

CURRENT PRACTICE IN
**Forensic
Medicine**

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EDITORS

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Edited by

John A.M. Gall and J. Jason Payne-James

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Jason Payne-James is an independent specialist in forensic and legal medicine with a range of research and clinical interests. He qualified in medicine in 1980 at the London Hospital Medical College. He has undertaken additional postgraduate

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Mercedes Salado Puerto first qualified as a biologist and completed her doctorate in the Department of Biological Anthropology, Universidad Autónoma de Madrid, Spain. As a forensic anthropologist, she was member of the Guatemalan forensic anthropology team from 1998 to 2003. Since 2003 she has been a member of the Argentine Forensic Anthropology Team (EAAF), where she currently works as identification coordinator. She specializes in the historical investigation of cases of political violence (analysis of written and oral sources, collecting ante-mortem data from relatives of the missing, interviewing witnesses, information management and databases), archaeological exhumation of individual and mass graves, and analysis of skeletal human remains in order to identify them and determine the cause of death. She has been involved in forensic investigations and training in Argentina, Bosnia-Herzegovina, Burundi, Colombia, Cyprus, Chile, Guatemala, Iran, Iraq, Morocco, Mexico, Nepal, Panama, Peru, South Africa, Sudan, Thailand, Timor-Leste, Togo, Uruguay and Vietnam. She has been lecturer in the postgraduate diploma in forensic anthropology and human rights (Universidad Nacional Mayor de San Marcos, Perú), the master's degree in forensic anthropology and bioarchaeology (Graduate School of the Pontificia Universidad Católica del Perú) and the doctorate in anthropology at the Universidad Nacional de Córdoba, Argentina. She is a member of the Latin American Association of Forensic Anthropology and the International Committee of the Red Cross Forensic Advisory Group.

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As an undergraduate, John Puntis attended Southampton School of Medicine before undertaking postgraduate paediatric specialty training in Birmingham, UK, where he developed an interests in neonatology, gastroenterology and clinical nutrition.

In 1990 he moved to Leeds as director of Neonatal Services for Yorkshire, and subsequently developed a multidisciplinary nutritional support team for the Leeds Children's Hospital. He now works as a paediatric gastroenterologist helping to provide a regional service to a population of around three million; this includes a home parenteral nutrition programme (currently with eighteen patients). John has been a member of the nutrition committee of the British Society of Paediatric Gastroenterology, Hepatology and Nutrition, the Royal College of Paediatrics and Child Health and the European Society of Paediatric Gastroenterology, Hepatology and Nutrition. His research interests have included the development of gastrointestinal function, non-invasive assessment of pancreatic exocrine function, and the delivery of safe and effective nutritional support. He is an associate editor of *Archives of Disease in Childhood*, and has published over 220 papers, letters and book chapters on gastroenterology and nutritional topics.

John Ralston

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Keith Rix is an honorary consultant forensic psychiatrist in the Norfolk and Suffolk NHS Trust. His involvement in the forensic field began in the 1960s when he lived in hostels in London with ex-offenders and assessed prisoners for hostel admission. He qualified in medicine in Aberdeen in 1975 and trained in psychiatry in Edinburgh and Manchester. In Manchester he was a lecturer in psychiatry and carried out his doctoral research under the supervision of Professor Sir David Goldberg. In 1983 he moved to Leeds as senior lecturer in psychiatry and became a visiting consultant psychiatrist at HM Prison, Leeds. He established the Leeds Magistrates' Court Mental Health Assessment and Diversion Scheme and the city's forensic psychiatry service. His research has included studies of drunkenness offenders in a Scottish burgh police court, arson and psychiatric disorder in prisoners. He has provided expert evidence to the courts for 30 years, including evidence on a *pro bono* basis in capital cases in the Caribbean and Africa. He is the author of *Expert Psychiatric Evidence*, editor of *A Handbook for Trainee Psychiatrists* and co-author, with his wife, Elizabeth Lumsden Rix, of *Alcohol Problems*. Until recently he was chair of the Fitness to Practise Panel of the Medical Practitioners Tribunal Service and a part-time lecturer in the Department of Law, De Montfort University, Leicester. He is now Visiting Professor of Medical Jurisprudence, Institute of Medicine, University of Chester. He also has an interest in medical ethics and has recently published on the subject of the involvement of health professionals in capital punishment.

Anny Sauvageau MD MSc FRCPC

Anny Sauvageau started her career as a forensic pathologist in Montreal in 2002. In 2009, she moved to Alberta where she was deputy chief medical examiner (2010) and chief medical examiner (2011–2014). She is an associate clinical professor at the University of Alberta and the University of Calgary, and is a well-known expert on asphyxia. Anny received her medical degree from the University of Montreal in 1996 and is board certified in anatomical pathology. She has a founder designation in forensic pathology from the Royal College of Physicians and Surgeons of Canada in recognition of her contribution to the development of this new specialty in Canada. She was vice president of the forensic pathology examination board of the Royal College of Physicians and Surgeons of Canada (2007–2012). She was also the founder of the residency programme in forensic pathology at the University of Alberta, and the programme director from 2011 to 2015. Anny is the author of more than 75 papers in peer-reviewed forensic journals and a highly sought international speaker. She is the founder of the working group on human asphyxia. Her innovative approach to forensic research has significantly improved understanding of the pathophysiology of hanging and other types of strangulation.

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Bob Sheridan is a life scientist with a background in physiology and pharmacology. After obtaining his doctorate in central nervous system neuropharmacology from the University of Bath, UK in 1982, he went to work as a postdoctoral CNS electrophysiologist in the Department of Psychiatry, University of Manchester and then in the Institute of Physiology, Ludwig-Maximilians University, Munich. In 1989, he moved from academia to the pharmaceutical industry, becoming a research pharmacologist at the Syntex Research Centre, Edinburgh, and later at Quintiles Research, Edinburgh. Since 1998, he has worked as a researcher at the UK Defence Science and Technology Laboratory (Dstl). His introduction to less-lethal weapons came in 2001, when Dstl were commissioned to assess the medical effects of conducted energy devices (CEDs) to support a review of this technology by the UK's independent Defence Scientific Advisory Council sub-committee on the Medical Implications of Less-Lethal Weapons (DOMILL). The TASER® CED was subsequently introduced into policing in England and Wales in 2003, Scotland in 2005 and Northern Ireland in 2008. Bob continued to provide impartial advice to DOMILL until it was replaced in 2012 by the Scientific Advisory Committee on the Medical Implications Less-Lethal Weapons (SACMILL). He continues to advise SACMILL on CEDs and other less-lethal weapons. He is a member of the police National (formerly ACPO) Less-Lethal Weapon Working Group, serves on the editorial board of the *Journal of Forensic and Legal Medicine*, and is a chartered biologist and Fellow of the Royal Society of Biology.

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three units for the master's in forensic medicine degree at Monash University, including a unit in child and adolescent sexual abuse and a unit in physical child abuse which were modified from an existing single unit of study in child abuse. Anne also produced a new unit titled 'Advanced issues in child abuse'. She has coordinated and taught all three units, and is currently teaching the advanced unit after handing on to others the teaching role for the first two units.

Over the last 25 years she has taught medical students at the University of Melbourne, local, national and international paediatricians, GPs, nurses, child protection workers, police and allied health professionals about the medical aspects of child abuse and neglect. She has published articles and book chapters, produced seminars and workshops and has also been involved in developing training programmes for building doctors' clinical expertise in the medical evaluation of suspected child abuse and neglect. She has presented at national and international conferences. She has been involved in several committees of the Royal Australasian College of Physicians, including the Division of Paediatrics Council when she was chair of the Chapter of Community Child Health. She is currently a member of the governing committee of the Australasian Association of Forensic Physicians and is a member of the Faculty Committee of the Faculty of Clinical Forensic Medicine, Royal College of Pathologists, Australasia. Anne has been involved in writing policy and developing practice guidelines regarding child abuse and neglect for the RACP and for the services in which she has been employed. She has served on several committees and advisory groups for local government, including the Victorian Child Death Review Committee. Her international work includes training and lecturing in Vietnam and Cambodia.

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Danny H. Sullivan MBBS, MBioeth, MHIthMedLaw, AFRACMA, FRCPsych, FRANZCP

Danny Sullivan is a consultant forensic psychiatrist. His medical qualification was from the University of Melbourne and he later obtained postgraduate degrees in bioethics and in medical law. He trained in psychiatry at the Maudsley Hospital, London and the Institute of Psychiatry, and was awarded the Laughlin Prize of the Royal College of Psychiatrists. Danny subsequently completed psychiatry training in Australia and has since worked at the Victorian Institute of Forensic Mental Health (Forensicare). He is responsible for the statewide community forensic mental health service and has interests in intellectual disability and in sexual offending. He has worked in a number of prisons in the United Kingdom and Australia. He provides expert psychiatric evidence in criminal and coronial jurisdictions. In addition, he holds honorary academic appointments and is involved in teaching and research, with fifteen book chapters and over twenty academic publications.

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Preface

Forensic medicine is a broad and evolving field in which many points of controversy and change occur between the publication of standard textbooks, often because of new research, new technology or new laws or regulations. There is considerable overlap between the clinical and pathological aspects of this area of practice. This is the second in a series of volumes aimed at providing a practical update on areas relevant to clinical practice and a focus for debate in the selected topics. Each chapter, written by specialists within their respective fields, is intended to give a stimulating and sometimes controversial general overview of the area under discussion with reference to the published literature. The chapters contain details of significant changes or significant points that the reader should be aware of. In some cases, the chapter covers areas that have not previously been fully discussed in existing textbooks or that are currently highlighted in forensic practice. The book aims to be of direct relevance to a multiprofessional, international audience and provide a guide to current approaches to the subject area, although examples may be drawn from specific jurisdictions to enable readers to relate to their own practice.

This volume contains a range of current, new and often controversial subjects, including chapters on DNA decontamination and forensic sample collection, the toxicity of novel psychoactive substances, the use of gastric contents in the timing of death, the physiological and physiological effects of controlled energy devices, the main risk factors of driving impairment, the risk factors for harm to health of detainees in short-term custody, autoerotic deaths, child maltreatment and neglect, and the investigation of potential non-accidental head injury in children. Also included are chapters on Excited Delirium Syndrome, automatism and personality disorders, each of which is relevant to everyday clinical practice. Two topics not generally covered in standard clinical forensic medical textbooks include a forensic anthropological approach to body recovery in potential crimes against humanity, and risk management and security issues for the forensic practitioner investigating potential crimes against humanity in a foreign country.

The opinions expressed in this volume are those of the chapter authors and do not necessarily represent those of the editors or the publishers. We hope that this new volume will once again stimulate discussion and reflection on practice, even if the reader may have different opinions from some of the views expressed here.

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1 DNA contamination – a pragmatic clinical view

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Contamination considerations

Forensic DNA testing has had a significant impact on the investigation of crimes. Initially developed in the mid-1980's by Alec Jeffreys and his group (Jeffreys, Wilson and Thein, 1985), the original 'fingerprinting' procedure has been modified and refined to the point where 'DNA profiling' has seemingly attained infallibility status (Koehler, 1993; National Research Council, 1996; Thompson, 1997). This status has been acquired and/or enhanced by its use in the exoneration of persons falsely convicted of various crimes and the subsequent successful conviction of the apparent perpetrators (Innocence Project, 1992). The concept of DNA infallibility in the investigation of crimes has, however, led to some significant miscarriages of justice. It is these exceptions that serve as a warning that the use of DNA profiling in criminal investigations requires a full understanding of the process by those employing the technology, and caution in the interpretation of the findings and their subsequent application in the legal system.

Different types of error may occur in any scientific procedure; DNA testing is no exception. DNA profiling is not and never has been infallible. There are many potential sources of error in its application. If it is to be applied competently within the legal system, all persons utilizing DNA data must have some understanding of the science behind it and of its potential sources of error. What is apparent is that many involved in the process, whether for specimen collection, testing, interpretation, application or presentation in court, have a poor understanding of DNA technology and its limitations, the potential for both error and misinterpretation, and the consequences of these when judged in a court of law. The sources of error are many and it is not our intention to examine them all. Instead, this chapter will address the issue of DNA contamination of items – other than their deliberate contamination – and consider this in relation, in particular, to the work of the forensic physician.

Does DNA contamination occur? The answer is yes, and there are some very high-profile cases to demonstrate its occurrence – probably it occurs far more frequently than we appreciate. The following examples of accidental contamination illustrate

how easy it is for contamination to occur and how DNA profiling results may too readily be accepted without question.

The 'Phantom of Heilbronn'

Between 2007 and 2009, the German police were confronted by an apparent unknown female criminal who had been linked by DNA to some 40 crime scenes over a fifteen-year period (BBC News, 2009; Himmelreich, 2009). These crimes, which included six homicides and various burglaries and break-ins, had occurred in locations across Austria, France and Germany. Dubbed the 'Phantom of Heilbronn' following the murder of a young policewoman, Michele Kiesewetter, in Heilbronn in 2007, the hunt for this mystery female intensified and a substantial reward of €300,000 was offered for information leading to her capture. In 2009, the German police eventually unearthed the identity of the murderer and thief. The answer had been in plain sight all the time and is a stark reminder of the fallibility of DNA profiling.

In March 2009, while investigating the case of an unidentified, burned, deceased male from 2002, the Phantom's DNA profile was detected when re-examining the fingerprints of a male asylum-seeker taken years earlier in order to reveal his DNA profile. The improbability of this finding resulted in the DNA test being repeated using a second swab to collect more DNA from the specimen. This time the Phantom's DNA was not identified. The problem was revealed as one of DNA contamination and the identified DNA was that of a female worker on a production line for cotton-tipped swabs – swabs used by various police forces across Europe to collect specimens at crime scenes.

Stefan König of the Berlin Association of Lawyers was reported (Himmelreich, 2009) to have made a very pertinent comment in relation to placing too much credence on identifying trace amounts of DNA:

What we need to avoid is the assumption that the producer of the traces is automatically the culprit. Judges tend to be so blinded by the shiny, seemingly perfect evidence of DNA traces that they sometimes ignore the whole picture. DNA evidence on a crime scene says nothing about how it got there. There is good reason for not permitting convictions on the basis of DNA circumstantial evidence alone.

His comment has proved to be correct and the question of how the DNA 'got there' is particularly pertinent.

The Jama case

On 21 July 2008 a young Somalian male, Farah Abdulkadir Jama, was found guilty and imprisoned for the rape of a 48-year-old female in a Melbourne nightclub on 15 July 2006. The sentence was for six years with a non-parole period of four years. It was alleged that Mr Jama had sexually assaulted the woman, who had been found unconscious in a toilet cubicle, locked from the inside. Although the woman had

consumed alcohol that evening, its effects were in excess of her expectations and raised the possibility that she had been drugged, presumably by her drink or drinks being spiked. Subsequent toxicological analysis did not support this assumption but did show a high blood alcohol level.

The normal procedure for cases of acute alleged sexual assault in the State of Victoria is that victims are taken to one of several designated examination suites located within some of the major metropolitan hospitals. Within these suites a forensic examination is undertaken by a forensic medical officer (FMO) or forensic physician (FP) and specimens are collected and provided to the police. This occurred in the case of this female and Mr Jama's DNA was identified during subsequent forensic laboratory testing of biological specimens from the alleged victim. As a result, Mr Jama was found guilty of a sexual assault and sentenced to imprisonment, of which he completed approximately fifteen months prior to his being acquitted by the Court of Appeal.

The case against Mr Jama had been based on a single piece of evidence: that his DNA had been identified on a single slide preparation and associated swab collected, among other swabs, from the alleged victim when examined by the FMO. The matching DNA was, in fact, recovered from only one of two swabs taken from the endo-cervical region of the woman and was not present on two other swabs taken from within the vagina. In addition, from the available information, his DNA did not appear to be on the alleged victim's clothing. Also of importance was that Mr Jama had never attended the nightclub in question (based on evidence tendered to the court) and there was no evidence to suggest otherwise.

Following Mr Jama's acquittal, the Hon. Justice Vincent undertook an enquiry on behalf of the Victorian Department of Justice (Vincent, 2010). He came to the opinion that the most likely cause for Mr Jama's DNA appearing on the swab was as a result of contamination within the clinical examination suite. Part of the reasoning supporting this was a failure to find any evidence to suggest that Mr Jama's DNA could have come in contact with material from the alleged victim after the swabs had been packaged in any other way, and, more significantly, that the FMO undertaking the examination of the alleged assailant had undertaken a similar examination of another woman about one day previously in the same examination suite. This female had engaged in a sexual activity with Mr Jama, an issue that had not led to any charges being placed.

It is of note that Mr Justice Vincent was of the view that:

Not only was the evidence against him [Mr Jama] ultimately revealed as inherently unreliable but, it came to be recognized, the likelihood that he had committed the offence allegedly was, at the risk of understatement, remote and found to have been perpetrated in circumstances where it seemed to be highly improbable that there was any crime at all.

Mr Justice Vincent went on to say:

In other words, the DNA evidence was, like Ozymandias' broken statue in the poem by Shelley, found isolated in a vast desert. And like the inscription on the statue's pedestal, everything around it belied the truth of its assertion. The statue, of course, would be seen by any reasonably perceptive observer, and viewed in its surroundings, as a shattered

monument to an arrogance that now mocked itself. By contrast, the DNA evidence appears to have been viewed as possessing an almost mystical infallibility that enabled its surroundings to be disregarded. ... After following this history of the proceedings against the unfortunate Mr Jama from their origins through to their disastrous conclusion of his conviction, I have been left with the deep impression that at virtually every point, and by almost everyone involved, it was handled with so little insight into the issues which it presented that no need was seen to explore further or conduct research into them. This was particularly so in the case of those involved in the legal processes. There were ample warning signs along the way that suggested that something was amiss but they were simply not read.

These views are very similar to Stefan König's in relation to the Phantom case (discussed above) and have echoes in the case that follows. In contrast, it was the disbelief of a police officer in the Adam Scott case (discussed below) that revealed another case of contamination.

Mr Justice Vincent made a number of recommendations for change in relation to the examination facilities, specimen collection equipment and the legal processes. Importantly, as the swabs and slides used for the collection of DNA evidence were taken from readily and generally accessed trolleys located within the examination suites rather than from dedicated DNA collection kits, recommendations made were that the specimen collection equipment be kept separate from the trolleys and in locked storage accessed only by the examining doctor. Further, it was recommended that any unused specimen collection equipment prepared for an examination be disposed of.

Following tabling of Mr Justice Vincent's report, the Victorian Department of Justice implemented many of the recommendations (Smith, 2011) as well as the development and implementation of a forensic medical examination kit. This is now in use in examination suites throughout Victoria.

LGG forensics

In October 2011, Mr Adam Scott was charged with the rape of a female in Manchester, UK. He was arrested and held in custody by the Greater Manchester Police based on DNA evidence alone, which had been obtained from a vaginal swab taken from the alleged victim. Mr Scott, who lived in Exeter, denied ever having been in Manchester and an analysis of his mobile telephone by the police indicated that he was not in the area of the reported rape. The detective leading the investigation voiced concern regarding the reliability of the DNA result and the matter was subsequently investigated (Rennison, 2012).

The case was reviewed and, after further DNA testing, the probable cause of what was in fact contamination leading to the incorrect charging of Mr Scott was that within the testing laboratory there had been an inappropriate reuse of a disposable plastic tray, which had then contaminated the sample from the female. Mr Scott's DNA was in the system within the laboratory as he had been involved in an incident involving British Transport Police, during which there was spitting, resulting in his DNA sample being submitted for assessment. Mr Scott's saliva DNA sample, with its high-level male DNA, from this earlier, unconnected incident had been present in one of the wells in the automated tray that was incorrectly reused in the laboratory, the

same one that the sample from the alleged female victim (actually containing only her DNA) was subsequently loaded. The error was revealed when the reports of this fully accredited laboratory were reviewed. Initially, retesting of the original DNA extract confirmed the original findings of Mr Scott's potential culpability. This extract, however, had already been contaminated due to the reuse of the plastic disposable tray. When this possibility was raised, a complete retesting of the original swabs from the female was undertaken; these showed that Mr Scott's DNA was not present at all. The charges against him were subsequently withdrawn.

Jaidyn Leskie

Jaidyn Leskie was a 14-month-old child. On 15 June 1997, he disappeared while in the care of a family friend. Five and a half months later, Jaidyn's body was found floating in Blue Rock Dam, Victoria, in the district in which he had lived and was being cared for. Various investigations were made into the child's death and the findings were reported by the Victorian State Coroner in 2006 (Johnstone, 2006). According to the report, a search of the dam where Jaidyn was found revealed the presence of a plastic bag containing some of his clothing. On the bib and tracksuit a female DNA profile was identified and, in subsequent investigations in the early 2000's, a search of the Victoria Police Forensic Services Centre's (VPFSC) database revealed a match to the DNA profile of a female rape victim. Police investigation indicated that this woman was unlikely to have had links to the toddler. This raised the possibility of contamination. The matter was addressed in the Coronial investigation and involved the testimony of experts on the subject from a variety of laboratories. The VPFSC argued that contamination was unlikely given the processes that they engaged in and considered that the profile from the bib and tracksuit was not the identified female but that of another, unidentified individual, the result of what they referred to as an 'adventitious match'.

The DNA aspect of the case was interesting as the VPFSC continued to maintain the adventitious match hypothesis rather than admit to a possible contamination within the laboratory, despite the fact that clothing from both cases – the child and the rape victim – was received within seven minutes of each other, and that the two cases were examined by the same person within a relatively short time-frame, albeit in different locations within the laboratory. It was possible that a single pair of scissors, wiped clean between each use with 70% alcohol (which, it should be noted, does not denature DNA), may have been used to cut cloth samples from each case. In light of the evidence, it is difficult to accept the adventitious match hypothesis.

The four cases outlined above illustrate how contamination can occur in different settings. The first is where contamination takes place in the preparation of materials being used for the purpose of DNA collection; the second is of the potential for cross-contamination in forensic examination suites; and the third and fourth show how contamination can occur within the laboratory setting. The last does occur occasionally and processes are implemented within laboratories in an effort to detect such contaminations. Irrespective of where the contamination occurs, however, it will

never be detected if those investigating it do not consider the possibility seriously and remain open to the potential for contamination to take place in their own domain.

DNA profiling

The first use of DNA in criminal justice was when Alec Jeffreys used his DNA ‘fingerprinting’ technique to assist in the identification and conviction of Colin Pitchfork for the rape and murder of two young women in Leicestershire in 1983 and 1986. The technique involved the identification of a particular DNA sequence through the use of a radioactive-labelled complementary sequence, or multi-locus probe, within variable lengths of DNA cut with restriction enzymes (known as restriction fragment length polymorphisms). Electrophoresis pulls the charged DNA through a gel matrix, with the fragments being spread out according to size. Fragments containing the bound probes can be visualized by exposure to an x-ray plate and are seen as dark bands, which can be likened to a bar code. The resulting pattern is known as a DNA fingerprint and is identical within an individual and essentially unique. This provides a powerful tool if the DNA fingerprint from a crime scene matches that of a suspect. However, despite its accuracy and reproducibility, the technique requires DNA in microgram amounts and is slow.

Two scientific developments have since changed DNA analysis into the DNA profiling technique used today:

- The polymerase chain reaction (PCR) has enabled picogram amounts of DNA to be multiplied, incorporating fluorescent probes into the copies, thus allowing visualization.
- The discovery of polymorphic mini-satellites within the genome. Mini-satellites are repeated sequences of 2–5 nucleotides (the building blocks of DNA) and are known as short tandem repeats (STRs). Probes are prepared that bind to the sequence outside the repetitive region and the PCR reaction makes copies of the repeat sequence. The STR and its flanking sequence are selected so that they are found only once in the genome. The different-sized molecules produced reflect the number of repeats and these are separated by means of electrophoresis. A DNA profile is a list of numbers of repeats, each obtained from a selected area of the genome.

Sample collection

Perhaps the most important part of the process in DNA profiling is at the beginning of the process. The integrity and preservation of collected DNA, representing what is believed to be material likely to be associated with the crime scene, is vital. With the ability to profile DNA from just a few cells, individuals responsible for law enforcement and crime scene management need to be acutely aware of the ease with which DNA on an item can be transferred to other areas, or contaminated with extraneous and unrelated DNA, as illustrated in the Jama case discussed above.

In addition to protective clothing and defined processes to avoid contamination, discussed in more detail below, the integrity and documentation of material collected directly from the crime scene requires:

- A detailed record of what was found, with annotated photographs, sketches and measurements.
- Items of evidence collected into clearly and appropriately labelled individual paper containers which are securely sealed to maintain a proper chain of custody. Plastic bags are to be avoided as any retained moisture can speed DNA degradation.
- Use of a double swabbing (wet then dry) technique to collect biological stains from items that cannot be moved. DNA-free water opened at the scene is used to rehydrate biological material followed by a dry swab to collect any remaining cells. The choice of swab material is important in the subsequent analysis, as is the need for the swab to be left to dry. DNA survives better when dry, avoiding hydrolytic cleavage of the sugar-base bond which leads to loss of the base and subsequent single strand breaks in the DNA molecule. (This is discussed in more detail below.) Drying also protects the material from any DNA-digesting enzymes that may be prevalent from contaminating bacterial growth.

DNA extraction and quantification

DNA extraction methods are designed to separate proteins and other cell components from the DNA molecule and to remove any inhibitors of the PCR process. All samples must be handled carefully to avoid contamination, regardless of what extraction method is used. (Laboratory protocols are considered below.)

Quantification is important as non-human DNA may be extracted, along with human material. It is important in the subsequent analysis to know how much human DNA is present. Methods are also available to inform the scientist about how much male DNA is present and to provide information about the presence of PCR inhibitors.

DNA amplification

This process not only multiplies the DNA present for analysis, but also selects the particular DNA sequence to be analysed and incorporates fluorescent chemicals in the subsequent amplicon to allow downstream analysis. Essentially, some 30 heating and cooling cycles in a reaction with the appropriate chemical constituents and a polymerase enzyme (required to assemble the DNA chain) doubles the selected sequence, producing in excess of several million copies of the targeted sequence. Direct amplification methods have been developed which can decrease the risk of contamination by avoiding the extraction processes described above.

PCR offers both advantages and disadvantages:

- Very small amounts of DNA can be analysed, down – potentially – to single cells.
 - This highlights the risk of the presence of contaminating cells which are then analysed along with the intended DNA, leading to significant problems in interpretation and underlines the importance of consumables and reagents being DNA-free (Gill, Rowlands, Bastisch et al., 2010). As the ‘Phantom’ case above illustrates, gross contamination can also result if manufacturers do not treat their products to remove any DNA. This happened in the United States where a DNA-concentrating device used in several laboratories was grossly contaminated with DNA from a female member of their staff (Butler, 2012).
 - Negative controls are used to support the absence of reagent contamination.
 - Particularly important in low template analysis, where amplification is increased or DNA detection is enhanced, is a repeat analysis which can help control for environmental or plastic consumable contamination.
- Fragmented DNA molecules can be analysed, down to a few hundred base-pairs.
- Different DNA sequences can be multiplied at the same time (multiplex PCR).
- Primers are selected that target only human-specific sequences.

However, amplification may fail if:

- The PCR process is inhibited due to the DNA molecule being damaged by exogenous factors. This is also the aim of cleaning processes.

The very high template amplicons that result following PCR also provide a high risk for contamination. It is for this reason that laboratories have a strict policy of separation of areas, equipment and protective clothing, and use one-way movement through the laboratory between pre- and post-amplification facilities.

DNA separation and detection

Electrophoresis is the process used to separate different-sized DNA molecules, such as those DNA template copies in the amplicon. The separation process has to be sufficient to allow the resolution of DNA template copies that differ by only a single base in their sequence length. This is achieved using very fine long capillaries.

DNA is introduced to the capillary through an electro-kinetic injection and the electric charge draws the material through. At a particular point along the capillary the fluorescent label (fluorophore), introduced into the template during amplification, is detected by laser excitation and a photosensitive detector. Smaller molecules arrive at the detector first and the time of detection is used to assign size (length) of