J.S., Curran, J.M. (2014) A comparison of statistical models for the analysis of complex forensic DNA profiles. *Science & Justice* 54(1): 66-70. |
| 25 | 18. | Taylor, D., Bright, J.-A., Buckleton, J. (2014) The 'factor of two' issue in mixed DNA profiles. *Journal of Theoretical Biology* 363: 300-306. |
| 34 | 19. | Taylor, D., Bright, J.-A., Buckleton, J. (2014) Interpreting forensic DNA profiling evidence without specifying the number of contributors. *Forensic Science International: Genetics* 13: 269-280. |
| 20 | 20. | Taylor, D., Bright, J.-A., Buckleton, J. (2014) Considering relatives when assessing the evidential strength of mixed DNA profiles. *Forensic Science International: Genetics* 13: 259-263. |

**Location Published**

*FSI Genetics* (26)
*J. Forensic Sci.* (5)
*Forensic Sci. Int.* (3)
*Sci. Justice* (3)
*PLoS ONE* (2)
*Appl. Stat.* (1)
*Electrophoresis* (1)
*J. Theor. Biol.* (1)
*Ann. Rev. Stats. Appl.* (1)
*Stat. Appl. Genet. Mol. Biol.* (1)

10

## Interpretation: Probabilistic Genotyping Software
(Category L – 44 articles) – Part 3

| Google Scholar Number of citations (8 Jan 2021) | | |
|---|---|---|
| 52 | 21. | Bright, J.-A., Taylor, D., Curran, J.M., Buckleton, J. (2014) Searching mixed DNA profiles directly against profile databases. *Forensic Science International: Genetics* 9: 102-110. |
| 32 | 22. | Bille, T.W., Weitz, S.M., Coble, M.D., Buckleton, J., Bright, J.-A. (2014) Comparison of the performance of different models for the interpretation of low level mixed DNA profiles. *Electrophoresis* 35: 3125-3133. |
| 20 | 23. | Steele, C.D., Greenhalgh, M., Balding, D.J. (2014) Verifying likelihoods for low template DNA profiles using multiple replicates. *Forensic Science International: Genetics* 13: 82-89. |
| 65 | 24. | Steele, C.D. and Balding, D.J. (2014) Statistical evaluation of forensic DNA profile evidence. *Annual Review of Statistics and Its Application* 1: 361-384. |
| 38 | 25. | Bright, J.-A., Evett, I.W., Taylor, D., Curran, J.M., Buckleton, J. (2015) A series of recommended tests when validating probabilistic DNA profile interpretation software. *Forensic Science International: Genetics* 14: 125-131. |
| 97 | 26. | Gill, P., Haned, H., Bleka, O., Hansson, O., Dørum, G. and Egeland, T. (2015) Genotyping and interpretation of STR-DNA: Low-template, mixtures and database matches-Twenty years of research and development. *Forensic Science International: Genetics* 18: 100-117. |
| 28 | 27. | Perlin, M.W., Hornyak, J.M., Sugimoto, G., Miller, K.W. (2015) TrueAllele® genotype identification on DNA mixtures containing up to five unknown contributors. *Journal of Forensic Sciences* 60(4): 857-868. |
| 23 | 28. | Greenspoon, S.A., Schiermeier-Wood, L., Jenkins, B.C. (2015) Establishing the limits of TrueAllele® Casework: A validation study. *Journal of Forensic Sciences* 60(5): 1263-1276. |
| 39 | 29. | Bleka, Ø., Benschop, C.C., Storvik, G. and Gill, P. (2016) A comparative study of qualitative and quantitative models used to interpret complex STR DNA profiles. *Forensic Science International: Genetics* 25: 85-96. |
| 64 | 30. | Bright, J.-A., et al. (2016) Developmental validation of STRmix, expert software for the interpretation of forensic DNA profiles. *Forensic Science International: Genetics* 23: 226-239. |

**Location Published**

*FSI Genetics* (26)
*J. Forensic Sci.* (5)
*Forensic Sci. Int.* (3)
*Sci. Justice* (3)
*PLoS ONE* (2)
*Appl. Stat.* (1)
*Electrophoresis* (1)
*J. Theor. Biol.* (1)
*Ann. Rev. Stats. Appl.* (1)
*Stat. Appl. Genet. Mol. Biol.* (1)

11

## Interpretation: Probabilistic Genotyping Software
(Category L – 44 articles) – Part 4

| Google Scholar Number of citations (8 Jan 2021) | | |
|---|---|---|
| 20 | 31. | Steele, C.D., Greenhalgh, M. and Balding, D.J. (2016) Evaluation of low-template DNA profiles using peak heights. *Statistical Applications in Genetics and Molecular Biology* 15(5): 431-445. |
| 17 | 32. | Haned, H., Gill, P., Lohmueller, K., Inman, K., Rudin, N. (2016) Validation of probabilistic genotyping software for use in forensic DNA casework: Definitions and illustrations. *Science & Justice* 56(2): 104-108. |
| 46 | 33. | Moretti, T.R., Just, R.S., Kehl, S.C., Willis, L.E., Buckleton, J.S., Bright, J.A., Taylor, D.A. and Onorato, A.J. (2017) Internal validation of STRmix for the interpretation of single source and mixed DNA profiles. *Forensic Science International: Genetics* 29: 126-144. |
| 12 | 34. | Bright, J.-A., Taylor, D., Gittelson, S., Buckleton, J. (2017) The paradigm shift in DNA profile interpretation. *Forensic Science International: Genetics* 31: e24-e32. |
| 85 | 35. | Meuwly, D., Ramos, D., Haraksim, R. (2017) A guideline for the validation of likelihood ratio methods used for forensic evidence evaluation. *Forensic Science International* 276: 142-153. |
| 48 | 36. | Bright, J.-A., et al. (2018) Internal validation of STRmix™ – a multi laboratory response to PCAST. *Forensic Science International: Genetics* 34: 11-24. |
| 3 | 37. | Slooten, K. (2018) The information gain from peak height data in DNA mixtures. *Forensic Science International: Genetics* 36: 119-123. |
| 6 | 38. | Swaminathan, H., Qureshi, M.O., Grgicak, C.M., Duffy, K. and Lun, D.S. (2018) Four model variants within a continuous forensic DNA mixture interpretation framework: effects on evidential inference and reporting. *PLoS ONE* 13(11): e0207599. |
| 8 | 39. | Benschop, C.C.G., Nijveld, A., Duijs, F.E. and Sijen, T. (2019) An assessment of the performance of the probabilistic genotyping software EuroForMix: Trends in likelihood ratios and analysis of Type I & II errors. *Forensic Science International: Genetics* 42: 31-38. |
| 10 | 40. | Bright, J.-A., et al. (2019) STRmix™ collaborative exercise on DNA mixture interpretation. *Forensic Science International: Genetics* 40:1-8. |

**Location Published**

*FSI Genetics* (26)
*J. Forensic Sci.* (5)
*Forensic Sci. Int.* (3)
*Sci. Justice* (3)
*PLoS ONE* (2)
*Appl. Stat.* (1)
*Electrophoresis* (1)
*J. Theor. Biol.* (1)
*Ann. Rev. Stats. Appl.* (1)
*Stat. Appl. Genet. Mol. Biol.* (1)

12

## Interpretation: Probabilistic Genotyping Software
(Category L – 44 articles) – Part 5

| Google Scholar Number of citations (6 Jan 2021) | | |
|---|---|---|
| 7 | 41. | You, Y. and Balding, D. (2019) A comparison of software for the evaluation of complex DNA profiles. *Forensic Science International: Genetics* 40: 114-119. |
| 0 (too new) | 42. | Lin, M.H., Bright, J.-A, Pugh, S.N., Buckleton, J.S. (2020) The interpretation of mixed DNA profiles from a mother, father, and child trio. *Forensic Science International: Genetics* 44: 102175. |
| 7 | 43. | Bauer, D.W., Butt, N., Hornyak, J.M., Perlin, M.W. (2020) Validating TrueAllele® interpretation of DNA mixtures containing up to ten unknown contributors. *Journal of Forensic Sciences* 65(2): 380-398. |
| 1 | 44. | Schuerman, C., Kalafut, T., Buchanan, C., Sutton, J., Bright, J.-A. (2020) Using the nondonor distribution to improve communication and inform decision making for low LRs from minor contributors in mixed DNA profiles. *Journal of Forensic Sciences* 65(4): 1072-1084. |

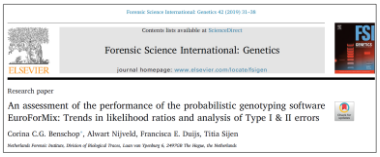
**Location Published**

*FSI Genetics* (26)
*J. Forensic Sci.* (5)
*Forensic Sci. Int.* (3)
*Sci. Justice* (3)
*PLoS ONE* (2)
*Appl. Stat.* (1)
*Electrophoresis* (1)
*J. Theor. Biol.* (1)
*Ann. Rev. Stats. Appl.* (1)
*Stat. Appl. Genet. Mol. Biol.* (1)

13

## Assessment of PGS Performance

**L39.** Benschop, C.C.G., Nijveld, A., Duijs, F.E. and Sijen, T. (2019) An assessment of the performance of the probabilistic genotyping software EuroForMix: Trends in likelihood ratios and analysis of Type I & II errors. *Forensic Science International: Genetics* 42: 31-38.


Forensic Science International: Genetics 42 (2019) 31–38

Contents lists available at ScienceDirect

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsigen

Research paper

An assessment of the performance of the probabilistic genotyping software EuroForMix: Trends in likelihood ratios and analysis of Type I & II errors

Corina C.G. Benschop*, Alwart Nijveld, Francisca E. Duijs, Titia Sijen

Also discussed in Chapter 9 "Validation" (pp. 277-308) of Peter Gill, Øyvind Bleka, Oskar Hansson, Corina Benschop and Hinda Haned (2020) *Forensic Practitioner's Guide to the Interpretation of Complex DNA Profiles* (Elsevier Academic Press, San Diego)

14

## Different Categories of Mixture Types Were Studied in Exploring the DNA Mixture Factor Space

**L39.** Benschop, C.C.G., Nijveld, A., Duijs, F.E. and Sijen, T. (2019) An assessment of the performance of the probabilistic genotyping software EuroForMix: Trends in likelihood ratios and analysis of Type I & II errors. *Forensic Science International: Genetics* 42: 31-38.

**Table 2**
Mixture proportions and amounts of DNA used per donor to create a total of 20 different mixtures per dataset.

| Mixture Type | Number of contributors | | | |
|---|---|---|---|---|
| | 2 | 3 | 4 | 5 |
| | Picograms DNA per contributor | | | |
| A: major 2x more than any minor | 300:150 | 300:150:150 | 300:150:150:150 | 300:150:150:150:150 |
| B: major 10x more than any minor | 300:30 | 300:30:30 | 300:30:30:30 | 300:30:30:30:30 |
| C: 2 majors with equal amount | 150:150 | 150:150:60 | 150:150:60:60 | 150:150:60:60:60 |
| D: major 5 to 2.5x more than minors | 150:30 | 150:30:60 | 150:30:60:30 | 150:30:60:30:30 |
| E: major 20 to 10x more than minors | 600:30 | 600:30:60 | 600:30:60:30 | 600:30:60:30:30 |
| Number of mixtures | 5 | 5 | 5 | 5 |

"Factor Space" in DNA Mixture Studies

1. **Total DNA amount**
2. **Sample quality**
3. **Number of contributors**
4. **Degree of allele overlap**
5. **Contributor component ratios**

15

## Multiple Donor Combinations Used to Create Different Degrees of Allele Sharing

L39. Benschop, C.C.G., Nijveld, A., Duijs, F.E. and Sijen, T. (2019) An assessment of the performance of the probabilistic genotyping software EuroForMix: Trends in likelihood ratios and analysis of Type I & II errors. *Forensic Science International: Genetics* 42: 31-38.

**Table 1**
Overview of the six donor combinations used for mixture preparation.

| Dataset number | Type of dataset | Number of contributors | | | |
|---|---|---|---|---|---|
| | | 2 | 3 | 4 | 5 |
| | | Donor combinations per dataset | | | |
| 1 | High allele sharing | a:b | a:b:c | a:b:c:d | a:b:c:d:e |
| 2 | Low allele sharing | f:g | f:g:h | f:g:h:i | f:g:h:i:j |
| 3 | Random | k:l | k:l:k | k:l:k:n | k:l:m:n:o |
| 4 | Random | p:q | p:q:r | p:q:r:s | p:q:r:s:t |
| 5 | Random | u:v | u:v:w | u:v:w:x | u:v:w:x:y |
| 6 | Random | z:aa | z:aa:ab | z:aa:ab:ac | z:aa:ab:ac:ad |

**Specific genotypes can be kept anonymous and still differentiate various degrees of allele sharing**

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## Summary and Key Takeaways

- There is **a growing literature on probabilistic genotyping software** (PGS)
- **Continuous PGS models (involving peak heights) use more information** from samples than discrete models (with only allele information) or binary approaches (that cannot cope with the possibility of allele drop-out)
- DNA mixture **factor space that is explored in the well-designed studies** includes (1) total DNA amount, (2) contributor component ratios, (3) number of contributors, (4) degree of allele overlap, and (5) sample quality

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## Thank you for your attention!

**John M. Butler**
john.butler@nist.gov

**Robin W. Cotton**
rwcotton@bu.edu

**Mechthild "Mecki" Prinz**
mprinz@jjay.cuny.edu

**Charlotte J. Word**
cjword@comcast.net

**Please put questions in the chat box while watching our pre-recorded presentations and we will answer them over the chat or through a live Zoom session at the end – you can also email any of us…**

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American Academy of Forensic Sciences
VIRTUAL WORKSHOP W19 (MVPs of Forensic DNA)
February 16, 2021

2021 AAFS VIRTUAL MEETING

*MVPs = Most Valuable Publications*

# MVPs on DNA Transfer and Activity Level Propositions

**John M. Butler, PhD**
*National Institute of Standards and Technology*

NIST FORENSIC SCIENCE
RESEARCH. STANDARDS. FOUNDATIONS.

**Module 9**

1

## Acknowledgments and Disclaimer

*Thank you to SWGDAM and Phil Danielson with the OSAC Literature Task Group for their starting materials in developing these MVPs (most valuable publications)*

**Points of view are mine** and do not necessarily represent the official position or policies of the National Institute of Standards and Technology.

Certain commercial entities are identified in order to specify experimental procedures as completely as possible. In no case does such identification imply a recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that any of the entities identified are necessarily the best available for the purpose.

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## Presentation Outline

- Principles with DNA Transfer and Activity Level Propositions
- MVPs of DNA Transfer (Category W)
  - #1 article and why
  - Number and types of publications in this category
- MVPs of Activity Level Propositions (some in Category N)
  - #1 article and why
- Summary and Key Takeaways

3

## Some Principles and Thoughts with DNA Transfer and Activity Level Propositions

1. The **importance of DNA transfer and activity propositions has increased** in recent years **with use of highly sensitive DNA testing methods**.
2. There is **a growing body of literature** on this topic in the past few years due to the desire to address not only the source of the DNA but how the DNA got there.
3. Several reviews highlight ***how little we know with certainty about how DNA transfers*** from the donor, the range of variables that affect transfer and persistence, the value of activity propositions, and a proposal for a more systematic approach to data collection.
4. The overall takeaway from this literature is that **sub-source DNA results in isolation cannot automatically be assumed to relate to the crime**. **What question(s) are we answering with a DNA result?**

4

## Levels in the Hierarchy of Propositions

| | Purpose | Questions Addressed | Results Used | Factors Considered |
|---|---|---|---|---|
| **Sub-source** | Investigation | Who could be the source of the DNA? | DNA profile | **Occurrence of DNA profile genotypes in the relevant population**; variability of results (e.g., presence or absence of alleles) assuming the DNA came from the POI |
| | Evaluation | **Is the DNA from the person of interest (POI)?** | | |
| **Source** | Investigation | Who could be the source of the biological fluid? | DNA profile; biological fluid presumptive tests | (Sub-source factors) + presumptive test false positive/ false negative rates (e.g., cross-reactivity, etc.) |
| | Evaluation | **Is the biological fluid from the POI?** | | |
| **Activity** | Evaluation | **Did the POI perform the given activity?** | DNA profile; biological fluid presumptive tests; relative quantity of DNA; where DNA was recovered; existence of multiple samples | (Source factors) + **DNA transfer, persistence, and recovery**; **DNA present for unknown reasons (i.e., background DNA)** |

**sub-sub-source** if only a portion of a DNA mixture is considered

DNA transfer impact

**W43.** Taylor et al. (2018) Evaluation of forensic genetics findings given activity level propositions: A review. *Forensic Sci Int Genet*. 2018;36:34-49.

Table adapted from **Reference N17** Gill et al. (2018) *Forensic Sci. Int. Genet.* 36: 189-202

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## DNA Transfer and Activity Level Reporting

(Category W – 57 articles; some Category M & N articles)

- **DNA Transfer:**
  - Systematic research and sharing data (W45, W49)
  - Contamination possibilities (W5, W7, W47)
  - Recovery from touch samples (W2, W8, W10, W54)
  - Shedder status (W3, W6, W31, W41)
  - Mechanisms (W23, W26, W48, W51)
  - Interlaboratory study on transfer (W35, W52)
  - Secondary transfer variables (W9, W13, W20, W29)
  - Primary vs secondary (W34, W50, W56)
  - Implications (W14, W15, W16, W18)
  - Environmental monitoring (W12, W19)
  - Persistence (W17, W37)
  - Review (W1, W4, W11, W48)

#1

key

  - On clothing (W28)
  - On firearms (W55)
  - On hands (W39, W40, W42)
  - On knives or tools (W36, W37)
  - Through laundry (W21, W27, W46)
  - Within a laboratory (W25)
  - Relative contributions of donors (W30)

- **Activity Level:**
  - Hierarchy of propositions (M3, M4, M5, N5, N10, N21, W32, W33)
  - Formation of propositions (N16)
  - Propositions of actor vs activity (W38)
  - ENFSI Evaluative Reporting (W22)
  - ISFG DNA Commission (N17, N22)
  - Bayesian Networks (N4, W44, W53, W57)
  - Review & sub-sub-source (W43)

#1

- **DNA Elimination Databases:**
  - Necessity for CSI, police, and lab (W24)

6

## #1 MVP on DNA Transfer

**W1.** **van Oorschot, R.A.H., Szkuta, B., Meakin, G.E., Kookshoorn, B., Goray, M. (2019) DNA transfer in forensic science: a review. *Forensic Science International: Genetics* 38: 140-166.**


Forensic Science International: Genetics

DNA transfer in forensic science: A review

Google Scholar
**Cited 75 times**
(8 Jan 2021)

- **Why is this article valuable?**
  - Cites 298 articles in covering the issues and history of research
  - Recognizes that offering sound opinions on DNA transfer and activity level questions requires dedicated training and competency testing

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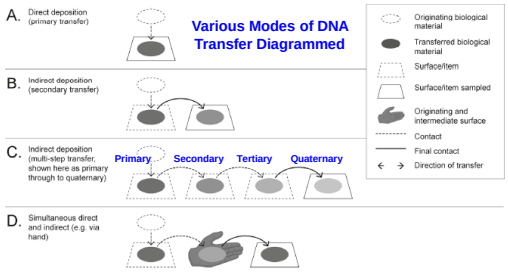
**W1.** **van Oorschot, R.A.H., Szkuta, B., Meakin, G.E., Kookshoorn, B., Goray, M. (2019) DNA transfer in forensic science: a review. *Forensic Science International: Genetics* 38: 140-166.**




**(A portion of) Fig. 1.** Diagrammatic illustration of various modes of transfer.

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## #1 MVP on Activity Level

**N22.** Gill, P., et al. (2020) DNA Commission of the International Society for Forensic Genetics: Assessing the value of forensic biological evidence - guidelines highlighting the importance of propositions. Part II: Evaluation of biological traces considering activity level propositions. *Forensic Science International: Genetics* 44: 102186.


Forensic Science International: Genetics

DNA commission of the International society for forensic genetics: Assessing the value of forensic biological evidence - Guidelines highlighting the importance of propositions. Part II: Evaluation of biological traces considering activity level propositions

Google Scholar
**Cited 13 times**
(20 Jan 2021)

- **Why is this article valuable?**
  - Discusses why, when and how to carry out an evaluation given **activity level propositions** using examples
  - Distinguishes between **results, propositions and explanations**

9

# Most Valuable Publications of Forensic DNA

(J.M. Butler, R.W. Cotton, M.K. Prinz, C.J. Word)

16 February 2021

## DNA Transfer and Activity Level Reporting

(Category W – 57 articles) – Part 1

| Google Scholar Number of citations (8 Jan 2021) | | | Location Published |
|---|---|---|---|
| 75 | 1. | **van Oorschot, R.A.H., Szkuta, B., Meakin, G.E., Kookshoorn, B., Goray, M. (2019) DNA transfer in forensic science: a review. *Forensic Science International: Genetics* 38: 140-166.** | *FSI Genetics* (32)<br>*Front. Genet.* (5)<br>*Int. J. Legal Med.* (4)<br>*Forensic Sci. Int.* (3)<br>*FSIG Suppl. Ser.* (3)<br>*J. Forensic Sci.* (2)<br>*Legal Med.* (2)<br>*Sci. Justice* (2)<br>*Aus. J. Forensic Sci.* (1)<br>*ENFSI* (1)<br>*FSMP* (1)<br>*Nature* (1) |
| 454 | 2. | van Oorschot, R.A. and Jones, M.K. (1997) DNA fingerprints from fingerprints. *Nature* 387: 767. | |
| 357 | 3. | Lowe, A., Murray, C., Whitaker, J., Tully, G. and Gill, P. (2002) The propensity of individuals to deposit DNA and secondary transfer of low level DNA from individuals to inert surfaces. *Forensic Science International* 129: 25-34. | |
| 347 | 4. | Wickenheiser, R.A. (2002) Trace DNA: a review, discussion of theory, and application of the transfer of trace quantities of DNA through skin contact. *Journal of Forensic Sciences* 47(3): 442-450. | |
| 32 | 5. | Port, N.J., Bowyer, V.L., Graham, E.A.M., Batuwangala, M.S., Rutty, G.N. (2006) How long does it take a static speaking individual to contaminate the immediate environment? *Forensic Science, Medicine, and Pathology* 2(3): 157-163. | |
| 231 | 6. | Phipps, M. and Petricevic, S. (2007) The tendency of individuals to transfer DNA to handled items. *Forensic Science International* 168(2-3): 162-168. | |
| 46 | 7. | Malsom, S., Flanagan, N., McAlister, C., Dixon, L. (2009) The prevalence of mixed DNA profiles in fingernail samples taken from couples who co-habit using autosomal and Y-STRs. *Forensic Science International: Genetics* 3(2): 57-62. | |
| 131 | 8. | Goray, M., Mitchell, R.J., van Oorschot, R.A.H. (2010) Investigation of secondary DNA transfer of skin cells under controlled test conditions. *Legal Medicine* 12(3): 117-120. | |
| 147 | 9. | Goray, M., Eken, E., Mitchell, R. J., van Oorschot, R.A.H. (2010) Secondary DNA transfer of biological substances under varying test conditions. *Forensic Science International: Genetics* 4(2): 62-67. | |
| 180 | 10. | Daly, D.J., Murphy, C., McDermott, S.D. (2012) The transfer of touch DNA from hands to glass, fabric and wood. *Forensic Science International: Genetics* 6(1): 41-46. | |

10

## DNA Transfer and Activity Level Reporting

(Category W – 57 articles) – Part 2

| Google Scholar Number of citations (8 Jan 2021) | | | Location Published |
|---|---|---|---|
| 154 | 11. | Meakin, G. and Jamieson, A. (2013) DNA transfer: review and implications for casework. *Forensic Science International: Genetics* 7: 434-443. | *FSI Genetics* (32)<br>*Front. Genet.* (5)<br>*Int. J. Legal Med.* (4)<br>*Forensic Sci. Int.* (3)<br>*FSIG Suppl. Ser.* (3)<br>*J. Forensic Sci.* (2)<br>*Legal Med.* (2)<br>*Sci. Justice* (2)<br>*Aus. J. Forensic Sci.* (1)<br>*ENFSI* (1)<br>*FSMP* (1)<br>*Nature* (1) |
| 41 | 12. | Ballantyne, K.N., Poy, A.L. and van Oorschot, R.A.H. (2013) Environmental DNA monitoring: beware of the transition to more sensitive typing methodologies. *Australian Journal of Forensic Sciences* 45(3): 323-340. | |
| 67 | 13. | Verdon, T.J., Mitchell, R.J. and van Oorschot, R.A. (2013) The influence of substrate on DNA transfer and extraction efficiency. *Forensic Science International: Genetics* 7(1): 167-175. | |
| 11 | 14. | Jackson, G. (2013) The impact of commercialization on the evaluation of DNA evidence. *Frontiers in Genetics* 4: 227. | |
| 18 | 15. | McKenna, L. (2013) Understanding DNA results within the case context: importance of the alternative proposition. *Frontiers in Genetics* 4: 242. | |
| 40 | 16. | Champod, C. (2013) DNA transfer: informed judgment or mere guesswork? *Frontiers in Genetics* 4: 30 | |
| 61 | 17. | van Oorschot, R.A.H., Glavich, G., and Mitchell, R.J. (2014) Persistence of DNA deposited by the origin user on objects after subsequent use by a second person. *Forensic Science International: Genetics* 8( 219-225. | |
| 55 | 18. | Cale, C.M., Earll, M.E., Latham, K.E., Bush, G.L. (2015) Could secondary DNA transfer falsely place someone at the scene of a crime? *Journal of Forensic Sciences* 61(1): 196-203. | |
| 29 | 19. | Lehmann, V.J.., Mitchell, R.J., Ballantyne, K.N., van Oorschot, R.A.H. (2015) Following the transfer of DNA: How does the presence of background DNA affect the transfer and detection of a target source of DNA? *Forensic Science International: Genetics*. 19: 68-75. | |
| 66 | 20. | Fonneløp, A.E., Egeland, T., Gill, P. (2015) Secondary and subsequent DNA transfer during criminal investigation. *Forensic Science International: Genetics* 17: 155-162. | |

11

## DNA Transfer and Activity Level Reporting

(Category W – 57 articles) – Part 3

| Google Scholar Number of citations (8 Jan 2021) | | | Location Published |
|---|---|---|---|
| 31 | 21. | Kamphausen, T., Fandel, S.B., Gutmann, J.S., Bajanowski, T., Poetsch, M. (2015) Everything clean? Transfer of DNA traces between textiles in the washtub. *International Journal of Legal Medicine* 129(4): 709-714. | *FSI Genetics* (32)<br>*Front. Genet.* (5)<br>*Int. J. Legal Med.* (4)<br>*Forensic Sci. Int.* (3)<br>*FSIG Suppl. Ser.* (3)<br>*J. Forensic Sci.* (2)<br>*Legal Med.* (2)<br>*Sci. Justice* (2)<br>*Aus. J. Forensic Sci.* (1)<br>*ENFSI* (1)<br>*FSMP* (1)<br>*Nature* (1) |
| 84 | 22. | ENFSI (2015) ENFSI Guideline for Evaluative Reporting in Forensic Science; available at http://enfsi.eu/wp-content/uploads/2016/09/m1_guideline.pdf. | |
| 59 | 23. | Goray, M. and van Oorschot, RA. (2015) The complexities of DNA transfer during a social settling. *Legal Medicine* 17(2): 82-91. | |
| 25 | 24. | Lapointe, M., Rogic, A., Bourgoin, S., Jolicoeur, C. and Séguin, D. (2015) Leading-edge forensic DNA analyses and the necessity of including crime scene investigators, police officers and technicians in a DNA elimination database. *Forensic Science International: Genetics* 19: 50-55. | |
| 26 | 25. | Taylor, D., Abarno, D., Rowe, E., Rask-Nielsen, L. (2016) Observations of DNA transfer within an operational Forensic Biology Laboratory. *Forensic Science International: Genetics* 23: 33-49. | |
| 15 | 26. | Jones, S., Scott, K., Lewis, J., Davidson, G., Allard, J. E., Lowrie, C., McBride, B.M., McKenna, L., Teppett, G., Rogers, C., Clayson, N., Baird, A. (2016) DNA transfer through nonintimate social contact. *Science & Justice* 56(2): 90-95. | |
| 22 | 27. | Noël, S., Lagacé, K., Rogic, A., Granger, D., Bourgoin, S., Jolicoeur, C., Séguin, D. (2016) DNA transfer during laundering may yield complete genetic profiles. *Forensic Science International: Genetics* 23: 240-247. | |
| 38 | 28. | Breathnach, M., Williams, L., McKenna, L., Moore, E. (2016) Probability of detection of DNA deposited by habitual wearer and/or the second individual who touched the garment. *Forensic Science International: Genetics* 20: 53-60. | |
| 29 | 29. | Helmus, J., Bajanowski, T., Poetsch, M. (2016) DNA transfer-a never ending story: a study on scenarios involving a second person as carrier. *International Journal of Legal Medicine* 130(1): 121-125. | |
| 34 | 30. | Oldoni, F., Castella, V., Hall, D. (2016) Shedding light on the relative DNA contribution of two persons handling the same object. *Forensic Science International: Genetics* 24: 148-157. | |

12

## DNA Transfer and Activity Level Reporting
(Category W – 57 articles) – Part 4

| Google Scholar Number of citations (8 Jan 2021) | | |
|---|---|---|
| 77 | 31. | Goray, M., Fowler, S., Szkuta, B., van Oorschot, R.A.H. (2016) Shedder status – an analysis of self and non-self DNA in multiple handprints deposited by the same individuals over time. *Forensic Science International: Genetics* 23: 190-196. |
| 4 | 32. | Biedermann, A. and Hicks, T. (2016) The importance of critically examining the level of propositions when evaluating forensic DNA results. *Frontiers in Genetics* 7: 8. |
| 33 | 33. | Biedermann, A., Champod, C., Jackson, G., Gill, P., Taylor, D., Butler, J., Morling, N., Hicks, T., Vuille, J., Taroni, F. (2016) Evaluation of forensic DNA traces when propositions of interest relate to activities: analysis and discussion of recurrent concerns. *Frontiers in Genetics* 7: 215. |
| 36 | 34. | Taylor, D., Biedermann, A., Samie, L., Pun, K.M., Hicks, T. and Champod, C. (2017) Helping to distinguish primary from secondary transfer events for trace DNA. *Forensic Science International: Genetics* 28: 155-177. |
| 21 | 35. | Steensma, K., Ansell, R., Clarisse, L., Connolly, E., Kloosterman, A.D., McKenna, L.G., van Oorschot, R.A.H., Szkuta, B. and Kokshoorn, B. (2017) An inter-laboratory comparison study on transfer, persistence and recovery of DNA from cable ties. *Forensic Science International: Genetics* 31: 95-104. |
| 41 | 36. | Meakin, G.E., Butcher, E.V., van Oorschot, R.A.H., Morgan, R.M. (2017) Trace DNA evidence dynamics: An investigation into the deposition and persistence of directly- and indirectly-transferred DNA on regularly-used knives. *Forensic Science International: Genetics* 29: 38-47. |
| 27 | 37. | Pfeifer, C.M. and Wiegand, P. (2017) Persistence of touch DNA on burglary-related tools. *International Journal of Legal Medicine* 131(4): 941-953. |
| 19 | 38. | Kokshoorn, B., Blankers, B.J., de Zoete, J., Berger, C.E.H. (2017) Activity level DNA evidence evaluation: On propositions addressing the actor or the activity. *Forensic Science International* 278: 115-124. |
| 3 | 39. | Stella, C.J., Mitchell, R.J., van Oorschot, R.A.H. (2017) Hand activities during robberies—Relevance to consideration of DNA transfer and detection. *Forensic Science International: Genetics Supplement Series* 6: e3-e5. |
| 49 | 40. | Szkuta, B., Ballantyne, K.N., van Oorschot, R.A.H. (2017) Transfer and persistence of DNA on the hands and the influence of activities performed. *Forensic Science International: Genetics* 28: 10-20. |

**Location Published**

*FSI Genetics* (32)
*Front. Genet.* (5)
*Int. J. Legal Med.* (4)
*Forensic Sci. Int.* (3)
*FSIG Suppl. Ser.* (3)
*J. Forensic Sci.* (2)
*Legal Med.* (2)
*Sci. Justice* (2)
*Aus. J. Forensic Sci.* (1)
*ENFSI* (1)
*FSMP* (1)
*Nature* (1)

13

## DNA Transfer and Activity Level Reporting
(Category W – 57 articles) – Part 5

| Google Scholar Number of citations (8 Jan 2021) | | |
|---|---|---|
| 61 | 41. | Kanokwongnuwut, P., Martin, B., Kirkbride, K.P., Linacre, A. (2018) Shedding light on shedders. *Forensic Science International: Genetics* 36: 20-25. |
| 27 | 42. | Szkuta, B., Ballantyne, K.N., Kokshoorn, B., and van Oorschot, R.A.H. (2018) Transfer and persistence of non-self DNA on hands over time: Using empirical data to evaluate DNA evidence given activity level propositions. *Forensic Science International: Genetics* 33: 84-97. |
| 41 | 43. | Taylor, D., Kokshoorn, B. and Biedermann, A. (2018) Evaluation of forensic genetics findings given activity level propositions: A review. *Forensic Science International: Genetics* 36: 34-49. |
| 22 | 44. | Taylor, D., Biedermann, A., Hicks, T. and Champod, C. (2018) A template for constructing Bayesian networks in forensic biology cases when considering activity level propositions. *Forensic Science International: Genetics* 33: 136-146. |
| 18 | 45. | Kokshoorn, B., Aarts, L.H.J., Ansell, R., Connolly, E., Drotz, W., Kloosterman, A.D., McKenna, L.G., Szkuta, B., van Oorschot, R.A.H. (2018) Sharing data on DNA transfer, persistence, prevalence and recovery: Arguments for harmonization and standardization. *Forensic Science International: Genetics* 37: 260-269. |
| 19 | 46. | Voskoboinik, L., Amiel, M., Reshef, A., Gafny, R., Barash, M. (2018) Laundry in a washing machine as a mediator of secondary and tertiary DNA transfer. *International Journal of Legal Medicine* 132(2): 373-378. |
| 8 | 47. | Goray, M., Pirie, E., van Oorschot, R. A. (2019) DNA transfer: DNA acquired by gloves during casework examinations. *Forensic Science International: Genetics* 38: 167-174. |
| 43 | 48. | Burrill, J., Daniel, B., Frascione, N. (2019) A review of trace "touch DNA" deposits: Variability factors and an exploration of cellular composition. *Forensic Science International: Genetics* 39:8-18. |
| 18 | 49. | Gosch, A. and Courts, C. (2019) On DNA transfer: the lack and difficulty of systematic research and how to do it better. *Forensic Science International: Genetics* 40: 24-36. |
| 0 (too new) | 50. | Rolo, M., Sampaio, L., Balsa, F., Bento, A.M., Gouveia, N., Serra, A., Brito, P., Lopes, V., Sao-Bento, M., Bogas, V., Cunha, P., Porto, M. J., Carneiro de Sousa, M.J. (2019) Assessment of individual shedder status and background DNA on objects: Direct or indirect transfer? *Forensic Science International Genetics Supplement Series* 7(1): 622-623. |

**Location Published**

*FSI Genetics* (32)
*Front. Genet.* (5)
*Int. J. Legal Med.* (4)
*Forensic Sci. Int.* (3)
*FSIG Suppl. Ser.* (3)
*J. Forensic Sci.* (2)
*Legal Med.* (2)
*Sci. Justice* (2)
*Aus. J. Forensic Sci.* (1)
*ENFSI* (1)
*FSMP* (1)
*Nature* (1)

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## DNA Transfer and Activity Level Reporting
(Category W – 57 articles) – Part 6

| Google Scholar Number of citations (8 Jan 2021) | | |
|---|---|---|
| 0 (too new) | 51. | Romero-García, C., Rosell-Herrera, R., Revilla, C.J., Baeza-Richer, C., Gomes, C., Palomo-Díez, S., Arroyo-Pardo, E., López-Parra, A.M. (2019) Effect of the activity in secondary transfer of DNA profiles. *Forensic Science International: Genetics Supplement Series* 7(1): 578-579. |
| 10 | 52. | Szkuta, B., Ansell, R., Boiso, L., Connolly, E., Kloosterman, A.D., Kokshoorn, B., McKenna, L.G., Steensma, K. and van Oorschot, R.A.H. (2019) Assessment of the transfer, persistence, prevalence and recovery of DNA traces from clothing: An inter-laboratory study on worn upper garments. *Forensic Science International: Genetics* 42: 56-68. |
| 3 | 53. | Taylor, D., Samie, L., Champod, C. (2019) Using Bayesian networks to track DNA movement through complex transfer scenarios. *Forensic Science International: Genetics* 42: 69-80. |
| 3 | 54. | Burrill, J., Daniel, B. & Frascione, N. (2020) Illuminating touch deposits through cellular characterization of hand rinses and body fluids with nucleic acid fluorescence. *Forensic Science International: Genetics* 46: 102269. |
| 0 (too new) | 55. | Gosch, A., Euteneuer, J., Preuss-Wossner, J., Courts, C. (2020) DNA transfer to firearms in alternative realistic handling scenarios. *Forensic Science International: Genetics* 48: 102355. |
| 2 | 56. | Samie, L., Taroni, F., Champod, C. (2020) Estimating the quantity of transferred DNA in primary and secondary transfers. *Science & Justice* 60(2): 128-135. |
| 0 (too new) | 57. | Samie, L., Champod, C., Taylor, D., Taroni, F. (2020) The use of Bayesian Networks and simulation methods to identify the variables impacting the value of evidence assessed under activity level propositions in stabbing cases. *Forensic Science International: Genetics* 48: 102334. |

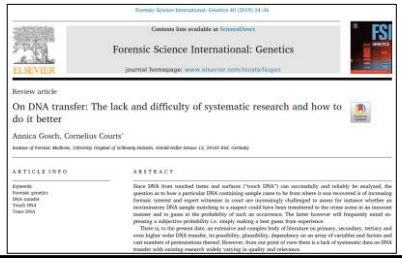
**Location Published**

*FSI Genetics* (32)
*Front. Genet.* (5)
*Int. J. Legal Med.* (4)
*Forensic Sci. Int.* (3)
*FSIG Suppl. Ser.* (3)
*J. Forensic Sci.* (2)
*Legal Med.* (2)
*Sci. Justice* (2)
*Aus. J. Forensic Sci.* (1)
*ENFSI* (1)
*FSMP* (1)
*Nature* (1)

15

## Catalog of Research on DNA Transfer Studies

**W49.** Gosch, A. and Courts, C. (2019) On DNA transfer: the lack and difficulty of systematic research and how to do it better. *Forensic Science International: Genetics* 40: 24-36


Forensic Science International: Genetics 40 (2019) 24–36

Contents lists available at ScienceDirect

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsigen

Review article

On DNA transfer: The lack and difficulty of systematic research and how to do it better

Annica Gosch, Cornelius Courts*

Institute of Forensic Medicine, University Hospital of Schleswig-Holstein, Arnold-Heller-Strasse 12, 24105 Kiel, Germany

ARTICLE INFO

*Keywords:*
Forensic genetics
DNA transfer
Touch DNA
Trace DNA

ABSTRACT

Since DNA from touched items and surfaces ("touch DNA") can successfully and reliably be analysed, the question as to how a particular DNA-containing sample came to be from where it was recovered is of increasing forensic interest and expert witnesses in court are increasingly challenged to assess for instance whether an incriminatory DNA sample matching to a suspect could have been transferred to the crime scene in an innocent manner and to guess at the probability of such an occurrence. The latter however still frequently entail us pressing a subjective probability i.e. simply making a best guess from experience.

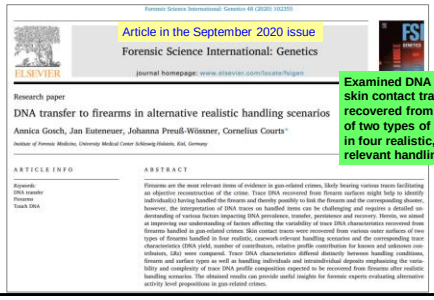
There is, to the present date, an extensive and complex body of literature on primary, secondary, tertiary and even higher order DNA transfer, its possibility, plausibility, dependency on an array of variables and factors and vast numbers of permutations thereof. However, from our point of view there is a lack of systematic data on DNA transfer with existing research widely varying in quality and relevance.

This German group developed an open resource and Microsoft Access database of **published research on DNA transfer** (called "DNA-TrAC")

– see Appendix A of their article

16

**W55.** Gosch, A., Euteneuer, J., Preuss-Wossner, J., Courts, C. (2020) DNA transfer to firearms in alternative realistic handling scenarios. *Forensic Science International: Genetics* 48: 102355.


Forensic Science International: Genetics 48 (2020) 102355

Article in the September 2020 issue

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsigen

Research paper

DNA transfer to firearms in alternative realistic handling scenarios

Annica Gosch, Jan Euteneuer, Johanna Preuß-Wössner, Cornelius Courts*

Institute of Forensic Medicine, University Medical Center Schleswig-Holstein, Kiel, Germany

ARTICLE INFO

*Keywords:*
DNA transfer
Firearms
Touch DNA

ABSTRACT

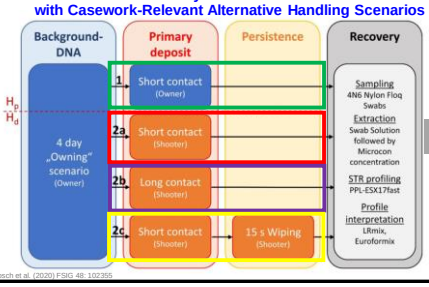
Firearms are the most relevant items of evidence in gun-related crimes, likely bearing various traces facilitating an objective reconstruction of the crime. Trace DNA recovered from firearm surfaces might help to identify individual(s) having handled the firearm and thereby possibly to link the firearm and the corresponding shooter, however, the interpretation of DNA traces on handled items can be challenging and requires a detailed understanding of various factors impacting DNA prevalence, transfer, persistence and recovery. Herein, we aimed at improving our understanding of factors affecting the variability of trace DNA characteristics recovered from firearms handled in gun-related crimes: Skin contact traces were recovered from various outer surfaces of two types of firearms handled in four realistic, casework-relevant handling scenarios and the corresponding trace characteristics (DNA yield, number of contributors, relative profile contribution for known and unknown contributors, LRs) were compared. Trace DNA characteristics differed distinctly between handling conditions, firearms and surface types as well as handling individuals and intraindividual deposits emphasizing the variability and complexity of trace DNA profile composition expected to be recovered from firearms after realistic handling scenarios. The obtained results can provide useful insights for forensic experts evaluating alternative activity level propositions in gun-related crimes.

**Examined DNA mixtures from skin contact traces of DNA recovered from three surfaces of two types of firearms handled in four realistic, casework-relevant handling scenarios**

17

**W55.** Gosch, A., Euteneuer, J., Preuss-Wossner, J., Courts, C. (2020) DNA transfer to firearms in alternative realistic handling scenarios. *Forensic Science International: Genetics* 48: 102355.

**First Research Study of DNA Transfer on Firearms with Casework-Relevant Alternative Handling Scenarios**




Only Owner (1st Handler)

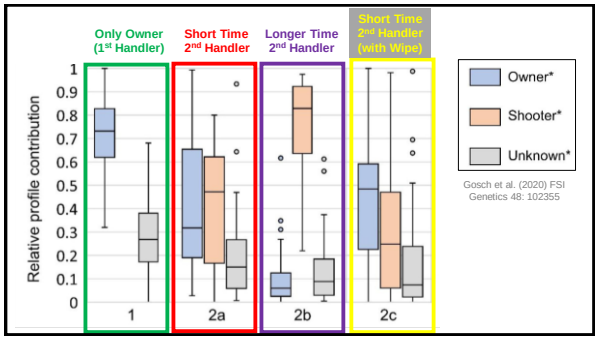
Short Time 2nd Handler

Longer Time 2nd Handler

Short Time 2nd Handler (with Wipe)

*Each repeated three times with two different owner/shooter pairs*

18




Gosch et al. (2020) FSI Genetics 48: 102355

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## Recent ISFG DNA Commission Articles

**N17. *Forensic Sci. Int. Genet.* (2018) 36: 189-202**

DNA commission of the International society for forensic genetics: Assessing the value of forensic biological evidence - Guidelines highlighting the importance of propositions
Part I: evaluation of DNA profiling comparisons given (sub-) source propositions

Peter Gill[a,b,c,1], Tacha Hicks[c,d,e,1], John M. Butler[f], Ed Connolly[g], Leonor Gusmão[h,i,k], Bas Kokshoorn[j], Niels Morling[k], Roland A.H. van Oorschot[l,m,n], Walther Parson[m,o], Mechthild Prinz[p], Peter M. Schneider[q], Titia Sijen[j], Duncan Taylor[r,s]

**N22. *Forensic Sci. Int. Genet.* (2020) 44: 102186**

DNA commission of the International society for forensic genetics: Assessing the value of forensic biological evidence - Guidelines highlighting the importance of propositions. Part II: Evaluation of biological traces considering activity level propositions

Peter Gill[a,b,c,1], Tacha Hicks[c,d,e,1], John M. Butler[f], Ed Connolly[g], Leonor Gusmão[h,i,k], Bas Kokshoorn[j], Niels Morling[k], Roland A.H. van Oorschot[l,m], Walther Parson[m,o], Mechthild Prinz[p], Peter M. Schneider[q], Titia Sijen[j], Duncan Taylor[r,s]

**(N17) 2018**
- Difference between **investigative and evaluative reporting** is explained
- Common pitfalls of **formulating propositions** are discussed
- **Challenges of low-level mixtures** are discussed

**(N22) 2020**
- Why, when and how to carry out evaluation given **activity level propositions** are addressed with examples
- Distinguishing between **results, propositions and explanations**

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## Summary and Key Takeaways

- Carefully consider and communicate to data users **what question is being answered** with your DNA results
- It is vital that results from one level of proposition are not used to move to another level without necessary information (e.g., DNA transfer rates)
  - Matching DNA (sub-source level) is not automatically relevant to the crime (activity level)
  - Matching DNA (sub-source level) is not proof of guilt (offense level)

Sub-source LR: **Who?** ≠ Activity LR: **How did it get there?**

**Who?** ≠ Offense LR: **Who did it?**

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American Academy of Forensic Sciences
VIRTUAL WORKSHOP W19 (MVPs of Forensic DNA)
February 16, 2021

2021 AAFS VIRTUAL MEETING

*MVPs = Most Valuable Publications*

# MVPs on Lineage Markers

**Robin W. Cotton, PhD**
*Boston University Biomedical Forensic Sciences*

BOSTON UNIVERSITY

**Module 10**

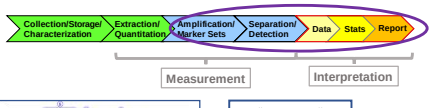
1

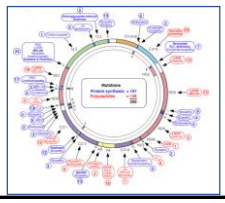
## Presentation Outline

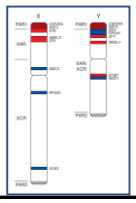
- Principles on use of Lineage Markers
- MVP's on Mitochondrial DNA Testing
  - #1 article and why
- MVP's on Y Chromosome & X Chromosome Testing
  - #1 article and why
- Special thanks to Hannah Reasbeck and Erin Ruigrok for all their work finding the number of cites for each paper and other library research work needed for my presentation.

2

## Steps in Forensic DNA Testing





3

## Critical Principles in Mitochondrial DNA, Y & X Chromosome Testing

- What is a haplotype?
- Biology, structure & inheritance of mitochondrial DNA
- Biology, structure and inheritance of the Y chromosome
- Considerations when amplifying Y STR loci in the presence of female DNA

- Criteria for appropriate application of these tools to casework?
- Use of specialized databases built for these markers?
- Understand how knowledge of genetics, mutation and evolutionary biology contribute to, and effect, the interpretation of data from these markers?

4

### Category P: Mitochondrial DNA Testing

| Journal Article | Source | Number of Citations |
|---|---|---|
| Wilson, M.R., DiZinno, J.A., et.al. (1995) Validation of mitochondrial DNA sequencing for forensic casework analysis. | Int. J of Legal Med. | 427 |
| Budowle, B., Allard, M.W., et.al. (2003) Forensics and mitochondrial DNA: Applications, debates, and foundations. | Rev. of Genomics, Human Genetics | 320 |
| #1 Parson, W., Gusmão, L., et.al. (2014) DNA Commission of the International Society for Forensic Genetics: revised and extended guidelines for mitochondrial DNA typing. | FSI Genetics | 176 |
| Melton, T. (2004) Mitochondrial DNA heteroplasmy. | FS Review | 73 |
| Huber, N., Parson, W., et.al. (2018) Next generation database search algorithm for forensic mitogenome analyses. | FSI Genetics | 35 |
| Peck, M.A., Sturk-Andreaggi, K., et.al. (2018) Developmental validation of a Nextera XT mitogenome Illumina MiSeq sequencing method for high-quality samples. | FSI Genetics | 19 |
| Holland, M.M., Makova, K.D., et.al. (2018) Deep-coverage MPS analysis of heteroplasmic variants within the mtGenome allows for frequent differentiation of maternal relatives. | Genes (Basel) | 19 |
| Pereira, V., Longobardi, A., et.al. (2018) Sequencing of mitochondrial genomes using the Precision ID mtDNA Whole Genome Panel. | Electrophoresis | 15 |
| Amorim, A., Fernandes, T., et.al. (2019) Mitochondrial DNA in human identification: a review. | Peer J | 14 |
| Brandhagen, M.D., Just, R.S., et.al. (2020) Validation of NGS for mitochondrial DNA casework at the FBI Laboratory. | FSI Genetics | 12 |
| van der Gaag, K.J., Hoogenboom, J., et.al. (2017) Validation and implementation of MPS mtDNA control region analysis for forensic casework: Determination of C-stretch lengths by the FDSTools noise correction feature. | FSI Genetics | 1 |

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## Mitochondrial DNA Testing; Section P

- Recommendations
- P1 (2014)

- Validation
- P2 (1995)

- Review
- P3 (2003)
- P4 (2004)
- P10 (2019)

- New data, polymorphisms
- P7 (2018)

- NGS
- P5 (2017)
- P6 (2018)
- P8 (2018) Search Algorithm
- P9 (2018)
- P11 (2020) Validation

6

## #1 MVP on Lineage Markers, Mitochondrial DNA


Forensic Science International: Genetics

DNA Commission of the International Society for Forensic Genetics: Revised and extended guidelines for mitochondrial DNA typing

W. Parson, L. Gusmão, D.R. Hares, J.A. Irwin, W.R. Mayr, N. Morling, E. Pokorak, M. Prinz, A. Salas, P.M. Schneider, T.J. Parsons

#1

*2014 Publication*
***Citations 176***
*Jan 16, 2021*

- **Why is this article valuable?**
- **This paper provides current guidelines for the analysis of mitochondrial DNA**

7

## Runner-Up in the Category of Mitochondrial DNA

#2

PeerJ

Mitochondrial DNA in human identification: a review

António Amorim, Teresa Fernandes and Nuno Taveira

- **Why is this article valuable?**
- **This paper is an excellent, and recent, review of the use of mitochondrial DNA testing.**

*2019 Publication*
***Citations 14***
*Jan 16, 2021*

8

## Y & X Chromosome Testing; Section Q

- Recommendations
  - Q1 (2020)
  - Q2 (2001)
  - Q5 (2006)
  - Q13 (2017-X)
  - Q16 (2018-stats)
- Mutations
  - Q7 (2010)
  - Q9 (2014)
- Review
  - Q3 (2003)
  - Q10 (2015)
  - Q14 (2017)
  - Q17 (2020-X)
- Y Chromosome Biology
  - Q4 (2003)
  - Q15 (2017)

Kits
Q6 (2006)
Q8 (2013)
Q11 (2015)
Q12 (2016)

9

## #1 MVP on Y-Chromosome & X-Chromosome Testing

***Forensic Science International: Genetics 2020* 48: 102308**


*2020 Publication*
***Cited 4 times***
*(Jan 16, 2021)*

- **Why is this article valuable?**
- **This paper provides guidance for the interpretation of Y-STR results**

10

## Thoughts

- Reviews are important for laboratories that do not do Mito and Y testing to ensure that application of Mito and Y are appropriately considered.
- Statistics for these markers are very specialized and need to be clearly understood.
- Neither of these important tools are significantly helpful for most complex mixtures.

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## Thank you for your attention!

**John M. Butler**
john.butler@nist.gov

**Robin W. Cotton**
rwcotton@bu.edu

**Mechthild "Mecki" Prinz**
mprinz@jjay.cuny.edu

**Charlotte J. Word**
cjword@comcast.net

**Please put questions in the chat box while watching our pre-recorded presentations and we will answer them over the chat or through a live Zoom session at the end – you can also email any of us…**

12

American Academy of Forensic Sciences
VIRTUAL WORKSHOP W19 (MVPs of Forensic DNA)
February 16, 2021

2021 AAFS VIRTUAL MEETING

*MVPs = Most Valuable Publications*

# MVPs on Phenotyping and New Technologies

**Mechthild K. "Mecki" Prinz, PhD**
*John Jay College of Criminal Justice*

**Module 11**

1

## Presentation Outline

- Forensic genetics beyond human identification
- MVPs on **DNA Phenotyping (Ancestry, Appearance, Age)**
  Number and types of publications in this category
- Discussion #1 article - 2015 review by Manfred Kayser
- MVPs on **New Technologies (Rapid DNA, Massive Parallel Seq.)**
  Number and types of publications in this category
- Discussion #1 article - 2015 contribution by John Butler
- Summary

2

## Forensic Genetics beyond Human Identification

- Appearance, age, and ancestry information can provide investigative leads.
- Research well established on pigmentation traits.
- MPS technology enables simultaneous detection of markers for human identification, kinship, appearance and ancestry.
- Forensic testing of coding genes requires additional legal and ethical framework.
- Rapid DNA applied to reference samples is a biometric tool beyond criminal casework.
- Rapid DNA may assist in smarter crime scene processing.

3

## DNA Phenotyping (Ancestry, Appearance, Age)
(Category U – 24 articles) – Part 1

| Google Scholar Number of citations (11 Jan 2021) | | Location Published |
|---|---|---|
| 246 | 1. **Kayser, M. (2015) Forensic DNA Phenotyping: Predicting human appearance from crime scene material for investigative purposes. *Forensic Science International: Genetics* 18: 33-48.** | *FSI Genetics (14)*<br>*New Genet & Soc (2)*<br>*Deut. Arzteblatt (1)*<br>*eLife (1)*<br>*Forensic Sci. Rev. (1)*<br>*Front. Genet. (1)*<br>*Genes (1)*<br>*Genome Biology (1)*<br>*Gerontology (1)*<br>*J. Invest. Dermat. (1)* |
| 258 | 2. Kayser, M. and Schneider, P.M. (2009) DNA-based prediction of human externally visible characteristics in forensics: motivations, scientific challenges, and ethical considerations. *Forensic Science International: Genetics* 3(3): 154-161. | |
| 381 | 3. Walsh, S., Liu, F., Wollstein, A., Kovatsi, L., Ralf, A., Kosiniak-Kamysz, A., Branicki, W., Kayser, M. (2013) The HIrisPlex system for simultaneous prediction of hair and eye colour from DNA. *Forensic Science International: Genetics* 7(1): 98-115. | |
| 145 | 4. Phillips, C. (2015) Forensic genetic analysis of bio-geographical ancestry. *Forensic Science International: Genetics* 18: 49-65. | |
| 49 | 5. Freire-Aradas, A., Phillips, C., Lareu, M.V. (2017) Forensic individual age estimation with DNA: From initial approaches to methylation tests. *Forensic Science Review* 29(2): 121-144. | |
| 96 | 6. Chaitanya, L., Breslin, K., Zuñiga, S., Wirken, L., Pośpiech, E., Kukla-Bartoszek, M., Sijen, T., Knijff, P., Liu, F., Branicki, W., Kayser, M., Walsh, S. (2018) The HIrisPlex-S system for eye, hair and skin colour prediction from DNA: Introduction and forensic developmental validation. *Forensic Science International: Genetics* 35: 123-135. | |
| 34 | 7. Vidaki, A. and Kayser, M. (2017) From forensic epigenetics to forensic epigenomics: broadening DNA investigative intelligence. *Genome Biology* 18(1): 238. | |
| 15 | 8. Vidaki, A. and Kayser, M. (2018) Recent progress, methods and perspectives in forensic epigenetics. *Forensic Science International: Genetics* 37: 180-195. | |

4

## DNA Phenotyping (Ancestry, Appearance, Age)
(Category U – 24 articles) – Part 2

| Google Scholar Number of citations (11 Jan 2021) | | Location Published |
|---|---|---|
| 34 | 9. Parson, W. (2018) Age estimation with DNA: from forensic DNA fingerprinting to forensic (epi)genomics: a mini-review. Gerontology 64(4): 326-332. | *FSI Genetics (14)*<br>*New Genet & Soc (2)*<br>*Deut. Arzteblatt (1)*<br>*eLife (1)*<br>*Forensic Sci. Rev. (1)*<br>*Front. Genet. (1)*<br>*Genes (1)*<br>*Genome Biology (1)*<br>*Gerontology (1)*<br>*J. Invest. Dermat. (1)* |
| 15 | 10. Naue, J., Hoefsloot, H.C.J., Kloosterman, A.D., Verschure, P.J. (2018) Forensic DNA methylation profiling from minimal traces: How low can we go? *Forensic Science International: Genetics* 33: 17-23. | |
| 12 | 11. Scudder, N., McNevin, D., Kelty, S.F., Walsh, S.J., Robertson, J. (2018) Forensic DNA phenotyping: Developing a model privacy impact assessment. *Forensic Science International: Genetics* 34: 222-230. | |
| 19 | 12. Pośpiech, E., Chen, Y., Kukla-Bartoszek, M., Breslin, K., ..., Kayser, M. (EUROFORGEN-NoE Consortium) (2018) Towards broadening Forensic DNA Phenotyping beyond pigmentation: Improving the prediction of head hair shape from DNA. *Forensic Science International: Genetics* 37: 241-251. | |
| 17 | 13. Wienroth, M. (2018) Governing anticipatory technology practices: forensic DNA phenotyping and the forensic genetics community in Europe. *New Genetics and Society* 37(2): 137-152. | |
| 35 | 14. Samuel, G. and Prainsack, B. (2019) Forensic DNA phenotyping in Europe: views "on the ground" from those who have a professional stake in the technology. *New Genetics and Society* 38(2): 119-141. | |
| 9 | 15. Schneider, P. M., Prainsack, B., & Kayser, M. (2019) The use of forensic DNA phenotyping in predicting appearance and biogeographic ancestry. *Deutsches Arzteblatt International* 116: 873–880. | |
| 7 | 16. Katsara, M.-A, and Nothnagel, M. (2019) True colors: A literature review on spatial distribution of eye and hair pigmentation. *Forensic Science International: Genetics* 39: 109-118. | |
| 7 | 17. Xiong, Z., Dankova, G., Howe, L.J., Lee, M.K., .. Kayser, M. (2019) Novel genetic loci affecting facial shape variation in humans. *eLIFE* 8: e49838. | |

5

## DNA Phenotyping (Ancestry, Appearance, Age)
(Category U – 24 articles) – Part 3

| Google Scholar Number of citations (11 Jan 2021) | | Location Published |
|---|---|---|
| 5 | 18. Peng, F., Zhu, G., Hysi, P.G., Eller, R.J., Chen, Y., Li, Y., Hamer, M.A., Zeng, C., Hopkins, R.L., Iacobus, C.L., Wallace, P.L., Uitterlinden, A.G., Ikram, M.A., Nijsten, T., Duffy, D.L., Medland, S.E., Spector, T.D., Walsh, S., Martin, N.G., Liu, F., Kayser, M. (2019) Genome-wide association studies identify multiple genetic loci influencing eyebrow color variation in Europeans. *Journal of Investigative Dermatology* 139(7): 1601-1605. | *FSI Genetics (14)*<br>*New Genet & Soc (2)*<br>*Deut. Arzteblatt (1)*<br>*eLife (1)*<br>*Forensic Sci. Rev. (1)*<br>*Front. Genet. (1)*<br>*Genes (1)*<br>*Genome Biology (1)*<br>*Gerontology (1)*<br>*J. Invest. Dermat. (1)* |
| 8 | 19. Liu, F., Zhong, K., Jing, X., Uitterlinden, A.G., Hendriks, A.E.J., Drop, S.L.S., Kayser, M. (2019) Update on the predictability of tall stature from DNA markers in Europeans. *Forensic Science International: Genetics* 42: 8-13. | |
| 5 | 20. Kukla-Bartoszeka, M., Pośpiech, E., Woźniak, A., Boroń, M., Karłowska-Pik, J., Teisseyre, P., Zubańska, M., Bronikowska, A., Grzybowski, T., Płoski, R., Spólnicka, M., Branicki, W. (2019) DNA-based predictive models for the presence of freckles. *Forensic Science International: Genetics* 42: 252-259. | |
| 0 | 21. Freire-Aradas, A., Pośpiech, E., Aliferi, A., Giron-Santamarie, L., Mosquera-Miguel, A., Pisarek, A., Ambroa-Conde, A., Phillips, C., Casares De Cal, M., Gomez-Tato, A., Spolnicka, M., Wozniak, M., Alvarez-Dios, J., Ballard, D., Syndercombe-Court, D., Branicki, W., Carracedo, Á., Lareu, M.V. (2020) A comparison of forensic age prediction models using data from four DNA methylation technologies. *Frontiers in Genetics* 11: 932. | |
| 0 | 22. Heidegger, A., Xavier, C., Niederstätter, H., de la Puente, M., Pośpiech, E., Pisarek, A., Kayser, M., Branicki, W., Parson, W. (VISAGE Consortium) (2020) Development and optimization of the VISAGE basic prototype tool for forensic age estimation. *Forensic Science International: Genetics* 48: 102322. | |
| 1 | 23. Palencia-Madrid, L., Xavier, C., de la Puente, M., Hohoff, C., Phillips, C., Kayser, M., Parson, W. (2020) Evaluation of the VISAGE basic tool for appearance and ancestry prediction using PowerSeq chemistry on the MiSeq FGx System. *Genes (Basel)* 11(6): 708. | |
| 2 | 24. Xavier, C., de la Puente, M., Mosquera-Miguel, A., Freire-Aradas, A., Kalamara, V., Vidaki, A., Gross, T.E., Revoir, A., Pośpiech, E., Kartasińska, E., Spólnicka, M., Branicki, W., Ames, C.E., Schneider, P.M., Hohoff, C., Kayser, M., Phillips, C., Parson, W. (VISAGE Consortium) (2020) Development and validation of the VISAGE AmpliSeq basic tool to predict appearance and ancestry from DNA. *Forensic Science International: Genetics* 48: 102336. | |

6

## DNA Phenotyping (Ancestry, Appearance, Age)

(Category U – 24 articles)

#1

- **Phenotyping:**
  - Reviews (U1, 2, 15)
  - Pigmentation (U3, 6, 16, 18)
  - Ethics(U11 - 14)
  - Other traits (U12, 17, 19, 20)

  *Other traits are freckles, facial features, hair shape, and height.*
- **Biogeographical Ancestry:**
  - Review (U4)
  - MPS assays for appearance and ancestry (U23, 24)
- **Epigenetics:**
  - Review (U7, 8)
- **Age:**
  - Review (U5, 9)
  - Technical issues (U10, 21)
  - MPS assay for age (U22)

7

## DNA Phenotyping (Ancestry, Appearance, Age)

U1. **Kayser, M. (2015) Forensic DNA Phenotyping: Predicting human appearance from crime scene material for investigative purposes. *Forensic Science International: Genetics* 18: 33-48.**


Contents lists available at ScienceDirect

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsig

Forensic DNA Phenotyping: Predicting human appearance from crime scene material for investigative purposes☆

Manfred Kayser*

*Google Scholar*
**Cited 246 times**
(11 Jan 2021)

From an active researcher

**Why is this article valuable?**

- Extensive review of pigmentation traits (eye, hair, skin)
- Current progress and future perspectives on other traits
- Ethical and legal concerns

8

## DNA Phenotyping (Ancestry, Appearance, Age)

U1. **Kayser, M. (2015) Forensic DNA Phenotyping: Predicting human appearance from crime scene material for investigative purposes. *Forensic Science International: Genetics* 18: 33-48.**

**Forensic DNA Phenotyping (FDP) ➡ Externally Visible Characteristics (EVC)**

**Important Points**

- All pigmentation traits show a strong effect of a few genes with a known connection to the melanin pathway.
- Complex traits, like height or facial features, are characterized by small additive effects of 100s of genes.
- Age needs to be co-determined to predict some traits, like face, baldness, grey hair
- Bio-geographical ancestry $\neq$ appearance
- Ethical and reporting concerns need to be addressed

9

## DNA Phenotyping (Ancestry, Appearance, Age)

**MVP list contains combination of review articles and seminal papers on new prediction capabilities.**

U4. Phillips, C. (2015) Forensic genetic analysis of bio-geographical ancestry. *Forensic Science International: Genetics* 18: 49-65.

**Covers marker selection (SNPs, Indels and STRs) and statistical approaches.**

U5. Freire-Aradas, A., Phillips, C., Lareu, M.V. (2017) Forensic individual age estimation with DNA: From initial approaches to methylation tests. *Forensic Science Review* 29(2): 121-144.

**Initial approaches, extensive review of DNA methylation detection and age informative methylation sites in different biological tissues.**

- The most recent contributions are from the European **VISAGE Consortium**.

10

## New Technologies (Rapid DNA, Massive Parallel Seq.)
(Category V – 35 articles) – Part 1

| Google Scholar Number of citations (12 Jan 2021) | | |
|---|---|---|
| 130 | 1. | Butler, J.M. (2015) The future of forensic DNA analysis. *Philosophical Transactions of the Royal Society of London Series B, Biological Sciences* 370: 20140252. |
| 308 | 2. | Gill, P. (2001) An assessment of the utility of single nucleotide polymorphisms (SNPs) for forensic purposes. *International Journal of Legal Medicine* 114: 204-210. |
| 145 | 3. | Gill, P., Werrett, D.J., Budowle, B., Guerrieri, R. (2004) An assessment of whether SNPs will replace STRs in national DNA databases--joint considerations of the DNA working group of the European Network of Forensic Science Institutes (ENFSI) and the Scientific Working Group on DNA Analysis Methods (SWGDAM). *Science & Justice* 44(1): 51-53. |
| 174 | 4. | Butler, J.M., Coble, M.D., Vallone, P.M. (2007) STRs vs SNPs: thoughts on the future of forensic DNA testing. *Forensic Science Medicine and Pathology* 3: 200-205. |
| 30 | 5. | Blackman, S., Dawnay, N., Ball, G., Stafford-Allen, B., Tribble, N., Rendell, P., Neary, K., Hanson, E.K., Ballantyne, J., Kalliafatis, B., Mandel, J., Mills, D.K., Wells, S. (2015) Developmental validation of the ParaDNA(®) Intelligence System-A novel approach to DNA profiling. *Forensic Science International: Genetics* 17: 137-148. |
| 21 | 6. | Date-Chong, M., Hudlow, W.R., Buoncristiani, M.R. (2016) Evaluation of the RapidHIT 200 and RapidHIT GlobalFiler® Express kit for fully automated STR genotyping. *Forensic Science International: Genetics* 23: 1-8. |
| 154 | 7. | Parson, W., Ballard, D., Budowle, B., Butler, J.M., Gettings, K.B., Gill, P., Gusmão, L., Hares, D.R., Irwin, J.A., King, J.L., Knijff, P., Morling, N., Prinz, M., Schneider, P.M., Neste, C.V., Willuweit, S., Phillips, C. (2016) Massively parallel sequencing of forensic STRs: Considerations of the DNA commission of the International Society for Forensic Genetics (ISFG) on minimal nomenclature requirements. *Forensic Science International: Genetics* 22: 54-63. |
| 80 | 8. | van der Gaag, K.J., de Leeuw, R.H., Hoogenboom, J., Patel, J., Storts, D.R., Laros, J.F.J., de Knijff, P. (2016) Massively parallel sequencing of short tandem repeats-Population data and mixture analysis results for the PowerSeq™ system. *Forensic Science International: Genetics* 24: 86-96. |
| 71 | 9. | Bruijns, B., van Asten, A., Tiggelaar, R., Gardeniers, H. (2016) Microfluidic devices for forensic DNA analysis: a review. *Biosensors (Basel)* 6(3): 41. |

**Location Published**

*FSI Genetics (15)*
*Forensic Sci. Int. (5)*
*Int. J. Leg. Med. (5)*
*Science & Justice (3)*
*Electrophoresis (2)*
*Phil. Trans. R.Soc. B (1)*
*J. Forensic Sci. (1)*
*For Sci Med Path (1)*
*PlosOne (1)*
*Biosensors (1)*

11

## New Technologies (Rapid DNA, Massive Parallel Seq.)
(Category V – 35 articles) – Part 2

| Google Scholar Number of citations (12 Jan 2021) | | |
|---|---|---|
| 25 | 10. | Della Manna, A., Nye, J.V., Carney, C., Hammons, J.S., Mann, M., Al Shamali, F., Vallone, P.M., Romsos, E.L., Marne, B.A., Tan, E., Turingan, R.S., Hogan, C., Selden, R.F., French, J.L. (2016) Developmental validation of the DNAscan™ Rapid DNA Analysis™ instrument and expert system for reference sample processing. *Forensic Science International: Genetics* 25: 145-156. |
| 16 | 11. | Mapes, A.A., Kloosterman, A.D., de Poot, C.J., van Marion, V. (2016) Objective data on DNA success rates can aid the selection process of crime samples for analysis by rapid mobile DNA technologies. *Forensic Science International* 264: 28-33. |
| 21 | 12. | Moreno, L.I., Brown, A.L., Callaghan, T.F. (2017) Internal validation of the DNAscan/ANDE Rapid DNA Analysis platform and its associated PowerPlex® 16 high content DNA biochip cassette for use as an expert system with reference buccal swabs. *Forensic Science International: Genetics* 29: 100-108. |
| 11 | 13. | Wiley, R., Sage, K., LaRue, B., Budowle, B. (2017) Internal validation of the RapidHIT® ID system. *Forensic Science International: Genetics* 31: 180-188. |
| 50 | 14. | Mehta, B., Daniel, R., Phillips, C., McNevin, D. (2017) Forensically relevant SNaPshot® assays for human DNA SNP analysis: a review. *International Journal of Legal Medicine* 131(1): 21-37. |
| 138 | 15. | Jäger, A.C., Alvarez, M.L., Davis, C.P., Guzmán, E., Han, Y., Way, L., Walichiewicz, P., Silva, D., Pham, N., Caves, G., Bruand, J., Schlesinger, F., Pond, S.J.K., Varlaro, J., Stephens, K.M., Holt, C.L. (2017) Developmental validation of the Miseq FGx Forensic Genomics System for targeted next generation sequencing in forensic DNA casework and database laboratories. *Forensic Science International: Genetics* 28: 52-70. |
| 28 | 16. | Sharma, V., Chow, H.Y., Siegel, D., Wurmbach, E. (2017) Qualitative and quantitative assessment of Illumina's forensic STR and SNP kits on MiSeq FGx. *PLoS ONE* 12(11): e0187932. |
| 44 | 17. | Phillips, C., Gettings, K.B., King, J.L., Ballard, D., Bodner, M., Borsuk, L., Parson, W. (2018) "The devil's in the detail": Release of an expanded, enhanced and dynamically revised forensic STR sequence guide. *Forensic Science International: Genetics* 34: 162-169. |
| 35 | 18. | Alonso, A., Barrio, P.A., Müller, P., Kircher, S., Berger, B., Martin, P., Bodner, M., Willuweit, S., Parson, W., Roewer, L., Budowle, B. (2018) Current state-of-art of STR sequencing in forensic genetics. *Electrophoresis* 39(21): 2655-2668. |

**Location Published**

*FSI Genetics (15)*
*Forensic Sci. Int. (5)*
*Int. J. Leg. Med. (5)*
*Science & Justice (3)*
*Electrophoresis (2)*
*Phil. Trans. R.Soc. B (1)*
*J. Forensic Sci. (1)*
*For Sci Med Path (1)*
*PlosOne (1)*
*Biosensors (1)*

12

## New Technologies (Rapid DNA, Massive Parallel Seq.)
(Category V – 35 articles) – Part 3

| Google Scholar Number of citations (12 Jan 2021) | | Location Published |
|---|---|---|
| 37 | 19. Bruijns, B., Tiggelaar, R., Gardeniers, H. (2018) Massively parallel sequencing techniques for forensics: a review. *Electrophoresis* 39(21): 2642-2654. | *FSI Genetics (15)* |
| 15 | 20. Morrison, J., Watts, G., Hobbs, G., Dawnay, N. (2018) Field-based detection of biological samples for forensic analysis: Established techniques, novel tools, and future innovations. *Forensic Science International* 285: 147-160. | *Forensic Sci. Int. (5)* |
| 28 | 21. Liu, Y.-Y. and Harbison, S. (2018) A review of bioinformatic methods for forensic DNA analyses. *Forensic Science International: Genetics* 33: 117-128. | *Int. J. Leg. Med. (5)* |
| 15 | 22. Buscaino, J., Barican, A., Farrales, L., Goldman, B., Klevenberg, J., Kuhn, M., Lin, F., Nguyen, P., Salceda, S., Schuerer, R., Smith, C., Troup, C., Tsosi, D., Vangbo, M., King, D. (2018) Evaluation of a rapid DNA process with the RapidHIT® ID system using a specialized cartridge for extracted and quantified human DNA. *Forensic Science International: Genetics* 34: 116-127. | *Science & Justice (3)* |
| 4 | 23. Shackleton, D., Pagram, J., Ives, L., Vanhinsbergh, D. (2018) Development and validation of the RapidHIT™ 200 utilising NGMSElect™ Express for the processing of buccal swabs. *Forensic Science International* 289: 244-252. | *Electrophoresis (2)* |
| 5 | 24. Shackleton, D., Gray, N., Ives, L., Malsom, S., Vanhinsbergh, D. (2019) Development of RapidHIT™ ID using NGM SElect™ Express chemistry for the processing of reference samples within the UK criminal justice system. *Forensic Science International* 295: 179-188. | *Phil. Trans. R.Soc. B (1)* |
| 6 | 25. Shackleton, D., Pagram, J., Andrews, N., Malsom, S., Ives, L., Vanhinsbergh, D. (2019) Development of enhanced sensitivity protocols on the RapidHIT™ 200 with a view to processing casework material. *Science & Justice* 59: 411-417. | *J. Forensic Sci. (1)* |
| 5 | 26. Wu, J., Li, J.L., Wang, M.L., Li, J.P., Zhao, Z.C., Wang, Q., Yang, S.D., Xiong, X., Yang, J.L., Deng, Y.J. (2019) Evaluation of the MiSeq FGx system for use in forensic casework. *International Journal of Legal Medicine* 133(3): 689-697. | *For Sci Med Path (1)* |
| 18 | 27. de Knijff, P. (2019) From next generation sequencing to new generation sequencing in forensics. *Forensic Science International: Genetics* 38: 175-180. | *PlosOne (1)* |
| | | *Biosensors (1)* |

13

## New Technologies (Rapid DNA, Massive Parallel Seq.)
(Category V – 35 articles) – Part 4

| Google Scholar Number of citations (12 Jan 2021) | | Location Published |
|---|---|---|
| 12 | 28. Oldoni, F. and Podini, D. (2019) Forensic molecular biomarkers for mixture analysis. *Forensic Science International: Genetics* 41: 107-119. | *FSI Genetics (15)* |
| 17 | 29. Bennett, L., Oldoni, F., Long, K., Cisana, S., Madella, K., Wootton, S., Chang, J., Hasegawa, R., Lagacé, R., Kidd, K., Podini, D. (2019) Mixture deconvolution by massively parallel sequencing of microhaplotypes. *International Journal of Legal Medicine* 133: 719-729. | *Forensic Sci. Int. (5)* |
| 3 | 30. Morgan, R., Illidge, S., Wilson-Wilde, L. (2019) Assessment of the potential investigative value of a decentralized rapid DNA workflow for reference DNA samples. *Forensic Science International* 294: 140-149. | *Int. J. Leg. Med. (5)* |
| 7 | 31. Mapes, A.A., Stoel, R.D., de Poot, C.J., Vergeer, P., Huyck, M. (2019) Decision support for using mobile Rapid DNA analysis at the crime scene. *Science & Justice* 59: 29-45. | *Science & Justice (3)* |
| 13 | 32. Carney, C., Whitney, S., Vaidyanathan, J., Persick, R., Noel, F., Vallone, P.M., Romsos, E.L., Tan, E., Grover, R., Turingan, R.S., French, J.L., Selden, R.F. (2019) Developmental validation of the ANDE™ rapid DNA system with FlexPlex™ assay for arrestee and reference buccal swab processing and database searching. *Forensic Science International: Genetics* 40: 120-130. | *Electrophoresis (2)* |
| 3 | 33. Romsos, E.L., French, J.L., Smith, M., Figarelli, V., Harran, F., Vandegriff, G., Moreno, L.I., Callaghan, T.F., Brocato, J., Vaidyanathan, J., Pedroso, J.C., Amy, A., Stoloff, S., Morillo, V.H., Czelycko, K., Johnson, E.D., de Tagyos, J., Murray, A., Vallone, P.M. (2020) Results of the 2018 Rapid DNA Maturity Assessment. *Journal of Forensic Sciences* 65(3): 953-959. | *Phil. Trans. R.Soc. B (1)* |
| 3 | 34. Ballard, D., Winkler-Galicki, J., Wesoly, J. (2020) Massive parallel sequencing in forensics : advantages, issues, technicalities, and prospects. *International Journal of Legal Medicine* 134: 1292–1303. | *J. Forensic Sci. (1)* |
| 0 | 35. Bleka, Ø., Just, R., Le, J., & Gill, P. (2020). An examination of STR nomenclatures, filters and models for MPS mixture interpretation. *Forensic Science International: Genetics*, *48*, 102319. | *For Sci Med Path (1)* |
| | | *PlosOne (1)* |
| | | *Biosensors (1)* |

14

## New Technologies (Rapid DNA, Massive Parallel Seq.)
(Category V – 35 articles)

- **Overview:**
  - Future of Forensic DNA Analysis (V1)

#1

- **Rapid DNA:**
  - Workflow and decisions (V11, 30, 31)
  - Microfluidic Overview (V9)
  - Collaborative Study (V33)
  - DNAScan ANDE (V10, 12, 32)
  - RapidHIT (V6, 13, 22-25)
- **Non-STR marker types:**
  - SNP markers (V2-4, V14)
  - Screening (V5, V20)
  - Allele specific PCR (V28)
  - Microhaplotypes (V29)
- **MPS for nuclear DNA:**
  - Reviews (V18, 19, 27, 34)
  - STR nomenclature (V7, 17)
  - Mixture Interpretation (V35)
  - Validation and assay evaluations (V8, 15, 16, 21, 26)

*More on STR sequence variation in section (O) on autosomal markers and kits.*

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## New Technologies (Rapid DNA, Massive Parallel Seq.)

V1. **Butler, J.M. (2015) The future of forensic DNA analysis. *Philosophical Transactions of the Royal Society of London Series B, Biological Sciences* 370: 20140252.**

PHILOSOPHICAL TRANSACTIONS B
rstb.royalsocietypublishing.org

The future of forensic DNA analysis

John M. Butler

National Institute of Standards and Technology, Gaithersburg, MD, USA

*Google Scholar*
**Cited 130 times**
(12 Jan 2021)

**Why is this article valuable?**

- Offers a 30-year retrospective
- Discusses future directions for different areas
- Not just technical aspects, also operational considerations
- Caution on limitations of data

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## New Technologies (Rapid DNA, Massive Parallel Seq.)

V1. **Butler, J.M. (2015) The future of forensic DNA analysis. *Philosophical Transactions of the Royal Society of London Series B, Biological Sciences* 370: 20140252.**

| marker | current practice (as of 2014) | future potential |
|---|---|---|
| autosomal STRs | core loci used to create DNA profile databases and to perform casework; data generated in laboratories with CE systems | expanded core set of loci enabling more international comparisons; data generated by NGS |
| Y-chromosome STRs | casework examination of 12–27 Y-STR loci with haplotype frequencies searched in population databases (e.g. YHRD.org); familial searching candidate pool restricted with Y-STR screening | larger population databases to improve haplotype frequency estimates; genetic genealogy database information combined with Y-STR casework data to help provide potential surname of perpetrator in some cases; rapidly mutating Y-STRs used to separate close male relatives |
| X-chromosome STRs | population data collected for 12+ loci but only used occasionally in kinship cases | X-STRs and X-SNP markers routinely used to help address challenging kinship questions with testing performed on NGS platform in parallel with autosomal STRs |
| mitochondrial DNA | control region Sanger sequencing with haplotype frequencies estimated through population database searches (e.g., EMPOP.org) | full mtGenome by NGS to produce the highest resolution possible; larger population databases to improve haplotype frequency estimates |
| bi-allelic markers (SNPs and InDels) | a few dozen SNPs examined with multiple SNaPshot assays on CE platforms for simple phenotype or biogeographic ancestry prediction; some population data collected with insertion/deletion (InDel) assays | hundreds of SNPs or InDels for biogeographic ancestry and phenotype predictions tested on NGS platform in parallel with STRs |

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## New Technologies (Rapid DNA, Massive Parallel Seq.)

V1. **Butler, J.M. (2015) The future of forensic DNA analysis. *Philosophical Transactions of the Royal Society of London Series B, Biological Sciences* 370: 20140252.**

Distinguishes new developments based on Olympic motto

**FASTER - HIGHER - STRONGER**

- **Faster** results – Rapid DNA
- **Higher** - Sensitivity
  - Information content
- **Stronger** conclusions – Probabilistic Genotyping
  - More Leads

Includes caution on interpretation and emphasizes stakeholder communication & work with crime scene.

18

## New Technologies (Rapid DNA, Massive Parallel Seq.)

**What to read to catch up:**

**Rapid DNA**

V33. Romsos, E.L., French, J.L., Smith, M., Figarelli, V., Harran, F., Vandegrift, G., Moreno, L.I., Callaghan, T.F., Brocato, J., Vaidyanathan, J., Pedroso, J.C., Amy, A., Stoiloff, S., Morillo, V.H., Czetyrko, K., Johnson, E.D., de Tagyos, J., Murray, A., Vallone, P.M. (2020) Results of the 2018 Rapid DNA Maturity Assessment. *Journal of Forensic Sciences* 65(3): 953-959.

**Collaborative exercise using both commercial integrated rapid DNA instruments.**

**Massive Parallel Sequencing**

V34. Ballard, D., Winkler-Galicki, J., Wesoly, J. (2020) Massive parallel sequencing in forensics : advantages, issues, technicalities, and prospects. *International Journal of Legal Medicine* 134: 1292-1303.

**Covers science, current commercial solutions, and future perspectives for both – STR and mtDNA typing.**

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## Summary

- Forensic DNA Phenotyping and New Technologies are two areas where forensic scientists must read original research papers and developmental validations.
- Field still developing and textbooks cannot keep up.

**At the same time**

- Rapid DNA instruments are slated for use outside of laboratory.
  - Quality assurance and data safety are a concern.
- MPS platforms capable of FDP already placed in DNA labs.
  - Guidance on implementation of this type of investigative genetic testing is needed.

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## Thank you for your attention!

**John M. Butler**
john.butler@nist.gov

**Robin W. Cotton**
rwcotton@bu.edu

**Mechthild "Mecki" Prinz**
mprinz@jjay.cuny.edu

**Charlotte J. Word**
cjword@comcast.net

**Please put questions in the chat box while watching our pre-recorded presentations and we will answer them over the chat or through a live Zoom session at the end – you can also email any of us…**

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American Academy of Forensic Sciences
VIRTUAL WORKSHOP W19 (MVPs of Forensic DNA)
February 16, 2021

2021 AAFS VIRTUAL MEETING

*MVPs = Most Valuable Publications*

# MVPs on Method Validation, Quality Control, and Human Factors

**John M. Butler, PhD**
*National Institute of Standards and Technology*

NIST FORENSIC SCIENCE
RESEARCH. STANDARDS. FOUNDATIONS.

**Module 12**

1

## Presentation Outline

- Definitions and principles involved with method validation, error rates, quality control, and human factors
- Review of the 23 articles in Category Y
  - #1 MVP on DNA Error Rates
  - #1 MVP on Human Factors
  - #1 MVP on Quality Control
  - #1 MVP on Method Validation

3

## Definitions and Principles

- **Quality Control:** a process by which entities review the quality of all factors involved in production (Wikipedia)
- **Human Factors:** the study of how people use technology; the goal of human factors is to reduce human error, increase productivity, and enhance safety and comfort with a specific focus on the interaction between the human and the thing of interest (Wikipedia)
- **Method Validation**: the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Results from method validation can be used to judge the quality, reliability and consistency of analytical results; it is an integral part of any good analytical practice. (L. Huber, 1998, *Validation and Qualification in Analytical Laboratories*)
  - Validation studies and experiments performed in a laboratory provide information to **make assessments regarding the degree of reliability for a specified method**
  - These studies are concluded and deemed sufficient when those performing them have *convinced themselves* that the results obtained are reliable for their application
  - A determination of whether the amount and type of data available is satisfactory or sufficient to the user of the information is something that **must be decided by the user of the information not the provider**.

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# Most Valuable Publications of Forensic DNA

(J.M. Butler, R.W. Cotton, M.K. Prinz, C.J. Word)

16 February 2021

## Method Validation, Quality Control, and Human Factors (Category Y – 23 articles)

- **Method Validation:**
  - Validation issues with low level DNA (Y3)
  - ENFSI validation guidelines (Y9)
  - STR validator program (Y10)
  - Standardizing validation process (Y13)
  - PROVEDIt data set (Y15)
  - #1 Collaborative validation approach (Y19)
- **Human Factors:**
  - Texas sharpshooter fallacy (Y4)
  - Subjectivity with mixtures (Y5)
  - Role of investigative facts (Y6)
  - Research about bias (Y7)
  - Confirmation bias (Y8)
  - Understanding the human element (Y11)
  - Strengthening decision making (Y14)
  - Workplace stress and well-being (Y16)
  - #1 Expert decision making (Y21)
- **Error Rates:**
  - #1 DNA error rates at NFI (Y1)
  - Perspective on errors, etc. (Y2)
  - Why needed yet so elusive (Y22)
- **Quality Control:**
  - ISFG DNA Commission – STRidER (Y12)
  - #1 STR data 2-years of QC with STRidER (Y20)
  - FBI Quality Assurance Standards (Y23)
- **DNA Contamination:**
  - Study from Swiss police services and labs (Y17)
  - Contamination minimization procedures (Y18)

5

## Method Validation, Quality Control, and Human Factors (Category Y – 23 articles)

| *Google Scholar* Number of citations (6 Jan 2021) | | |
|---|---|---|
| 69 | 1. | **Kloosterman, A., Sjerps, M., & Quak, A. (2014) Error rates in forensic DNA analysis: Definition, numbers, impact and communication. *Forensic Science International: Genetics* 12: 77-85.** |
| 128 | 2. | Budowle, B., Bottrell, M.C., Bunch, S.G., Fram, R., Harrison, D., Meagher, S., Oien, C.T., Peterson, P.E., Seiger, D.P., Smith, M.B., Smrz, M.A., Soltis, G.L., Stacey, R.B. (2009) A perspective on errors, bias, and interpretation in the forensic sciences and direction for continuing advancement. *Journal of Forensic Sciences* 54(4): 798-809. |
| 46 | 3. | Buckleton, J. (2009) Validation issues around DNA typing of low-level DNA. *Forensic Science International: Genetics* 3(4): 255-260. |
| 108 | 4. | Thompson, W.C. (2009) Painting the target around the matching profile: the Texas sharpshooter fallacy in forensic DNA interpretation. *Law, Probability and Risk* 8(3): 257-276. |
| 264 | 5. | Dror, I.E and Hampikian G. (2011) Subjectivity and bias in forensic DNA mixture interpretation. *Science and Justice* 51: 204-208. |
| 73 | 6. | Thompson, W.C. (2011) What role should investigative facts play in the evaluation of scientific evidence? *Australian Journal of Forensic Sciences* 43(2-3): 123-134. |
| 8 | 7. | Dror, I. E. (2012). Cognitive forensics and experimental research about bias in forensic casework. *Science & Justice* 52(2): 128-130. |
| 486 | 8. | Kassin, S.M., Dror, I.E., Kukucka, J. (2013) The forensic confirmation bias: Problems, perspectives, and proposed solutions. *Journal of Applied Research in Memory and Cognition* 2(1): 42-52. |
| -- | 9. | ENFSI (2014) Guidelines for the single laboratory validation of instrumental and human based methods in forensic science. Available at http://enfsi.eu/wp-content/uploads/2017/06/Guidance-QCC-VAL-002.pdf. |
| 11 | 10. | Hansson, O., Gill, P., Egeland, T. (2014) STR-validator: an open-source platform for validation and process control. *Forensic Science International: Genetics* 13: 154-166. |
| 55 | 11. | Dror, I.E. (2015) Cognitive neuroscience in forensic science: understanding and utilizing the human element. *Philosophical Transactions of the Royal Society of London Series B, Biological Sciences* 370: 20140255. |

**Location Published**

*FSI Genetics* (8)
*J. Forensic Sci.* (3)
*Sci. Justice* (3)
*Anal. Chem.* (1)
*Australian J. Forensic Sci.* (1)
ENFSI (1)
*FSI Synergy* (1)
*Genes* (1)
*JARMAC* (1)
*Law Prob. Risk* (1)
*Phil. Trans. Royal Soc. B* (1)
FBI website (1)

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## Method Validation, Quality Control, and Human Factors (Category Y – 23 articles)

| *Google Scholar* Number of citations (6 Jan 2021) | | |
|---|---|---|
| 91 | 12. | Bodner, M., Bastisch, I., Butler, J.M., Fimmers, R., Gill, P., Gusmão, L., Morling, N., Phillips, C., Prinz, M., Schneider, P.M., Parson, W. (2016) Recommendations of the DNA Commission of the International Society for Forensic Genetics (ISFG) on quality control of autosomal Short Tandem Repeat allele frequency databasing (STRidER). *Forensic Science International: Genetics* 24:97-102. |
| 5 | 13. | Peters, K.C., Swaminathan, H., Sheehan, J., Duffy, K.R., Lun, D.S., Grgicak, C.M. (2017) Production of high-fidelity electropherograms results in improved and consistent DNA interpretation: Standardizing the forensic validation process. *Forensic Science International: Genetics* 31: 160-170. |
| 28 | 14. | Jeanguenat, A.M., Budowle, B., Dror, I.E. (2017) Strengthening forensic DNA decision making through a better understanding of the influence of cognitive bias. *Science & Justice* 57(6): 415-420. |
| 25 | 15. | Alfonse, L.E., Garnett, A.D., Lun, D.S., Duffy, K.R. and Grgicak, C.M. (2018) A large-scale dataset of single and mixed-source short tandem repeat profiles to inform human identification strategies: PROVEDIt. *Forensic Science International: Genetics* 32: 62-70. |
| 28 | 16. | Jeanguenat, A.M. and Dror, I.E. (2018) Human factors effecting forensic decision making: workplace stress and well-being. *Journal of Forensic Sciences* 63(1) 258-261. |
| 13 | 17. | Basset, P. and Castella, V. (2018) Lessons from a study of DNA contaminations from police services and forensic laboratories in Switzerland. *Forensic Science International: Genetics* 33: 147-154. |
| 4 | 18. | Basset, P. and Castella, V. (2019) Positive impact of DNA contamination minimization procedures taken within the laboratory. *Forensic Science International: Genetics* 38: 232-235. |
| 0 (too new) | 19. | Wickenheiser, R. and Farrell, L. (2020) Collaborative versus traditional method validation approach: Discussion and business case. *Forensic Science International: Synergy* 2: 230-237. |
| 0 (too new) | 20. | Bodner, M. and Parson, W. (2020) The STRidER report on two years of quality control of autosomal STR population datasets. *Genes (Basel)* 11(8): 901. |
| 16 | 21. | Dror, I.E. (2020) Cognitive and human factors in expert decision making: six fallacies and the eight sources of bias. *Analytical Chemistry* 92(12): 7998-8004. |
| 3 | 22. | Dror, I.E. (2020) The error in "error rate": why error rates are so needed, yet so elusive. *Journal of Forensic Sciences* 65(4): 1034-1039. |

**Location Published**

*FSI Genetics* (8)
*J. Forensic Sci.* (3)
*Sci. Justice* (3)
*Anal. Chem.* (1)
*Australian J. Forensic Sci.* (1)
ENFSI (1)
*FSI Synergy* (1)
*Genes* (1)
*JARMAC* (1)
*Law Prob. Risk* (1)
*Phil. Trans. Royal Soc. B* (1)
FBI website (1)

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Google Scholar Number of citations (6 Jan 2021) **88**

## Method Validation, Quality Control, and Human Factors (Category Y – 23 articles)

**Y23.** FBI (2020) Quality Assurance Standards for Forensic DNA Testing Laboratories. Available at https://www.fbi.gov/file-repository/quality-assurance-standards-for-forensic-dna-testing-laboratories.pdf/view.

### The FBI QAS 2020 Standards

1. Scope and Applicability
2. Definitions
3. Quality Assurance Program
4. Organization and Management
5. Personnel
6. Training
7. Facilities and Evidence Control
8. Validation
9. Analytical Procedures
10. Equipment
11. Reports
12. Review
13. Proficiency Testing
14. Corrective Action
15. Audits
16. Professional Development
17. Outsourcing Ownership

**Location Published**

*FSI Genetics* (8)
*J. Forensic Sci.* (3)
*Sci. Justice* (3)
*Anal. Chem.* (1)
*Australian J. Forensic Sci.* (1)
ENFSI (1)
*FSI Synergy* (1)
*Genes* (1)
JARMAC (1)
*Law Prob. Risk* (1)
*Phil. Trans. Royal Soc. B* (1)
FBI website (1)

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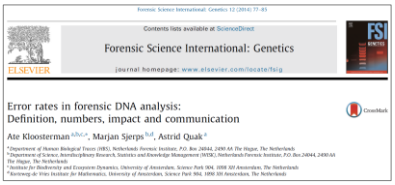
## #1 MVP on DNA Error Rates

**Y1.** **Kloosterman, A., Sjerps, M., & Quak, A. (2014) Error rates in forensic DNA analysis: Definition, numbers, impact and communication. *Forensic Science International: Genetics* 12: 77-85**


Contents lists available at ScienceDirect

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsig

Error rates in forensic DNA analysis:
Definition, numbers, impact and communication

Ate Kloosterman[a,b,c,*], Marjan Sjerps[b,d], Astrid Quak[a]

Google Scholar **Cited 69 times** (6 Jan 2021)

- **Why is this article valuable?**
  - Compares error rates in their laboratory to medical laboratory errors and discusses sources of error and how to communicate potential impact

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## Compared Reported DNA Error Rates in Their Forensic Laboratory with Medical Laboratory Literature Data

**Y1.** **Kloosterman, A., Sjerps, M., & Quak, A. (2014) Error rates in forensic DNA analysis: Definition, numbers, impact and communication. *Forensic Science International: Genetics* 12: 77-85**

| | | year | # tests | # errors | 1 in | % |
|---|---|---|---|---|---|---|
| Medical Laboratory Literature Data | Plebani & Carraro [33] | 1997 (3 mo.) | 40,490 | 189 | **214** | 0.47% |
| | Carraro & Plebani [34] | 2007 (3 mo.) | 51,746 | 160 | **323** | 0.31% |
| | Stahl et al. [34] | 1998 (3 yr.) | 676,564 | 4,135 | **164** | 0.61% |
| | Hofgärtner & Tait [35] | 1999 (1 yr.) | 88,394 | 293 | **302** | 0.33% |
| | | | # notifications | | | |
| Kloosterman et al. 2014 *FSI Genetics* | NFI DNA casework | 2008 | 66,391 | 328 | **202** | 0.49% |
| | NFI DNA casework | 2009 | 82,896 | 329 | **252** | 0.40% |
| | NFI DNA casework | 2010 | 89,977 | 435 | **207** | 0.48% |
| | NFI DNA casework | 2011 | 100,407 | 526 | **191** | 0.52% |
| | NFI DNA casework | 2012 | 132,456 | 572 | **232** | 0.43% |

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## Not All Quality Issue Notifications (aka "Errors") Are Equal

**Y1.** Kloosterman, A., Sjerps, M., & Quak, A. (2014) Error rates in forensic DNA analysis: Definition, numbers, impact and communication. *Forensic Science International: Genetics* 12: 77-85

**Table 3**
Types of quality issue notifications (QINs) at the NFI in the years 2008–2012. In 2011 it was decided to no longer incorporate the type c QIN: opportunities for improvement ($n = 2$ in 2011 and $n = 10$ in 2012) in the yearly totals of this overview.

| | 2008 | 2009 | 2010 | 2011 | 2012 |
|---|---|---|---|---|---|
| a. External origin | 23 | 10 | 23 | 54 | 100 |
| b. External contamination | 3 | 0 | 5 | 24 | 22 |
| c. Room for improvement | 11 | 6 | 3 | (2) | (10) |
| d. Positive response | 19 | 9 | 11 | 6 | 17 |
| e. Clerical (no adverse outcome) | 29 | 25 | 92 | 77 | 82 |
| f. Not related to case work | 13 | 9 | 20 | 10 | 5 |
| g. Other (NFI related) | 230 | 270 | 281 | 355 | 346 |
| Total | 328 | 329 | 435 | 526 | 572 |

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## #1 MVP in Human Factors

**Y21.** Dror, I.E. (2020) Cognitive and human factors in expert decision making: six fallacies and the eight sources of bias. *Analytical Chemistry* 92(12): 7998-8004


Google Scholar
**Cited 16 times**
(6 Jan 2021)

"DNA analysis depends and hinges on the humans conducting it, and how their biases may impact the DNA results"

- **Why is this article valuable?**
  - Provides a clear description of sources of bias that can influence decisions
  - Describes some approaches to combat and minimize bias
  - Concludes: "Biases, often without our awareness or consciousness, impact how we sample and perceive data…"

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**Y21.** Dror, I.E. (2020) Cognitive and human factors in expert decision making: six fallacies and the eight sources of bias. *Analytical Chemistry* 92(12): 7998-8004

**Table 1. Six Fallacies about Cognitive Bias Commonly Held by Experts**

| Fallacy | Incorrect belief |
|---|---|
| 1. Ethical Issues | It only happens to corrupt and unscrupulous individuals, an issue of morals and personal character. |
| 2. Bad Apples | It is a question of competency and happens to experts who do not know how to do their job properly. |
| 3. Expert Immunity | Experts are impartial and are not affected because bias does not impact competent experts doing their job with integrity. |
| 4. Technological Protection | Using technology, instrumentation, automation, or artificial intelligence guarantees protection from human biases. |
| 5. Blind Spot | Other experts are affected by bias, but not me. I am not biased; it is the other experts who are biased. |
| 6. Illusion of Control | I am aware that bias impacts me, and therefore, I can control and counter its affect. I can overcome bias by mere willpower. |

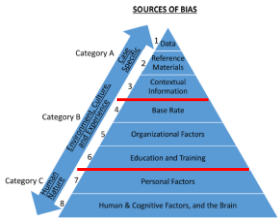


Figure 1. Eight sources of bias that may cognitively contaminate sampling, observations, testing strategies, analysis, and conclusions, even by experts. They are organized in a taxonomy within three categories: starting off at the top with sources relating to the specific case and analysis (Category A), moving down to sources that relate to the specific person doing the analysis (Category B), and at the very bottom sources that relate to human nature (Category C).

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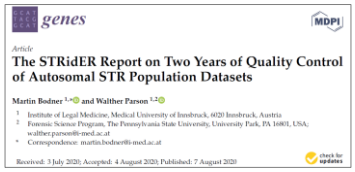
## #1 MVP in Quality Control

**Y20.** Bodner, M. and Parson, W. (2020) The STRidER report on two years of quality control of autosomal STR population datasets. *Genes (Basel)* 11(8): 901


genes

MDPI

Article

The STRidER Report on Two Years of Quality Control of Autosomal STR Population Datasets

Martin Bodner [1,*] and Walther Parson [1,2]

1 Institute of Legal Medicine, Medical University of Innsbruck, 6020 Innsbruck, Austria
2 Forensic Science Program, The Pennsylvania State University, University Park, PA 16801, USA; walther.parson@i-med.ac.at
* Correspondence: martin.bodner@i-med.ac.at

Received: 3 July 2020; Accepted: 4 August 2020; Published: 7 August 2020

check for updates

Google Scholar
**Cited 0 times**
**→ too new**
(6 Jan 2021)

- **Why is this article valuable?**
  - Examines quality of STR datasets containing 177,595 individual genotypes submitted in the first two years of STRidER quality control (2017-2019) to explore types of errors observed

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## What is STRidER?

STRidER
STRs for identity ENFSI Reference database, v2/R2

https://strider.online/

**STRidER serves the STR community in forensics and beyond in inter-related ways:**

- **The high-quality autosomal STR allele frequency database can be directly queried**
- Allele frequency tables of STR loci from diverse populations can be downloaded and used for third party software
- **Centralized STR data quality control is offered prior to publication**
- Accepted datasets will become rapidly available online and receive a unique and traceable STRidER accession number
- Allele frequencies are calculated from datasets
- **Individual STR genotypes are not accessible on STRidER to comply with privacy regulations**

**Y12.** Bodner, M., Bastisch, I., Butler, J.M., Fimmers, R., Gill, P., Gusmão, L., Morling, N., Phillips, C., Prinz, M., Schneider, P.M., Parson, W. (2016) Recommendations of the DNA Commission of the International Society for Forensic Genetics (ISFG) on quality control of autosomal Short Tandem Repeat allele frequency databasing (STRidER). *Forensic Science International: Genetics* 24:97-102.

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**Y20.** Bodner, M. and Parson, W. (2020) The STRidER report on two years of quality control of autosomal STR population datasets. *Genes (Basel)* 11(8): 901

**Table 2.** Statistics of errors found in the 165 autosomal STR datasets generated by capillary electrophoresis (CE) and submitted to STRidER in its first two years.

| | | | n | (%) |
|---|---|---|---|---|
| **Datasets Revealing Errors** | | | **158** | **95.8** |
| Error categories | (i) | Identical genotypes | 63 | 38.2 |
| | (ii) | Non-ascending allele pairs | 58 | 35.2 |
| | (iii) | Allele nomenclature errors | 29 | 17.6 |
| | (iv) | Allele calling errors | 17 | 10.3 |
| | (v) | Incomplete genotypes | 16 | 9.7 |
| | (vi) | Errors in locus nomenclature | 10 | 6.1 |
| | (vii) | Aneuploidy | 9 | 5.5 |
| | (viii) | No raw data/shuffled data | 9 | 5.5 |
| | (ix) | Identical identifiers | 7 | 4.2 |
| | (x) | Information mismatch | 5 | 3.0 |
| | (xi) | Locus swapping | 3 | 1.8 |
| | (xii) | Loss of intermediate alleles | 2 | 1.2 |
| **Datasets Revealing No Errors** | | | **7** | **4.2** |

Note: Sum of datasets allocated to categories is larger than 100% because of datasets that harbored multiple error categories. Results as per six months after the initial two-year period.

**Errors were observed in 96% of the datasets submitted in the first two years!**

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## #1 MVP in Method Validation

**Y19.** Wickenheiser, R. and Farrell, L. (2020) Collaborative versus traditional method validation approach: Discussion and business case. *Forensic Science International: Synergy* 2: 230-237.


Forensic Science International: Synergy

Collaborative versus traditional method validation approach: Discussion and business case

Ray Wickenheiser [a,*], Laurel Farrell [b]

[Open Access]
https://doi.org/10.1016/j.fsisyn.2020.08.003

- **Why is this article valuable?**
  - Encourages the community to work towards a collaborative validation approach where we can share data and learn from each other ("utilization of published validation data increases efficiency through shared experiences…")

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## Thank you for your attention!

**John M. Butler**
john.butler@nist.gov

**Robin W. Cotton**
rwcotton@bu.edu

**Mechthild "Mecki" Prinz**
mprinz@jjay.cuny.edu

**Charlotte J. Word**
cjword@comcast.net

**Please put questions in the chat box while watching our pre-recorded presentations and we will answer them over the chat or through a live Zoom session at the end – you can also email any of us…**

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American Academy of Forensic Sciences
VIRTUAL WORKSHOP W19 (MVPs of Forensic DNA)
February 16, 2021

2021 AAFS VIRTUAL MEETING

# Wrap-Up and Workshop Conclusion

**John M. Butler, PhD**
*National Institute of Standards and Technology*

NIJ FORENSIC SCIENCE
RESEARCH. STANDARDS. FOUNDATIONS.

**Module 13**

1

## Development of Expert Knowledge

DNA analysts benefit from at least three different levels of expert knowledge:

1. **Education in basic science** covering biochemistry, biology, chemistry, genetics, molecular biology, population genetics, and statistics
2. **Training in forensic science** and specific methods and protocols used in their laboratory to develop competency needed to perform casework
3. **Continued education and professional development** to keep up-to-date as the field evolves and new methods become available

***#3 involves knowing the ever-growing scientific literature***

2

## Thoughts and Observations on the Literature

1. New articles and advances are regularly being published
   - Keep an open mind and remember that science is open-ended
2. Limitations of some publications
   - Claims made do not always correspond to available data
   - How can we encourage more data sharing?
3. The community seems to make more use of articles on methodology as compared to interpretation
   - For example, Goggle Scholar found fewer citations to PGS articles than to PCR articles (in part because PGS efforts are more recent)
4. Training is challenging as there is simply too much to know in a constantly evolving field
   - Suggestion that an analyst learns to think through what is happening to DNA molecules at each step of the process
5. Not only did we not cover every article in our MVP reference list, but we also didn't even touch on some entire categories!

3

## Reference List Provided with Slide Handouts
### *497 References Across 26 Topics (A-to-Z)*
(480) Articles + (17) Informative Textbooks

**A (4)** Plain Language Guides to Forensic DNA Analysis
**B (24)** Serology and Body Fluid Identification
**C (25)** Collection and Storage of Biological Material — Module 4
**D (18)** DNA Extraction/Purification, Differential Extraction
**E (10)** DNA Quantitation, Degraded DNA
**F (13)** PCR Amplification, Inhibition, and Artifacts — Module 5
**G (12)** Capillary Electrophoresis Separation and Detection
**H (7)** Assessing Sample Suitability and Complexity, Low-Template DNA
**I (12)** Estimating the Number of Contributors
**J (12)** Data Interpretation, Mixture Deconvolution, Interlaboratory Studies
**K (11)** Interpretation: Binary Approaches (CPI, RMP, LR) — Module 7
**L (44)** Interpretation: Probabilistic Genotyping Software (Discrete, Continuous)
**M (8)** Report Writing and Technical Review — Module 8

**N (22)** Court Testimony, Communication of Results, Juror Comprehension Studies
**O (29)** Autosomal STR Markers and Kits — Module 5
**P (11)** Mitochondrial DNA Testing — Module 10
**Q (17)** Y-Chromosome and X-Chromosome Testing
**R (14)** DNA Databases and Investigative Genetic Genealogy
**S (11)** Statistical Analysis — Module 6
**T (11)** Population Genetics
**U (24)** DNA Phenotyping (Ancestry, Appearance, Age) — Module 11
**V (35)** New Technologies (Rapid DNA, Massively Parallel Sequencing)
**W (57)** DNA Transfer and Activity Level Reporting
**X (15)** Non-Human DNA Testing — Module 9
**Y (23)** Method Validation, Quality Control, Human Factors
**Z (11)** General Forensic Science Topics — Module 12

Module 2 Value of a Knowledge Base  Module 3 Training Standards  Module 13 Wrap-Up

4

## Many Categories are in Our Literature List But Not Covered in This Workshop

**A1.** Sense about Science (2017) *Making Sense of Forensic Genetics*. A 40-page plain language guide available at https://senseaboutscience.org/activities/making-sense-of-forensic-genetics/.

**B1.** Gaensslen, R.E. (1983) *Sourcebook in Forensic Serology, Immunology, and Biochemistry*. U.S. Department of Justice, National Institute of Justice: Washington, D.C.

**H1.** Gill, P., Whitaker, J., Flaxman, C., Brown, N., Buckleton, J. (2000) An investigation of the rigor of interpretation rules for STRs derived from less than 100 pg of DNA. *Forensic Science International* 112(1): 17-40.

**I1.** Buckleton, J.S., Curran, J.M. and Gill, P. (2007) Towards understanding the effect of uncertainty in the number of contributors to DNA stains. *Forensic Science International: Genetics* 1(1): 20-28.

**J1.** Gill, P., Sparkes, R. and Kimpton, C. (1997) Development of guidelines to designate alleles using an STR multiplex system. *Forensic Science International* 89(3): 185-197.

**M1.** Association of Forensic Science Providers (2009) Standards for the formulation of evaluative forensic science expert opinion. *Science & Justice* 49: 161-164.

**N1.** Eldridge, H. (2019) Juror comprehension of forensic expert testimony: a literature review and gap analysis. *Forensic Science International: Synergy* 1: 24-34.

**R1.** Struyf, P., De Moor, S., Vandeviver, C., Renard, B., van der Beken, T. (2019) The effectiveness of DNA databases in relation to their purpose and content: A systematic review. *Forensic Science International* 301: 371-381.

**X1.** Linacre, A., Gusmão, L., Hecht, W., Hellmann, A.P., Mayr, W.R., Parson, W., Prinz, M., Schneider, P.M., Morling, N. (2011) ISFG: recommendations regarding the use of non-human (animal) DNA in forensic genetic investigations. *Forensic Science International: Genetics* 5(5): 501-505.

**Z1.** National Academy of Sciences, Committee on the Conduct of Science (1989) On being a scientist. *Proceedings of the National Academy of Sciences of the United States of America* 86(23): 9053-9074.

A = Plain Language Guides
B = Serology & Body Fluid ID
H = Low-template DNA
I = Number of contributors
J = Data Interpretation
M = Report Writing
N = Court Testimony
R = DNA Databases
X = Non-Human DNA
Z = General Forensic Science

***The #1 article in each of these categories is listed here***

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## *Making Sense of Forensic Genetics* (2017)

concepts clearly explained in 40 pages


- Developed by European Forensic Genetics Network of Excellence (EuroForGen-NoE) and published with Sense about Science
- **Free PDF file** available for download https://senseaboutscience.org/wp-content/uploads/2017/01/making-sense-of-forensic-genetics.pdf
- *Final point made*: "As DNA profiling continues to grow more sensitive, and it is used in more investigations, **the need for accurate communication between scientists and nonscientists only grows** - both **to ensure that their expectations of the technology are realistic, and its limits are properly understood**…"

Translated into Spanish, Portuguese, and Polish (so far)

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## (17) Informative Textbooks on Forensic DNA

1. National Research Council (1996) *The Evaluation of Forensic DNA Evidence*. National Academy Press: Washington, D.C.
2. Evett, I.W. and Weir, B.S. (1998) *Interpreting DNA Evidence: Statistical Genetics for Forensic Scientists*. Sinauer Associates: Sunderland MA.
3. Inman, K. and Rudin, N. (2001) *Principles and Practice of Criminalistics: The Profession of Forensic Science*. CRC Press: Boca Raton.
4. Fung, W.K. and Hu, Y.-Q. (2008) *Statistical DNA Forensics: Theory, Methods and Computation*. Wiley: Chichester, UK.
5. Butler, J.M. (2010) *Fundamentals of Forensic DNA Typing*. Elsevier Academic Press: San Diego.
6. Goodwin, W., Linacre, A., Hadi, S. (2011) *An Introduction to Forensic Genetics Second Edition*. Wiley: Chichester, UK.
7. Butler, J.M. (2012) *Advanced Topics in Forensic DNA Typing: Methodology*. Elsevier Academic Press: San Diego.
8. Shewale, J.G. and Liu, R.H. (Editors) (2013) *Forensic DNA Analysis: Current Practices and Emerging Technologies*. CRC Press: Boca Raton.
9. Gill, P. (2014) *Misleading DNA Evidence: Reasons for Miscarriages of Justice*. Elsevier Academic Press: San Diego.
10. Butler, J.M. (2015) *Advanced Topics in Forensic DNA Typing: Interpretation*. Elsevier Academic Press: San Diego.
11. Balding, D. J. and Steele, C. D. (2015). *Weight-of-evidence for Forensic DNA Profiles Second Edition*. Wiley: Chichester, UK.
12. Buckleton, J.S., Bright, J.-A., Taylor, D. (Editors) (2016) *Forensic DNA Evidence Interpretation Second Edition*. CRC Press: Boca Raton.
13. Robertson, B., Vignaux, G.A., Berger, C.E.H. (2016) *Interpreting Evidence: Evaluating Forensic Science in the Courtroom Second Edition*. Wiley: Chichester, UK.
14. Jamieson, A. and Bader, S. (Editors) (2016) *A Guide to Forensic DNA Profiling*. Wiley: Chichester, UK.
15. Amorim, A. and Budowle, B. (Editors) (2017) *Handbook of Forensic Genetics: Biodiversity and Heredity in Civil and Criminal Investigation*. World Scientific Publishing: London.
16. Bright, J.-A. and Coble, M. (2020) *Forensic DNA Profiling: A Practical Guide to Assigning Likelihood Ratios*. CRC Press: Boca Raton.
17. Gill, P., Bleka, Ø., Hansson, O., Benschop, C., Haned, H. (2020) *Forensic Practitioner's Guide to the Interpretation of Complex DNA Profiles*. Elsevier Academic Press: San Diego.

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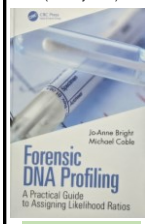
## Recent Books to Assist with DNA Mixture Interpretation

CRC Press
(January 2020)




**Hardback:** 258 pages
109 references cited

**Table of Contents**

1. DNA Profiling Interpretation
2. Statistics and Proposition Setting
3. LR Single-Source Examples and Population Genetics
4. Binary LR for Mixtures
5. LRs Considering Relatives as Alternative Contributors
6. Probabilistic Genotyping: Semicontinuous Models
7. Probabilistic Genotyping: Continuous Models
8. **Considerations on Validation of PGS**

Appendix 1: Allele Frequencies
Appendix 2: Model Answers

Elsevier Academic Press
(June 2020)


**Paperback:** 530 pages
362 references cited

**Table of Contents**

1. Forensic Genetics Basics
2. DNA Profiles
3. Allele Drop-out
4. Low-template DNA
5. LRmix Model Theory
6. LRmix Studio
7. Continuous Model Theory
8. EuroForMix
9. **Validation**
10. DNAxs
11. *SmartRank* & *CaseSolver*
12. Interpretation & Reporting
13. Complex DNA Profiling by Massively Parallel Sequencing

Appendix A: Genotype Probabilities
Appendix B: Probabilistic Models

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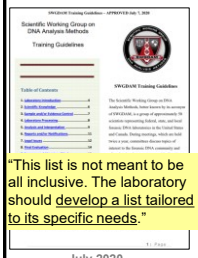
## New SWGDAM Training Guidelines (July 2020)




"This list is not meant to be all inclusive. The laboratory should develop a list tailored to its specific needs."

**Recommended References** (**129** + 6 websites)

*The following resources may be helpful to the trainer in defining the breadth and scope of the materials for the trainee's reading. This list is not meant to be all inclusive. The laboratory should develop a list tailored to its specific needs.*

1. General Forensic DNA and Autosomal STRs (**42**)
2. Mixture Interpretation/Population Genetics/ Probabilistic Genotyping/Statistics (**40**)
3. Mitochondrial DNA (**37**)
   - General Mitochondrial DNA Information (**6**)
   - Heteroplasmy (**15**)
   - Maternal Inheritance (**1**)
   - Population Studies (**1**)
4. Y STRs (**10**)
5. Informational Websites (**6**)

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## Reference Lists Compared

| | | MVPs Feb 2021 | OSAC 10-26-20 | SWGDAM July 2020 |
|---|---|---|---|---|
| | **Informative Textbooks on Forensic DNA** | **17** | **16** | **5 + 2** |
| | | | | 6 websites |
| A | Plain Language Guides to Forensic DNA Analysis | 4 | 3 | -- |
| B | Serology and Body Fluid Identification | 24 | 15 + 2 | -- |
| C | Collection and Storage of Biological Material | 25 | 19 | -- |
| D | DNA Extraction/Purification, Differential Extraction | 18 | 14 | 1 |
| E | DNA Quantitation, Degraded DNA | 10 | 9 + 1 | 1 |
| F | PCR Amplification, Inhibition, and Artifacts | 13 | 10 | 3 |
| G | Capillary Electrophoresis Separation and Detection | 12 | 12 | 6 |
| H | Assessing Sample Suitability and Complexity, Low-Template DNA | 7 | 8 | -- |
| I | Estimating the Number of Contributors | 12 | 12 | -- |
| J | Data Interpretation, Mixture Deconvolution, Interlaboratory Studies | 12 | 12 | 2 + 4 |
| K | Interpretation: Binary Approaches (CPI, RMP, LR) | 11 | 9 | 3 |
| L | Interpretation: Probabilistic Genotyping Software (Discrete, Continuous) | 44 | 41 | 7 + 11 |
| M | Report Writing and Technical Review | 8 | 8 | -- |
| N | Court Testimony, Communication of Results, Juror Comprehension | 22 | 21 | 3 |
| O | Autosomal STR Markers and Kits | 29 | 27 | 4 |
| P | Mitochondrial DNA Testing | 11 | 10 + 1 | 3 + 32 |
| Q | Y-Chromosome and X-Chromosome Testing | 17 | 13 | 4 + 2 |
| R | DNA Databases and Investigative Genetic Genealogy | 14 | 14 | -- |
| S | Statistical Analysis | 11 | 9 | 3 + 2 |
| T | Population Genetics | 11 | 10 | 1 |
| U | DNA Phenotyping (Ancestry, Appearance, Age) | 24 | 20 | -- |
| V | New Technologies (Rapid DNA, Massively Parallel Sequencing) | 35 | 31 | -- |
| W | DNA Transfer and Activity Level Reporting | 57 | 54 | -- |
| X | Non-Human DNA Testing | 15 | 15 | -- |
| Y | Method Validation, Quality Control, and Human Factors | 23 | 23 | 1 + 5 |
| Z | General Forensic Science Topics | 11 | 11 | 1 |
| | Historical | | 19 | 1 |
| | TOTAL | **497** | **448** | **135** |

Different Reference Lists

- (**497**) this AAFS 2021 Most Valuable Publications Workshop
- (**448**) OSAC 10-26-20 version
- (**135**) SWGDAM Training Guidelines

- OSAC list shares a common origin with our MVP list
  - some differences exist
- SWGDAM 2020 Training Guidelines Reference List
  - Historical references (19)
  - More mtDNA articles (>30)
  - No coverage of DNA transfer and many other potentially valuable topics

*Underlined numbers reflect those found only in that list*

**3 articles in common with our MVP list + 32 mtDNA articles only in the SWGDAM list**

SWGDAM includes 19 articles I have classified as "historical"

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## ISFG DNA Commission Articles

- Several of the #1 MVPs are ISFG DNA Commission articles:

**DNA mixture interpretation**

- **K1.** Gill, P., Brenner, C.H., Buckleton, J.S., Carracedo, A., Krawczak, M., Mayr, W.R., Morling, N., Prinz, M., Schneider, P.M. and Weir, B.S. (2006) DNA Commission of the International Society of Forensic Genetics: Recommendations on the interpretation of mixtures. *Forensic Science International* 160: 90-101.

**mtDNA**

- **P1.** Parson, W., Gusmão, L., Hares, D.R., Irwin, J.A., Mayr, W.R., Morling, N., Pokorak, E., Prinz, M., Salas, A., Schneider, P.M., Parsons, T.J. (2014) DNA Commission of the International Society for Forensic Genetics: revised and extended guidelines for mitochondrial DNA typing. *Forensic Science International: Genetics* 13: 134-142.

**Y-STRs**

- **Q1.** Roewer, L., Andersen, M.M., Ballantyne, J., Butler, J.M., Caliebe, A., Corach, D., D'Amato, M.E., Gusmão, L., Hou, Y., de Knijff, P., Parson, W., Prinz, M., Schneider, P.M., Taylor, D., Vennemann, M., Willuweit, S. (2020) DNA commission of the International Society of Forensic Genetics (ISFG): Recommendations on the interpretation of Y-STR results in forensic analysis. *Forensic Science International: Genetics* 48: 102308.

**Non-human DNA testing**

- **X1.** Linacre, A., Gusmão, L., Hecht, W., Hellmann, A.P., Mayr, W.R., Parson, W., Prinz, M., Schneider, P.M., Morling, N. (2011) ISFG: recommendations regarding the use of non-human (animal) DNA in forensic genetic investigations. *Forensic Science International: Genetics* 5(5): 501-505.

- These are freely available on the ISFG website:
  - https://www.isfg.org/Publications/DNA+Commission

ISFG International Society for Forensic Genetics

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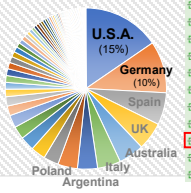
## International Society for Forensic Genetics (ISFG)

https://www.isfg.org/files/ISFG_50Years_Brochure.pdf

ISFG
50 Years
International Society for Forensic Genetics
1968 - 2018




**12 Working Groups**

- German
- English
- French
- Italian
- Spanish and Portuguese
- Chinese
- Korean
- Arabian Speaking
- CaDNAP
- DNA Commission
- EDNAP
- Polish

**Biennial Meetings**

Prague (2019)

**#1 Journal on Forensic DNA**


**President**: John M. Butler, Gaithersburg • **Vice President**: Walther Parson, Innsbruck • **Secretary**: Peter M. Schneider, Cologne
**Treasurer**: Marielle Vennemann, Münster • **Representative of the Working Groups**: Leonor Gusmão, Rio de Janeiro

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## The Next ISFG Meeting is in the U.S.

https://www.isfg2021.org/

ISFG
WASHINGTON, DC
ISFG 2022

*Delayed to 2022 due to pandemic*

***Once in a Lifetime Opportunity*** **– The best scientific meeting in the field with top researchers in forensic genetics coming to the United States for the first time in the 21st Century**

**16 Pre-Congress Workshops**
**To be held August 29-30, 2022**

**DNA Mixtures (Basic)**
**DNA Mixtures (Advanced)**
Kinship Analysis
Y-STRs
**Court Testimony**
NGS Bioinformatics 101

NGS Methods | mtDNA Casework
NGS STR Markers | Phenotyping
**DNA Transfer | Evaluative Reporting**
**Probability and Statistics | Validation**
Biogeographical Ancestry | Publication

https://www.isfg.org/Meeting


*Previous Meetings:* Münster (2001), Archacon (2003), San Miguel, Azores (2005), Copenhagen (2007), Buenos Aires (2009), Vienna (2011), Melbourne (2013), Krakow (2015), Seoul (2017), Prague (2019)

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## Some Improvements That Would Be Beneficial to the Forensic DNA Community

1. **An agreed upon, defined body of knowledge** for DNA mixture interpretation and a means to update and remove outdated information as methods evolve
2. **Access to appropriate relevant literature** for technical leaders and analysts
3. **Dedicated time in the workday to read the literature** so that technical leaders and analysts can keep up-to-date with developments
4. **Uniformly documented knowledge assessment**
5. **A method to acknowledge competence in a specific area** to allow true expertise in testimony (e.g., DNA transfer and activity assessments, see van Oorschot et al. 2019)
6. **Additional training for technical leaders in experimental design and data analysis** to assist with validation studies and protocol development

**This workshop is intended as a start**

From deliberations and discussions of NIST team members and Resource Group in connection with the Scientific Foundation Review on DNA Mixture Interpretation

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## Some Final Thoughts

1. No selection criteria or reference list will be perfect or complete
   - continuing research and future review articles add knowledge to our field
   - some references could be removed to focus content in various categories
2. We would love to hear your ideas on how to best maintain an updated list to benefit the community
   - Are there other categories that should be included in MVP lists?
3. How could a national/international MVP list benefit future training?
   - Would it be worth conducting an ASCLD or AAFS survey on this topic?
   - If we understand the need, then we can lay the groundwork for future possibilities in funding
   - Funding would need to be continuing and sustained to be effective (not year-to-year) – would forensic laboratories support a subscription fee of some kind?

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## Thank you for your attention!

**John M. Butler**
john.butler@nist.gov

**Robin W. Cotton**
rwcotton@bu.edu

**Mechthild "Mecki" Prinz**
mprinz@jjay.cuny.edu

**Charlotte J. Word**
cjword@comcast.net

**Please put questions in the chat box while watching our pre-recorded presentations and we will answer them over the chat or through a live Zoom session at the end – you can also email any of us...**

**Acknowledgments and Disclaimer**

**Points of view are the presenters** and do not necessarily represent the official position or policies of the National Institute of Standards and Technology.

Certain commercial entities are identified in order to specify experimental procedures as completely as possible. In no case does such identification imply a recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that any of the entities identified are necessarily the best available for the purpose.

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# Q & A

John, Robin, Mecki, and Charlotte

Approximately 4:40 to 5:00pm (Central)

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