

Feature

Low Template DNA

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Faced with a statement from a forensic scientist that the probability of DNA from the crime scene coming from someone other than the accused is less than 1 in 1 billion, the familiar reaction (if defending) is urgently to advise a guilty plea, or (if prosecuting) to assume that that guilty plea will soon be forthcoming. This understandable reaction will often fail to do justice to the evidence. Of course, as with other areas of expert evidence, not all DNA results are equal, with some being rather more equal than others. Practitioners need to familiarise themselves with what lies behind apparently compelling random match probabilities and likelihood ratios.

An essential first step, when served with apparently compelling DNA evidence, is to request the underlying scientific results. These will assist in the asking of the right questions – such as whether this is “low template” DNA (LTDNA), what the precise amount of DNA material recovered was, whether it is below the “stochastic threshold”, and what process or processes were used to obtain and subsequently analyse the results? Practitioners will need to be able to make sense of the graphs generated by the analysis process and also of the reporting scientist’s handwritten profiling results. Whether the results are said to be from a single source or mixture of different contributors and, if so, how many, is a further important enquiry.

Low template DNA

Routinely, DNA samples are subjected to profiling using a variety of commercially available test kits with SGM+ and Identifiler being the most commonly used in this jurisdiction. These kits used by the various forensic services providers amplify the sample and target 10 (SGM+) or 15 (Identifiler) specific areas (loci) on the DNA. These loci are “hypervariable” between individuals, and at each locus an individual has two components (alleles) – one inherited from each parent. Both kits also target an additional area, the amelogenin locus, for the purpose of ascertaining if the contributor(s) to a sample are male or female. The results are shown on graphs (electrophoretograms or EPGs) which are then analysed by a reporting scientist. The height of the peaks on the graph are measured in RFUs² and will typically be in the order of 1500 RFUs when analysing optimal samples (i.e. where there is an optimal amount of DNA material to analyse, typically 0.5 ng or more). Interpretation of results from optimal samples (single source, high quality and quantity of DNA) is uncontroversial, and rarely gives rise to any meaningful debate.

It is however different in relation to “low template” DNA samples (LTDNA). Essentially, low template is the umbrella term for sub-optimal quantities of DNA (below 0.5 nano-

gram). Results from LTDNA are frequently uninformative when using the kits as recommended by their manufacturer as the processes have been deliberately designed to yield no results when small amounts of starting DNA are used. To attempt to address this, different forensic science providers have developed new techniques to make the tests more sensitive. These include Low Copy Number (LCN—where the usual 28 cycles of amplification are increased, often to 34) and Enhancement (where the sample is chemically “cleaned up”). The results are again shown graphically, but peak heights will often be lower – 50 RFUs, and below are not uncommon.

This is where interpretation of results can become subjective – with reporting scientists legitimately differing as to whether a peak found at a particular locus is indeed DNA rather than background noise, contamination (DNA from a source other than the crime sample) or stutter (a commonly encountered false “peak” generated by the analytical process). These are known collectively as “stochastic” (random) effects, and it is widely recognised that these effects increase as the amount of the DNA sample decreases.

The importance of this is readily apparent. Matches to the suspect’s known DNA profile will provide the basis for the random match probability/likelihood ratio statistics which will be put confidently by the Crown in front of a jury. But if an apparently “matching” peak may in fact be something else, then those statistics may give a positively misleading view of the probative value of the DNA evidence.

Inherent in the field of LTDNA are the issues of “drop in” and “drop out”. Essentially “drop in” is a peak which, although DNA, may not be from the crime stain, and arises from contamination. “Drop out” on the other hand is where there is either one or no apparent peak at one or more of the loci tested (or where the peak is considered to be too low to be “called” as a DNA component). These “empty” loci are sometimes referred to as “voids”.

Such phenomena frequently produce partial or incomplete profiles. (A full profile on SGM+ will have a total of 20 peaks – 2 alleles at each of the 10 loci tested). The question then arises as to whether the voids may invalidate the statistical analysis put on the peaks found. Is the sample a single profile, or is it a mixture? If it is a mixture, how many persons contributed to that mixture? What if there has been “drop-out” of DNA which did not match the suspect, and might have exonerated him or her? What if a peak attributed to and matching the suspect is in fact “drop in” from another source?

These questions are not purely academic. Conventional statistical analysis of LTDNA in this jurisdiction struggles to provide reliable statistics where there is evidence of two-person mixtures. With three possible contributors, the statistics break down. So one scientist may read a set of LTDNA results as representing a single person’s profile, while another may interpret the same results as a mixture.

¹ Both at Doughty Street Chambers. They were instructed on behalf of the defence in the Broughton trials and appeal.

² Relative Fluorescent Units are the units of measurement for the amount of the DNA component detected after processing of the sample.

Attempts have been made to minimise the risk inherent in profiling from LTDNA results. Commonly “consensus” has been used as one approach. Each test is run at least twice, and a peak is only called as genuine if it is replicated in two or more tests. But to the argument that this favours a suspect, (by excluding what may be a genuine match seen only once) can be put a counter-argument. The consensus approach would also ignore a non-matching component seen only once. If the profile is said to be from a single contributor, that non-matching component should in fact exculpate the suspect.

A further issue with LTDNA arises from the ease with which a profile can be transferred from one surface to another – sometimes both unwittingly and innocently. For example A could shake hands with B, and a minute quantity of A's DNA may transfer to B's hand. If B then touched a knife, A's DNA could transfer from B's hand onto that knife. Some scientific studies have suggested that the knife may *only* show indications of A's DNA being present on that knife. No one can say with confidence that an LTDNA profile comes from a person who came in contact with an object most recently or frequently. So a person's DNA profile could be found at a crime scene with which that person has never actually been associated.

New methods of analysis

There have recently been efforts to take the subjectivity out of the analysis of LTDNA results through the use of computerised data analysis and modelling. Here, the role of the reporting scientist is reduced to inputting all the data generated into a computer programme, which then provides “objective” statistics for random match probability and likelihood ratios. These may well represent a considerable advance on the currently employed methodology, but, as ever, new solutions throw up new (and old) concerns.

An example—the Broughton retrial

In a recent retrial at Oxford Crown Court (*R. v Broughton*, June–July 2010), at which the defendant, an animal rights activist, was convicted of conspiracy to commit arson, Professor David Balding was called by the Crown to give evidence based on LTDNA results which he had analysed using software that he had written. He is Professor of statistical genetics at University College London, and a well-known and internationally respected expert in the field of statistical analysis of DNA results. He has in the past also been a critic of the subjective analysis of profiling results, in particular in relation to the way drop-out has been largely ignored when formulating statistics (see, for example *Bates, post*). The algorithms underlying his computer code had been published in a peer-reviewed journal,³ and the court decided that his methods had been sufficiently validated to permit their introduction into evidence. His evidence gave powerful support to the Crown's case that the accused was the principal source of the LTDNA from the crime scene. This was strongly disputed by the defence.

Laurence Mueller, Professor of ecology and evolutionary biology at the University of California, Irvine USA, was called by the defence. Although he considered Balding's approach to be a “significant advance”, he pointed out that the statistics generated were dependent on subjective es-

timates of values for drop-in and drop-out. He (along with Dan Krane, Professor of Biological Sciences, Wright State University, Dayton, Ohio USA, also called by the defence) considered that there was no way these values could ever be accurately estimated. Mueller demonstrated that by increasing the probability of drop-out having occurred, the statistics favouring the Crown's hypothesis decreased – to the extent of eventually becoming positively exculpatory of the defendant. Realistic values for drop-in and drop-out were hotly contested by the respective experts, and it was accepted that such values could only be roughly estimated – but this case did illustrate that subjectivity has yet to be taken out of this area of forensic science, and that respected experts can still differ widely as to the correct interpretation to be placed on LTDNA results.

Whatever its potential shortcomings, the authors consider it likely that the “Balding” method will soon replace the primacy given to the opinion of the reporting scientist, and as such practitioners will need to gain an understanding of his computer model, and of its potential strengths and weaknesses. Also on the horizon (though ruled inadmissible as insufficiently validated in the Broughton trial), is a proprietary computer programme (“The True Allele”) created by Dr Mark Perlin (Director of Cybergenetics Corp, Pittsburgh USA). The workings of this programme rely on complex mathematical models analysing all the data however marginal, and it claims to be able to provide meaningful statistics even from complex mixtures. Professor Mueller (on a *voire dire* to determine admissibility in the Broughton retrial) described the methods as an interesting and potentially important advance for the objective interpretation of LTDNA, but had real concerns as to its forensic use due principally to his view that it had yet to be sufficiently validated by peer review.

Legal framework

The admissibility of DNA evidence was first addressed by the Court of Appeal in *Doheny* [1997] 1 Cr.App.R. 369 in which the Court held that when adducing DNA evidence in a criminal trial the expert should also provide his calculations of the random occurrence ratio and the prosecution should provide the defence with the means of calculation to allow adequate scrutiny of the results. The expert should explain the match between the DNA samples, but should not give their opinion on the chances of the defendant having left the crime stain.

In *Bates* [2006] EWCA Crim 1395 the Court considered the position in regard to partial profile DNA evidence. It was common ground that a partial profile was less satisfactory than a full profile, because the information missing from a partial profile could potentially establish conclusively that the person under investigation had not contributed to the sample. The appellant argued that the effect of the decision in *Doheny* was that only statistical evidence could properly be placed before the jury in relation to DNA analysis, and that in the case of a partial profile the inability to take account of the potential exculpatory effect of missing information, or “voids”, invalidated any match probability. It was further submitted that it was inappropriate to invite the jury to assess for themselves the evidential value of a partial profile, and that of the missing voids, as it would require them to weigh up something which was inherently unquantifiable. The Court rejected these arguments and

³ Balding, D.J. and Buckleton, J., ‘Interpreting low template DNA profiles’, *Forensic Science International: Genetics* 4 (2009) 1–10.

held that *Doheny* was not authority for the proposition that only match probability calculations which took into account the statistical value of every possibility were admissible in evidence, and stated that in principle there was no reason why evidence based on a partial profile should not be admissible, provided that the jury were made aware of its limitations and were given sufficient explanation to enable them to evaluate it.

Over the last 18 months Lord Justice Thomas has presided over a series of appeals in which the Court has addressed admissibility issues relating to low template DNA. The most important decision was in *Reed* [2009] EWCA Crim 2698; [2010] 1 Cr.App.R. 23. In *Reed* the Court considered both the reliability and admissibility of low template DNA and the procedure for determining its admissibility. In regard to the first issue, it concluded that low template DNA could be used to obtain profiles capable of reliable interpretation if the quantity of DNA that could be analysed was above the stochastic threshold:

- i) Low Template DNA can be used to obtain profiles capable of reliable interpretation if the quantity of DNA that can be analysed is above the stochastic threshold – that is to say where the profile is unlikely to suffer from stochastic effects (such as allelic drop out mentioned at paragraph 48) which prevent proper interpretation of the alleles.
- ii) There is no agreement among scientists as to the precise line where the stochastic threshold should be drawn, but it is between 100 and 200 picograms.
- iii) Above that range, the LCN process used by the FSS can produce electrophoretograms which are capable of reliable interpretation. There may, of course, be differences between the experts on the interpretation, for example as to whether the greater number of amplifications used in this process has in the particular circumstances produced artefacts and the effect of such artefacts on the interpretation. Care may also be needed in interpretation where the LCN process is used on larger quantities than that for which it is normally used. However a challenge to the validity of the method of analysing Low Template DNA by the LCN process should no longer be permitted at trials where the quantity of DNA analysed is above the stochastic threshold of 100-200 picograms in the absence of new scientific evidence. A challenge should only be permitted where new scientific evidence is properly put before the trial court at a Plea and Case Management Hearing (PCMH) or other pre-trial hearing for detailed consideration by the judge in the way described at paragraphs 129 and following below.
- iv) As we have mentioned, it is now the practice of the FSS to quantify the amount of DNA before testing. There should be no difficulty therefore in ascertaining the quantity and thus whether it is above the range where it is accepted that stochastic effects should not prevent proper interpretation of a profile.
- v) There may be cases where reliance is placed on a profile obtained where the quantity of DNA analysed is within the range of 100-200 picograms where there is disagreement on the stochastic threshold on the present state of the science. We would anticipate that such cases would be rare and that, in any event, the scientific disagreement will be resolved as the science of DNA profiling develops. If such a case arises, expert evidence must be given as to whether in the particular case, a reliable interpretation can be made. We would anticipate that such evidence would be given by persons who are expert in the science of DNA and supported by the latest research on the subject. We would not anticipate there being any attack on the good faith of those who sought to adduce such evidence (at para.74).

The Court in *Reed* also emphasised that it was essential that the court exercise its powers of case management to establish a firm degree of control over the admissibility of low template DNA evidence. The evidence of the possibilities and the evaluation of the evidence must be clearly set out in full in the terms in which it was to be given. Where there was a challenge to its admissibility, the court had to rule

on the issue of admissibility in advance, or at the outset of the trial:

In cases involving DNA evidence:

- i) It is particularly important to ensure that the obligation under Rule 33.3(1)(f) and (g) [of the Criminal Procedure Rules] is followed and also that, where propositions are to be advanced as part of an evaluative opinion (...), that each proposition is spelt out with precision in the expert report.
- ii) Expert reports must, after each has been served, be carefully analysed by the parties. Where a disagreement is identified, this must be brought to the attention of the court.
- iii) If the reports are available before the PCMH, this should be done at the PCMH; but if the reports have not been served by all parties at the time of the PCMH (as may often be the case), it is the duty of the Crown and the defence to ensure that the necessary steps are taken to bring the matter back before the judge where a disagreement is identified.
- iv) It will then in the ordinary case be necessary for the judge to exercise his powers under Rule 33.6 and make an order for the provision of a statement.
- v) We would anticipate, even in such a case, that ... much of the science relating to DNA will be common ground. The experts should be able to set out in the statement under Rule 33.6 in clear terms for use at the trial the basic science that is agreed, in so far as it is not contained in one of the reports. The experts must then identify with precision what is in dispute – for example, the match probability, the interpretation of the electrophoretograms or the evaluative opinion that is to be given.
- vi) If the order as to the provision of the statement under Rule 33.6 is not observed and in the absence of a good reason, then the trial judge should consider carefully whether to exercise the power to refuse permission to the party whose expert is in default to call that expert to give evidence. In many cases, the judge may well exercise that power. A failure to find time for a meeting because of commitments to other matters, a common problem with many experts as was evident in this appeal, is not to be treated as a good reason (at para.131).

In *Broughton* [2010] EWCA Crim 549, a case concerning the consideration of low template DNA (“LTDNA”), the Court considered the position as to the admissibility of DNA evidence in cases where the quantity of DNA recovered was so low as to be below the stochastic threshold. The Court recognised that at very low levels of DNA the dangers presented by the possibility of stochastic effects are increased. It further recognised that there will be occasions where the profiles generated are unreliable and/or the probative value more debatable. However it did not accept that these were reasons for ruling out LTDNA evidence in such cases altogether. The Court did recognise that in the appellant’s case the profiles had been derived from unqualified samples of DNA of less than 100pg and that this had raised entirely legitimate grounds for scientific dispute which the appellant had been right in testing before the judge.

In *Weller* [2010] EWCA Crim 1085 Lord Justice Thomas reinforced the necessity in every DNA case for there to be detailed consideration by the parties and the judge of the DNA evidence and refinement of the issues in dispute. Where there was no dispute that DNA was the DNA of a particular person, it was essential that that was put before the jury as admitted and agreed expert evidence. That would enable the jury to perform its essential function of assessing what was in issue without being troubled by matters that were not.

The Court in *Weller* also identified the importance of the practical experience of DNA experts in low template cases. The Court of Appeal held that a court in assessing whether there was a sufficiently reliable scientific basis for expert evidence to be given and a jury in evaluating

evidence would be entitled to take into account the experience of experts. It was noted that experts often of necessity relied on unpublished papers and their own practical experience. In a judgment which was critical of the lack of practical experience of the DNA expert instructed by the appellant it was observed that an attack on the reliability of low template DNA evidence (concerning transfer in this case) run purely by reference to published papers and without the practical experience upon which others had reached a judgment, was likely to fail. Lord Justice Thomas stated that courts should look to really experienced experts in such cases.

More general principles and practical guidance for the management and presentation of complex scientific and medical evidence were set down by the Court of Appeal in *Henderson* [2010] EWCA Crim 1269; [2010] 2 Cr.App.R. 24. In a hearing of three joined appeals involving shaken baby syndrome, the Court of Appeal gave detailed guidance on the management of expert evidence and on the content of the summing up. The Court sought to address the problem of how to manage expert evidence so that a jury could be directed in a way which would, so far as possible, ensure that any verdict they reached was justified on a logical basis. Concluding that a jury could only approach conflicting expert evidence if that evidence was marshalled and controlled before it was presented to the jury, the Court reinforced the views of the Court expressed in *Reed* and emphasised the need for compliance with the r.33 regime contained in the Criminal Procedure Rules. But *Henderson's* greater importance for DNA cases was in relation to the Court's comments in regard to the summing up. Whilst specifically directed towards shaken baby syndrome cases, the Court's observations plainly have application to wider cases involving consideration of complex and developing scientific evidence. Firstly, the Court stated that in cases where developing medical science was relevant, the jury should be reminded that special caution was needed where expert opinion evidence was fundamental to the prosecution; and secondly, the Court felt that a jury needed directions on how to approach conflicting expert evidence and that the judge should guide them by identifying those reasons which would justify either accepting or rejecting any conflicting expert opinion on which either side relied.

In practice

Gangbos? Part 4 (ss.34–50) of the Policing and Crime Act 2009 introduced a new form of injunction designed to prevent gang-related violence. The statutory provisions were significantly amended by sections 34–39 of the Crime and Security Act 2010, and were eventually brought into force on January 31, 2011: see Policing and Crime Act 2009 (Commencement No.7) Order 2010/2988.

Conclusion

Though it takes some uncovering, the debate within the forensic community over the use and reliability of LTDNA still rages,⁴ especially in the USA.⁵ There, the admissibility of LTDNA (sometimes referred to as “touch DNA”, “low copy DNA” or “low-level DNA”) in criminal cases has been far more controversial than in the UK. Eminent experts including Dr Bruce Budowle (Department of Forensic and Investigative Genetics, University of North Texas Health Science Center, Fort Worth, Texas, USA and ex head of the FBI's DNA laboratory) have spoken out against the forensic use of LTDNA as evidence in criminal trials.⁶ Professors Krane and Mueller continue to be sceptics, and broadly take the view that “fit for purpose”⁷ should mean limiting that purpose to the generating of intelligence leads. US courts have recently taken differing and contradictory approaches to admissibility. The decisions known to the authors have been from courts equivalent to our Crown Court, not appellate decisions, and they arise from litigation within the state, as opposed to federal, court system. In a case before a New York court,⁸ LCN evidence was ruled admissible, while in California⁹ the court was concerned with the dangers inherent in LTDNA—stating that “based on the evidence before the court, that there is no general acceptance in the scientific community as to the procedures to be used once you're dealing with a LCN sample, there's no general acceptance as to how to interpret the results that you would get when you begin with a LCN sample, and there's no general acceptance as to the statistics that can be applied to those results.” The court went on to rule the test results in that case inadmissible.

In this jurisdiction, challenges to admissibility will now be relatively rare, given the guidance from the Court of Appeal in *Reed*. But if LTDNA evidence is to be properly understood by juries and not given undue weight in a particular case, then lawyers will need to understand the limitations and shortcomings of this type of evidence. Indeed not all DNA evidence is equal.

⁴ See, e.g. <http://www.nature.com/news/2010/100317/full/464347a.html>.

⁵ Professor Allan Jamieson of the Forensic Institute, Glasgow has been a lone critical voice within this jurisdiction.

⁶ Low copy number typing has yet to achieve “general acceptance”. See Budowle, B., Eisenberg, A.J., and van Daal, A., in *Forensic Science International: Genetics Supplement Series* (2009).

⁷ See the Caddy Review, commissioned by the Forensic Regulator to examine the science following the collapse of the Omagh bombing case (*Hoey* [2007] NICC49), which concluded that the science supporting the delivery of LTDNA analysis was sound and that the main UK service providers had adequately validated their processes.

⁸ *People v Hemant Magnath*, 2010 NY Slip Op 20037 [27 Misc 3d 405]. February 8, 2010.

⁹ See *People v Hector Espino*, NA 076620 (Los Angeles County Superior Court), March 18, 2009. See also *People v Days*, 2009 N.Y. Slip Op. 52667(U) (December 2009).