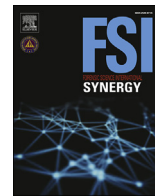




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Interpol review of forensic biology and forensic DNA typing 2016–2019

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ABSTRACT

This review paper covers the forensic-relevant literature in biological sciences from 2016 to 2019 as a part of the 19th Interpol International Forensic Science Managers Symposium. The review papers are also available at the Interpol website at: <https://www.interpol.int/content/download/14458/file/Interpol%20Review%20Papers%202019.pdf>.

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1. Introduction

This review explores developments during the years 2016–2019 in forensic biology and forensic DNA analysis of biological evidence. Topics covered include expansion of the core short tandem repeat (STR) markers used in the U.S. national DNA database, rapid DNA testing, investigative genetic genealogy, next-generation sequencing (NGS), DNA mixture interpretation involving probabilistic genotyping software (PGS), DNA transfer and activity level evaluations, forensic biology and body fluid identification, DNA phenotyping, privacy and ethical issues, published guidance documents to assist quality control, and contamination avoidance efforts. Several special issues and additional review articles are noted as well.

An INTERPOL Global DNA Profiling Survey conducted in early 2017 found that 69 member countries have a national DNA database with an estimated 35 million DNA profiles among those countries that responded to the survey (see link to report at <https://www.interpol.int/en/How-we-work/Forensics/DNA>). Some 84 member countries use DNA in police investigations with 73 countries performing Y-chromosome STR analysis and 31 countries using mitochondrial DNA.

The forensic DNA review presented at the 17th International Forensic Science Managers Symposium (available at <https://www.interpol.int/en/How-we-work/Forensics/Forensic-Symposium>) in October 2013 reviewed 114 articles from 2010 to 2013 spanning topics such as autosomal STRs, Y-STRs, single nucleotide polymorphisms (SNPs), insertion/deletion (InDel) markers, body fluid identification, and new genomic platforms [1]. The DNA review

presented in October 2016 examined 75 articles from 2013 to 2016 focused on rapid DNA, analysis of complex DNA profiles including mixtures and low template DNA, and forensic applications of next-generation sequencing [2].

This review examines >230 articles published in 35 different scientific journals including *Forensic Science International: Genetics*, *Electrophoresis*, *Genes*, *Journal of Forensic Sciences*, *Forensic Science International*, *PLoS ONE*, *Science*, and the *International Journal of Legal Medicine*. We note that during this same time frame (January 2016 to July 2019) over 700 articles on forensic genetics were published in *Forensic Science International: Genetics* alone – thus, we recognize that our review is neither comprehensive nor exhaustive.

2. Core loci expansion

In January 2017, the FBI required expansion of the U.S. core loci for entry into the Combined DNA Index System (CODIS) National DNA Index System (NDIS) (see <https://www.fbi.gov/services/laboratory/biometric-analysis/codis>), and the original 13 CODIS core STR markers grew to an expanded set of 20 STRs [3]. To the original core 13 STR loci (D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D21S11, CSF1PO, FGA, TH01, TPOX, vWA), the following seven loci have been added: D1S1656, D2S441, D2S1338, D10S1248, D12S391, D19S433, D22S1045.

Commercial kits that amplify more than 20 STR loci have now been adopted by many countries worldwide. These new kits enable more international sharing of DNA data with increased compatibility between STR data going into national DNA databases. A total of 15 STRs are now in common among most STR kits employed in various countries around the world: D1S1656, D2S441, D2S1338, D3S1358, D8S1179, D10S1248, D12S391, D16S539, D18S51, D19S433, D21S11, D22S1045, FGA, TH01, and vWA.

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3. Rapid analysis of STR markers

In an effort to generate faster DNA results to speed decisions in investigations, rapid DNA instruments have been created that can produce a DNA profile in less than 2 h. Full automation of the STR typing process consists of DNA extraction, amplification, separation, detection, and allele calling from reference sample buccal swabs (“swab in – profile out”).

Commercially available rapid DNA instruments include (1) the DNAscan/ANDE 4C (4-color) now called ANDE 6C (Accelerated Nuclear DNA Equipment 6-color) Rapid DNA System, originally developed by NetBio (Waltham, MA), which has become ANDE (Longmont, CO) (<https://www.ande.com/>), and (2) the RapidHIT 200 and (3) RapidHIT ID instruments, originally developed by IntegenX (Pleasanton, CA) and now sold by Thermo Fisher Scientific (South San Francisco, CA) (see <https://thermofisher.com/rapidDNA>). These instruments are capable of being deployed at police booking stations, border crossings, and embassies as well as in traditional forensic laboratories. Several publications have examined the cost of decentralized or mobile rapid DNA operations [4–6].

The Rapid DNA Act of 2017 was signed into U.S. law on August 18, 2017 [7]. This act authorizes the FBI Director to “issue standards and procedures for the use of rapid DNA instruments and resulting DNA analyses.” With the passage of the Rapid DNA Act of 2017, U.S. law enforcement booking station environments have been given the “green-light” to process single-source reference samples – and the FBI has established prerequisites for rapid DNA in a booking environment (see <https://www.fbi.gov/services/laboratory/biometric-analysis/codis/rapid-dna>). Several groups have published policy statements emphasizing the need for these automated rapid DNA systems to only be used on single-source samples and not crime scene evidence containing mixtures [8–10].

A number of articles have been published on rapid DNA instruments since 2016. Table 1 summarizes 13 published developmental validation, internal validation, or evaluation studies [11–23]. In these publications, success rates (i.e., did a sample produce a result for the required STR markers?) are often reported as first-pass success rates when run in a fully automated (auto) mode. Some studies report a follow-up human (manual) review of first-pass inconclusive results. Prior to 2017, 13 CODIS core loci were required to obtain a successful rating. Since then, a determination of “successful” results depends on obtaining the expanded CODIS 20 STRs.

A new assay named “FlexPlex27” has also been developed that generates rapid DNA data for the expanded CODIS core loci and all additional STR loci currently required for international databasing [24]. FlexPlex27 co-amplifies 23 autosomal loci (D1S1656, D2S1338, D2S441, D3S1358, D5S81, D6S1043, D7S820, D8S1179, D10S1248, D12S391, D13S317, D16S539, D18S51, D19S433, D21S11, D22S1045, FGA, CSF1PO, Penta E, TH01, vWA, TPOX, and SE33), three Y-chromosomal loci (DYS391, DYS576, and DYS570), and Amelogenin [24].

The Australia New Zealand Policing Advisory Agency (ANZPAA) National Institute of Forensic Science (NIFS) published a 28-page detailed technical evaluation report on the DNAscan System [25]. In February 2017, the Swedish National Forensic Centre published a 40-page review of their experiences with the RapidHIT system and issues identified when processing crime scene samples [17]. A 2018 maturity assessment organized by the National Institute of Standards and Technology (NIST) involved nine laboratories from U.S. federal and state laboratories, police agencies, and commercial vendors [26]. This NIST study found an overall success rate of 90% (with 240 tested swabs) in generating data with the 20 core STR loci now required for NDIS entry.

4. Investigative genetic genealogy

In April 2018, the use of investigative genetic genealogy drew international attention with the identification and arrest of alleged “Golden State Killer” Joseph DeAngelo [27,28]. Genetic data from distant relatives in public genetic genealogy databases have aided dozens of other cold case investigations since then [29] and increased discussions around genetic privacy (e.g., Refs. [30–35]).

The direct-to-consumer (DTC) genomic industry has grown rapidly in the past few years. Gathering samples from individuals primarily within the United States, companies including 23andMe, Ancestry, FamilyTree DNA, and My Heritage have collectively amassed ancestry DNA data from millions of individuals who have submitted a DNA test seeking assistance in understanding their family heritage [36]. Connections to distant relatives up to third- or fourth-cousins have been demonstrated with this type of DNA data that typically involves examining information from >500,000 SNP markers [37].

Using a dataset of 1.28 million DTC results, researchers found that 60% of these long-range familial searches return a relative with a total length of 100 centimorgans (cM) or more, which is around the level of a third cousin or closer relative [38]. Since a majority of the individuals with DTC genetic ancestry results contain a Northern European genetic background, these types of searches will not be as effective with individuals from genetic heritages from other parts of the world. If a genetic database needs to cover only 2% of the target population to provide a third-cousin match, then a population genetics model predicts that in a database of approximately 3 million U.S. individuals of European descent more than 99% of the people of this ethnicity would have a least a single third-cousin match and more than 65% would be expected to have at least one second-cousin match [38].

Following the arrest of the alleged Golden State Killer, researchers at the Center for Medical Ethics and Health Policy at the Baylor College of Medicine in Houston, Texas conducted an online survey in May 2018 to study public opinion of law enforcement using DTC genetic data [39]. A majority of the 1,587 respondents to this survey “supported police searches of genetic websites [like GEDmatch] that identify genetic relatives (79%) and disclosure of DTC genetic testing customer information to police (62%), as well as the creation of fake profiles of individuals by police on genealogy websites (65%)” [39]. However, the authors note: “As more people become familiar with the vulnerabilities of personal genetic services, opinions may shift regarding the acceptability of police access to data that are generated by and shared with these services” [39].

A letter to the editor of the journal *Science* notes several factors that mitigate the threat to privacy: (1) “only data voluntarily uploaded and explicitly made public are searched” as “investigations have relied on data that individuals have chosen to download from a testing company’s database and upload to GEDmatch,” (2) “no one is legally required to contribute to a genetic genealogy database, and because the samples are not in the possession of government agencies, these searches are substantially different from familial searching of law enforcement databases,” (3) “raw genetic data are not disclosed to law enforcement” – rather “search results display only the length and chromosomal location of shared DNA blocks, which are used to determine approximate kinship relationships between individuals,” and (4) “genetic genealogy is for lead generation, not conviction” [40].

GEDmatch, the publicly available genetic genealogy website that enabled the Golden State Killer arrest, changed its rules in May 2019 [41]. GEDmatch now requires that participants opt-in rather than opt-out of being included in potential law enforcement searches. It will take time to regrow the capabilities of long-range

Table 1
Summary of rapid DNA instrument validation and evaluation studies published from 2016 to 2019.

Publication	Instrument	STR Primer Set	Tests Performed and Success Rates Reported
[11]	DNAscan/ ANDE 4C	PowerPlex 16	Evaluation of swabs from drinking containers, blood and buccal cells on FTA paper, blood and buccal cells on untreated paper, dried blood on ceramic tile, dried blood and dried semen on clothing, chewing gum, cigarette butt, cellphones, and bones to assess reproducibility, accuracy/concordance, sensitivity, precision, resolution, and peak height ratios
[12]	DNAscan/ ANDE 4C	PowerPlex 16	SWGAM developmental validation (across 8 laboratories, >2300 swabs): species specificity, sensitivity, stability, inhibitors, reproducibility, mixtures, precision, accuracy, and concordance; success rate (1362 samples with 13 CODIS core loci) = 84% (auto) → 91% (manual)
[13]	RapidHIT 200	GlobalFiler Express	Evaluation of 34 known buccal samples and 23 negative controls; success rate = 50% (auto)
[14]	DNAscan/ ANDE 4C	PowerPlex 16	SWGAM internal validation: contamination assessment, consistency and reliability, sizing precision, peak height ratio determination, noise and average peak height assessment, stutter percent calculation, sensitivity and interpretation threshold calculations, and stability studies; success rate (193 samples) = 75% (auto) for 13 CODIS core loci with no incorrect calls
[15]	RapidHIT ID	GlobalFiler Express	SWGAM internal validation: contamination assessment, reliability, swab re-analysis, sensitivity, inhibitor, mixture, swab stability, precision, concordance and reliability, swab substrate, standards/controls, and bridge studies; success rate (50 samples) = 72% (auto) → 90% (manual)
[16]	RapidHIT ID	GlobalFiler Express	SWGAM developmental validation: thermal cycling parameters, mock inhibition, species specificity, sensitivity, concordance and carryover, swab retrieval and re-extraction, repeatability and reproducibility, electrophoresis sizing accuracy, stutter calculations and precision studies
[17,18]	RapidHIT 200	NGM SElect Express	28 runs performed in total (with 7 samples each); problems encountered with hardware, software, and consumables; found the system was not suitable for crime scene samples in its current design; success rate (155 samples) = 77% gave complete DNA profiles with samples involving (1, 2, or 5) μ L blood spotted on swabs
[19]	RapidHIT ID	GlobalFiler Express and NGM SElect Express	Evaluation of thermal cycling parameters, sensitivity, carryover contamination risks, repeatability and reproducibility, mixtures, and mock crime scene samples
[20]	RapidHIT ID	GlobalFiler Express	SWGAM internal validation: known and database-type samples, reproducibility, precision, sensitivity, stochastic effects, mixtures, contamination assessment, and concordance studies
[21]	ANDE 6C	FlexPlex (6-dye, 27plex STR assay)	SWGAM developmental validation (across 6 labs, 2045 swabs, 13 instruments): species specificity, limit of detection, stability, inhibitors, reproducibility, reference material, mixtures, precision, concordance, signal strength, peak height ratio, stutter, non-template addition, resolution, and contamination assessment; first-pass success rate (1338 samples with 20 CODIS core loci) = 92% ; successfully interpreted >2000 samples with over 99.99% concordant alleles; data package led to receiving NDIS approval in June 2018
[22]	RapidHIT 200	NGM SElect Express	Development studies that included process optimization, sensitivity, repeatability, contamination checks, inhibition, swab age, concordance, and overall performance; success rate (124 samples) = 84.5% gave a full profile
[23]	RapidHIT 200	NGM SElect Express	Protocol adjustments that extended the overall run times were made to enhance slightly sensitivity with mock crime scene samples (dilutions of blood and cell line DNA)

familial searches that existed when more than a million profiles could be compared.

5. Next-generation sequencing

Next-generation sequencing (NGS), also known as massively parallel sequencing (MPS) in the forensic community, has been used for many years to perform high-throughput DNA sequencing for biotechnology discovery purposes. Compared to existing capillary electrophoresis (CE) methods, which only measure the length of the overall PCR product, NGS provides an additional dimension to the data. With NGS of STR markers, sequences of targeted PCR amplicons for the STR alleles and their accompanying stutter products are produced. In addition, both STR and SNP markers can be analyzed in the same test – and with more markers than is possible with CE. This higher information content per sample opens up potential new applications including biogeographical ancestry, phenotyping of externally visible characteristics, and finer details on STR alleles to possibly improve mixture component resolution. A special issue of the journal *Electrophoresis* on novel applications of MPS in forensic DNA analysis was published in November 2018 [42].

As discussed in a recent state-of-art review [43], two primary MPS platforms are used in forensic DNA analysis: (1) MiSeq FGx Forensic Genomics Systems (Illumina, San Diego, CA, USA) and (2) Ion Torrent PGM or Ion S5 (ThermoFisher Scientific, Waltham, MA, USA).

A survey of 33 European laboratories from 25 countries found that 17 had purchased at least one MPS instrument [44]. The top

four challenges for implementation of MPS were identified as (1) lack of consistent nomenclature and reporting standards, (2) lack of compatibility with existing national DNA database infrastructure, (3) lack of population data to support statistical calculations, and (4) lack of an adequate legislative framework. Some comments on policy and legal issues for law enforcement with MPS data were also shared [45].

Some eight considerations with minimal STR allele nomenclature requirements were spelled out by the DNA Commission of the International Society for Forensic Genetics [46]. An international collaborative effort known as STRSeq is cataloging sequence diversity observed at common STR markers [47]. Based on experience with examining many STR allele sequences, a revised sequence guide for forensic STRs used in MPS has been prepared [48] and new approaches for compacting sequence information are being developed [49].

MPS sequence population data have been published for 23 autosomal STR loci in Koreans [50], for the Yavapai Native Americans from West-Central Arizona using the MiSeq FGx system [51], and for U.S. population data across 22 autosomal STR loci [52], across 27 autosomal STR loci [53] and with the complex locus SE33 [54]. Illumina's MiSeq FGx Forensic Genomics System has been the subject of a developmental validation study [55], an assessment of forensic STR and SNP kits [56], the validation of a mitochondrial DNA sequencing method [57], and an evaluation for use in case-work [58]. The ForenSeq DNA Signature Prep Kit has also been evaluated [59] along with the effects of the Ion PGM Hi-Q sequencing chemistry on sequence data quality [60].

A group from the Netherlands examined 45 mixtures, which

consisted of 5 two-person mixtures at ratios of 1:99, 5:95, 10:90, 50:50, 80:20, 90:10, 95:5, and 99:1 [61]. New STR markers are being considered as well to potentially assist in future DNA mixture interpretation with MPS [62,63].

In May 2019, the NDIS Board of the FBI Laboratory began accepting data from approved NGS kits for upload to the U.S. national DNA database (see Section 4.4 of the NDIS Operational Procedures Manual at <https://www.fbi.gov/file-repository/ndis-operational-procedures-manual.pdf/view>). However, it is noted in the NDIS manual: “The CODIS software is not capable of storing, searching, or maintaining information on X STRs or identity SNPs ... Only DNA records relating to the required CODIS Core Loci and NDIS accepted loci ... shall be uploaded, stored and searched at NDIS.”

6. DNA mixture interpretation and probabilistic genotyping software

DNA mixtures arise from the combination of DNA from more than one individual. Mixture are common, and even expected, in many forensic investigations (e.g., sexual assaults, mixed bloodstains, handled items). Deciphering the various components present in a mixture and assigning an appropriate weight to the evidence can be challenging. Improper use of DNA mixture interpretation approaches led to closure of several U.S. forensic DNA laboratories in 2015 and 2016. To assist in the appropriate use of one of the commonly used mixture interpretation approaches, some rules for the combined probability of inclusion (CPI) were spelled out [64].

The past few years have seen an increase in the use of probabilistic genotyping software (PGS) to assist DNA mixture interpretation. Generally, PGS systems use either (1) “discrete” (sometimes called “semi-continuous”) models that use the presence or absence of peaks along with probabilities of allele drop-out or drop-in or (2) “continuous” (sometimes called “fully-continuous”) models that take peak heights into account as well as the presence or absence of peaks along with probabilities of allele drop-out or drop-in. Fig. 1 describes the general steps in mixture interpretation along with

user inputs required for PGS systems.

A review article describing PGS and available software programs was published in early 2019 [65]. Table 2 summarizes the various PGS systems available as of July 2019 [66–79].

To inform human identification strategies, a set of over 25,000 single-source and mixed-source DNA profiles, known as the PROVEDIt (Project Research Openness for Validation with Empirical Data) dataset, was produced and made publicly available [80]. In the past three years, several interlaboratory studies involving DNA mixture interpretation have been organized and published by the ISFG Spanish-Portuguese Working Group [81,82], the Netherlands Forensic Institute [83], the U.S. National Institute of Standards and Technology (NIST) MIX05 and MIX13 studies [84], and New Zealand’s Institute of Environmental Science and Research [85]. The NIST MIX13 data were examined with four PGS systems [86].

Some suggestions were made for validation of PGS systems [87], and several validation studies were published for the PGS system STRmix including a developmental validation by the developers [88], an FBI Laboratory internal validation [89], and a 31-laboratory compilation of 2825 mixture results [90]. A machine learning-based assessment for estimating the number of contributors was described [91], and the challenges of estimating the number of contributors with low levels of DNA were explored [92]. Variation of results with four different continuous PGS models were studied [93] and responses to court admissibility challenges with STRmix were provided [94].

7. DNA transfer and activity level evaluations

The recognition of the importance of DNA transfer and activity propositions (see Ref. [95]) has increased with use of highly sensitive DNA testing methods. It reflects the growing interest in this area that four thorough reviews were published in the last year. They focus on separate areas of interest. The mechanism on how DNA is transferred from a subject is explored with comprehensive sources from medical as well as forensic literature [96]. The most prolific researchers in the area describe variables affecting transfer

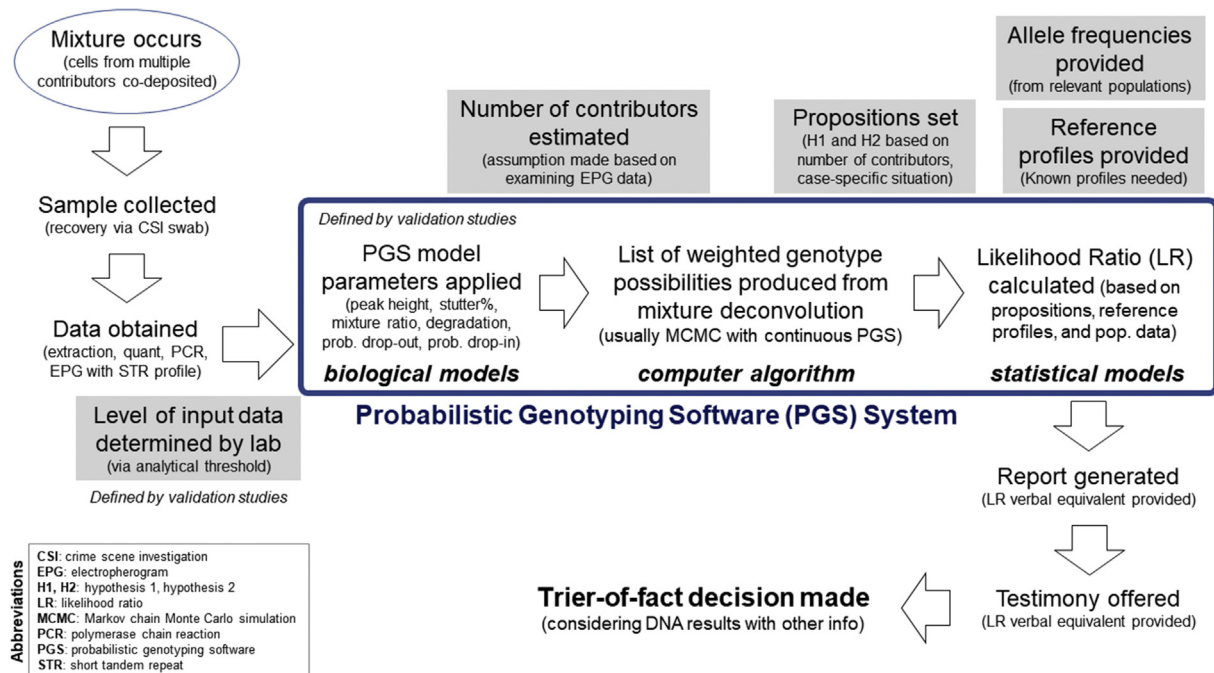


Fig. 1. Overview of DNA mixture interpretation and input required from users (see grey boxes) of probabilistic genotyping software (PGS) systems.

Table 2
Summary of probabilistic genotyping software (PGS) systems available as of July 2019 (see also Table S1 in Ref. [65]). PGS are listed in alphabetical order by program name.

Program Name	Type (Model)	Creator(s)	Availability	References
1 CEESit	Continuous	Catherine Grgicak	Open-source software: https://lftdi.camden.rutgers.edu/	[66]
2 DNAmixtures	Continuous	Therese Graversen	Open-source software: http://dnamixtures.r-forge.r-project.org/	[67]
3 DNA Mixture Solution	Continuous	Charles Brenner	Commercial product: http://dna-view.com/dnaview.htm	[68]
4 eDNA	Discrete & Continuous	Available through subscription service: Bullet (Semi-continuous; uses LRmix math) and BulletProof (Fully-continuous; uses EuroForMix math) http://ednalims.com/probabilistic-genotyping/		
5 EuroForMix	Continuous	Øyvind Bleka, Peter Gill	Open-source software: http://www.euroformix.com/	[69]
6 FST	Discrete	NYC OCME	Proprietary to NYC OCME Department of Forensic Biology	[70]
7 GenoProof Mixture 3	Continuous	Frank Götz	Commercial product: https://www.qualitytype.de	[71]
8 Kongoh	Continuous	Sho Manabe	Open-source software: https://github.com/manabe0322/Kongoh/releases	[72]
9 Lab Retriever	Discrete	David Balding; maintained by Norah Rudin and colleagues	Open-source software: https://scieg.org/lab-retriever/	[73]
10 likeLTD	Discrete & Continuous	David Balding	Open-source software: https://sites.google.com/site/baldingstatisticalgenetics/software/likeltd-r-forensic-dna-r-code	[74]
11 LiRa/LiRa-HT	Discrete/Continuous	Roberto Puch-Solis	Proprietary to LGC (now Eurofins)	[75]
12 LRmix; LRmix studio	Discrete/Discrete	Hinda Haned, Peter Gill; Jeroen de Jong	Open-source software: https://sites.google.com/site/forensicdnastatistics/PCR-simulation/lrmix; http://lrmixstudio.org/	[76]
13 MaSTR	Continuous	Teresa Snyder-Leiby	Commercial product: https://softgenetics.com/MaSTR.php	[77]
14 STRmix	Continuous	Duncan Taylor, Jo-Anne Bright, John Buckleton	Commercial product: https://strmix.esr.cri.nz/	[78]
15 TrueAllele	Continuous	Mark Perlin	Commercial product: http://www.cybgen.com	[79]

of DNA in a review that is a valuable compendium of the studies available to date [97]. In spite of the growing interest in the field, many gaps remain to be filled. It is difficult to compare studies one with another because of the different criteria used by different researchers to measure association. An argument for harmonization and sharing of data was made in order to assist in addressing activity level questions [98]. The most recent review makes suggestions to rationalize how data should be compiled as a way of helping practitioners to use available data [99].

Activity propositions have been proposed by many as the most appropriate approach to dealing with small quantities of DNA. A review on this topic discusses the advantages of using such propositions because of the increasing sensitivity of analytical systems which has shifted the focus of the court from questions about the source of the DNA to the mechanism of how it got there [95]. A discursive paper identifying the value of this approach and identifying the drawbacks of alternatives was published earlier [100]. The subject of the need for critically examining the appropriate level of propositions was also addressed [101] while another publication provided a more prescriptive approach to the use of activity propositions [102]. The value of relying on empirical data to formulate activity likelihood ratios rather than using generic values was outlined [103].

Published studies which sought to gain more understanding on the factors affecting DNA transfer can be divided into two main categories: ones where a particular variable was studied and ones which loosely simulated casework by exploring factors affecting transfer.

When the effect of pressure was studied, an increase in DNA from both donor and unknown sources was recorded [104]. The impact of sex and age was explored and it was noted that shedder

status changed in 77% of the cases [105]. A method to track shedder status was proposed [106]. While earlier DNA transfer studies sought to explore shedder status, the studies published in this period by researchers who have regularly published in this field were concerned with more sophisticated questions such as an analysis of self and non-self on handprints transferred onto glass plates [107] and whether the last person to handle an item can be detected in the DNA profile produced from that item [108]. Both of these factors are reported as being related to the shedder status of the participants. When researchers set out to shed light on the relative DNA contribution of two persons handling the same object, the effects of substrate as well as shedder status were noted [109]. Sweatbands were used to study how long an item had to be worn by a second person to leave detectable DNA behind – and it was concluded that it is highly unlikely to use a piece of clothing even for a short period without leaving DNA behind [110]. The influence of activities on transfer was also studied [103] as well as potential transfer from various parts of a hand' [111].

The possibility of a second person as a carrier was studied in various ways. DNA was found to transfer from donor to cotton to plastic or cotton via a second person 40% of the time in 180 samples examined [112]. An investigation into the deposition and persistence of directly and indirectly transferred DNA on regularly used knives sought to check whether intrinsic qualities of profiles could distinguish between directly and indirectly transferred DNA [113]. Burglary tools were the subject of another persistence study from which nature of contact, substrate and user characteristics were identified as variables [114]. The owner was detected in 47% of cases but not always as the major profile in a detectable mixture result [114].

Knives were studied by a number of groups to address various

questions arising in casework. DNA from persons standing close to the stabber were not detected in 83% of cases even though two-, three-, and four-person mixtures were recovered [115]. The question was raised as to whether secondary transfer could falsely place someone at the scene of a crime by examining knives handled by individuals who had shaken hands with someone beforehand [116]. A follow-up letter to the editor commented on the duration of the 2-min handshake used and noted eight aspects of the experimental setup in the Cale et al. study that were considered optimal for detecting DNA deposited through secondary transfer [117].

Two studies considered mock assault situations. The possibility of detecting DNA following skin-to-skin contact noted that the amount of DNA falls off rapidly from skin but is detectable on clothes worn up to 24 h after the simulated assault [118]. The high number of non-self-alleles detected in control areas in this study supports an earlier study on the implications of shedder status and background DNA on direct and secondary transfer in an attack scenario [119]. These authors noted that background DNA from the environment can be confused with crime samples [119].

Clothing have sometimes been considered as a reference source for the wearer. Experiments to distinguish wearer from toucher were reported [120]. Another study of wearer and non-wearer on the collars and cuffs of upper garments illustrated how varied the results of such studies are [121]. While the wearer was detected in all interpretable profiles and present as a major most of the time, no DNA was recovered from the cuffs of two garments and in one instance a non-wearer contributed more than a wearer [121].

A number of groups explored the effects of laundry or washing on detection of DNA. Following various temperatures and actions, DNA could be recovered from clothes exposed to water for more than one week [122]. Transfer during laundry was studied, and it was demonstrated for both spermatozoa and vaginal secretions that sufficient amounts of DNA may transfer during laundry to yield complete genetic profiles [123]. Thus, clothing in a washing machine can act as a mediator of secondary and tertiary DNA transfer as it was recorded that DNA profiles were detected on 22% of samples on which no DNA was present prior to laundry [123]. In another study, tertiary transfer between washings via the washing machine drum was not detected [124].

The ease with which DNA transfers unto a person's external clothing during a regular day was illustrated by examining 10 cm × 10 cm areas located on back, front and shoulders of an individual's clothing during a regular day's activity [125]. In a separate set of experiments the possibility of laundering being a source of transfer was studied and DNA recovered from 74% of UV treated cotton swatches [125].

A study of recovery of STR profiles from bite marks underlined the stability of human nuclear DNA not only on inert surfaces but also on biological surfaces and their forensic usefulness even when bite marks are stored 21 days under adverse but realistic conditions at a crime scene [126]. Whether it was possible to distinguish social contact from sexual activity was considered by searching for female profiles on the inside of male underpants [127]. Real-time PCR and Y-STR were used to assess the transfer and persistence of male DNA under female fingernails following controlled scratching experiments [128].

Co-extraction of DNA/RNA was used to explore palmer surface of hands and fingers in order to gain understanding of foreign material. Non-skin cellular material was observed in 15% of palms [129]. The prevalence of human cell material in public and private objects was studied using DNA and RNA [130]. This study noted that high levels are not related to the number of contributors and confirmed findings from other studies that the major DNA on an individual may not be the owner.

8. Forensic biology and body fluid identification

RNA continues to be the main focus for the identification of body fluids, but the number of papers on various techniques could be seen as an indication that the ideal solution to body fluid identification is not yet available. The result of a 2017 EuroForGen-NOE and EDNAP laboratories collaborative exercise demonstrated moderate-to-high count values in the body fluid or tissue of interest with little-to-no counts in non-target body fluids [131]. The authors propose that results of this collaborative mRNA massively parallel sequencing (MPS) exercise support targeted mRNA sequencing as a reliable body fluid identification method that could be added to the repertoire of forensic MPS panel [131]. Some of the same authors discuss a probabilistic model that predicts the origin of a stain. The model differs from the ones previously suggested in that it incorporates quantitative information (NGS read counts) rather than just presence/absence of markers [132].

MicroRNA (miRNA) molecules have been shown to have high tissue specificity and are less susceptible to degradation as a result of their small size, which infers great advantages to their potential role for identifying forensically relevant body fluids. A study identified the miRNeasy mini kit as the optimal method for the extraction of miRNAs from body fluids and validates a selection of miRNAs previously suggested as potential biomarkers [133].

Differential expression of 15 preselected miRNAs in tissues of brain, kidney, lung, liver, heart muscle, skeletal muscle and skin were assessed. miRNA expression profiling could be used to reliably differentiate between organ tissues. Authors claim this method, which is compatible with and complementary to forensic DNA analysis, is applicable to realistic forensic samples, e.g., mixtures, aged and degraded material as well as traces generated by mock stabbings and experimental shootings at ballistic models [134].

Development of HyBeacon probes for specific mRNA detection using body fluids as a model system describes HyBeacons, linear oligonucleotides which incorporate fluorescent dyes covalently linked to internal nucleotides, which were previously used with PCR and isothermal amplification to interrogate SNPs and STRs. Here their use is explored for the identification of expressed gene sequences through mRNA profiling. Each assay shows a high degree of specificity to the target body fluid mRNA suggesting there is no requirement to remove genomic DNA prior to analysis. Of the five assays developed, four were able to detect between 10 and 100 copies of target cDNA, the fifth 1000 copies of target [135].

Human specificity of mRNA as an organ typing assay was assessed against organ tissue RNAs of various animals and human specificity was confirmed [136].

Improving body fluid identification in forensic trace evidence led to construction of an immunochromatographic test array to rapidly detect up to five body fluids simultaneously. Immunochromatographic strip tests are promoted as easy to use, user-independent, quick, and inexpensive. These researchers constructed a combined immunochromatographic strip test array based on commercially available tests. With this test it was possible to identify the components of a mixture, the test was easily incorporated into standard laboratory work, and its sensitivity and specificity were shown to be comparable to those of conventional strip tests [137]. The same authors earlier proposed a different solution: "Independent validation of body fluid-specific CpG markers and construction of a robust multiplex assay." Potential forensic use of tissue-specific DNA methylation markers were used in a study where 13 promising markers were evaluated to identify suitable candidate markers for the development of a robust and reliable multiplex assay [138].

Various other techniques are available in the literature including development of a protein microarray chip with enhanced

fluorescence for identification of semen and vaginal fluid [139], identification and detection of protein markers to differentiate between forensically relevant body fluids [140], Phadebas paper as a presumptive screening tool for saliva on forensic exhibits [141], feasibility of a handheld near infrared device for the qualitative analysis of bloodstains [142], multiple reaction monitoring tandem mass spectrometry approach for the identification of biological fluids at crime scene investigations [143], development of a quantitative validation method for forensic investigation of human spermatozoa using a commercial fluorescence staining kit (SPERM HY-LITER Express) [144], expansion of microbial forensics [145], and differentiation of body fluid stains on fabrics using external reflection Fourier transform infrared spectroscopy (FT-IR) and chemometrics [146].

9. DNA phenotyping

Continuing research into the genetic components of age, ancestry, and appearance have improved DNA phenotype capabilities. The VISAGE (Visible Attributes Through Genomics) Consortium (see <http://www.visage-h2020.eu/>) is a European Union (EU)-funded research and innovation program working to predict a person's appearance, age, and bio-geographical ancestry from DNA samples. The VISAGE Consortium consists of 13 partners from 8 EU member states (The Netherlands, Poland, Spain, Austria, Germany, United Kingdom, France, and Sweden) and involves efforts in eight areas: (1) project management and coordination, (2) epigenetic markers, (3) prototype tools based on massively parallel sequencing, (4) integrative statistical framework with prototype software, (5) societal, ethical, and regulatory dimensions of constructing composite sketches from DNA for forensic applications, (6) implementing the construction of composite sketches from DNA in the routine forensic DNA service environment, (7) training of relevant target groups and publicly disseminating project outcomes, and (8) ethics requirements.

In November 2018, VISAGE researchers published a 123-page report entitled “The regulatory landscape of forensic DNA phenotyping in Europe” that examines the regulatory and legal frameworks for phenotyping in the 8 EU member states participating in the project and highlights country-specific legal questions (see http://www.visage-h2020.eu/Report_regulatory_landscape_FDP_in_Europe2.pdf). VISAGE researchers have published reviews on progress in forensic epigenetics [147–149] and spatial distribution of eye and hair pigmentation across European populations and beyond [150]. The benefits and problems associated with forensic DNA phenotyping were studied through analysis of 36 interviews with various stakeholders including forensic scientists, police officers, lawyers, government agencies, and social scientists [151].

The HRISplex-S system for eye, hair, and skin color prediction has been subjected to developmental validation studies [152]. Additional research studies have examined the effect of gender on eye color prediction [153], the ability to predict eye and hair color from World War II skeletal remains [154], the impact of age-dependent hair color darkening during childhood [155], the prediction of head hair shape from DNA [156], the performance of four models for eye color prediction in an Italian population sample [157], and the development of new prediction models for skin color, tanning, and freckling from DNA in Polish populations using linear regression, random forest, and neural network approaches [158]. The predictability of tall stature from DNA markers has been explored in European samples [159] and genome-wide association studies conducted to identify loci influencing eyebrow color variation [160].

DNA methylation studies have explored the outcome of DNA methylation analysis using simulated low amounts of DNA [161],

evaluated MPS methods for forensic methylation profiling [162], and investigated epigenetic discrimination of identical twins using buccal swabs, saliva, and cigarette butts [163].

10. Privacy and ethical issues

In September 2017, a report entitled “Establishing Best Practice for Forensic DNA Databases” was prepared following consultation by Forensic Genetics Policy Initiative (see <http://dnapolicyinitiative.org/report/>). While acknowledging the benefits of DNA databasing, it debates the various ethical and privacy issues involved in storing DNA data and makes recommendations for best practice. A response to this forensic genetics policy initiative's report discusses the blurring of boundaries of DNA-based information inside and outside forensic databases [164], which is a debate that is likely to grow in light of investigative genetic genealogy developments. The scope of the original report involves DNA databases held for criminal investigation so it does not capture additional issues that arise when data are used for other purposes beyond for what it was originally collected, such as genetic genealogy databases used in law enforcement investigations.

The social and ethical responses to the history of innovations in forensic genetics and their application to criminal investigations were reviewed [165]. Four major ethical concerns form a focus of the paper (dignity, privacy, justice, and social solidarity), and key features of forensic genetics practice are examined in the light of these concerns. The different views about benefits and risks was acknowledged in a study of what influences public views on forensic DNA testing in the criminal field [166]. Results suggested that public views on forensic DNA testing are influenced by the level of education, age, and exposure to law enforcement occupations although not in a straightforward manner.

Concerns about the contrast of the ability of technology and the proportionality of its use for fixed functions is illustrated by a letter “Approaching ethical, legal and social issues of emerging forensic DNA phenotyping (FDP) technologies comprehensively: Reply to ‘Forensic DNA phenotyping: Predicting human appearance from crime scene material for investigative purposes’” [167] where the authors discuss the tension between the ability of technology and the ethics of increasing the power of the state albeit to prevent crime.

Another article looks at the adoption of phenotyping from a privacy perspective, using this to inform and critique the application of a Privacy Impact Assessment to this emerging technology [168]. Noting the benefits and limitations, the authors develop a number of themes that would influence a model Privacy Impact Assessment as a contextual framework for forensic laboratories and law enforcement agencies considering implementing forensic DNA phenotyping for operational use [168].

Genetic markers for trait prediction ability has mainly been assessed in European and North American populations. This has prompted research investigating the discriminatory power of these markers in other populations, especially those exhibiting admixture. South Africa is such a population and there are numerous ethical and social considerations discussed in a recent article [169].

11. Guidance documents

A growing number of standards and guidance documents are being published by various organizations around the world. Table 3 lists 34 such documents in the past three years. While these documents may be designed to be specific for certain regions, there is value in knowing what others are doing and learning from them as science knows no boundaries.

During the time period of this review (2016–2019), the

Table 3
Guidance documents related to forensic DNA published from 2016 to 2019.

Organization	Publication Date	Title
SWGDM	December 2016	Recommendations for the Efficient DNA Processing of Sexual Assault Evidence Kits https://docs.wixstatic.com/ugd/4344b0_4daf2bb5512b4e2582f895c4a133a0ed.pdf
SWGDM	December 2016	Validation Guidelines for DNA Analysis Methods https://docs.wixstatic.com/ugd/4344b0_813b241e8944497e99b9c45b163b76bd.pdf
SWGDM	January 2017	Contamination Prevention and Detection Guidelines for Forensic DNA Laboratories https://docs.wixstatic.com/ugd/4344b0_c4d4dbba84f1400a98aa2e48f2bf291.pdf
SWGDM	January 2017	Interpretation Guidelines for Autosomal STR Typing by Forensic DNA Testing Laboratories https://docs.wixstatic.com/ugd/4344b0_50e2749756a242528e6285a5bb478f4c.pdf
SWGDM	July 2018	Recommendations of the SWGDAM Ad Hoc Working Group on Genotyping Results Reported as Likelihood Ratios https://docs.wixstatic.com/ugd/4344b0_dd5221694d1448588dcd0937738c9e46.pdf
SWGDM	April 2019	Addendum to “SWGDM Interpretation Guidelines for Autosomal STR Typing by Forensic DNA Testing Laboratories” to Address Next Generation Sequencing https://docs.wixstatic.com/ugd/4344b0_91f2b89538844575a9f51867def7be85.pdf
SWGDM	April 2019	Interpretation Guidelines for Mitochondrial DNA Analysis by Forensic DNA Testing Laboratories https://docs.wixstatic.com/ugd/4344b0_f61de6abf3b94c52b28139bfff600ae98.pdf
SWGDM	January 2018	Quality Assurance Standards for Forensic DNA Testing Laboratories (draft) https://docs.wixstatic.com/ugd/4344b0_d4c50d6204b240d3ab23e388b5f6591a.pdf
SWGDM	February 2019	FBI Quality Assurance Standards Audit for Forensic DNA Testing Laboratories (draft) https://docs.wixstatic.com/ugd/4344b0_7b03780db7244a5b9a93b3bdd59345b5.pdf
SWGDM	February 2019	Quality Assurance Standards for DNA Databasing Laboratories (draft) https://docs.wixstatic.com/ugd/4344b0_bf68274461f3425888adce9399115099.pdf
SWGDM	February 2019	FBI Quality Assurance Standards Audit for DNA Databasing Laboratories (draft) https://docs.wixstatic.com/ugd/4344b0_990aee2783af4a82b4d21358e0bd1c53.pdf
US DOJ	September 2018	Department of Justice Uniform Language for Testimony and Reports for Forensic Autosomal DNA Examinations Using Probabilistic Genotyping Systems https://www.justice.gov/olp/page/file/1095961/download
US DOJ	September 2018	Department of Justice Uniform Language for Testimony and Reports for Forensic Mitochondrial DNA Examinations https://www.justice.gov/olp/page/file/1095966/download
US DOJ	September 2018	Department of Justice Uniform Language for Testimony and Reports for Forensic Y-STR Data Examinations https://www.justice.gov/olp/page/file/1095976/download
US DOJ	September 2018	Department of Justice Uniform Language for Testimony and Reports for Forensic Serological Examinations https://www.justice.gov/olp/page/file/1095971/download
ASB	September 2018	Standard for Validation Studies of DNA Mixtures, and Development and Verification of a Laboratory's Mixture Interpretation Protocol https://asb.aafs.org/wp-content/uploads/2018/09/020_Std_e1.pdf
ISO/TC 272	February 2016	ISO 18385:2016 Minimizing the Risk of Human Contamination in Products Used to Collect, Store and Analyze Biological Material for Forensic Purposes – Requirements https://www.iso.org/standard/62341.html?browse=tc
ISO/TC 272	August 2018	ISO 21043–1:2018 Forensic Sciences – Part 1: Terms and Definitions https://www.iso.org/standard/69732.html?browse=tc
ISO/TC 272	August 2018	ISO 21043–2:2018 Forensic Sciences – Part 2: Recognition, Recording, Collecting, Transport and Storage of Items https://www.iso.org/standard/72041.html?browse=tc
ISO/CASCO	November 2017	ISO/IEC 17025:2017 General Requirements for the Competence of Testing and Calibration Laboratories https://www.iso.org/standard/66912.html
ENFSI	May 2017	Best Practice Manual for the Internal Validation of Probabilistic Software to Undertake DNA Mixture Interpretation http://enfsi.eu/wp-content/uploads/2017/09/Best-Practice-Manual-for-the-internal-validation-of-probabilistic-software-to-undertake-DNA-mixture-interpretation-v1.docx.pdf
ENFSI DNA WG	April 2017	DNA Contamination Prevention Guidelines http://enfsi.eu/wp-content/uploads/2017/09/DNA-contamination-prevention-guidelines-v2.pdf
ENFSI DNA WG	April 2017	DNA Database Management Review and Recommendations http://enfsi.eu/wp-content/uploads/2017/09/DNA-databasemanagement-review-and-recommendations-april-2017.pdf
UKFSR	October 2017	Codes of Practice and Conduct for Forensic Science Providers and Practitioners in the Criminal Justice System (Issue 4) https://www.gov.uk/government/publications/forensic-science-providers-codes-of-practice-and-conduct-2017
UKFSR	March 2016	Validation: Use of Casework Material (FSR-P-300) https://www.gov.uk/government/publications/protocol-using-casework-material-for-validation-purposes
UKFSR	July 2016	Sexual Assault Referral Centres and Custodial Facilities: DNA Anti-Contamination https://www.gov.uk/government/publications/sexual-assault-referral-centres-and-custodial-facilities-dna-anti-contamination
UKFSR	July 2016	Crime Scene DNA: Anti-Contamination Guidance https://www.gov.uk/government/publications/crime-scene-dna-anti-contamination-guidance
UKFSR	September 2018	Software Validation for DNA Mixture Interpretation (FSR-G-223) https://www.gov.uk/government/publications/software-validation-for-dna-mixture-interpretation-fsr-g-223
UKFSR	October 2018	DNA Mixture Interpretation (FSR-G-222) https://www.gov.uk/government/publications/dna-mixture-interpretation-fsr-g-222
ISFG DNA Commission	January 2016	Massively parallel sequencing of forensic STRs: Considerations ... on minimal nomenclature requirements [46] https://www.isfg.org/files/d5ccd549ee232596c75ad8a0b435190e7dba3035.parson2016_str_recommendations.pdf
ISFG DNA Commission	June 2016	Recommendations ... on quality control of autosomal short tandem repeat allele frequency databasing (STRider) [170] https://www.isfg.org/files/db9864824b44997f1014a62a0321f0d25ef6cf98.bodner2016_strider.pdf
ISFG DNA Commission	September 2016	Recommendations on the validation of software programs performing biostatistical calculations for forensic genetic applications [171] https://www.isfg.org/files/225be64835df624d1ddac70b95a2e7354f916fbb.coble_software_validation_isigen2016.pdf
ISFG DNA Commission	May 2017	Guidelines on the use of X-STRs in kinship analysis [172] https://www.isfg.org/files/eea3394d1595b83aeb59e093725518fb94691e78.tillmar2017_x_str_recommendations.pdf
ISFG DNA Commission	July 2018	Assessing the value of forensic biological evidence – guidelines highlighting the importance of propositions. Part I: evaluation of DNA profiling comparisons given (sub-) source propositions [173]
OSAC	Ongoing	Numerous documents under development (see text)

International Society for Forensic Genetics (ISFG) DNA Commission has published recommendations or considerations on STR allele sequencing nomenclature [46], quality control of autosomal STR allele frequency databasing [170], validation of software programs performing biostatistical calculations for forensic genetic applications [171], guidelines on using X-chromosome STRs in kinship analysis [172], and guidance on evaluating DNA profiling comparisons given (sub-) source propositions and emphasizing differences between investigations and evaluations of complex DNA data [173].

The International Organization for Standardization (ISO) now has a technical committee on forensic science (ISO/TC 272; see <https://www.iso.org/committee/4395817.html>) that issued its first standard on minimizing the risk of human DNA contamination. In addition, the ISO Committee on Conformity Assessment (ISO/CASCO) updated the ISO/IEC (International Electrotechnical Commission) 17025:2017 standard on general requirements for testing laboratories that are used to audit most forensic DNA laboratories around the world.

In the United States, the Scientific Working Group on DNA Analysis Methods (SWGDM), the Organization of Scientific Area Committees for Forensic Science (OSAC), the American Academy of Forensic Sciences (AAFS) Standards Board (ASB), and the Department of Justice (US DOJ); see <https://www.justice.gov/olp/uniform-language-testimony-and-reports>) have released various guidance documents over the past three years. In Europe, the European Network of Forensic Science Institutes (ENFSI), the ENFSI DNA Working Group (WG), and the UK Forensic Science Regulator (UKFSR) have been active as well with preparing best practice manuals (see <http://enfsi.eu/documents/best-practice-manuals/>), codes of practice, and guidance on a variety of topics.

11.1. SWGDAM activities

The Federal Bureau of Investigation (FBI) Laboratory funds the Scientific Working Group on DNA Analysis Methods (SWGDM) to serve as a forum for discussing, sharing, and evaluating forensic biology methods, protocols, training, and research (see <https://www.swgdam.org/>). SWGDAM provides recommendations to the FBI Director on the Quality Assurance Standards used to assess U.S. forensic DNA laboratories involved in the National DNA Index System (NDIS) performing DNA databasing and forensic casework.

SWGDM meets semiannually in January and July. Work products are developed in various committees and working groups including the Autosomal STR Committee, the CODIS Committee, the Laboratory Operations Committee, the Lineage Marker Committee, the Quality Assurance Committee, the Next Generation Sequencing Working Group, and the Rapid DNA Committee. Other groups are empaneled as needed to address specific topics as needed.

11.2. OSAC activities

In the United States, with Congressional funding, the Organization of Scientific Area Committees for Forensic Science (OSAC) was launched in 2014. OSAC is administered by the National Institute of Standards and Technology (NIST) to facilitate development of technically-sound documentary standards and adoption of these standards across the forensic science community (see <https://www.nist.gov/topics/organization-scientific-area-committees-forensic-science>). More than 550 members and several hundred affiliates from dozens of government agencies (federal, state, and local), academic institutions, and the private sector contribute their expertise in scientific research, measurement science, statistics, law, policy, and practice across 25 subcommittees organized by forensic discipline. In forensic DNA, there are active efforts ongoing with three OSAC subcommittees under the direction of the Biology/

DNA Scientific Area Committee (SAC).

Draft documents are developed as work products in each subcommittee and then provided to a Standards Developing Organization (SDO) to be formalized into documentary standards. The most widely used SDOs by OSAC include the American Academy of Forensic Sciences Standards Board (ASB) and ASTM International. OSAC publishes a monthly standards bulletin to update readers on forensic science standards in development at OSAC as well as in various SDOs (see <https://www.nist.gov/topics/forensic-science/organization-scientific-area-committees-osac/osac-newsroom/osac-standards>). A quarterly newsletter provides further updates on OSAC activities (see <https://www.nist.gov/topics/forensic-science/organization-scientific-area-committees-osac/osac-newsroom/osac-newsletter>). After an approval process, documents are posted to an OSAC Registry of Approved Standards that can be found at <https://www.nist.gov/topics/forensic-science/organization-scientific-area-committees-osac/osac-registry/osac-approved>.

The OSAC Biological Methods Subcommittee has sent 13 work products to an SDO. These documents include guidance on training programs, validation, and preventing, monitoring, and mitigating DNA contamination. In addition, another 14 standards, best practice recommendations, and technical reports are under development as of July 2019 (see <https://www.nist.gov/topics/forensic-science/biological-methods-subcommittee>).

The OSAC Biological Data Interpretation and Reporting Subcommittee has sent five work products to an SDO covering validation of probabilistic genotyping systems, forensic DNA interpretation and comparison protocols, assigning propositions for likelihood ratios in forensic DNA interpretations, and best practice recommendations for validation of forensic DNA software. In addition, another 14 standards are under development as of July 2019 on training, setting analytical and stochastic thresholds, statistical interpretation of autosomal STRs, reporting of DNA results containing a contaminant or failed control, next generation sequencing/massively parallel sequencing, use of elimination databases, use of rapid DNA at the crime scene, reporting DNA conclusions, and best practices for DNA testimony (see <https://www.nist.gov/topics/forensic-science/biological-data-interpretation-and-reporting-subcommittee>).

The OSAC Wildlife Forensics Subcommittee has sent eight work products to an SDO and six of them have been published so far. These documents cover general standards for wildlife forensics, morphology, report writing, validation of STR analysis, validation of new sequencing primers, wildlife forensic DNA standards procedures, protein serology method for taxonomic identification, and training in mitochondrial DNA analysis for taxonomic identification. In addition, another nine standards are under development as of July 2019 on use of public databases, sampling of reference samples from live mammals, geographic assignment of individual animals, validation of wildlife sequences in public databases, reference collections, genetic methods to determine an individual of potential hybrid origin, development and use of in-house sequence databases for taxonomic assignment of wildlife, development and use of allele frequency and population genetics databases, and best practices for building new STR panels in wildlife forensics (see <https://www.nist.gov/topics/forensic-science/wildlife-forensics-subcommittee>).

Finally, the OSAC Lexicon contains over 400 forensic DNA terms defined by the Biology/DNA Scientific Area Committee (see <http://lexicon.forensicosac.org/Term/Home/Index>).

11.3. ASB activities

The AAFS Standards Board (ASB) began operations in 2016 with

12 consensus bodies (i.e., committees) covering activities in anthropology, bloodstain pattern analysis, disaster victim identification, DNA, dogs and sensors, firearms and toolmarks, footwear and tire, forensic document examination, friction ridge, medicolegal death investigation, toxicology, and wildlife forensics. These consensus bodies meet virtually on a regular basis to create standards and best practices to assist the forensic science community. Published documents typically build on the materials prepared by corresponding OSAC subcommittees (see <https://www.asbstandardsboard.org/published-documents/>).

In 2018, the DNA Consensus Body completed ANSI/ASB Standard 020 “Standard for Validation Studies of DNA Mixtures, and Development and Verification of a Laboratory’s Mixture Interpretation Protocol” (available at https://asb.aafs.org/wp-content/uploads/2018/09/020_Std_e1.pdf). More than a dozen other DNA standards are currently under development.

11.4. ENFSI DNA working group activities

The ENFSI DNA Working Group meets annually to promote quality management systems, develop uniform guidelines, exchange information and expertise, promote research collaborations, provide education and training, assess needs, implement new methods, and support organization of collaborative exercises (see <http://enfsi.eu/about-enfsi/structure/working-groups/dna/>). Members come from over 50 organizations across 35 European countries. Recent documents produced by the group include DNA contamination prevention guidelines, DNA database management review and recommendations, and a best practice manual for the internal validation of probabilistic genotyping software used in DNA mixture interpretation (see Table 3).

12. Contamination avoidance and DNA success rates

The widespread use of DNA detection methods with increased sensitivity has led to various advisory groups offering guidance on contamination avoidance including SWGDAM and the UK Forensic Science Regulator (see Table 3).

As noted in Table 3, the UK Forensic Science Regulator has added guidance for contamination avoidance at crime scenes to the previous guide for the laboratory. In addition there is specific guidance on anti-contamination measures for Forensic Medical Examination in Sexual Assault Referral Centres and Custodial Facilities (see <https://www.gov.uk/government/collections/forensic-science-providers-codes-of-practice-and-conduct>). This document has illustrations of the various paths that can give rise to contamination either by direct transfer or indirect transfer both secondary and tertiary. In April 2017, the ENFSI DNA Working Group also issued guidelines for contamination avoidance (see Table 3).

The scientific literature contains various references highlighting what additional concerns increased sensitivity raises for contamination avoidance. Studies considered several possible sources of contamination. For example, fingerprint brushes were researched [174,175] as were gloves as potential sources [176]. The possibility of a cleaning sponge being a source of transfer was another concern [177]. A study of contamination in police stations highlighted a number of previously unrecorded incidents and raised the possibility of DNA on the outside of bags contaminating exhibits [178]. Two other studies focused on the need for contamination avoidance by police and laboratories [179,180] and later one group reported on the positive impact of such a program [181]. Indirect transfer as a source of contamination and its database-assisted detection in Austria is another example of the need for elimination databases to reduce the impact of contamination [182].

A case history of miscarriage of justice resulting in part from

contamination has been described [183,184]. A very comprehensive study of transfer of DNA within a Biology laboratory is a must read for any quality manager concerned with practices to minimize contamination [185].

An evaluation of 2260 crime samples from the Netherlands found that approximately 50% resulted in no DNA profiling results, 13% in complex DNA profiles and 37% in results that met their quality criteria for DNA database storage [186]. Recovered DNA quantity was a key factor in generating a successful DNA profile. The authors observed that 23% of their extracts contained more than 100 pg/μL of DNA with 958 DNA extracts measuring a concentration of 6 pg/μL or less, of which only 46 of these low-level extracts provided any meaningful DNA profiling data [186]. It was suggested that knowledge of success rates can assist in optimizing the DNA analysis process and sample selection criteria by the police and the laboratory, and that “a thorough selection of DNA traces for analysis, based on DNA success rates, will lead to fewer unnecessary analysis activities and will therefore shorten turnaround times and reduce backlogs” [186].

13. Recent special issues and review articles of note

Over the past three years, several special issues specific to forensic DNA were published in the journals *Electrophoresis*, *Genes*, *Forensic Science International: Genetics*, and *Forensic Science International*.

In October 2016, *Electrophoresis* published a special issue (volume 37, issue 21, pages 2725–2902) on “Forensic Analysis” (see <https://onlinelibrary.wiley.com/toc/15222683/2016/37/21>). Guest editor Bruce McCord organized 20 research articles into six subtopics: (1) DNA quantification and extraction [187,188], (2) body fluid identification using methylation analysis [189–191] and micro-RNA sequencing [192], (3) DNA typing using autosomal STRs [193,194] and rapidly mutating Y-STRs [195], (4) massively parallel sequencing [196–198], (5) toxicology and drug detection, and (6) sample characterization. Levels of PCR inhibitors, such as indigo, phenol, EDTA, bile salts, melanin, and tannic acid, were assessed with direct analysis in real time (DART) mass spectrometry [199].

In November 2018, *Electrophoresis* published another special issue (volume 39, issue 21, pages 2633–2833) on “Novel Applications of Massively Parallel Sequencing (MPS) in Forensic Analysis” (see <https://onlinelibrary.wiley.com/toc/15222683/2018/39/21>). Guest editors Bruce McCord and Steven Lee organized these 20 articles into five subtopics: (1) reviews and applications of STR technologies, (2) regional and global population sequence variation of STRs, (3) SNPs for identity, ancestry and phenotyping, (4) mitochondrial DNA applications, and (5) future directions in MPS [42]. Reviews of MPS techniques [200] and current state-of-the-art STR sequencing [43] were followed by applications in paternity testing [201], evaluation of performance on degraded samples [202,203], and a look at kits for streamlined analysis of routine reference samples [204]. Population data were described for the SE33 locus on 1036 U.S. population samples [54], for 58 STRs and 94 identity SNPs with 209 Korean individuals [205], for 58 STRs with the 944 individuals in the CEPH [Centre d’Etude du Polymorphisme Humain] human genome diversity panel containing 51 globally distributed populations [206], and for 15 STRs with 554 unrelated Chinese Northern Han individuals [202,203]. SNP marker work in this issue included ancestry analysis with 165 SNPs in two Chinese minority populations [207], development of a SNP panel for predicting biogeographical ancestry and phenotype [208] and an automated workflow for analysis of MPS data from forensic SNP assays [209], and implementing a biogeographical ancestry service for forensic casework [210]. The mitochondrial DNA applications portion of this special issue includes articles on sequencing the

mtGenome using the Precision ID mtDNA Whole Genome Panel [211], performance with degraded DNA samples using the Ion Torrent platform [212], and the bioinformatic removal of nuclear mitochondrial DNA pseudogene variants from NGS data using a mitotiling strategy [213]. For the future directions section of the issue, articles included an evaluation of MPS for forensic DNA methylation profiling [162], a DNA methylation assay based on pyrosequencing for determination of smoking status [214], a discussion of selecting microhaplotypes optimized for different purposes [215], and a bacterial DNA quantification assay for NGS library preparation of human biological samples [216].

A special issue on forensic genomics was organized in *Genes* [217] by guest editors Manfred Kayser and Walther Parson with 11 articles published between November 2017 and December 2018 (see https://www.mdpi.com/journal/genes/special_issues/Forensic_Genomics). Topics for these open access articles include performing molecular analysis of the RNA transcriptome for human organ tissue identification to assist in investigations of traumatic injury [218], investigating the epigenetic discrimination of identical twins using reference sample buccal swabs and saliva and cigarette butts common to forensic evidence [163], recovering fragmented nuclear DNA from human hair shafts [219], using high-throughput sequencing to recover nuclear DNA from a 4000-year-old Egyptian mummy head [220], examining mitochondrial DNA heteroplasmy with MPS to help distinguish maternal relatives [221], dating juvenile blow flies to assist forensic entomology with postmortem interval estimation [222], predicting the postmortem interval using microbiome data [223], applying NGS probe capture enrichment techniques to examine the whole mitochondrial genome and 426 nuclear SNPs on individual telogen hairs [224], and demonstrating that flanking region variation can impact rates of stutter product formation in STR markers [225].

Forensic Science International: Genetics published a virtual special issue (see <https://www.journals.elsevier.com/forensic-science-international-genetics/special-issues>) on trends and perspectives in forensic genetics 2018 (guest editor: Manfred Kayser) with 11 articles spread across the September 2018, November 2018, and January 2019 issues of the journal [226] covering activity level propositions [95], DNA transfer [97], probabilistic genotyping software [65], match probabilities for Y-STR profiles [227], microhaplotype markers [228], next generation sequencing [229], new database research algorithms for mitogenome analyses [230], human identification with targeted microbiome markers [231], estimating the postmortem interval using microbes [232], large scale DNA identifications from the International Commission on Missing Persons [233], and recent activities in forensic epigenetics [149].

Forensic Science International organized a virtual special issue on cold cases (guest editors: Rob Davis and James Adcock) with articles spread across the May, June, July, and August 2019 issues of the journal [234] covering topics such as considering the benefits versus the costs of DNA testing in sexual assault cases [235], genetic genealogy for cold case and active investigations [29], and assisting missing persons cases with genetic genealogy database searches [236].

A recent review article in *Analytical Chemistry* with 246 references (of which 225 are from the 2016 to 2018 time frame) covers a variety of topics including serology, DNA extraction and sample recovery, genotyping methods using STRs, mixtures and probabilistic genotyping, methods for estimating the number of contributors, X-STR and Y-STR markers, SNPs, InDels, mitochondrial DNA, ancient DNA, nonhuman DNA, endangered species and wildlife forensics, drug sourcing with DNA, MPS advances, and forensic applications of the microbiome [237].

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Declaration of Competing Interest

The authors declare that they have no competing interests.

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