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Molecular markers used in forensic genetics

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Abstract

Forensic genetics is a field that has become subject to increasing interest in recent years. Both the technology and the markers used for forensic purposes have changed since the 1980s. The minisatellite sequences used in the famous Pitchfork case introduced genetics to the forensic sciences. Minisatellite sequences have now been replaced by more sensitive microsatellite markers, which have become the basis for the creation of genetic profile databases. Modern molecular methods also exploit single nucleotide polymorphisms, which are often the only way to identify degraded DNA samples. The same type of variation is taken into consideration in attempting to establish the ethnicity of a perpetrator and to determine phenotypic traits such as the eye or hair colour of the individual who is the source of the genetic material. This paper contains a review of the techniques and molecular markers used in human and animal forensic genetics, and also presents the potential trends in forensic genetics such as phenotyping.

Keywords

Forensic genetics, STR, SNP, forensic DNA phenotyping

Introduction

The main aim of investigating authorities is to identity those individuals responsible for committing crimes, and this goal is increasingly achieved with the aid of science. During an investigation, forensic science helps to reconstruct the course of events that occurred when the crime was committed. One way that this is achieved is by comparing samples from the crime scene with material derived from suspects, ultimately resulting in an opinion that is presented in court. Opinions, depending on the strength of the evidence on which they are based, may vary in their value for the justice system, from those based on circumstantial evidence to extremely powerful opinions that play an important role in the successful identification of criminals and exoneration of innocent people.

Contemporary forensics is becoming a highly interdisciplinary science, drawing from numerous fields such as dactyloscopy, traceology, biology, veterinary forensics[1] and computer forensics. A relatively young branch of forensic science is forensic genetics, which brings the the higher confidence in results than previous methods based on morphomethrical traits. Identification analysis was previously based on morphometric characteristics, fingerprints or blood groups, which constituted a certain breakthrough. However, blood-group antigens were not highly stable, and their level of polymorphism was low. Analyses were gradually expanded to include the polymorphism of different proteins, and thus by the 1970s, electrophoresis of proteins such as plasminogen or transferrin had become the dominant technique. Numerous proteins were discovered which could be used for forensic purposes, mainly red and white blood-cell enzymes such as phosphoglucomutase 1 or esterase D.[2] A true breakthrough occurred in the second half of the 1980s research on non-coding fragments of the myoglobin gene showed that they were highly polymorphic between individuals.[3] This

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observation led to the emergence of a new branch of forensic science: forensic genetics.

Minisatellites: The first markers in forensic genetics

The use of forensic genetics for identification purposes largely results from two rules. First, a single cell with a cell nucleus contains the complete genetic information about an organism. The second rule, which forms the basis of all branches of criminalistics, is Locard's exchange principle, formulated in 1928.[4] According to this principle, contact between two objects leads to an exchange of substances between them, so that the perpetrator both takes material away from the scene of the incident and leaves traces of his or her presence. In this manner, the biological matter left behind, containing complete information on the organism of the perpetrator, enables individual identification.

During its 30-year existence, forensic genetics has made use of a variety of genetic markers. The first of these were minisatellites: sequences differing in the number of repetitions of the core motif composed of 9–80 nucleotides. Differences in sequence length between individuals provided the basis for using polymorphisms in identification tests. The method was developed by Jeffreys et al.,[2] who analysed the hypervariable regions contained in introns of human myoglobin and demonstrated the presence of highly variable regions of DNA. They analysed variable number tandem repeat (VNTR) minisatellite sequences using the restriction fragment length polymorphism technique. Using this technique in combination with hybridisation techniques, they succeeded in obtaining an unique individual genetic fingerprint (DNA fingerprint).[5] The key was the probe that bound to an internal sequence of minisatellites that is also present in other minisatellites. The next step was the development of single locus probes. Jeffreys was a pioneer in forensic genetics who first used molecular identification for forensic purposes in the famous Pitchfork case in which Colin Pitchfork was the first person to be convicted on the basis of the results of DNA tests.[6]

The next breakthrough achievement, not only in forensic science but in all of molecular biology, was the development of the polymerase chain reaction (PCR) technique. This technique was originally used for the amplification of sequences containing VNTRs. The products were separated in polyacrylamide gel, making it possible to avoid hybridisation and contact with radioactive isotopes. Due to the relatively short time needed to conduct the analyses and the possibility of testing degraded or trace quantities of DNA, variants of the PCR technique still play a major role in contemporary forensic analysis.[7]

The method of individual identification based on VNTR polymorphism, despite its relatively high power of discrimination, was not without disadvantages. A significant problem was that a large quantity of un-degraded biological material was required for analysis, which is not always possible in practice. Therefore, further research was needed to find new markers which would increase the reliability of analyses and make it possible to test partially degraded material occurring in trace quantities.

Short tandem repeat polymorphism: The gold standard of contemporary forensic genetics

A new group of markers was microsatellites, that is, short tandem repeats (STRs) in which the core motif consisted of two to seven nucleotides. STR sequences are much shorter than minisatellites and therefore trace quantities of degraded material can be analysed.

STR markers were first used in the early 1990s and were quickly adopted for the purposes of individual identification.[8] The first multiplex system (QUAD), amplifying four STR markers, was designed by Forensic Science Service in the UK and was introduced to forensic practice in 1994. Microsatellites gained in popularity mainly due to their high level of polymorphism, providing results which constituted very strong evidence.

Variants of short, repeating DNA sequences are also found on the sex chromosomes – Y-STRs and X-STRs – and on autosomes. In paternity testing, the X chromosomal STRs can be used when the disputed child is a girl, and also when the alleged father is absent (then the markers of the X chromosome, present in the mother of the defendant and the child, allow for inference about paternity) and in case of incest.

Autosomal STRs are of greater importance for individual identification, while the information contained on the X and Y chromosomes is useful in determining the sex of the individual who was the source of the material. Human sex identification is mostly based on the amelogenin gene (AMEL) because of the differences in amplicons sizes observed between the X- and Y-specific gene.[9] However, there is a possibility of mutation in the amelogenin fragment range, which results in an erroneous indication of ancestry. Therefore, additional sex-identifying markers are used. Besides the AMEL locus, Y indel or Y-STR systems are included in the latest sets of Globalfiler, PowerPlex Fusion 6C, which not only increase the

probability of identity but eliminate possible misinterpretation resulting from the AMEL mutation.

Analysis of Y-STRs is also used during analysis of mixtures of biological material, for example in the case of rape or gang rape, as well as in cases in which there is a negligible amount of male DNA amid a large quantity of DNA from a woman.

Y-STR polymorphisms have been used in dragnets in some countries. Due to the uniparental inheritance of Y chromosome genetic material, the Y-STR profile of related individuals is identical. This means that a group of suspects can be narrowed down to individuals who share the same Y chromosome haplotype, and among these, the individual whose autosomal profile matches the evidence can be identified as a possible suspect. In Poland, this method was first used in the case of a rapist and murderer from Świnoujście. The analysis was based on nine polymorphic loci of the Y chromosome. Tests were performed on 400 suspects of whom one had an identical DNA haplotype in all Y-STR loci and 9/10 identical autosomal alleles, which suggested that the donor of the reference material and the perpetrator were related. Additional analysis confirmed that the profile of the evidence material was identical to the genetic profile of the brother of the man who was primary genotyped during dragnet. Analysis confirmed an exact match in all 19 tested loci (10 autosomal STRs, and 9 Y-STRs).[10]

Due to the fact that as the number of loci analysed increases, the random match probability (the probability of two unrelated individuals having the same genetic profile) decreases, microsatellites were analysed using a modification of PCR known as multiplex PCR.[11] This technique involves simultaneous amplification of many loci to obtain a panel of microsatellites. A standard analysis usually includes 15–16 STR loci ranging from 90 to 500 base pairs (bp) and various sets of primers.[12] In recent years, there has been a tendency to increase the number of loci amplified in order to avoid adventitious matches as databases grow, and nowadays the standard in many cases is extended up to 24 STR loci. The personal identification kit market has become dominated by three companies. One of these is Promega, offering the PowerPlex ESX/ESI and the PowerPlex Fusion system based on current five-dye technology amplifying 24 polymorphic loci. The former, with 16 loci and the amelogenin sex indicator (in which the length of the sequence depends on the sex of the individual), is a commonly used kit in forensic laboratories.

Even better power of discrimination may be obtained with the PowerPlex® Fusion 6C System, which is 27-locus, six-dye, and multiplex. In the case of the PowerPlex Fusion, the probability of identity value (probability that two individuals selected at random will have an identical genotype at the tested locus) is 6.58×10^{-29}, whereas probability of identity value for the PowerPlex® Fusion 6C is estimated to be 2.30×10^{-32}.[13] Another company offering personal identification kits is Thermo Fisher Scientific whose GlobalFiler kit, amplifying 24 loci – 21 autosomal STR loci, the Y chromosome STR locus and the Y indel – and the sex-determining marker-amelogenin, has a probability of identity about 10^{-26}.[14] The Investigator 24plex QS Kit[15] by Qiagen, which is also based on amplification of 24 STR sequences, contains an additional control in the form of a quality sensor.

The widespread use of STR polymorphism as the leading method in forensic analysis results not only in the appearance of new identification kits with ever-increasing powers of discrimination, but also in the creation and expansion of genetic profile databases. In 1995, a nationwide DNA profile database was established in the UK: the UK National Criminal Intelligence DNA Database (NDNAD).[16] The standard which underlies databases in the United States is the Combined DNA Index System (CODIS), developed in 1993 by the Federal Bureau of Investigation, which serves mainly to gather and compare DNA profiles. Until recently, the system consisted of seven STR loci (also used in European countries in accordance with the recommendations of the European Network of Forensic Science Institutes (ENFSI), six additional loci and the amelogenin gene locus.[17] In January 2017, seven more markers were added to CODIS, which is currently based on a panel of 20 polymorphic loci.[18] The system in use in Europe is based on 12 STRs in accordance with ENFSI recommendations from 2009.[19] The number of loci needed to upload to a criminal justice DNA database varies greatly from country to country.

One of the most commonly used individual identification kits which meets these requirements is the AmpF/STR® NGM™ PCR kit, which contains 10 STR loci from the previous SGM Plus kit, five new loci recommended by ENFSI and the highly polymorphic SE33 if needed. The profiles obtained form the basis for the creation of databases; the condition for deposition of a profile in a database is identification of at least eight complete STR loci. DNA profile databases are used to compare deposited profiles with material secured at the crime site. To estimate the probative value of a given profile, forensic genetics uses statistical computations carried out on population databases. These analyses make it possible to determine the frequency of individual alleles in a population, which is a significant factor in the choice of polymorphic regions for identification purposes and determines the probative value of an expert opinion.

STR polymorphism can also be used for the individual identification of animals in cases of crimes such as poaching, animal cruelty and collecting and smuggling endangered species. Highly polymorphic microsatellite markers enable determination if two analysed samples derived from the same individual. There are STR reagent kits that have been designed for individual identification of companion animals such as dogs and cats. The Canine Genotypes Panel 2.1 Kit (Thermo Fisher Scientific), amplifying 18 polymorphic loci and sex determining locus ZFX/Y is considered an informative and robust tool for the identity and parentage testing of dogs, and can be applied in such forensic cases as a dog attack or animal abuse.[20,21] Commercial kits are also available for cats (the MeowPlex Kit amplifying 11 STRs markers and a sex identification marker) that can be used when cat hairs are evidence associated with crime scenes.[22] The identification of animals based on STR markers also plays a growing role in solving crimes against wildlife, including the ivory black market,[23] poaching and smuggling of endangered species.[24]

Single nucleotide polymorphisms as a supplement for STRs

The polymorphism of STR sequences is increasingly being supplemented with a different type of DNA polymorphism involving single nucleotide mutations in DNA. Polymorphic nucleotides arise as a result of point mutations, which may be indels (insertions or deletions) or substitutions. Single nucleotide polymorphisms (SNPs), despite having much lower discriminatory power than STR markers, are a good solution in the case of identification of degraded genetic material. Butler et al.[12] estimated that a level of discrimination corresponding to a panel of 15 STRs can be obtained by analysing 40–60 SNPs. To increase the power of discrimination and reduce analysis time, SNP multiplexes are developed to enable simultaneous analysis of >100 polymorphic loci.[25] A huge amount of SNPs may be analysed by massively parallel sequencing (MPS), which is technology based on next-generation sequencing (NGS). MPS enables combinations of several traditional electrophoresis assays into one single MPS assay. The single analysis may be used to detect >160 ancestry informative SNPs simultaneously. Therefore, this technique constitute a potential tool for the identification and inference about the biogeographical ancestry of the sample donor.[26] However, despite the higher number of analysed loci, the power of discrimination of SNP panels is lower than in STR. Therefore, SNPs are a supplement for STR markers rather than a replacement.

SNP panels are increasingly validated and introduced in population studies, making it possible to determine the suitability of a kit for forensic purposes. A certain compromise between STRs and SNPs is making use of the deletion–insertion polymorphism (DIP) based on the analysis of short amplicons as in the case of SNP while applying the techniques used in STR analyses. Simultaneous amplification of 30 markers in a Czech population indicated a combined power of discrimination at a level of 99.9999999999%.[27] The kit has also been validated on individuals in the Polish and Taiwanese[28] populations, and in each case, DIP was shown to be potentially useful in identification tests.

SNP markers are very successfully used in analyses of samples from disaster victims or archaeological finds which contain a small quantity of degraded genetic material, mainly mtDNA.[7] In analysis of STR markers, longer fragments are used in which a high level of degradation makes it impossible to obtain a complete profile. One solution enabling analysis of low-quality samples is the miniSTR method. The use of specific primers which bind closer to the microsatellite sequence results in shorter amplicons than in the case of basic STR markers, which means that miniSTRs can be used to analyse degraded DNA.[29] This solution is used practically in the PowerPlex® ESX 17 System partially based on miniSTR (amplicon size <125 bp) and midiSTR (amplicon size 125–185 bp), which shows higher tolerance to common inhibitors and enables genotyping of degraded DNA samples. Shorter products of amplifications of different loci are obtained by use of the PowerPlex® ESI 17 Pro System, which amplifies six of the original seven European Standard Set loci as shorter amplicons (size <250 bp).[30] These two STR systems can be used to complement each other when analysing degraded and challenging samples.

Analysis of SNPs in the case of biological traces often involves mitochondrial DNA, which may enable the identification and analysis of relatedness when nuclear DNA is not available. Mitochondria, as semi-autonomous organelles, are surrounded by a double membrane, and the DNA contained in them has a circular form, which makes mtDNA more resistant to degradation than nuclear DNA. Because of these properties, genetic material can be obtained from biological samples that have been subjected to various physical and chemical factors, from bones that are several thousand years old or from hair lost post-mortem.[31] An important characteristic of the mtDNA molecule is its uniparental, maternal inheritance. In the human mitochondrial genome, containing >16.5 kbp, SNP analysis is performed on non-coding fragments containing hypervariable regions, as well as fragments encoding respiratory chain enzymes.

Hypervariable regions situated on the D-loop in the control region have the highest degree of polymorphism.[32] Three hypervariable regions are distinguished – HV1, HV2 and HV3 – within which AC polymorphic dinucleotide repeats occur, appearing in the genome from three to seven times.[33] All polymorphic mtDNA sequences obtained are compared to the revised Cambridge Reference Sequence, also known as the revised Anderson sequence.[34] According to the guideliness for mitochondrial DNA typing, recommended by the DNA Commission of the International Society of Forensic Genetics, the entire control region is sequenced in order to obtain the most reliable results.[35]

One area of forensic science associated with mitochondrial polymorphism, and with the DNA barcoding based on it, is wildlife forensics, which deals with crimes committed against protected species. Species identification is performed using sequence analysis of the *cytochrome b* gene, which is highly conserved within the same species.[36]

In the case of sequencing of the *cyt b* gene in various vertebrate species, including fish, amphibians, reptiles, birds and mammals, the use of one pair of universal primers has proved sufficient for species identification. There are validated primers for *cyt b*, and these span areas of sufficient variation to differentiate even closely related species. BLAST software is used to compare the DNA sequences obtained with other *cyt b* sequences deposited in the online database GenBank. This means that in this type of test, the species can be identified in the absence of reference material. Polymorphisms in the 12S rRNA and 16S rRNA genes, cytochrome oxidase I (*COI*), and in the case of plants *trnL* and *rbcl*, which are also used for species identification, are used in a similar manner. *COI* was also adopted by the Barcode of Life consortium to analyse biodiversity and is supported by the database Barcode of Life Database.

Uniparental inheritance also takes place in the case of the Y chromosome, and therefore it can be used to analyse relatedness. The nucleotide sequence of the Y chromosome determines the male sex and is inherited exclusively through the paternal line. In additional to determination of relatedness, both Y-SNP and Y-STR polymorphisms enable identification of the male DNA fraction present in a mixture, mainly in the case of sexual offences. SNPs are the result of point mutations taking place during human evolution, and therefore Y chromosomes with the same single nucleotide mutations and having a common origin are termed haplogroups and can potentially be used to provide information about the possible geographical ancestry of the perpetrator of a crime.[37]

SNPs play a very important role in forensic genetics not only in identifying individuals, but also due to the possibility of determining their phenotypic characteristics.[38,39] Eye colour is determined using a commercial panel, IrisPlex, developed on the basis of six SNP polymorphisms,[40] and when expanded to include an additional 18 polymorphisms, the kit (HIrisPlex*)* can also be used to predict hair colour.[41] The new kit (HIrisPlex-S), includes additional SNPs (17 skin colour prediction SNPs) enabling the prediction of the skin colour in addition to the colour of the eyes and hair.[42] Intensive research on the effect of SNPs on phenotypic traits is ongoing, and databases with SNP variants are being created, such as SNPedia. The large number of SNP profiles contained within it, characteristic of given genotypic effects, provides excellent support for identification tests.

Technological progress and the development of forensic genetics

Forensic genetics is an extremely dynamic and rapidly evolving field. Besides the search for new markers, new techniques have been developed which have resulted in modifications of PCR aimed at simplifying the analysis, increasing its reliability and at the same time reducing its time and cost. The sensitivity of analysis of microsatellite sequences from minimal quantities of DNA, obtained for example from trace evidence, is improved not only by optimising isolation protocols but also by introducing DNA purification and concentration kits, making it possible to identify alleles which cannot be detected using classical protocols.[43]

The PCR method is the basis for numerous new methods, some of which meet the requirements of law-enforcement agencies. One such solution is the direct PCR method, which integrates isolation and amplification processes and makes it possible to obtain genetic profiles of individuals from trace quantities of degraded DNA material, and also to identify DNA rapidly from reference material. Research shows that as a result of the washing which is necessary during isolation and the irreversible binding of some of the DNA to the silica resin, 50–90% of the initial quantity of DNA is lost.[44] By combining the isolation and amplification steps and by using polymerases with greater tolerance for inhibitors, potential losses can be avoided and the sensitivity of the method increased. This makes it possible to obtain genetic profiles from trace evidence and from poor sources of genetic material such as fingernail clippings or scrapings or single hairs that have fallen out in the telogen stage.[45,46]

Another trend is the reduction of analysis time, which in the case of standard laboratory analyses is at least two days (12 hours of laboratory work). Direct PCR kits reduce analysis time and produce a

result within just 25 minutes.[47] The essence of the accelerated reaction is modified time and temperature conditions, optimised buffers and the use of polymerases that amplify the target sequence at a higher rate.[48] A practical and innovative means of using rapid technology is the ParaDNA Intelligence System, which enables rapid analysis of five STR loci and the amelogenin gene in about 75 minutes. The system consists of a plate with reagents for amplification and visualisation of reaction products and an apparatus which allows the analysis to be conducted outside the laboratory.[49] A similar solution with a much greater power of discrimination is RapidHIT technology, which produces a profile based on 15 STR sequences within 90 minutes.[50] These solutions unquestionably simplify and speed up analysis, but it is still good practice to verify the result in a fixed laboratory.

After amplification, the length of PCR products is analysed to determine the number of repeats of particular alleles. Estimation of amplicons length is made by sized-based separation in gel or via capillary electrophoresis (CE). Nowadays, CE is the gold standard in forensic genetics, as amplified loci are separated not only by size but also by dye colour, enabling the analysis of more polymorphic loci in one run with higher precision. Obtained data are analysed by software, which determines STR size.

The trend of NGS appearing throughout molecular biology also has potential application in forensic genetics. The possibility of learning an entire genome sequence enables not only detection of new markers, but also simultaneous analysis of numerous types of polymorphisms (STR, SNP or indel polymorphisms). The high-throughput technology of NGS has many advantages, the most important of which include the favourable ratio of the cost of the analysis to the amount of information obtained, the possibility of simultaneously analysing multiple microsatellite loci on both autosomes and heterosomes and the ability to analyse DNA mixtures.[51] An innovative tool based on NGS technology is ForenSeq DNA Signature Prep Kit (Illumina). This kit allows simultaneous testing of 96 samples DNA in the range of 58 STRs (27 autosomal STRs, seven XSTR and 24 Y haplotype markers) and 94 identity-informative SNPs, or 32 samples containing the above-mentioned markers plus 56 ancestry-informative SNPs and 22 phenotypic-informative SNPs (two ancestry-informative SNPs are also used for phenotype prediction).[52]

NGS is also useful in other areas of forensic genetics. It can be used for epigenetic studies[53] or analysis of environmental samples (metagenomics).[54]

Directions of research are not limited to individual identification, but also involve attempts to characterise a potential perpetrator on the basis of his or her genetic information. Transcriptome analyses can be used to determine what kind of biological trace the genetic material is from. RNA was considered to be very non-persistent, and its stability usually ranged from a few minutes to a few days, whereas miRNA molecules remain stable for several weeks and can be used to obtain a profile even from degraded material.[55] However, an increasing amount of research has confirmed the utility of mRNA, even in cases of samples >20 years old. In particular, β-haemoglobin has been identified as a very persistent mRNA molecule that can be suitable for profiling old blood stains.[56]

Analyses performed using multiplex reverse transcription (RT)-PCR can determine the origin of specific transcripts,[57] and additional quantitative RT-PCR analysis enables determination of their level. Data obtained in this manner are used to establish an expression profile based on which the source of the biological material can be concluded, and with an additional analysis of the degree of degradation of the transcript, an attempt can be made to determine information such as time of death.[58]

Analysis of changes in the mRNA profile can be used to determine the circumstances leading to a death. A study by Ikematsu et al. on two groups of mice, of which the first were killed by slow suffocation and the second by decapitation, indicates that gene expression varies depending on the cause of death.[59] Analyses of mRNA can be used to determine the time when injuries occurred based on expression of cytokines, interleukins, metalloproteases and chemokines involved in the healing process.[60]

A relatively new aspect which is increasingly applied in forensic genetics is epigenetics. One type of epigenetic modification is methylation, which takes part in the regulation of gene expression. The methylation pattern of individual genes can be used to determine what kind of tissue the genetic material comes from, and it can be combined with an individual identification panel.[61] A much more interesting application of this DNA modification is its use to determine the approximate age of the individual the trace comes from. The ageing markers thus far used in forensic biology include accumulation of D-aspartic acid[62] or telomere shortening.[63] Observation of the relationship between the degree of methylation and the age of the organism has set a new trend, which is becoming increasingly popular, resulting in an increasing number of potential marker genes of the ageing process in both humans and animals.[64,65] Determination of age is one of the elements of a new trend in forensic genetics known as forensic DNA phenotyping, which aims to determine the perpetrator's external phenotypic traits or 'externally visible characteristics'.[66] The greatest obstacle in the case of phenotyping seems to be the fact that

phenotypic characteristics are determined by multiple genes, and expression also often depends on environmental factors. This means that determination of the age of a perpetrator is subject to error, as chronological age may differ from biological age. Therefore, presently it is not possible to obtain high probable results but merely an estimated phenotype.

In the UK, genetic profiles of individuals were previously stored in databases, even after the suspects were found innocent. However, issue of storage of profiles from unconvicted individuals led to a challenge in the European Court of Human Rights in 2008 and led to a change in the UK law so that profiles from those found not guilty are deleted in most cases. The British database currently contains profiles for nearly 10% of UK residents,[16] some of which come from volunteers. The British NDNAD does not store profiles from volunteers. These may be taken for varying reasons, such as elimination in a case. They are held separately and can only be used in relation to the case for which they are collected, and none of them go on the reference named DNA database. Despite some social controversy, genetic profile databases are an important tool for law-enforcement agencies. Since 1998, the British NDNAD has assisted in solving >300,000 crimes,[67] and has also proven useful in identifying missing persons or disaster victims. Although profile databases have come under mostly unexemplified criticism, taking into account the ease and speed of movement between countries or continents, the integrated profile database system seems to be a necessity whose contributions argue in favour of maintaining and developing it.

Conclusions

Forensic genetics is an extremely dynamic field. The development of new techniques and improvement of existing methods provide crucial support for the forensic sciences and law-enforcement agencies. Molecular analyses conducted for forensic purposes cover increasingly large regions of the genome, while non-coding regions remain the primary source of polymorphic sequences used for identification purposes. Ethically controversial analyses of coding fragments aimed at determining phenotypes on the basis of information encoded in DNA are also becoming increasingly common. The trend towards reducing analysis time and increasing sensitivity necessitates the development of new technological solutions, resulting in techniques such as direct PCR, which are increasingly popular among both researchers and law-enforcement agencies. In addition to developments in technology, databases are also growing as they gather more and more profiles. Three decades of forensic genetics have not only enabled the development of this field in multiple directions, but also solidified its position among the forensic sciences.

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