

## **DNA typing in forensic medicine and in criminal investigations: a current survey**

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Since 1985 DNA typing of biological material has become one of the most powerful tools for personal identification in forensic medicine and in criminal investigations [1–6]. Classical DNA “fingerprinting” is increasingly being replaced by polymerase chain reaction (PCR) based technology which detects very short polymorphic stretches of DNA [7–15]. DNA loci which forensic scientists study do not code for proteins, and they are spread over the whole genome [16, 17]. These loci are neutral, and few provide any information about individuals except for their identity. Minute amounts of biological material are sufficient for DNA typing. Many European countries are beginning to establish databases to store DNA profiles of crime scenes and known offenders. A brief overview is given of past and present DNA typing and the establishment of forensic DNA databases in Europe.

**P**ersonal identification and determination of paternity are the two major subjects of forensic DNA analysis. In contrast to clinical genetics, forensic DNA typing examines the properties of non-coding loci spread over the entire human genome. Because of noncoding loci not being expressed, for example, as proteins or ribozymes, DNA typing seldom reveals information about an individual except for his mere identity or his relatedness to other individuals (the others must also be examined). However, in 1997 many European countries are beginning to accumulate enormous databases storing DNA profiles of biological stains at crime scenes and DNA profiles of the body liquids of known and accused offenders. DNA typing today is performed by a method that differs substantially from classical “genetic fingerprinting.” We focus here on the advantages, disadvantages, and possibilities of classical and modern DNA typing as used in forensic practice.

### **Modern vs. classical DNA typing procedures**

DNA typing and “genetic fingerprinting” are based upon the variability of several noncoding DNA stretches in the human genome. Such variable stretches are composed of core units of a fixed nucleotide sequence that are repeated between 2 and 10 000 times, depending on the type of polymorphism. The targets for classical DNA fingerprinting are loci up to 10 kb long. The core units of these repeats are composed of hundreds of nucleotides which can be repeated hundred times. This type of repetitive DNA is called variable number of tandem repeat DNA (VNTR).

VNTRs are displayed by cutting genomic DNA with restriction enzymes such as *HaeIII*, *HinfI*, or *HindIII*,

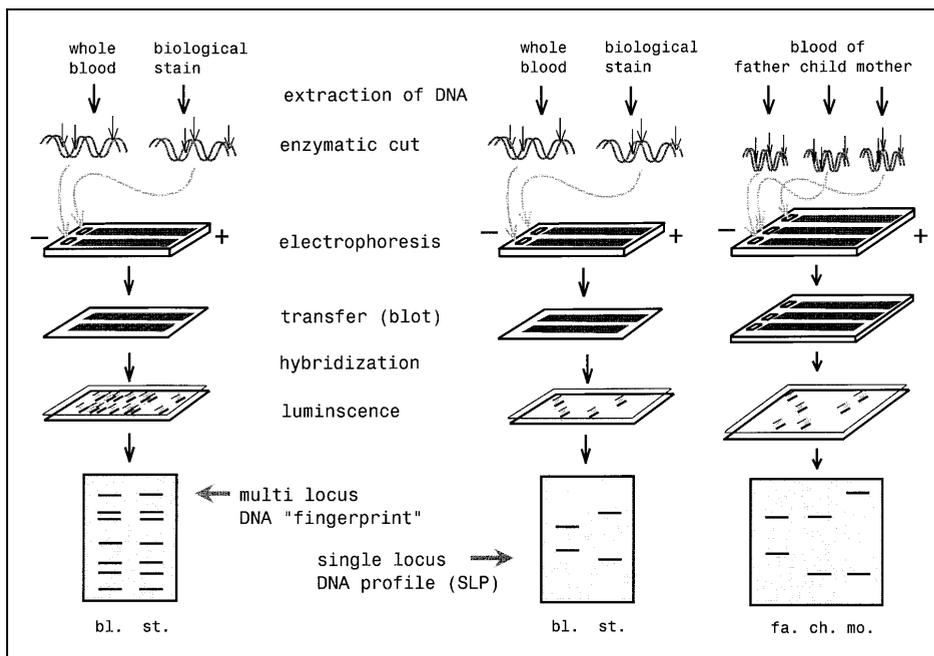


Fig. 1. Schematic representation of classical DNA typing procedures. *Left*, use of low-stringency probe results in multi-locus pattern. Here: match between whole blood (reference) and stain found at crime scene. *Middle*, highly specific probes detect allele(s) at only one locus. Here: no match between reference and stain. *Right*, paternity case; here: profile of child shows one allele of mother and one of father in question – therefore the father in question is biological father

separating the DNA fragments electrophoretically in a gel, and then detecting the variable fragments by the use of short DNA stretches that bind specifically to variable loci (probes). The probes can be labeled with alkaline phosphatase (for chemoluminescent reactions which produce light of 477 nm wavelength) or are composed partially of radioactive nucleotides. In each case a film that is sensitive to X-rays shows dark bands at the detected positions. Depending on the specificity of the probes, a single stretch of variable DNA (high specificity, single-locus probe) or many stretches (low-specificity, multilocus probes) are detected (Fig. 1).

Single-locus probes are used for paternity analysis. One variable allele of the mother and one of the father are passed on to each of their children. Comparing some five single-locus DNA profiles produces strong evidence for identity and for paternity/maternity.

After a period of initial disputes (and scientific inaccuracies) [18–20] DNA fingerprinting became firmly established in forensic science [21–24]. When police recognized the value of DNA analysis, the biological material to be examined changed from liquid blood to dried blood stains, minute amounts of epithelia, hair shafts, bones, and dried sperm. In contrast to whole blood used for paternity testing, biological stains at crime scenes are often exposed to UV (sun)light, humidity, and decay. In addition, clinicians have begun to request analyses of tissue which has been stored for years in paraffin or denaturing preservatives.

A problem in all of these cases is that, depending on the often unknown conditions of storage, DNA is often broken up into pieces (fragmentation is the only relevant type of degradation in forensic science), which means that even small amounts of short-length DNA can be processed in the laboratory. Classical DNA “fingerprints” are produced using at least 5–10 µg nondegraded DNA. Thus, classical technology often cannot be used for analysis of stains containing much lower amounts of DNA and/or only degraded DNA. For example, our experience shows that 1 ml ejaculate contains 150–300 µg DNA, while only 0.01–3 ng DNA can be extracted from 1 ml of a postcoital vaginal swab. A plucked hair with root contains up to 30 ng genomic DNA, but a hair shaft contains maximally 0.1 ng DNA. Especially in (low amounts of) degraded DNA short continuous stretches of DNA are more likely than longer stretches. Forensic DNA typing therefore often requires the use of techniques that allow the detection of short but informative repetitive loci.

Such loci were found 5 years after the introduction of DNA fingerprinting [10–12]. These are composed of core units three, four, or five nucleotides long. The core units are repeated up to a limited complete length of 80–400 basepairs. Both short tandem repeats (STRs) and VNTRs are spread over the entire genome and are situated exclusively in (noncoding) introns, which represent 90% of the human genome. Every person’s genome contains hundreds of STR loci (the exact number is not yet known). For every locus a limited amount of possible alleles – generally five to ten –

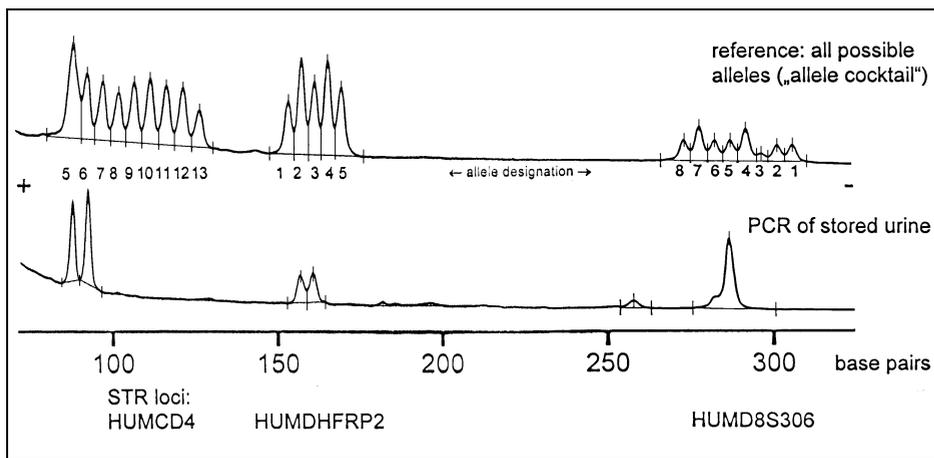


Fig. 2. Simultaneous amplification of three STR loci from DNA recovered from stored urine of a doping control at the Olympic Games in Atlanta. (Urine sample from the Deutsche Sporthochschule, Cologne, Dept. of Dr. Schänzer)

is observed (e.g., [25]). Any person may be either homozygous or heterozygous at each STR site. Comparison of the alleles of a person's body liquid (usually blood, but even saliva is sufficient) to a biological stain at a crime scene or to a body liquid of a child can determine paternity or identity.

STR loci are now the first choice for stain analysis and criminal investigation practice in Europe. In the United States, where STRs are used under restrictions, it was the recent criminal proceeding of the O.J. Simpson case that impressively demonstrated for the public the power of STRs in typing small amounts of DNA [26–28].

To display STRs it is necessary to amplify them using PCR. Up to nine STR loci can be amplified in a single reaction, run by primers detecting specifically a single STR locus (e.g., [29]). Due to the shortness of STRs, PCR of a single STR system often requires only 50 pg template DNA (e.g., [30, 31]). For a single human diploid cell containing approximately 6 pg DNA, at least eight cells are usually sufficient for a preliminary forensic DNA analysis. In rare cases even single cells – mainly sperm – can be typed.

After electrophoretic separation the PCR products are made visible by staining them with silver in a developing bath or by semiautomatic laser detection of the primers, which in this case must be labeled fluorescently [14, 32–34] (Fig. 2). With the increasing throughput rates and the fast, reliable, and comfortable processing of data, semiautomatic detection using DNA sequencing machines is becoming the technique of choice for DNA analysis of stains.

### Size determination, statistics, and significance of data

Forensic DNA analysis is based on the frequency of every DNA fragment at the tandemly repeated re-

gions being known. Knowing the frequency of a certain STR allele or of a fragment length (classical DNA fingerprinting) in a population enables the forensic biologist to calculate how often an allele combination appears in a given population. Because of their high variability, i.e., high numbers of rare alleles, classical VNTR loci alone often lead to much higher exclusion (or inclusion) probabilities than single modern STR systems alone, which often have quite common and widespread alleles. Thus the detection of an allele combination in only a single STR system in a biological stain seldom constitutes conclusive proof of identity. If, however, alleles in stain are observed *not* to be identical to those of a person's reference body fluid, in extreme cases even one STR profile can exclude the person from the suspicion of having left the stain.

Today STR multiplex systems have a discrimination power (i.e., matching probability) greater than a combination of five classical single locus DNA fingerprints [29]. For example, a third-generation multiplex PCR developed at the Forensic Science Service (FSS) in Birmingham matches persons with a probability of  $1:10^{15}$  to a stain. Compared to a total of  $5 \times 10^9$  humans living on earth, a matching probability of  $1:10^{15}$  means that a person with a given STR profile clearly and uncontentiously must be the person who left the biological stain at a given crime scene (exception: genetically identical twins).

For the sake of brevity we mention here only a few references to statistics and allele frequency databases:

- In forensic science the recently developed "exact test" [30, 35] is used to check Hardy-Weinberg-Castle equilibrium of alleles (e.g., [36]).
- Population databases demonstrating how often a specific allele can be expected exist for whites, blacks, eastern Germans, persons in and around Cologne, Lower Franconia (for European populations, e.g., [30, 31, 37–44]) and so on.

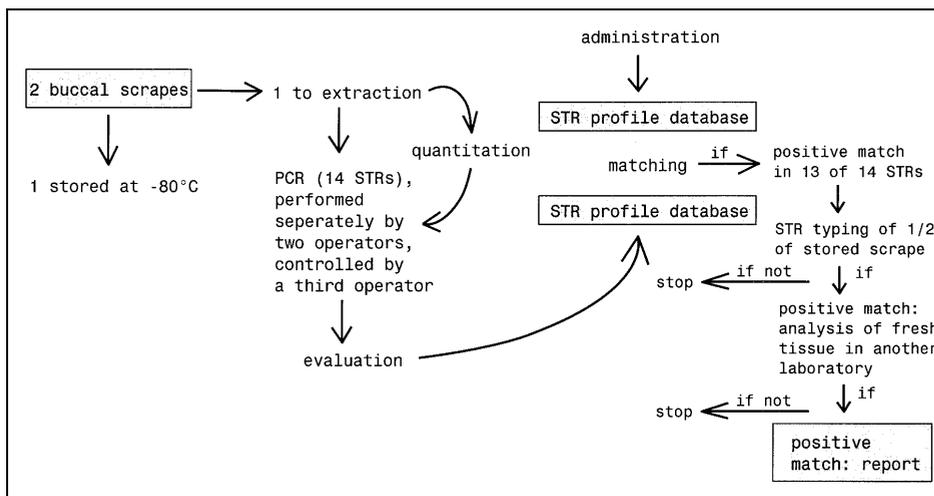


Fig. 3. Processing of samples, storage and matching of STR data at the DNA database at the Forensic Science Service, Birmingham

- STR allele length determination may have implications for the study of human evolution. For example, shorter alleles at STR loci are sometimes more frequent in Asians than the corresponding alleles in whites [45] (on the linking of population genetics and forensic population data see [46, 47]). However, the origin of mutations in STRs is still unclear (e.g., [48]), and further studies on STRs and evolution must yet be performed.
- Validation protocols and interlaboratory blind trials allow complete comparability, experimental reproduction, and quality control of any DNA typing results [49–51]. Blind trials are being performed throughout Europe by the European DNA Profiling Group and within single countries, for example, in German-speaking countries by the German DNA Profiling Group, which is organized by the German Society of Legal Medicine.

## Databases and personal rights

Many European countries (e.g., Denmark, Norway, Sweden, Belgium, the Netherlands, Italy, and Finland) plan to establish DNA databases to store and compare STR profiles of unknown biological stains and known offenders. In Austria there are several local databases, none of which covers the whole country. In the Netherlands a DNA database has existed since 1994. For various reasons Ireland, France, and Germany are not establishing STR profile databases [52].

The United Kingdom is one of the countries in which key research on STR typing was performed, and in April 1995 its Home Office established the first and largest STR database at the FSS in Birmingham [53] (Fig. 3).

In 1993 the Royal Commission on Criminal Justice encouraged the forensic use of DNA profiles, and after a pilot study in 1994 work on database began in June 1994, with the official establishment in March 1995. A criminal justice bill was enacted there which defines hair roots and buccal scrapes as “nonintimate samples.” In the United Kingdom the collecting of such samples is now allowed if the suspect’s offense may lead to imprisonment. (Also in other European countries which are establishing DNA databases, biological material of a suspect is allowed to be processed only if the alleged crime is severe enough to lead to a possible imprisonment for 1 year or more.)

At the FSS the DNA profiles are screened automatically for matches between profiles of (a) person to person(s), (b) person to scene(s), and (c) scene to scene(s). By January 1997 the FSS had matched 1258 individuals to crime scenes and 953 scenes to other scenes. A total of 110 278 STR profiles are now stored, and a further one million will be stored in the FSS database in the coming years [53, 54, 100] meaning that DNA-typing data of a substantial proportion of the nations entire population will be stored. In the future 135 000 DNA samples are planned to be tested per year, i.e., 650 anonymous (bar coded) samples will be processed every workday, at a cost of U.K. £40 per sample.

It must be stressed that both the FSS and all other forensic DNA laboratories take every precaution to avoid confusion of typing results which might lead to wrong matches that could incriminate innocent persons. The typing procedure of the FSS is illustrated in Fig. 3. When a match is found, DNA typing is performed again by a scientist or technician who does not know which sample he is processing (bar code, no information about former typing re-

sult). If a new PCR of the stored biological material confirms the match, fresh material is taken from the alleged suspect and analyzed in another laboratory. Only after a third confirmation of PCR results in 13 of 14 STR systems is the match reported to the responsible authority. (German courts generally consider five or six STRs to be sufficiently strong evidence of identity.) At the FSS practical quality controls are performed continually for all machines, employees, and scientists.

From the point of view of a forensic scientist, it is evident that neither wrong typing results nor misuse of the stored DNA data is possible under the very secure precautions now taken. (Other persons may imagine situations in which DNA databases can be misused.) The principal reason why Germany and France do not allow collection of "nonintimate" biological samples and not store STR profiles in a database is not the fear of misuse or mistyping but the view that *any* sampling of body tissue followed by storage of data violates the individual's privacy. Ireland is not establishing a database because of its lack of infrastructure and the low number of cases examined by DNA typing (around 50 cases per year). In Belgium the special situation of more than a dozen children being sexually abused and murdered in the summer of 1996 led to the establishment of a DNA database within 24 h in August 1996 [55]. Until then the law explicitly prohibited a DNA database. In contrast to the United Kingdom, some countries plan to destroy STR profiles after a certain time, for example, after 30 years in the Netherlands. Even in countries which do not have (or plan) a DNA database law it may be very difficult to sample biological material from suspected persons. For example, in the Netherlands 20 administrative and/or organizational steps are necessary to sample reference material, including the obtaining of full informed consent of the suspect [56]. In some countries a person's refusal to allow sampling can be used legally to incriminate him, but in most countries (including Germany [57]) it is forbidden to use such refusal as an evidence for guilt.

## Further implications of STR typing

Despite the very high-security standards of DNA typing and forensic DNA databases, some experts believe that more fundamental problems may arise which are not directly connected to the actual typing technique and database organization. In regard to the sampling procedure it is sometimes asked whether it is more important to apprehend criminals or to leave

innocent persons undisturbed [58, 59]. Depending on the matching procedure, it is clear that databases such as that of the FSS cannot wrongly match a person to a crime (exception: identical twins). If fewer STR loci were stored and compared, matches might be made that are apparently correct (i.e., for the STRs looked at) but in fact incorrect (i.e., wrong for other STRs).

A third fear arose when a linkage was observed between heritable diseases and some repetitive DNA loci. Actually the observation of certain changes in repetitive DNA stretches allows a preliminary prediction about a person contracting a given disease. Forensic DNA analysis uses principally STR loci composed of tri-, tetra-, and pentameric core units. In contrast, most known repetitive DNA stretches that are linked to diseases have a dimeric substructure. In only one case has a very weak correlation been suggested between a multigenic disease and a STR [99]. At the current state of knowledge it is impossible to obtain any information about a person by forensic DNA typing except for his identity. In addition, two other factors prevent the generating of unwanted genetic data. First, as a matter of principle no repetitive loci are used in forensic medicine that are thought to be correlated with diseases. Second, personal traits and behavior as well as most genetic diseases are in fact caused by a multitude of genes that interact. Such interactive processes cannot be linked to the single repetitive loci examined by forensic DNA-typing procedures. (It is worth mentioning that blood groups, which are routinely determined in general medicine, sometimes have a clear linkage and thus predictive power for genetic diseases.)

In only three cases does forensic DNA analysis (but not analysis of STRs) lead to more than an abstract identity code: (a) after detection of alleles characteristic of a specific ethnic group, (b) by estimating age, which is currently established by detecting a certain deletion in the genome which increases with advancing age [60], and (c), by meta-analysis of paternity data compared to sociodemographic factors. Despite the occasional possibility of meta-analysis (comparison of data already collected but not collated) this does not lead to further conclusions [61]. For example, it has been found that the exclusion probability regarding paternity is higher when only one marriage partner pays for the DNA paternity test than when the two marriage partners share the cost. Determination of sex by detecting Y-chromosomal markers by analysis of STRs and other diagnostic stretches of DNA is a regular part of the forensic identification procedure ([62–64]; by unusual use of a gene [65]). Figure 4 presents an overview of the aims of forensic DNA typing today.

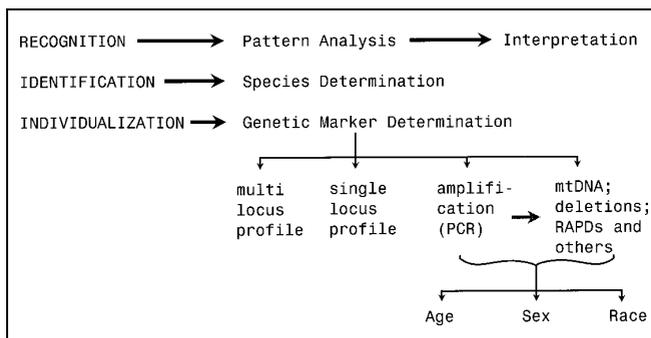


Fig. 4. Present state and aims of DNA-typing techniques. Due to considerable technical restrictions in routine forensic DNA typing, age, sex and race are not determined, although it might be possible sometimes. (Modified after [98])

The German Parliament is currently drafting regulations mandating the anonymous processing of forensically analyzed samples. Forensic scientists object to this [66] because of the need for information regarding the possible relatedness of suspect and victim, which has a great effect on the probability of inclusion/exclusion. In addition, attorneys may begin to dispute the correct coding of probes (in Germany this has not yet happened), thus prolonging of lawsuits [66].

## A challenge: degraded samples

Forensic scientists confront a wide range of biological materials, most of which suffer, for example, from bad quality and quantity of DNA (e.g., [5]). Today's use of STRs allows the typing of such materials. Investigations have been carried out on: cigarette ends [67], skeletal remains [68], urine [69–71] (Fig. 2), tissue on a gun muzzle and on bullets [5, 72], dismembered and decayed body parts [73], paraffin embedded tumor tissue [5], dirt under fingernails [74], epithelia of an offender from the victim's neck after strangling [75], mummified newborns [76], blowflies preserved in ethanol [77], burned corpses [78], dentin [79], dried chewing gum [80], body parts after mass disasters [81, 82], human feces [83], and skeletonized flood victims [84].

However, the challenge posed by DNA degradation sometimes exceeds the scope of current forensic science. Damaged DNA templates (very old bones, hair shafts) and minute amounts of cells occasionally lead to the elimination of single or, in the worst case, all alleles, and occasionally one even obtains nonreproducible results [5]. In contrast to the sometimes weak STR-typing results produced with extremely

bad samples, the DNA typing of STRs leads to clear results in almost all cases, even with macroscopically degraded material.

## DNA typing in forensic zoology

It is beyond the scope of this contribution to detail the extensive research in zoology and veterinary medicine on population genetics, paternity, and classification of animals now being performed by DNA typing. The major application of DNA typing of animals in forensic science is species determination. A number of techniques are presently being used in stain analysis (e.g., a hair of a dog on an automobile bumper versus dog's whole blood as reference material) and in forensic entomology, which determines postmortem intervals by analyzing the developmental status of certain hexapod species on corpses (e.g., [85, 86]). These include STR typing, multilocus and single-locus profiles, and random amplified polymorphic DNA analysis (e.g., [87–92]).

## Concluding remarks

Because of their shortness and the low detection limit – 50 pg DNA is often sufficient for reliable amplification – STRs are often the only target for DNA typing of forensic specimens. Due to their structural similarity, simultaneous amplification of STR loci (multiplex PCR; e.g., [29, 71, 93–96]) followed by semi-automatic detection on a DNA sequencer is possible and allows many materials to be individualized within 24 h. Complex STR systems composed of seven or more alleles generally have a higher discriminatory power than those containing fewer than seven alleles. Because of their high number of (inter-) alleles, complex STR systems often need to be resolved electrophoretically to 1 basepair (e.g., [44]).

STRs often allow mixtures of DNA from different individuals to be detected to a ratio of 1 + 5 (e.g., [31, 97]); in addition, denatured, old, and degraded DNA is accessible for individualization.

STR typing has thus become a common and safe part of the routine forensic analysis of biological samples in Europe and is replacing both traditional serological analyses of blood groups and classical multilocus and single-locus DNA fingerprinting. Interlaboratory blind trials and validation protocols today allow complete comparability, experimental reproduction, and quality control of forensic DNA typ-

ing results. STR typing is being used in an ever increasing number of cases, and the forensic interpretation of STRs is now widely accepted in Europe by both criminal investigators and attorneys.

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