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viewpoint

Coding or non-coding, that is the question

Having solved the last technical hurdles to extract DNA information from virtually any biological material, forensic biologists now have to ponder the ethical and social questions of using information from exonic DNA • *by Mark Benecke*

Currently, we are witnessing one of the most extensive biological forensic investigations. Private and public laboratories across the USA are using a wide range of techniques to extract DNA from tiny stains in order to identify the victims of the September 11 terrorist attack on the World Trade Center. They try to match the DNA from the crime scene with genetic 'fingerprints' of the victims' toothbrushes, razor blades and other personal items, or compare it against the DNA of the victims' relatives. Although the World Trade Center investigation is more a logistic than a scientific or technological challenge, the sheer number of victims and the interest of the media following the terrorist attack have drawn reporters' interest once again to forensic biology including DNA-based identification techniques (see, for example, Lipton and Glanz, 2002).

The latest scientific and technological advances in biological forensic science were presented at this year's meeting of the German-speaking 'genetic fingerprint' community at the University of Bonn in mid-March. Forensic biologists demonstrated that they have finally solved the technical problems of recovering DNA from nearly any biological sample, no matter how small it is. DNA can now be extracted from practically any biological substance left at a scene of crime or an accident, including teeth, blood, sperm, saliva, bones, hair, urine and faeces. A colleague from the Bundeskriminalamt (BKA)—the German Federal Bureau of Investigation—even presented a recipe for DNA extraction out of telogenic hair shafts, which had previously been thought to be devoid of genetic material. Colleagues from another police laboratory showed that they had mastered extracting DNA from single epithelial cells, which they first need to recover under a microscope. They now perform this technique on a routine basis, for example to trace a person who wound up a watch but then left it at a crime scene. There is a good chance that the grooves of the watch's winding crown will contain some epithelial cells that can be used for DNA typing.

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And as if all that was not enough to satisfy a modern Sherlock Holmes, it is now also possible to extract DNA from biological material that has previously been treated with colour stains. For example, microscopic preparations of tumour tissue for clinical examinations can be successfully typed, even after many years of storage at room temperature on a dusty shelf. Genetic fingerprints of such stained tissue are used to prove that a tumour spread from one person to another via an organ or a bone marrow transplant. Even if the original organ donor has since died, his or her tissue will often still be preserved on microscopic slides in the hospital.

In cases where clinical diagnosis had been based on incorrect or sloppy histological examination of samples, it is often necessary to identify the true donor of the tissue preserved on the slide. If the case is brought to court, such identification helps to determine the extent of possible indemnifications. What is more, if DNA typing of the samples is performed before medical treatment, it might save patients from unnecessary surgery caused by a mix-up of samples.

Colour reactions and stainings are also used in criminal investigations, mostly to obtain fingerprint patterns (from real fingers) from a crime scene. Surprisingly, neither superglue vapours nor any of these stains—amido black, leucomalachite green, Hungarian Red or luminol—interfere with DNA typing. The technical climax was reached when a DNA profile was developed out of a real fingerprint (see, for example, Schmitt and Benecke, 1997; Olaisen *et al.*, 1998; Sensabaugh *et al.*, 2000).

In the beginning, it was not only the physical extraction of the DNA that had limited our access to DNA profiles of biological stains, or possible offenders, or unidentified bodies—we also had to find the best target sequences that contained restricted yet useful, genetic information on individuals (see, for example, Benecke, 1997). Alec Jeffreys and members of his laboratory at the University of Leicester in the UK found a way when they discovered that certain repetitive stretches of DNA differ in their length. These repetitive loci were used as targets for the first generation of genetic fingerprints (Gill *et al.*, 1985; Jeffreys *et al.*, 1985). Genomic DNA was cut by restriction enzymes, sorted by electrophoresis, blotted and then hybridised against a radioactive probe. This probe was not too specific, and therefore bound against multiple repetitive sites, leading to the bar-code pattern that many people know, and now identify, as a DNA fingerprint (Figure 1). The more bands are shared between two individuals, the closer they are related.

The mutation rate of these repetitive DNA stretches, formerly known as parts

of the intronic 'junk DNA', is low. A child inherits half of the bands from its mother and the other half from its father, which made this type of DNA fingerprinting particularly useful for paternity testing. The method went straight to the courtroom in its very first year of use: in an immigration case in the UK, the relationship of a child to an adult female needed to be established. A multi-locus restriction fragment length polymorphism (RFLP) of variable loci proved that the child was indeed related to the female, and immigration was therefore approved. In Jeffreys' initial paper, the term 'fingerprinting' was introduced as a quite British pun; now it is part of our everyday language. All forensic scientists agreed that with DNA typing—as genetic fingerprints are called by forensic biologists—a new era in criminal investigation had begun.

Quickly, investigators started using sperm samples from rape cases to trace the offenders. Condoms and clothing, such as Monica Lewinsky's blue dress, could now collect DNA for us since sperm heads are perfect DNA preservation capsules. Even eating or drinking during a crime became a risk for offenders since epithelial cells in saliva left on breadcrumbs and bottle openings dry out quickly, thereby preserving the DNA. It also works the other way. This February, Arvin McGee was released from the Joseph Harp Correctional Center in Lexington, KT, after more than 14 years. DNA typing of a stored sperm stain showed that he did not commit the rape for which he was sentenced to 298 years in prison. Dozens of further cases of wrong juridical decisions could be changed by use of DNA typing (see, for example, Connors *et al.*, 1996; Klug, 2002).

Until around 1991, forensic biologists needed large amounts of high-molecular DNA for the multi-locus RFLP 'fingerprints'. *In praxi*, this meant that relatively large biological stains had to be recovered. A further drawback in the early years was a lack of safe biostatistics and laboratory standards. Both had to be established before DNA evidence was fully accepted, at least in US courts (see, for example, Lander and Budowle, 1994).

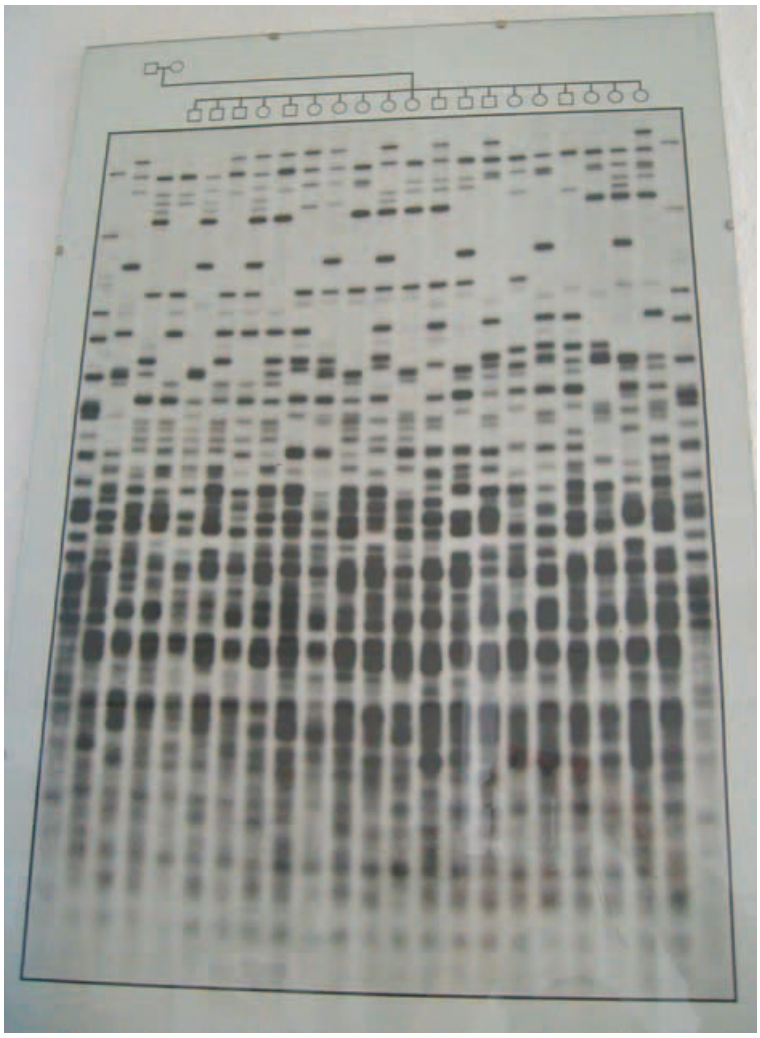

Fig. 1. Photo of a multiplex gel on the wall of a DNA typing laboratory: RFLPs of variable, repetitive intronic region against the probe (CAC)5/(GTG)5 (courtesy of Peter Nürnberg, Institute of Medical Genetics, Humboldt University, Berlin, Germany).

Dozens of cases of wrong juridical decisions could be changed by use of DNA typing

Only 5 years after the discovery of genetic fingerprints, when forensic applications as well as paternity testing by DNA typing had become successful and widely accepted, a technical switch took place. Instead of further dealing with complex properties of multiple loci—size, biostatistics and inheritance—laboratories now performed RFLPs of just one variable locus per probe as it was much easier to determine their fragment length. Since the 'single locus' probes could easily be washed off the nylon membrane, and then another probe for another 'single' locus could be hybridised against the RFLP, the information content of single-locus DNA typing remained high. However, the amounts of DNA needed—around 10 µg—were the same as with multi-locus DNA fingerprints.

This was the innocent golden age of DNA typing. A friend of mine, a technical assistant, still loves the classical single-locus method. If we do not watch her, she still performs paternity tests in this old-fashioned way, equipped with a sponge and some books as weights for the Southern blot.

It was not only an ethical decision to use intronic, non-coding regions of our genome as a target for DNA typing, but simply the presence of suitable DNA stretches in those regions. In the 1980s and early 1990s, it was not possible to use point mutations as indicators for differences in DNA stretches of any type: sequencing was far too expensive for forensic purposes. In contrast, the variations in repetitive DNA stretches could be detected by relatively cheap hybridisation methods. It was only by chance that those sequences were introns, and not genes. Of course it is true that genes are unlikely to carry enough molecular variation to distinguish one individual from another. However, it is also true that analysis of exons was technically out of reach for forensic scientists.

Therefore, when the police started establishing DNA-profile databases in various countries (see, for example, Barinaga 1988; McGourty, 1989), we were fortunate enough to open-heartedly

state that no information about a person's body or mind was filed into those data collections. This led to the present situation where stressful yet only basic restrictions apply to the collection of genetic fingerprints in criminal cases. DNA profiles can be collected in all European countries if a person has committed a severe crime, or if a person is under (very) reasonable suspicion. In the database, the person's DNA profile is compared against genetic fingerprints from recent as well as older criminal cases. If a match comes up, a link can be established between the possible offender and a crime, or a crime scene.

In other cases, DNA typing is just a tool for identification. DNA profiles from unidentified bodies or body parts are collected and stored even without any specific consent. Eventually, all genetic fingerprints, irrespective of their origin, are destroyed after a fixed interval of time just like any other information in the legal system of democratic countries.

Fig. 2. Mugshot of Peter Kuerten, the homicidal 'vampire of Düsseldorf': a photograph contains much more information about a person's physical, social and mental condition than a DNA fingerprint from non-coding, intronic regions (photo: Archive Benecke).

Why is it that an ID photograph can be taken without consent whereas a genetic fingerprint needs special legal treatment?

Still, some restrictions weigh heavily in terms of the actual workload they generate. In Germany, for example, a judge must grant permission for every single biological stain to be entered as a genetic fingerprint into the central database of the BKA. This causes severe bureaucratic hassles and delays. Obviously, the whole procedure is an unnecessary burden for the policemen and women who prefer to work on the case rather than filing applications for genetic fingerprints. Police unions such as the German 'Bund Deutscher Kriminalbeamter' are convincingly lobbying that a genetic fingerprint contains no more information than a regular ink fingerprint taken from skin. They state that the banding pattern of a genetic fingerprint is nothing more than a bar code that is unique for just one person on earth—with the only exception of monozygotic twins who need to be distinguished by more advanced methods on the molecular level (Schlieper *et al.*, 2002). Since regular fingerprints from skin belong to the routine repertoire of forensic work, it is hard to understand why genetic fingerprinting needs an extra confirmation from a judge.

To stress this point in political discussions and seminars, I usually present an old black-and-white mugshot (Figure 2). Clearly, the photograph reveals a lot more about the person's physical, social and maybe even mental state than the anonymous patterns in genetic fingerprints. Why is it then that an ID photograph can be taken without consent whereas a genetic fingerprint needs special legal treatment?

The field of forensic DNA typing had still not lost its innocence when we started using short tandem repeats (STRs, very short yet still intronic DNA fragments) as targets for our DNA profiles. STRs are composed of small core units (2–4 bp) that are repeated in tandem fashion around five to a few dozen times. Each repetition number is called an allele. Thus, a 10-fold repetition of the core unit at locus X is called 'allele 10 of locus X'. The combination of STR alleles at several loci is unique for an individual, even if some or many alleles can be shared with others. If full proof of a match between the genetic fingerprint from a biological sample from a crime scene and the DNA profile of an offender is needed, sometimes the combination of STR alleles from up to 12–15 loci might be needed (Figure 3). This, however, will lead to the perfectly safe statement that 'no other person on this continent/on earth shares the same allele composition'.

The major advantage of STRs is that even if the DNA is degraded, for example in aged biological stains, PCR amplification of the short stretches is still successful. As in the old-fashioned RFLPs, the length of the fragments is then determined and compared. No information about the individual human being, apart from an intronic bar code, plus gender, is detected in this procedure.

Later, in the 1990s, genetic association studies showed that very few loci near malfunctioning genes could be used as weak markers for that malfunction. Alarm bells started ringing concerning possible misuse of this information. Chorea Huntington seemed to be a prime example—neighbouring chromosomal changes were detected before the actual gene was found. However, such loci are never used in DNA typing. Extremely few single STR alleles show extremely weak associations with neighbouring gene malfunctions. Only one allele was found to be associated with a very slightly increased probability of a phenotypic, multigenic disease, such as constitution of schizophrenia. In current genetic fingerprinting, all such associations are so ridiculously weak that serious protest could never form.

This is illustrated well by the possible association between certain alleles of an STR named *Tho1* and diabetes type 1 (Bennett and Todd, 1996; Stead *et al.*, 2000). *Tho1* alleles are used routinely in DNA typing, and for a minute, the manufacturers of genetic fingerprint kits started to feel the heat over the possible association between an exonic illness and an intronic allele. Fortunately, it takes just a pen and a piece of paper to brush off possible

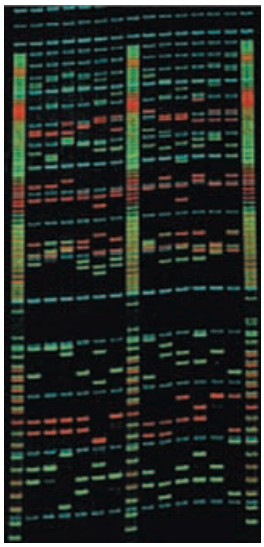

Fig. 3. A multiplex PCR of 16 short tandem repeat loci. The PCR products overlap and are therefore labelled in different fluorescent colours.

concerns: four out of 1000 Europeans will eventually get diabetes type 1. If you carry one of the 'risk' alleles in the intronic *Tho1* region, your chances of getting diabetes type 1 is 0.13 out of 1000. If I find out that you are carrying the alleged risk allele in my laboratory during DNA typing, I could—but I am not allowed to—calculate your total risk for diabetes as 0.4 × 1.3 = 0.52%. In plain language: in the worst case scenario, one allele of your possible genetic fingerprint might tell me that your general risk of getting diabetes type 1 is increased from 0.4 to 0.52%. All other alleles will not tell me anything about you, or your potential risk for illnesses. Abuse of such information is impossible because it simply has no practical predictive value.

Our well-protected innocence may, however, disappear in the near future. The day will come when somebody asks us if we could check for variations in genes instead of introns. In Europe it will be impossible to check for genes in criminal investigations; our laws will simply prohibit this for years to come. But is there not a certain charm to the idea that one could recover the colour of skin, eyes and hair from a decomposed body's remaining DNA? Would it not be useful if one could then more easily create a good phantom portrait of that dead person? Is a DNA test for a possible illness out of a biological stain a bad thing if it helps to track down a rapist and killer or a small girl? Microarrays with thousands of hybridisation spots for exonic variations will become available in the next few years: can we resist the temptation to cross the genetic borderline and examine the genes from biological stains and possible offenders? Why should we not trust that the laws in our fully functional democracy will prevent abuse of such information? Why should we assume that such investigations are not right for our society? (For attitudes towards genetic testing for illnesses, see, for example, Illes *et al.*, 2000, 2001).

Some older members of my favourite forensic academy do not even dare to face these questions. When I recently handed around 300 questionnaires on the topic of exonic DNA typing at a congress, one of the senior professors stood up and declared that it was 'by far too early to discuss those matters'. He recommended 'not to fill out the questionnaire, and not to answer any questions related to DNA typing of exons'. What my ageing colleague did not know was that before his outburst, the first answers had started trickling in. Most colleagues agreed that, as long as it does not come to the detection of dispositions such as depression, obesity or schizophrenia, some other properties of the body, such as colour of the eyes, skin and hair, might be useful in future criminal proceedings.

Soon, the day will come when somebody asks us if we could check for variations in genes instead of introns

As a human geneticist, you may wonder where the problem lies. What is wrong with using biological parameters in the hunt for killers, rapists and other serious offenders? As a parent of an abused child, you may ask the same. How could DNA data stored in a federal crime laboratory be used for anything other than catching the offender? As a person who takes care of possible data misuse, you will of course ask for severe controls concerning restricted access to genetic data—and you will get them without hesitation.

As a forensic biologist, you should at least start thinking about the matter. Neither will research stop, nor will people stop committing crimes. So the forensic community should grasp the nettle, and for once step out of the laboratories to talk to the people before the people start talking.

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What to call human cloning

The technical terminology increasingly used in the cloning debate sidesteps the ethical questions raised • *by Dónal P. O'Mathúna*

On April 4, 2002, the Italian physician Severino Antinori announced that a woman was 8 weeks pregnant with a cloned human fetus (Daniel, 2002). On April 15, 2002, Brigitte Boisselier, scientific director of Clonaid, a firm linked to the Raelian movement, announced that they had developed human clones to the blastocyst stage and planned to implant them into women. Later that month, Antinori told Italian state television that three cloned pregnancies existed in the world at that moment, two in Russia and one in an Islamic state. If these claims prove to be true, and the fetuses survive full-term, debates over the ethics of human cloning will no longer be theoretical exercises. We will have to consider how we treat cloned humans.

Judging by the vociferous condemnation of these reports, most people regard human cloning as immoral and would like to outlaw the procedure. On April 10, 2002, US President George W. Bush urged the US Senate to ban human cloning completely because it treats human life as a commodity, and stated that 'no human life should be exploited or extinguished for the benefit of another'. The US Senate is closely divided on the issue of cloning and although it is expected to ban 'reproductive cloning', it is still debating whether to allow research involving 'therapeutic cloning'.

While both these terms remain widely used, some scientists are urging their abandonment because of the negative public response they generate. Indeed, in a recent report, the US National Academy of Sciences (NAS) chose to call therapeutic cloning 'nuclear transplantation to produce stem cells' (NAS, 2002). This exemplifies the desire on the part of some within the scientific community to eliminate the term 'cloning' from the discussion about the production of embryonic stem cells by somatic cell nuclear transfer (SCNT). But the NAS report admitted that exactly the same methodology is involved in both reproductive and therapeutic procedures, except that in the former the cloned blastocysts are implanted into a uterus and in the latter they are experimented upon; thus by their own definition, SCNT produces cloned embryos. 'Clones contain identical sets of genetic material in the nucleus [...] of every cell in their bodies. Thus, cells from two clones have the same DNA and the same genes in their nuclei' (NAS, 2002).

> One controversial and morally questionable action —embryo destruction—is being used to justify another controversial and morally questionable action—human cloning

Another influential group of scientists proposed that cloning 'is properly associated with the ultimate outcome or objective of the research, not the mechanism or techniques used to achieve that objective.' Yet they define cloning as a 'term that refers to producing a copy of some biological entity—a gene, an organism, a cell' (Vogelstein *et al.*, 2002). By their definition—and the common use of this term in scientific language—cloning is a procedure regardless of its objective.

Dissociating the term cloning from the generation of human embryos is an attempt to influence the ethical debate while simultaneously avoiding discussion of central ethical problems. The term 'therapeutic cloning' certainly creates problems of its own: Vogelstein *et al.* (2002) noted that Antinori tries to justify reproductive cloning by claiming that it is therapeutic for those suffering from male infertility. They point out that he has simply relabelled a controversial technique, but

> If destroying human embryos is one of those inherently unethical means, the ends of technological progress or therapeutic benefit should be pursued by other means