

Opinion piece



Cite this article: Butler JM. 2015 The future of forensic DNA analysis. *Phil. Trans. R. Soc. B* **370**: 20140252.
<http://dx.doi.org/10.1098/rstb.2014.0252>

Accepted: 26 February 2015

One contribution of 15 to a discussion meeting issue 'The paradigm shift for UK forensic science'.

Subject Areas:

molecular biology, genomics, genetics

Keywords:

forensic science, DNA, short tandem repeat, DNA databases

Author for correspondence:

John M. Butler
e-mail: john.butler@nist.gov

The future of forensic DNA analysis

John M. Butler

National Institute of Standards and Technology, Gaithersburg, MD, USA

The author's thoughts and opinions on where the field of forensic DNA testing is headed for the next decade are provided in the context of where the field has come over the past 30 years. Similar to the Olympic motto of 'faster, higher, stronger', forensic DNA protocols can be expected to become more rapid and sensitive and provide stronger investigative potential. New short tandem repeat (STR) loci have expanded the core set of genetic markers used for human identification in Europe and the USA. Rapid DNA testing is on the verge of enabling new applications. Next-generation sequencing has the potential to provide greater depth of coverage for information on STR alleles. Familial DNA searching has expanded capabilities of DNA databases in parts of the world where it is allowed. Challenges and opportunities that will impact the future of forensic DNA are explored including the need for education and training to improve interpretation of complex DNA profiles.

1. Introduction

Since its introduction in the mid-1980s [1], forensic DNA testing has played an important role in the criminal justice community through aiding conviction of the guilty and exoneration of the innocent. Remains from missing persons and victims of mass disasters have been re-associated and identified through linking reference samples to recovered remains [2]. New technologies are regularly introduced and validated to expand the capabilities of laboratories working to recover DNA results with improved sensitivity and informativeness. Forensic laboratories have embraced automation for sample preparation and data interpretation in order to meet increasing throughput demands. Short tandem repeat (STR) typing continues to be the primary workhorse in forensic DNA analysis although other genetic markers are used for specific applications [3,4].

(a) DNA capabilities

DNA analysis provides capabilities not found in most of the other forensic disciplines. When biological material is transferred between perpetrator and victim in violent crimes such as murder and rape, DNA recovered from the crime scene has power to potentially identify the perpetrator. Theoretically when testing sufficient genetic markers, probabilistic 'individualization' of a DNA profile is statistically achievable except with identical twins—and even twins can sometimes be separated with additional genetic information [5]. Use of genetic markers that are inherited independent of one another enables application of the product rule (and subpopulation adjustments [6]), where statistical rarity of results at each marker can be combined across multiple genetic markers. Hence the weight of evidence can be expressed in quantitative terms that can be quite rare (1 in trillions or rarer) thanks to the multiplicative nature of independent multi-locus results. These calculations involve a genetic principle known as Hardy–Weinberg equilibrium [7]. DNA statistical interpretation rests on a solid scientific foundation, much of which originally came from academic efforts outside of the forensics arena. However, as will be discussed later, interpretations of complex DNA mixture profiles can be subjective and inconsistent between analysts and across laboratory protocols.

Interpretation of all forensic evidence involves comparing question (Q) results with known (K) references. This Q-to-K comparison depends on the quality of results obtained from crime scene evidence (Q) and the availability

of suitable reference samples (K). When samples from a suspect or multiple suspects are available during forensic DNA analysis, the Q-to-K comparison is fairly straightforward through examining the Q and K samples at the same genetic markers. When no suspects are available, DNA databases, which have been developed over the past 20 years to provide potential reference profiles from known previous offenders, can be searched to try to find a match to the unknown Q profile. DNA database profiles essentially enable throwing a wide net at an unknown Q profile to try to identify the perpetrator.

Another important capability of DNA is that by the nature of its inheritance pattern, where half of an individual's genetic code comes from his or her mother and half comes from his or her father, close biological relatives can be used for reference points. In other words, the possibility exists to reach beyond information available in a sample itself due to the capabilities of established genetic trait transmission principles. Missing persons and disaster victims can be identified from kinship associations if no direct reference sample is available for comparison purposes [8]. For example, if a child is missing, then the child's mother and father can supply DNA for comparison purposes that may be used to link their DNA profiles to putative sample(s) from their child.

Superb sensitivity is available with forensic DNA testing due to amplification of target regions with the polymerase chain reaction (PCR). DNA results can be obtained from as little as a single cell depending on methods used [9]. However, this exquisite sensitivity is both a blessing and a curse. When high-sensitivity techniques are used, a very real possibility exists for contamination from DNA coming from someone not associated with the crime sample under investigation. If consumables such as swabs or tubes are not DNA-free, then the manufacturer's DNA may be detected and even lead investigators down the wrong path [10]. A number of measures, such as examining negative controls, are typically used to prevent drawing incorrect conclusions if DNA contamination occurs.

Quality assurance measures have been developed over the years for DNA testing to promote confidence in results obtained. The DNA testing quality infrastructure is probably more advanced than that of many other forensic disciplines, because of experience from organizations like the European DNA Profiling Group (EDNAP) and the European Network of Forensic Science Institutes (ENFSI) in Europe and the Federal Bureau of Investigation's DNA Advisory Board (DAB) and Scientific Working Group on DNA Analysis Methods (SWGDM) in the USA.

Forensic DNA technology development has directly benefited from the Human Genome Project (HGP) and subsequent scientific efforts and discoveries in genetics and genomics. Genomic information from HGP provided the STR genetic markers for testing and more recent studies have supplied population data for assessing genetic variation. Tools, talent and technology have spilled over into the forensic DNA field from the much larger DNA sequencing market. Current DNA typing with STR markers involves use of fluorescent dyes to label PCR products and capillary electrophoresis (CE) to rapidly separate and detect these dye-labelled PCR products. The dyes and the CE methods used in forensic DNA testing were initially developed for DNA sequencing purposes. Future advances in forensic DNA analysis will probably mirror genomic technology development.

(b) Author's experience

I have sought throughout my career to understand past and present activities in the field through writing textbooks and providing training materials to practitioners. I have also tried to help shape the future of forensic DNA analysis through performing relevant research. My first contributions to forensic DNA analysis involved pioneering quantitation methods for mitochondrial DNA [11] and CE separation methods for genotyping STR markers [12]. Twenty years of research, 150 publications, more than 300 presentations and workshops, and five textbooks have contributed to an informed perspective on forensic DNA analysis. Over the past decade, I have given more than a dozen presentations on my thoughts regarding the future of forensic DNA (<http://www.cstl.nist.gov/strbase/NISTpub.htm>). The Applied Genetics Group in the US National Institute of Standards and Technology, which I had the privilege of leading from 2000 to 2013, has actively worked to improve the future of forensic DNA.

In 2007, two colleagues and I shared our thoughts on the types of genetic markers that would play a role in the future and why [13]. Those thoughts regarding continued use of STR markers with single nucleotide polymorphism (SNP) markers remaining supplemental solely for ancestry and phenotype application have proved prophetic in many ways. In my Fundamentals textbook, I included an entire chapter on 'Future Trends' [14]. I hope that the thoughts presented in this article can provide some useful input into where we are as a field and where we need to go in the foreseeable future.

2. The past reviewed

Accurately predicting the future is always challenging due to unforeseen innovation. However, by examining the past and understanding present challenges, it is often possible to extrapolate to reasonable predictions for the future. Appreciating the trajectory of activities in any field of endeavour enables those acting in it to know where they are going and to work to improve the future. The Research and Development Working Group of the National Commission on the Future of DNA Evidence in 2000 [15], Peter Gill in 2002 [16] and Lutz Roewer in 2013 [17] provided their perspectives at the time on the past, present and future of forensic DNA.

(a) Major themes and time periods for forensic DNA analysis

Table 1 summarizes my views regarding primary activities in each phase of forensic DNA analysis development over the past three decades (see also [18]). The first decade of forensic DNA analysis involved what I would term the 'exploration' phase, where different methodologies were attempted involving restriction fragment length polymorphism (RFLP) followed by more rapid and sensitive PCR assays. However, the early PCR assays did not have the specificity (i.e. power of discrimination) that original RFLP methods provided with its many possible alleles and genotypes.

By the beginning of the second decade of DNA typing, efforts solidified around STRs, as these genetic markers provided both a high degree of sensitivity through PCR amplification and specificity when results from multiple STR loci were combined in multiplex PCR amplifications. This decade is what I refer to as the 'stabilization and standardization'

Table 1. Forensic DNA analysis phases and activities by decade. RFLP, restriction fragment length polymorphism; VNTR, variable number of tandem repeats; PCR, polymerase chain reaction; STR, short tandem repeat; EDNAP, European DNA Profiling Group (a working group of the International Society for Forensic Genetics); ENFSI, European Network of Forensic Science Institutes; SWGDAM, Scientific Working Group on DNA Analysis Methods.

phase	time frame	description of activities
exploration	1985–1995	beginnings and first publications; different methods tried including multi- and single-locus VNTRs with RFLP and early PCR assays such as DQ α and single-locus STR markers; need for standardization and quality control results in formation of EDNAP and SWGDAM
stabilization and standardization	1995–2005	national databases launched for UK (1995), USA (1998) and many European countries; standardization to multiplex STR systems and CE; initial autosomal STR and Y-STR kits released; selection of core loci for US and Europe; implementation of FBI Quality Assurance Standards in the USA; ENFSI begins role in Europe to aid standardization and quality assurance
growth	2005–2015	rapid growth of DNA databases; expanded core loci in Europe and USA lead to new STR kits; Y-STR use on the rise; extended applications being pursued (e.g. rapid DNA instruments, familial searching, NGS research into STR allele variability)
sophistication	2015–2025 and beyond	expanding set of tools with capabilities for rapid DNA testing outside of laboratories, greater depth of information from allele sequencing, higher sensitive methodologies applied to casework, and probabilistic software approaches to complex evidence; need to confront privacy concerns increases as knowledge of genomic information improves

phase. The UK Forensic Science Service (FSS) led the application of STR markers to forensic casework with a first-generation quadruplex consisting of TH01, vWA, FES/FPS and F13A1 [19]. Collaborative studies conducted by EDNAP helped to ensure consistency of early methods between laboratories [20]. A second-generation multiplex (SGM) followed a few years later and examined six STR loci (TH01, vWA, FGA, D8S1179, D18S51 and D21S11) and the sex-typing marker amelogenin [21]. National DNA databases began to be developed, with the first one being the UK National DNA Database in April 1995 that involved the SGM loci [22]. The USA launched its National DNA Index System (NDIS) in October 1998 with 13 core STR loci (TH01, vWA, FGA, D8S1179, D18S51, D21S11, CSF1PO, TPOX, D3S1358, D5S818, D7S820, D13S317 and D16S539) [23]. Once core loci were decided upon, commercial kits began to be used for autosomal STR and Y-chromosome STR markers. Analysis platforms, which originally involved slab gel electrophoresis with silver-stain or fluorescence detection, eventually stabilized on CE with laser-induced fluorescence detection that permitted sensitive, automated and high-throughput analysis. Quality assurance measures were put into place during this decade by the FBI's DNA Advisory Board in the USA and ENFSI in Europe [24].

The third decade of DNA analysis involved a 'growth' phase. The US NDIS began this decade with around two million DNA profiles at the end of 2004. Ten years later, NDIS is 12 million profiles larger (≈ 14 million total profiles) due to expansion of DNA database collection state laws and laboratory capacity thanks to significant federal government funding. Similar growth patterns have occurred in other national DNA databases around the world. With larger DNA database sizes, an expanded set of core loci have been proposed and adopted by the European Union [25] and the USA [26].

The next decade and the future beyond it can be labelled as a 'sophistication' phase, where expanding tool sets provide new capabilities for DNA analysis and use. Laboratories must navigate the flood of sample submissions that often come due to the success of DNA to solve crimes and the

ability to conduct more tests from a single item than previously feasible. DNA testing will become more rapid, more informative and more sensitive. Probabilistic methods of expressing complex results will require software and deeper thinking about the meaning of data obtained. An ever-increasing understanding of human genomic information will require confronting genetic privacy concerns now and in the future.

(b) Research leadership

Research and development is crucial to the future of any field. During its existence, the UK Forensic Science Service played an important role in the development and application of forensic DNA techniques [27]. The pioneering research efforts of Peter Gill, Ian Evett, Gillian Tully and others led the way for development of new DNA markers, methodologies and interpretation frameworks. Other important centres of research in forensic DNA over the past several decades include University of Innsbruck (Austria), University of Copenhagen (Denmark), University of Santiago de Compostella (Spain), NIST Applied Genetics Group (USA), University of North Texas Health Sciences Center (USA) and the Institute of Environmental Science and Research (ESR, New Zealand). These groups regularly publish their research in *Forensic Science International: Genetics*, *Forensic Science International*, *International Journal of Legal Medicine*, *Legal Medicine* and *Journal of Forensic Sciences*.

3. The present considered

Understanding the present situation and challenges can aid looking to the future of forensic DNA analysis.

(a) Genetic marker systems

The primary genetic tests in use today involve autosomal STR markers—especially those core STRs that are used in national

Table 2. Current practice and future potential for genetic markers used in forensic DNA analysis.

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

Table 3. Summary of USA state DNA database laws and qualifying offences for DNA collection. Adapted from table 8.7 in [3] with recent information from a personal communication with Dawn Herkenham (Leidos; 2014). For additional information, see <http://www.ncsl.org/research/civil-and-criminal-justice/dna-laws-database.aspx> and <http://www.dnaresource.com>.

offences	no. of states				
	1999	2004	2008	2010	2014
sex crimes	50	50	50	50	50
all violent crimes	36	48	50	50	50
burglary	14	47	50	50	50
all felons	5	37	47	49	50
juveniles	24	32	32	32	31
arrestees	1	4	14	25	32 ^a
familial searching performed	—	—	2	2	7 ^b

^aThirty-two states, US Department of Justice, US Department of Defense and Puerto Rico have laws authorizing the collection of a DNA sample from a specified category of arrestees or persons arraigned for specific offences (including VT, MN and CA where collection of DNA from arrestees has been deemed unconstitutional by a court of law).

^bSeven states with familial searching efforts are CA, CO, VA, TX, WY, WI, MI.

DNA databases. Current commercial kits typically amplify in the range of 15–22 autosomal STR loci in order to cover European and/or USA core loci. Table 2 shows the current practice and future potential for various genetic marker systems used in forensic DNA (see [3] for more information on these markers). A primary benefit for STRs is that their multiple alleles enable detection and interpretation of evidentiary DNA mixtures more effectively than bi-allelic markers.

(b) DNA database growth and use in the USA

The number of samples (both known references and crime scene specimens) involved in DNA databases means that

genetic markers used to generate the DNA profiles in those databases will drive the future of DNA testing. Over the past 15 years, DNA collection laws have expanded to provide a greater number of samples for testing purposes. Table 3 reviews the growth in the number of states over the years that have required DNA collection for specific categories of offences. Note that while only sex crimes were required by all 50 states back in 1999, the range of crimes requiring mandatory DNA collection has steadily increased. In 1999, only five states required that everyone convicted of a felony offence would need to provide a DNA sample. All 50 states now collect DNA from all convicted felons. Likewise, the number of states permitting collection of DNA from

those arrested of a crime has increased from one in 1999 to 32 in 2014.

This growth has come because developers of DNA databases and software, suppliers of DNA testing kits and victim advocates have been effective in lobbying federal and state legislatures for new laws in the USA. With significant federal funding since 2004, DNA testing in the USA has grown to a level of around 1.5 million samples processed each year. Private laboratories, such as Bode Technology Group, Cellmark and Myriad Genetics, supplied the bulk of the initial DNA profiles generated because public laboratories did not have the needed capacity. More recently (through federal funding support), public forensic laboratory capabilities have grown in many regions to match the needs of convicted offender and arrestee DNA testing. FBI records indicate that over 250 000 criminal investigations have been aided through DNA testing as of late 2014 (<http://www.fbi.gov/about-us/lab/biometric-analysis/codis/ndis-statistics>).

(c) Critical challenges faced today

The success of DNA testing has brought significant growth, which in turn has brought new challenges particularly in the areas of sample backlogs and data interpretation. Laboratory automation and expert system data review can ease sample backlogs as can restrictive case acceptance policies. In some cases, law enforcement investigators may conduct 'swab-a-thons' at crime scenes and submit numerous items to try to solve a case through a DNA database hit rather than thinking carefully about which items may be most probative.

Data interpretation uncertainties are highest and errors are most likely to be made in situations with DNA mixtures from three or more individuals, especially with low-template DNA 'touch' samples. Work with these types of samples has been enabled in recent years with an increase in DNA detection sensitivity. With greater sensitivity comes the need for greater responsibility in data interpretation. Unfortunately, inconsistencies with handling DNA interpretation of complex mixtures adds to the challenge of obtaining reproducible results from multiple analysts and/or forensic laboratories.

4. The future predicted

The Olympic motto of *Citius, Altius, Fortius*—which is Latin for faster, higher, stronger (<http://www.olympic.org/>)—provides a framework for our discussion of where I believe the future of forensic DNA is headed. DNA testing protocols can be expected to get *faster* with rapid DNA instrumentation. Improved sensitivity and technology in recent years has enabled *higher* amounts of data to be recovered from biological evidence. Conclusions that are *stronger* can be drawn in many cases with probabilistic approaches under development. Each of these areas is discussed in greater detail later.

(a) Faster results

In the past few years, instrumentation providing a fully automated DNA profiling system has been introduced [28–31]. These rapid DNA instruments integrate the steps of DNA extraction, rapid PCR amplification of 15 or more STR loci, DNA separation, detection, sizing and genotyping. The initial versions of these rapid DNA systems can provide swab-in to profile-out results in less than 90 min for five buccal swab

reference samples, but at a reagent expense that is currently about 10 times that of conventional laboratory testing. These systems are being marketed for generating DNA data in police booking station environments where a suspect may be held in custody for about 4 h while mug shots and fingerprints are taken. In order for these rapid DNA devices to reach their full potential in deployed environments outside of forensic laboratories, real-time DNA database searches will be needed and communication improved across the various elements of the criminal justice system. The legal framework to permit arrestee testing must also be maintained in order to collect DNA profiles in a police booking station environment. As these rapid methods are implemented in situations outside of a typical laboratory environment, it is important to not sacrifice quality for speed.

How fast will DNA profiles be able to be generated in the future? There are technological limits with any technique, which in the case of PCR amplification is a combination of the polymerase biochemistry, primer binding kinetics and the thermal cycling device. The fastest full 15-locus STR profiles generated to-date require 14 min of multiplex PCR amplification with 28 cycles of heating and cooling, which can lead to DNA profiles being produced in a laboratory setting in less than an hour [32]. While instruments may exist to heat and cool small volumes of liquid more quickly, sufficient time for polymerase extension of multiple DNA templates is required to obtain useful DNA profiles. As PCR amplification becomes faster, DNA separation and detection may become the rate-limiting step.

(b) Higher sensitivity and information content

The future of *higher* amounts of information in forensic DNA involves improved detection sensitivity, higher information content from expanded sets of core STR loci and possibly supplemental genetic markers, and deeper information from sequence analysis of alleles.

More data are available from biological samples due to improved sensitivity in PCR assays and information content of profiles generated. A consequence of improving DNA test sensitivity in recent years is the generation of more complicated DNA profiles for interpretation. It is important to keep in mind that just because a DNA profile can be obtained from as little as a single cell does not mean that the source of the profile is relevant to the crime event being investigated [10].

In terms of sensitivity, fundamental limits exist with PCR amplification due to stochastic (random) variation in sampling each allele at a locus [33]. These stochastic effects lead to variation in peak heights and peak height ratios for heterozygous samples during replicate PCR amplification. Even though two alleles are present at an STR locus in equal amounts in the DNA template, stochastic variation in the early rounds of PCR can lead to selective amplification of one allele over the other, which in extreme situations leads to allele drop-out (i.e. failure to detect the allele). Studies with single-source samples have shown that stochastic effects such as elevated stutter and allele drop-out occur at around 15–20 cells or 100–125 pg [33,34]. Allele drop-in may also occur when the number of PCR amplification cycles is increased to improve sensitivity [16,35]. When stochastic variation occurs, it becomes increasingly difficult to confidently pair alleles into genotypes and to correctly separate individual contributors in DNA mixtures. Thus, with stochastic

effects ever-present in low-level DNA PCR amplifications, allele drop-out and potential allele sharing from multiple contributors lead to greater uncertainty in the specific genotype combinations that can be reliably assumed. Combining results from replicate amplifications can help identify the true alleles in a profile [36,37].

Uncertainty in an evidentiary Q profile due to potential allele or locus drop-out can make it difficult to confidently exclude a specific suspect when a Q-to-K comparison is conducted. Probabilistic genotyping approaches (see next section) are being developed to try to explain observed data in some cases using computer simulations that estimate relative contributions of potential contributors. Some laboratories in the future may decide to establish a complexity threshold in order to halt efforts on poor-quality data (see ch. 7 in [4]). Because sensitive DNA detection technology has the potential to outpace reliable interpretation, the forensic DNA community needs to be vigilant in efforts to appropriately interpret challenging evidence without pushing too far. Clearly communicating the limitations of interpretation approaches used by investigators and officers of the court is crucial to avoid improper use of DNA.

Higher information content will be present in future DNA profiles that incorporate an expanded number of required core loci for inclusion in national DNA databases [25,26]. International data exchange will benefit from having more STR loci in common across various databases [26,38]. However, more information collected means more analysis and interpretation time and effort. While mixture *detectability* will improve with use of more STR loci, mixture *interpretation* will take longer with the larger PCR multiplexes since there will be more data to review. Although the capability may exist in future assays to simultaneously collect information from autosomal STRs, Y-STRs, X-STRs and numerous SNPs as has been proposed [39], it is unclear what the benefits would be of routinely attempting to collect far more information from evidentiary samples than would be available to compare to with typical reference profiles that only contain core STR loci. Routinely adding extensive information to reference samples in the hopes that this information may be of benefit in the future also means investments in interpretation time and effort—and may not yield a return on this investment unless evidence samples are examined at all or a substantial number of the tested STR and SNP loci. Because forensic DNA analysis involves a Q-to-K comparison, an optimal investment of effort is achievable when the same loci are examined in the Q and the K profiles to provide as many points of comparison as possible.

Next-generation sequencing (NGS), also known as massively parallel sequencing, provides the opportunity to collect information from numerous STRs and SNPs simultaneously [40]. In addition, sequence analysis of STR alleles provides a deeper depth of information by characterizing internal sequence variation for same size alleles that cannot be distinguished with CE analysis. For example, eight different sequence versions of allele 21 were identified in D12S391 among 197 samples examined [41]. The single detected peak in apparent homozygotes using CE may be resolvable into heterozygous alleles with NGS at some hypervariable STR loci [41,42]. In addition, mtDNA genome sequences can be generated with NGS [43]. However, the orders-of-magnitude difference between mtDNA, which may contain thousands of copies per cell, and nuclear DNA, with its two copies of each allele per cell, will probably preclude

the routine combination of autosomal STRs and mtDNA into a single, robust NGS assay.

Only time will tell whether future routine DNA testing proceeds down the path of collecting and analysing a deeper depth of allele information available from NGS methods. The additional collected information with NGS requires specialized bioinformatics tools to process the data and a more complicated allele nomenclature scheme, and comes at a cost of more analysis time and more data storage space. What is interesting for research studies may not always be practical or necessary for routine application to forensic cases.

(c) Stronger conclusions with challenging samples

Improving the ability to decipher and interpret DNA results from challenging samples provides probably the largest opportunity for future advances in forensic DNA analysis. Challenges can come through poor-quality samples containing degraded DNA or PCR inhibitors that fail to produce an informative Q profile. Smaller PCR amplicons in the form of miniSTRs [44,45] or SNPs [46] have been shown to help recover information from damaged DNA templates. The DNA target size can be reduced with some STR markers in NGS assays versus what can be done when CE is the detection platform where limitations exist in terms of dye colour space.

DNA database searches that fail to produce a matching K reference profile can be essentially expanded through loosening the stringency of the search and using genetic inheritance principles to produce a ‘familial’ search in an attempt to identify close biological relatives of the source of the unknown Q profile [47]. Familial searching typically experiences low success rates due to a lack of close relatives of the true perpetrator in the database. In addition, true relatives in the database may be missed due to the search strategy used or specific inheritance patterns that leave a true sibling (in the database) with different alleles compared with the Q search profile (see appendix 2 in [3]). False positives when the Q profile has common alleles that are shared by unrelated people can result in long candidate lists. Y-STR testing on male samples from a ranked candidate list can help filter false positives [48]. While familial searching is unlikely to be conducted on a national level in the USA [49], it has produced some successes in the UK through aiding identification of 41 perpetrators in 188 police investigations [50]. Genetic privacy concerns are often raised in opposition to familial searching [51], and so the future of this technique is probably limited due to practical and privacy issues.

When no K reference sample is available for comparison following a database search, it may be possible that future DNA capabilities will include prediction of external visible characteristics [52], such as eye colour or hair colour [53]. Biogeographic ancestry estimation may be helpful in some case scenarios [54]. Microbial DNA may also play a role in future investigations to lend support to circumstances involving microbial transfer when touching a computer keyboard [55] or having sexual intercourse [56]. This analysis will likely be performed with NGS or other high-throughput methods in the future.

Complex DNA mixtures, containing genetic data from more than two individuals, especially if any of the individuals are related, offer one of the largest challenges for the future of forensic DNA analysis. Probabilistic genotyping offers a way to strengthen conclusions with challenging DNA mixture results

that might otherwise be declared inconclusive under a binary approach to interpretation (see appendix 4 in [4] for a detailed example). Software programs have been developed to perform probabilistic genotyping using what has been termed ‘semi-continuous’ (where only allele information is used) or ‘fully continuous’ (where alleles and peak height information are used) [57–63].

Supporting stronger conclusions with probabilistic approaches on complex evidence will require validation of software used to understand its limitations [64]. Scientists using these software tools must not treat them as ‘black boxes’ generating reports that they themselves do not understand. Equally important will be the need for effective communication of the meaning of results obtained to those who will receive and use the laboratory reports to make decisions. These reports must appropriately communicate limitations of methods, models, assumptions made and interpretation applied with the case results.

(d) Other factors

I believe in many ways we are at an interesting juncture in forensic DNA. We have over the past few years developed great technological capabilities that in some ways have outpaced our ability to appropriately interpret evidence results obtained. We have emphasized methods for enhanced sensitivity while not working equally hard to improve our understanding of possible genotypes that may compose the evidence results (i.e. the specificity of results). Thus, for forensic DNA typing to move forward responsibly we must improve the framework and consistency of DNA evidence interpretation [4]. To aid efforts in moving forward in DNA interpretation, I support the 13 recommendations that Peter Gill makes in his recent book ‘Misleading DNA evidence: reasons for miscarriages of justice’ [10].

Cost will play a role in the future use of DNA in forensic science. Important factors influencing cost include competition, centralization and communication. More competition is needed in many areas of the forensic DNA supply chain in order to drive prices down. Likewise, automation within laboratories and supply manufacturing lines is crucial to reduce potential quality challenges either from potential sample swaps or consumable contamination. To accomplish this type of automation cost-effectively, economies of scale are needed. The future will probably be better served by central, focused laboratories than by many small operations with redundant capabilities. While these thoughts would seem to suggest that rapid DNA testing at police stations will be unsuccessful, it is important to realize that the current political climate supports local funding over sending money to maintain central services, which will not necessarily have the local needs as a specific priority.

Technological innovation of the future is unlikely to be fully exploited by the forensic science community for several reasons. First, public laboratories are dependent on tax-payer support, and politicians will fight to keep these funds in local coffers rather than sending them to central, specialized facilities. Crime is largely committed by local perpetrators [65]. In a large country like the USA, we have many local laboratories with efforts that may be uncoordinated and in some cases redundant to neighbouring areas. Smaller countries, such as the UK, may be able to support more centralized laboratory systems. Thus, there is not a one-size-fits-all solution for the future of forensic DNA.

A second reason that technological innovation may be slow or non-existent relates to validation requirements for methods used in forensic applications. Slow technology adoption by the forensic community can result in few companies wanting to invest in long-term efforts to bring new products to market where a return-on-investment may be slower than it is in other fields. The scrutiny that products and manufacturing processes can undergo during adversarial legal battles may also keep potential companies out of the forensic DNA marketplace.

Another important reason that forensic DNA may not involve cutting-edge technology is the need to maintain connection to legacy information from results already collected. The fact that the USA has 14 million STR profiles (most with the 13 core STR loci selected in 1997) in its national DNA database means that switching to completely different genetic markers, such as SNPs or InDels, is not feasible (see also [15,66]). Switching to another set of genetic markers for established DNA databases does not make sense when doing so would, in the case of the USA, render obsolete a billion dollar investment. Thus, genetic markers used in future DNA databases will most probably include current core STR loci plus additional ones to avoid adventitious matches [25,26].

Knowledge and understanding of our DNA sequence information are constantly evolving. Human genomes are more complex than originally imagined and research studies are continuing to uncover additional information (e.g. [67]). While currently used STR markers show no significant value for predicting genetic diseases [68], the information landscape is continually changing with additional data being gathered.

Early uses of DNA databases involved using a large net to gather as many reference profiles as possible from previous offenders and more recently from those arrested for specific crimes. In the USA, laws have steadily increased the number of offences requiring DNA collection for testing purposes (table 3). However, I would predict that technology advances will not be the rate-limiting step in the future of DNA database growth. Rather the appetite of the general population to accept DNA use and continue to make significant resource investments in this effort will be. Public forensic laboratories will probably need to market the value of their work more in a fiscally challenging future. UK database growth has slowed in recent years due to privacy concerns raised. Other national DNA databases are likely to face similar challenges. I would not be surprised to find continued support for the position argued by Robert Gaensslen on this topic in a 2006 article that ‘absent specific legislative guidance, . . . the ‘default’ position [should] be more protective of individual privacy rights’ [69, p. 375].

5. Concluding thoughts

Successful research is an important key to the future in any scientific endeavour and is dependent on sufficient, sustainable funding. Beyond just publishing scientific articles and presenting work to colleagues at conferences, training of practitioners and stakeholders is essential for effective implementation of new techniques and ideas. Keys to success include resources and recognition of the value of training support in terms of funding for the presenters and setting aside time for the participants. Continuing education needs to mean more to participants than just checking a box that helps a laboratory

pass an audit. Quality work results when scientists have a desire to learn and try to think critically about casework results rather than mindlessly following a protocol.

The future of forensic DNA will have an impact on other areas of forensic science. As DNA has become successfully used in the past few decades to solve crimes, funding and personnel resources have been reduced in other areas such as trace evidence—and sometimes completely eliminated from a forensic laboratory's capabilities, which may bring long-term consequences. In my new role at NIST as Special Assistant to the Director for Forensic Science (which I have had since April 2013), I have an opportunity to look beyond DNA. Many challenges exist as we seek to improve forensic science efforts across various disciplines. I am involved in setting up and running the US National Commission on Forensic Science and the Organization of Scientific Area Committees [70]. There are many lessons from DNA that can be applied to other areas in forensic science. We should seek to understand issues, challenges and potential solutions outside of our focused, discipline-specific view and bring potential solutions back to our own areas of expertise. Improving communication across various forensic disciplines is just as important as improving communication between laboratories, law enforcement and the legal community. International cooperation and standards setting will enable forensic DNA testing of the future to become even more robust than it currently is.

Better communication across stakeholders in the criminal justice system is a primary need in order to maximize the value of DNA testing efforts. More than a billion dollars has been invested over the past decade to grow the USA national DNA database to its current state of almost 14 million profiles. While many crimes are solved with DNA database matches, I think it is safe to say that many more probably could be solved if the communication between investigators and forensic DNA scientists were improved. With fiscal

restrictions for the foreseeable future, significant investments in DNA databases may be a thing of the past. Forensic scientists will have to work smarter with the resources available to them. I believe it is also important to appreciate that a DNA test, even with the best technology available, is only as good as the sample collected. Thus, working closely with crime scene investigators is essential in gathering quality evidence. As we learn from the past and perform well in the present, we will create a future where forensic DNA analysis can play an important role in criminal justice systems around the world.

Competing interests. I declare I have no competing interests.

Funding. The author is supported by internal funds from the National Institute of Standards and Technology as part of its forensic science efforts.

Acknowledgments. I have been fortunate to work with many excellent scientists, colleagues and friends over my career. I am grateful for the encouragement and mentorship of Ralph Allen, Bruce McCord and Dennis Reeder early in my career. I have enjoyed working with members of the NIST Applied Genetics Group including Pete Vallone, Mike Coble, Becky Hill, Margaret Kline and Dave Diewer. We have conducted a lot of interesting research together over the years and I miss regular association with them as I have moved into a new role at NIST. I am thankful for the opportunity to serve as an Associate Editor for *Forensic Science International: Genetics* over the past 8 years. Ideas expressed in this article have come from reading the literature, writing my *Forensic DNA Typing* textbooks and learning from others at numerous conferences over the past two decades. Points of view in this document are those of the author and do not necessarily represent the official position or policies of the US Department of Justice or the National Institute of Standards and Technology. Certain commercial equipment, instruments and materials 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 materials, instruments or equipment identified are necessarily the best available for the purpose.

References

- Gill P, Jeffreys AJ, Werrett DJ. 1985 Forensic application of DNA 'fingerprints'. *Nature* **318**, 577–579. (doi:10.1038/318577a0)
- Clayton TM, Whitaker JP, Maguire CN. 1995 Identification of bodies from the scene of a mass disaster using DNA amplification of short tandem repeat (STR) loci. *Forensic Sci. Int.* **76**, 7–15. (doi:10.1016/0379-0738(95)01787-9)
- Butler JM. 2012 *Advanced topics in forensic DNA typing: methodology*. San Diego, CA: Elsevier Academic Press.
- Butler JM. 2015 *Advanced topics in forensic DNA typing: interpretation*. San Diego, CA: Elsevier Academic Press.
- Weber-Lehmann J, Schilling E, Gradl G, Richter DC, Wiehler J, Rolf B. 2014 Finding the needle in the haystack: differentiating 'identical' twins in paternity testing and forensics by ultra-deep next generation sequencing. *Forensic Sci. Int. Genet.* **9**, 42–46. (doi:10.1016/j.fsigen.2013.10.015)
- Balding DJ, Nichols RA. 1994 DNA profile match probability calculation: how to allow for population stratification, relatedness, database selection and single bands. *Forensic Sci. Int.* **64**, 125–140. (doi:10.1016/0379-0738(94)90222-4)
- Crow JF. 1999 Hardy, Weinberg and language impediments. *Genetics* **152**, 821–825.
- Prinz M, Carracedo A, Mayr WR, Morling N, Parsons TJ, Sajantila A, Scheithauer R, Schmitter H, Schneider PM. 2007 DNA Commission of the International Society for Forensic Genetics (ISFG): recommendations regarding the role of forensic genetics for disaster victim identification (DVI). *Forensic Sci. Int. Genet.* **1**, 3–12. (doi:10.1016/j.fsigen.2006.10.003)
- Findlay I, Taylor A, Quirke P, Frazier R, Urquhart A. 1997 DNA fingerprinting from single cells. *Nature* **389**, 555–556. (doi:10.1038/39225)
- Gill P. 2014 *Misleading DNA evidence: reasons for miscarriages of justice*. San Diego, CA: Elsevier Academic Press.
- Butler JM, McCord BR, Jung JM, Wilson MR, Budowle B, Allen RO. 1994 Quantitation of PCR products by capillary electrophoresis using laser fluorescence. *J. Chromatogr. B* **658**, 271–280. (doi:10.1016/0378-4347(94)00238-X)
- Butler JM, McCord BR, Jung JM, Allen RO. 1994 Rapid separation of the short tandem repeat HUMTH01 by capillary electrophoresis. *BioTechniques* **17**, 1062–1070.
- Butler JM, Coble MD, Vallone PM. 2007 STRs versus SNPs: thoughts on the future of forensic DNA testing. *Forensic Sci. Med. Pathol.* **3**, 200–205. (doi:10.1007/s12024-007-0018-1)
- Butler JM. 2010 Future trends. In *Fundamentals of forensic DNA typing*, ch. 18. pp. 423–438. San Diego, CA: Elsevier Academic Press.
- National Commission on the Future of DNA Evidence. 2000 The future of forensic DNA testing: predictions of the Research and Development Working Group. Washington, DC: National Institute of Justice. See <https://www.ncjrs.gov/pdffiles1/nij/183697.pdf>.
- Gill P. 2002 Role of short tandem repeat DNA in forensic casework in the UK—past, present, and future perspectives. *BioTechniques* **32**, 366–385.
- Roewer L. 2013 DNA fingerprinting in forensics: past, present, future. *Investig. Genet.* **4**, 22. (doi:10.1186/2041-2223-4-22)

18. Butler JM. 2010 Historical methods. In *Fundamentals of forensic DNA typing*, ch. 3. pp. 43–78. San Diego, CA: Elsevier Academic Press.
19. Kimpton C, Fisher D, Watson S, Adams M, Urquhart A, Lygo J, Gill P. 1994 Evaluation of an automated DNA profiling system employing multiplex amplification of four tetrameric STR loci. *Int. J. Legal Med.* **106**, 302–311. (doi:10.1007/BF01224776)
20. Andersen J *et al.* 1996 Report on the third EDNAP collaborative STR exercise. European DNA Profiling Group. *Forensic Sci. Int.* **78**, 83–93. (doi:10.1016/0379-0738(95)01871-9)
21. Sparkes R *et al.* 1996 The validation of a 7-locus multiplex STR test for use in forensic casework. (I). Mixtures, ageing, degradation and species studies. *Int. J. Legal Med.* **109**, 186–194. (doi:10.1007/BF01225517)
22. Werrett DJ. 1997 The national DNA database. *Forensic Sci. Int.* **88**, 33–42. (doi:10.1016/S0379-0738(97)00081-9)
23. Budowle B, Moretti TR, Niezgoda SJ, Brown BL. 1998 CODIS and PCR-based short tandem repeat loci: law enforcement tools. In *Proc. Second European Symp. on Human Identification*. See <http://www.promega.com/~media/files/resources/conference%20proceedings/ishi%2002/oral%20presentations/17.pdf>.
24. Gill P, Sparkes R, Fereday L, Werrett DJ. 2000 Report of the European Network of Forensic Science Institutes (ENFSI): formulation and testing of principles to evaluate STR multiplexes. *Forensic Sci. Int.* **108**, 1–29. (doi:10.1016/S0379-0738(99)00186-3)
25. Gill P, Fereday L, Morling N, Schneider PM. 2006 The evolution of DNA databases—recommendations for new European STR loci. *Forensic Sci. Int.* **156**, 242–244. (doi:10.1016/j.forsciint.2005.05.036)
26. Hares DR. 2012 Expanding the CODIS core loci in the United States. *Forensic Sci. Int. Genet.* **6**, e52–e54. (doi:10.1016/j.fsigen.2011.04.012). [Erratum in: *Forensic Sci. Int. Genet.* **6**, e135].
27. Budowle B, Kayser M, Sajantila A. 2011 The demise of the United Kingdom's Forensic Science Service (FSS): loss of world-leading engine of innovation and development in the forensic sciences. *Investig. Genet.* **2**, 4. (doi:10.1186/2041-2223-2-4)
28. Hopwood AJ *et al.* 2010 Integrated microfluidic system for rapid forensic DNA analysis: sample collection to DNA profile. *Anal. Chem.* **82**, 6991–6999. (doi:10.1021/ac101355r)
29. Tan E, Turingan RS, Hogan C, Vasantgadkar S, Palombo L, Schumm JW, Selden RF. 2013 Fully integrated, fully automated generation of short tandem repeat profiles. *Investig. Genet.* **4**, 16. (doi:10.1186/2041-2223-4-16)
30. LaRue BL, Moore A, King JL, Marshall PL, Budowle B. 2014 An evaluation of the RapidHIT[®] system for reliably genotyping reference samples. *Forensic Sci. Int. Genet.* **13**, 104–111. (doi:10.1016/j.fsigen.2014.06.012)
31. Hennessy LK, Mehendale N, Chear K, Jovanovich S, Williams S, Park C, Gangano S. 2014 Developmental validation of the GlobalFiler[®] Express kit, a 24-marker STR assay, on the RapidHIT[®] System. *Forensic Sci. Int. Genet.* **14**, 247–258. (doi:10.1016/j.fsigen.2014.08.011)
32. Butts EL, Vallone PM. 2014 Rapid PCR protocols for forensic DNA typing on six thermal cycling platforms. *Electrophoresis* **35**, 3053–3061. (doi:10.1002/elps.201400179)
33. Walsh PS, Erlich HA, Higuchi R. 1992 Preferential PCR amplification of alleles: Mechanisms and solutions. *PCR Methods Appl.* **1**, 241–250. (doi:10.1101/gr.1.4.241)
34. Butler JM, Hill CR. 2010 Scientific issues with analysis of low amounts of DNA. Profiles in DNA 13. See http://www.promega.com/profiles/1301/1301_02.html.
35. 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 Sci. Int.* **112**, 17–40. (doi:10.1016/S0379-0738(00)00158-4)
36. Taberlet P. 1996 Reliable genotyping of samples with very low DNA quantities using PCR. *Nucleic Acids Res.* **24**, 3189–3194. (doi:10.1093/nar/24.16.3189)
37. Benschop CCG, van der Beek CP, Meiland HC, van Gorp AGM, Westen AA, Sijen T. 2011 Low template STR typing: effect of replicate number and consensus method on genotyping reliability and DNA database search results. *Forensic Sci. Int. Genet.* **5**, 316–328. (doi:10.1016/j.fsigen.2010.06.006)
38. Martín P, de Simón LF, Luque G, Farfán MJ, Alonso A. 2014 Improving DNA data exchange: validation studies on a single 6 dye STR kit with 24 loci. *Forensic Sci. Int. Genet.* **13**, 68–78. (doi:10.1016/j.fsigen.2014.07.002)
39. Ge J, Eisenberg A, Budowle B. 2012 Developing criteria and data to determine best options for expanding the core CODIS loci. *Investig. Genet.* **3**, 1. (doi:10.1186/2041-2223-3-1)
40. Seo SB, King JL, Warshauer DH, Davis CP, Ge J, Budowle B. 2013 Single nucleotide polymorphism typing with massively parallel sequencing for human identification. *Int. J. Legal Med.* **127**, 1079–1086. (doi:10.1007/s00414-013-0879-7)
41. Gelardi C, Rockenbauer E, Dalsgaard S, Børsting C, Morling N. 2014 Second generation sequencing of three STRs D3S1358, D12S391 and D21S11 in Danes and a new nomenclature for sequenced STR alleles. *Forensic Sci. Int. Genet.* **12**, 38–41. (doi:10.1016/j.fsigen.2014.04.016)
42. Rockenbauer E, Hansen S, Mikkelsen M, Børsting C, Morling N. 2014 Characterization of mutations and sequence variants in the D21S11 locus by next generation sequencing. *Forensic Sci. Int. Genet.* **8**, 68–72. (doi:10.1016/j.fsigen.2013.06.011)
43. Parson W *et al.* 2013 Evaluation of next generation mtGenome sequencing using the Ion Torrent Personal Genome Machine (PGM). *Forensic Sci. Int. Genet.* **7**, 543–549. (doi:10.1016/j.fsigen.2013.06.003)
44. Butler JM, Shen Y, McCord BR. 2003 The development of reduced size STR amplicons as tools for analysis of degraded DNA. *J. Forensic Sci.* **48**, 1054–1064.
45. Coble MD, Butler JM. 2005 Characterization of new miniSTR loci to aid analysis of degraded DNA. *J. Forensic Sci.* **50**, 43–53. (doi:10.1520/JFS2004216)
46. Romanini C *et al.* 2012 Typing short amplicon binary polymorphisms: supplementary SNP and Indel genetic information in the analysis of highly degraded skeletal remains. *Forensic Sci. Int. Genet.* **6**, 469–476. (doi:10.1016/j.fsigen.2011.10.006)
47. Bieder FR, Brenner CH, Lazer D. 2006 Finding criminals through DNA of their relatives. *Science* **312**, 1315–1316. (doi:10.1126/science.1122655)
48. Myers SP *et al.* 2011 Searching for first-degree familial relationships in California's offender DNA database: validation of a likelihood ratio-based approach. *Forensic Sci. Int. Genet.* **5**, 493–500. (doi:10.1016/j.fsigen.2010.10.010)
49. SWGDAM. 2014 Recommendations from the SWGDAM Ad Hoc Working Group on Familial Searching. See <http://swgdam.org/SWGDAM%20Recs%20on%20Familial%20Searching%20APPROVED%2010072013.pdf>.
50. Maguire CN, McCallum LA, Storey C, Whitaker JP. 2014 Familial searching: a specialist forensic profiling service utilizing the National DNA Database to identify unknown offenders via their relatives—the UK experience. *Forensic Sci. Int. Genet.* **8**, 1–9. (doi:10.1016/j.fsigen.2013.07.004)
51. Suter SM. 2010 All in the family: privacy and DNA familial searching. *Harvard J. Law Technol.* **23**, 309–399.
52. Kayser M, Schneider PM. 2009 DNA-based prediction of human externally visible characteristics in forensics: motivations, scientific challenges, and ethical considerations. *Forensic Sci. Int. Genet.* **3**, 154–161. (doi:10.1016/j.fsigen.2009.01.012)
53. Walsh S, Liu F, Wollstein A, Kovatsi L, Ralf A, Kosiniak-Kamysz A, Branicki W, Kayser M. 2013 The HliisPlex system for simultaneous prediction of hair and eye colour from DNA. *Forensic Sci. Int. Genet.* **7**, 98–115. (doi:10.1016/j.fsigen.2012.07.005)
54. Phillips C *et al.* 2009 Ancestry analysis in the 11-M Madrid bomb attack investigation. *PLoS ONE* **4**, e6583. (doi:10.1371/journal.pone.0006583)
55. Fierer N, Lauber CL, Zhou N, McDonald D, Costello EK, Knight R. 2010 Forensic identification using skin bacterial communities. *Proc. Natl Acad. Sci. USA* **107**, 6477–6481. (doi:10.1073/pnas.1000162107)
56. Tridico SR, Murray DC, Addison J, Kirkbride KP, Bunce M. 2014 Metagenomic analyses of bacteria on human hairs: a qualitative assessment for applications in forensic science. *Investig. Genet.* **5**, 16. (doi:10.1186/s13323-014-0016-5)
57. Gill P *et al.* 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 Sci. Int. Genet.* **6**, 679–688. (doi:10.1016/j.fsigen.2012.06.002)
58. Balding DJ. 2013 Evaluation of mixed-source, low-template DNA profiles in forensic science. *Proc. Natl*

- Acad. Sci. USA* **110**, 12 241–12 246. (doi:10.1073/pnas.1219739110)
59. Gill P, Haned H. 2013 A new methodological framework to interpret complex DNA profiles using likelihood ratios. *Forensic Sci. Int. Genet.* **7**, 251–263. (doi:10.1016/j.fsigen.2012.11.002)
 60. Taylor D, Bright JA, Buckleton J. 2013 The interpretation of single source and mixed DNA profiles. *Forensic Sci. Int. Genet.* **7**, 516–528. (doi:10.1016/j.fsigen.2013.05.011)
 61. Perlin MW, Belrose JL, Ducean BW. 2013 New York State TrueAllele® casework validation study. *J. Forensic Sci.* **58**, 1458–1466. (doi:10.1111/1556-4029.12223)
 62. Kelly H, Bright J-A, Buckleton JS, Curran JM. 2014 A comparison of statistical models for the analysis of complex forensic DNA profiles. *Sci. Justice* **54**, 66–70. (doi:10.1016/j.scijus.2013.07.003)
 63. Steele CD, Balding DJ. 2014 Statistical evaluation of forensic DNA profile evidence. *Annu. Rev. Stat. Appl.* **1**, 20.1–20.24. (doi:10.1146/annurev-statistics-022513-115602)
 64. Bright JA, Evett IW, Taylor D, Curran JM, Buckleton J. 2015 A series of recommended tests when validating probabilistic DNA profile interpretation software. *Forensic Sci. Int. Genet.* **14**, 125–131. (doi:10.1016/j.fsigen.2014.09.019)
 65. Kent J, Leitner M, Curtis A. 2006 Evaluating the usefulness of functional distance measures when calibrating journey-to-crime distance decay functions. *Comput. Environ. Urban Syst.* **30**, 181–200. (doi:10.1016/j.compenvurbysys.2004.10.002)
 66. Gill P, Werrett DJ, 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 (SWGDM). *Sci. Justice* **44**, 51–53. (doi:10.1016/S1355-0306(04)71685-8)
 67. ENCODE Project Consortium. 2012 An integrated encyclopedia of DNA elements in the human genome. *Nature* **489**, 57–74. (doi:10.1038/nature11247)
 68. Katsanis SH, Wagner JK. 2013 Characterization of the standard and recommended CODIS markers. *J. Forensic Sci.* **58**(Suppl.), S169–S172. (doi:10.1111/j.1556-4029.2012.02253.x)
 69. Gaensslen RE. 2006 Should biological evidence or DNA be retained by forensic science laboratories after profiling? No, except under narrow legislatively-stipulated conditions. *J. Law Med. Ethics* **34**, 375–379. (doi:10.1111/j.1748-720X.2006.00042.x)
 70. Butler JM. 2014 The National Commission on Forensic Science and the Organization of Scientific Area Committees. In *Proc. 25th Int. Symp. on Human Identification*. See <http://www.nist.gov/forensics/ncfs.cfm>.