Paper

Analysis of Polymorphic DNA Sequences in the Identification of Individuals and its Possible Use in Biometric Systems

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Abstract—The article presents the achievements in DNA forensic science practice, the latest developments as well as future trends. The article concerns also other than forensic DNA applications as well as expectations, concerns and obstacles. DNA identification technology unlike other biometric techniques requires the collection of biological material and the identification is not performed in real time. DNA utilized in most of forensic identification, is present almost in every living cell in the body. What is more, each cell of the same body has the same DNA molecule which means that it is possible to compare the DNA sampled from different sources, for example saliva with blood or semen from the same person. Rapid development and reliability of DNA technology contribute to the fact that the analysis of polymorphic DNA sequences constitutes a very important evidence used in the court. The unique properties of DNA and rapid development of DNA analytical devices allow to claim that DNA may assume a more important position amongst biometric data in the future.

Keywords—DNA identification, DNA matching, genetic marker, genetic profiling.

1. Introduction

Currently, a growing significance of biometry can be observed in forensic analyzes using biological characteristics as a basis for human identification. Forensics has become a field in which the advances in biometry have met the demand for scientific evidence in criminal case processing for the purposes of justice.

In Argentina in 1892, a novel technique was used to identify a murderer. The perpetrator was apprehended thanks to the "fingerprints" left on the crime scene. This was the first time that fingerprints were used as a proof confirming one's identity [1]. Ninety years later in Leicester county, two girls were murdered. As in the previous case, the perpetrator was found using a novel technique, although at that time the method used was completely different. The Leicestershire murderer was captured thanks to a test allowing the identification of individual differences in the genetic material left on the crime scene [2]. That "genetic fingerprint", just as the proof found by Argentinian investigators, indicated unique personal characteristics of an individual. These two events – the oldest and the latest discovery in the field of forensic science – confirm the extensive development in this area that continuously aims to search for more efficient methods of identification. Modern forensic genetics has met this need and developed effective and reliable methods for human identification based on DNA analysis. The significant technological advances in this field and widespread acceptance in the scientific community make the results of DNA tests a highly estimated evidence used in the proceedings of criminal justice. Surveys carried out among judges of regional and district courts, regional prosecutors and attorneys of appeal districts demonstrated that experts' opinions supported by genetic test results are the strongest evidence with the highest level of confidence that affects the decisions of criminal justice officials, as compared with other evidence using biological characteristics as the basis for human identification in forensics [3].

The great advances in genetic testing were encouraged by technical inventions, such as DNA sequencing and oligonucleotide synthesis. The use of such tools as restriction enzymes, vectors or hybridization with molecular probes permitted the practical use of DNA tests.

This paper is organized as follows. Section 2 describes the possibilities in obtaining samples for the purpose of DNA testing. Section 3 presents the structure of DNA and the human genome structure and precedes the Section 4, where DNA polymorphism sequences are described. Section 5 is dedicated to using the results of the DNA analysis in databases and international data exchange. Section 6 discusses the use of DNA tests for the judicial purposes. Section 7 describes how expert opinion and his or her conclusions should be understood. In Section 8 the future of DNA technology in the context of forensic science is discussed. Section 9 presents the future perspective of DNA polymorphism in biometric systems. Finally the paper is concluded in Section 10 where expectations and obstacles in using DNA as a biometric data have been emphasized.

2. Biological Traces

The frequent use of DNA tests in forensic science stems from, e.g., the possibility of testing a wide range of bio-

logical material, such as blood, saliva, semen, hairs, bones, teeth and soft tissues, and in the recent years, the so-called contact traces, i.e., traces of sweat and sebum (also responsible for leaving fingerprint patterns), left upon the contact of a person with an object. The latter also include other biological traces, not noticeable with a "naked eye", unwittingly transferred from different body parts, such as traces of nose secretion or tears left on hands, traces made by nail biting, scratching, etc. [4]. DNA analysis made obsolete advanced tests related to blood groups and serum protein polymorphisms that required large quantities of biological material to enable identification of no more than a group of people potentially leaving the examined trace evidence. The study material is no longer proteins, but the directly inherited material – DNA. This has significantly reduced the waste of valuable material, since to conduct a complete genetic analysis leading to the identification of a person, as little as approximately 0.25 ng DNA is required. Such quantity of DNA can be found in approximately 40 nucleated cells.

3. Human Genome

Most human cells have two types of DNA constituting two separate genomes: nuclear and mitochondrial. In the process of a person forensic identification, nuclear DNA is the primary biometric trait used. Each living cell of the human body (except for mature erythrocytes) is a source of identical DNA molecules that virtually do not change over the entire life. Therefore, it is possible to compare the DNA of cells originating from materials such as sperm found at the crime scene with blood cells or cells observed in saliva, taken as reference material. Moreover, nuclear DNA is very well protected by nuclear membrane and the DNA molecule itself is quite stable and resistant to environmental factors. It is therefore often possible to analyze DNA in biological material reused after many years, coming from the cases discontinued due to failure to identify the perpetrator.

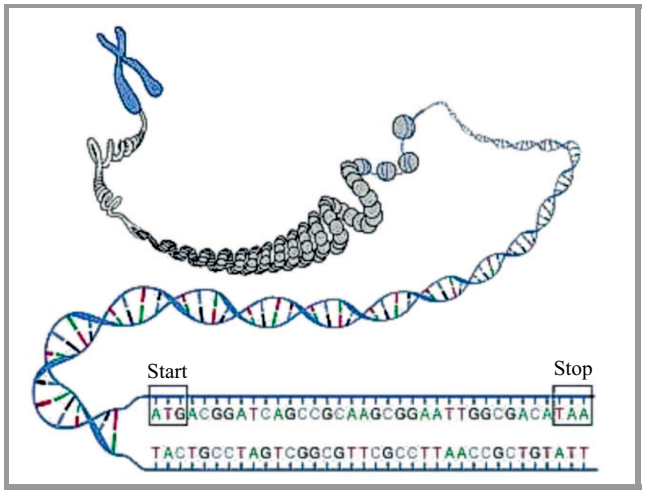

Fig. 1. The DNA structure [5].

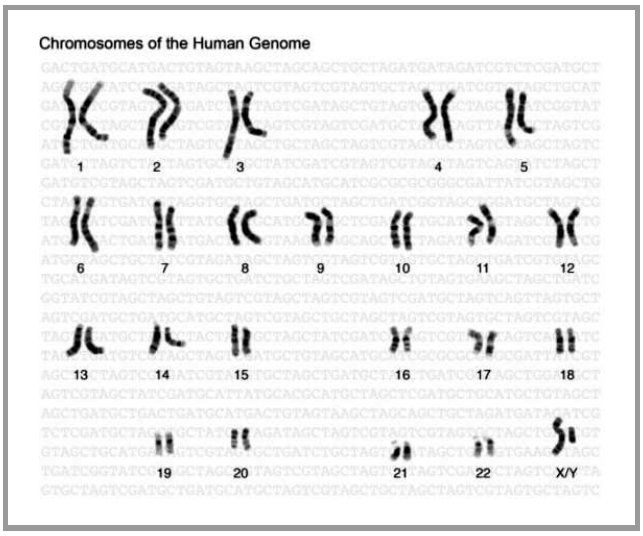

Fig. 2. The set of 46 human chromosomes [6].

Human genomic DNA is divided into 23 structural-functional units called chromosomes (Fig. 1). Most cells of the human body carry 46 chromosomes. Reproductive cells are the exception. The spermatozoon and the ovum have one complete set, i.e., 23 chromosomes. At the time of fertilization, the ovum and the spermatozoon fuse, which results in the formation of a cell containing two complete sets, i.e., 46 chromosomes, identical to those originally inherited from the parents (Fig. 2).

The matching chromosomes forming a pair (the so-called homologous chromosomes), one inherited from the mother, the other from the father, have approximately the same set of genes. The physical structure of DNA is highly ordered, i.e., every gene or marker (DNA fragment used in studies) has its defined localization on the chromosome called *locus* (pl. *loci*) [7] (Fig. 3). Specific DNA sequences identified at any particular locus always have their counterparts on the homologous chromosome. The sequences may be identical or may represent two different variants of the same sequence inherited in loci from both parents. This is one of the key facts underlying the variations in human DNA.

4. Polymorphic DNA Sequences

In human, genetic information is encoded by approx. 3 billion pairs of nitrogenous bases (bp) [8], representing the letters of the DNA "alphabet". The human genome is composed of nearly 25 thousand genes. During the 3 billion years of evolution from the simplest organisms to the currently living mammals and plants, genomic DNA content increased by approx. 1,000 times. Initially, it was believed that the increase in the content of DNA reflected the increase in complexity, whereby newly developed and often complex metabolic and developmental traits had to be encoded by new genes. However, it was discovered that the quantity of DNA in the genome is often not necessarily correlated with the position of an organism on the "evolutionary ladder". Currently, it is known that as little as 1.2%

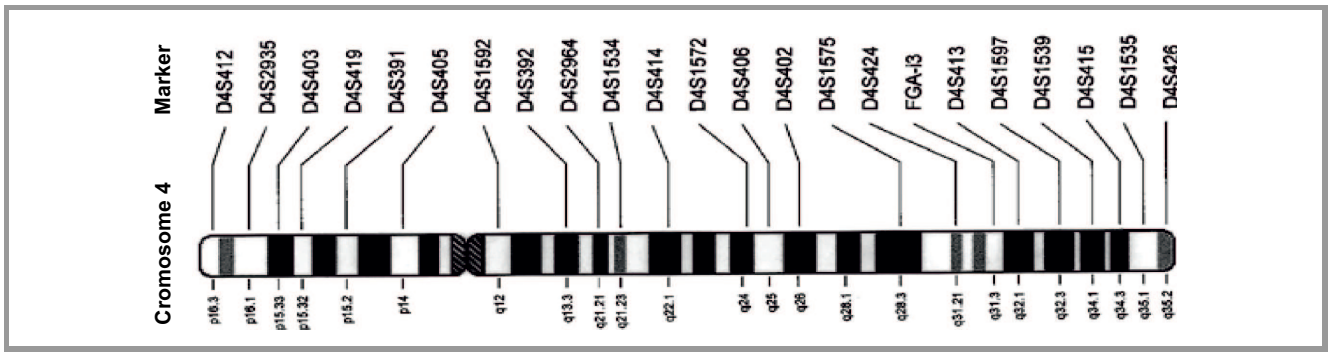

Fig. 3. Examples of localization of markers on chromosome 4 [10].

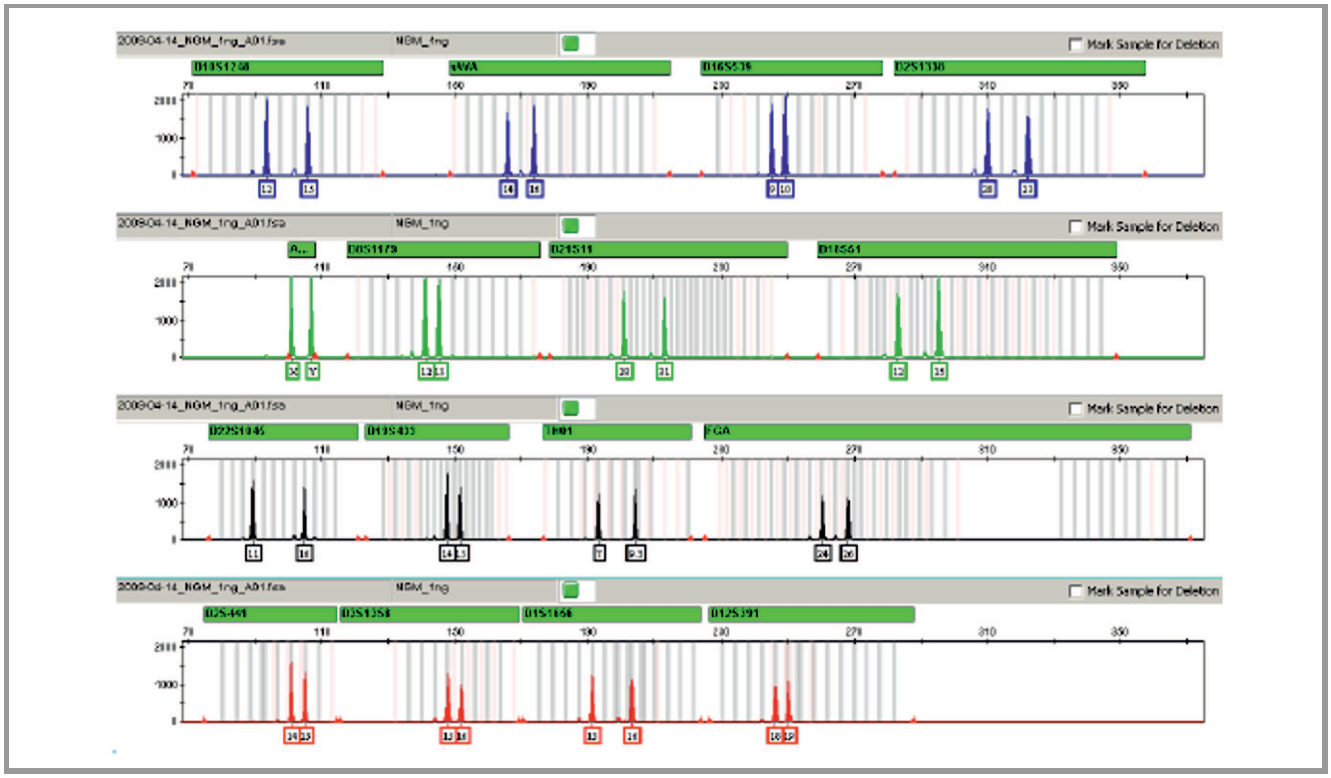

Fig. 4. DNA profile example [11].

of the human genome are coding sequences (exons), used in the cell as matrices for protein synthesis [9]. Molecular analysis of the humans and other organisms genome has shown that it is mostly composed of noncoding sequences: mainly intronic sequences and repetitive intergenic sequences. It appears that many of those sequences are:

- repeated multiple times throughout the entire genome,
- characteristic of particular individuals in the population,
- characterized by great polymorphism [12], i.e., a high degree of variability,
- ideally suited for the identification of individuals.

The source of this polymorphism is the various number of repetitions of specific sequences, the so-called repetitive units, found at specific loci on chromosomes. Simultaneous analysis of a few such loci in an individual allows obtaining a characteristic pattern known as the "genetic fingerprint". This pattern is so unique as to allow the crime perpetrator or a corpse identification, as well as paternal testing. The human profile is recorded in an alphanumeric format that contains locus (marker) name and two numeric values corresponding to the number of repetitive units copies located at that locus on the two homologous chromosomes inherited from the mother and the father (Fig. 4).

The probability of existence of two individuals with the same sequence pattern, and thus the same genetic profile, decreases along with the increasing number of loci used in the analysis. The naming of markers and genetic vari-

Table 1
Tabular representation of DNA profiles determined from trace evidence and reference material, i.e. obtained from a particular person. A lack of match between the DNA profiles at any particular locus excludes the person from whom the sample was obtained as the originator of the DNA isolated from the trace evidence

Markers	AMG	D3S1358	D19S443	D2S1338	D2S1015	D16S539	D18S51	D1S1656	D10S1248	D2S441	TH01	VWA	D21S11	D12S391	D8S1179	FGA
DNA profile from trace evidence	XY	14.15	13.14	16.17	15.18	11.12	12.16	11.15.3	13.15	10.14	6.9	15.19	31.32.2	17.22	13.13	20.22
DNA profile from reference material	XY	15.16	14.14	24.25	11.19	9.12	15.18	11.17.3	14.15	13.11	9.9.3	17.20	28.32.2	20.23	11.12	22.23

ants is universal, and the profiles are determined according to uniform sets of loci. One of them is the European Standard Set (ESS) proposed in a resolution of the Council of the European Union of November 30th 2009, consisting of eleven DNA markers: TH01, VWA, FGA, D21S11, D3S1358, D8S1179, D18S51, D1S1656, D2S441, D10S1248, D12S391 and D22S1045.

5. DNA Databases

Thanks to the standardized forensic tests and unified terminology, it became possible to compare the results obtained in different laboratories and to collect and process genetic profiles in police DNA databases operating in many countries throughout the world. Currently, the aforementioned ESS is the basis for the exchange of genetic profiles stored in DNA databases between the member states of the European Union within the framework of an international police cooperation. In the USA, in order to be incorporated into national DNA databases, a genetic profile is required to contain 13 identified loci. In this case, the probability of encountering two random, unrelated individuals having the same DNA profile is approximately 10^{-10}. According to the information presented by the European Network of Forensic Science Institutes (ENFSI), 12 million DNA profiles of suspects and unidentified biological traces from crime scenes are currently stored in the European DNA databases of 28 countries, which to date has led to more than 3 million positive "trace-person" and "trace-trace" matches [13]. As such national databases grow worldwide, their importance as one of the most effective tools in the fight against crime increases.

6. DNA Tests for Judicial Purposes

In contrast to other biometric systems, the identification system based on DNA analysis involves multiple steps and includes, e.g.:

- genomic DNA extraction,
- amplification of certain DNA sequences and their labeling with fluorescent dyes based on the PCR technique,
- electrophoresis of labeled DNA fragments in variable electrical field.

As DNA fragments pass through the analyzer detection window during electrophoresis, the dyes are excited by a laser beam. The emitted fluorescence is read by a CCD camera and the collected data are processed by software into a digital representation [14].
The person identification is performed by comparing the DNA profile of the investigated trace evidence with the reference material DNA profile (usually represented by a buccal swab) obtained from a specific person. The lack of match between the biological trace and the reference material, even at a single locus, excludes the person from whom the sample was obtained as the originator of the DNA isolated from the trace evidence (Table 1).
A match between the DNA profiles of the investigated and reference sample at all tested loci does not mean conclusively that both tested samples originate from the same person. This is due to the fact that genetic tests conducted for the purposes of criminal justice are based on the analysis of only a few DNA fragments and not the whole genome, therefore there is a mathematical probability that a genetic profile determined this way may be repeated in another unrelated person in the population.

7. Opinion of an Expert in the Field of Genetic Tests

The methodology of genetic tests in forensics requires the use of statistical tests [15]. Without a reliable mathematical analysis of the obtained results, the conclusions are worthless and the expert's opinion does not fulfill the requirements of scientific evidence [16]. This means that a positive result of a DNA test, i.e. a positive match between the profile of the trace evidence and the profile of the reference material, should be interpreted in relation to the distribution of a particular genotype in a given population. The estimation of the predictive value of DNA identification tests can be performed using several tests, e.g.:

- a test for the probability of obtaining the same profile in an unrelated, randomly selected person from the population, i.e., Match Probability (MP),

- a test for the rate of occurrence of the sample profile in the population,
- a test for the probability of inclusion or exclusion of a randomly selected person from the population as the source of the DNA found in the trace evidence,
- the calculation of the Likelihood Ratio (LR) [17].

Standard statistical calculations employ population data developed for each racial or ethnic group in the country of origin of the perpetrator. The probability value reported in the experts' opinions is assessed by the judge and it reflects the strength of the collected evidence.

In the case of genetic tests, experts increasingly use LR (in line with the Bayesian approach) which, from the point of view of criminal justice, seems to be the best method of assessing the evidence, based on the analysis of the odds ratio of individual events, according to the following formula:

$$\mathrm{LR} = \frac{Pr(\mathrm{E}|\mathrm{H_p})}{Pr(\mathrm{E}|\mathrm{H_d})}, \tag{1}$$

where: E – evidence, $\mathrm{H_p}$ – prosecution hypothesis, $\mathrm{H_d}$ – defense hypothesis.

The task of the judicial expert is the evaluation and interpretation of scientific evidence (E) in the context of various hypotheses (H) that are considered. In the case of genetic tests in forensics, the scientific evidence is a match between the DNA profile of the trace evidence from the crime scene and the DNA profile of the suspect. Scientific evidence that determines a relationship between the suspect and the trace evidence is evaluated in the context of two opposing hypotheses, conventionally called the prosecution hypothesis ($\mathrm{H_p}$), which assumes that the suspect is the source of the trace evidence, and the defense hypothesis ($\mathrm{H_d}$), which implies that the source of the trace evidence is another person unrelated to the suspect. In practice, forming hypotheses depends on the circumstances of the crime and the conducted test type. If the circumstances of the crime are not known to the expert and, in particular, the expert does not know where the trace evidence was collected, good contact and cooperation between the expert and the prosecution are essential in order to obtain complete information.

LR allows the assessment whether scientific evidence represented by genetic test results supports the prosecution hypothesis or that of the defense. In other words, it enables the estimation of how much more probable is obtaining a match between the profiles, provided that the accused is the source of the trace evidence, as opposed to the alternative hypothesis that the accused is not the source and the trace evidence is coming from another unknown person. The LR value greater than 1 means that the scientific evidence supports the hypothesis of the prosecution. The higher the LR value, the higher the reliability of the evidence obtained in the test.

In the case of interpretation of mixed DNA profiles, i.e. originating from at least two people, the formulas are more complex and take into account, e.g. the number of people from whom the mixed material originates.

In the case of determining the so-called incomplete DNA profiles (with no amplification products at several loci), resulting from a small quantity of DNA template or degraded DNA, i.e. damaged by the environmental conditions, the applicable models have to take this fact into account. If a DNA profile obtained from the evidence is incomplete, the chance of a random match between profiles from unrelated people may be relatively high, and the predictive value of DNA identification tests may be low. This is also the case for the mixed DNA profiles. The value of such DNA tests will be lower than in the case of single profiles. The use of this type of statistical models, assessing the predictive value of DNA identification tests, is usually allowed by an adequate available data quantity, including the already mentioned population databases and specialized commercial computer programs for performing complex calculations (e.g. LRmix developed by P. Gilla and H. Haned [18]).

8. The Future of DNA Testing in Forensic Science

Scientific research on the potential uses of coding DNA regions in forensics, that would allow developing some form of a "genetic portrait of the perpetrator", has been conducted for many years [19]. Among the new emerging opportunities is, e.g. genetic prediction of physical characteristics, such as red hair pigmentation, which may be very helpful in the investigation [20]. The research group led by Branicki in cooperation with researchers from Rotterdam have developed a test called HIrisPlex allowing the prediction of eye and hair color [21]. Other ongoing studies address the genes determining the body height [22], age (with the accuracy of approx. 4–5 years) [23] and ethnic origin, or even the structural features of the face [24]. Also available are bibliographic data on the genes responsible for alopecia [25], hence the scenario of determining the perpetrator appearance based on trace biological evidence left at the crime scene appears increasingly realistic.

9. The Use of DNA Polymorphism in Biometric Systems

Genetic test are the future of biometrics. Nonetheless, the low level of social acceptance for using DNA as a biometric marker does not allow that use for purposes other than forensics, i.e. identification of crime perpetrators for

the criminal justice purposes. The reason for this low acceptance is the general knowledge that a DNA molecule carries a large amount of additional information on, e.g., physical appearance, susceptibility to diseases or basic attributes of human character. Another complication in the implementation of DNA into biometric systems is the time necessary for conducting tests and analyzing the results that is unsatisfactorily long as compared with other biometric techniques in which reading and comparing the data occurs almost instantly. Commercial companies are trying to solve this issue, introducing new products to the market. In the recent years, a technique called "Rapid DNA" has become popular and helped to reduce the identification time to 90 minutes [26]. Another important issue seems to be the vulnerability to theft and exchanging the samples of biological material. Although the DNA code cannot be falsified, obtaining samples from a particular person without their knowledge is possible.

10. Conclusions

Genetic tests that allow establishing a person's DNA profile constitute an effective and reliable method of individual identification. However, in order to be able to use it successfully in business environments, as an element of access control systems and person authentication, a device capable of quickly and automatically generating and comparing DNA profiles, employing noninvasive sampling of biological material, e.g., sweat and sebum found on the fingers or palms, would need to be invented. Moreover, the issue of sample safety would need to be solved together with a complete samples destruction after profile determination. Although there is no doubt that the tests allowing the analysis of DNA polymorphism revolutionized individual identification in forensic science and forensic medicine, serious methodology – related limitations do not allow the current use of such tests in biometric systems. The future of DNA in biometrics invariably depends on the technological progress in several fields, including sequencing techniques and automatic profile comparison, which may contribute to the use of DNA profiling, with its precision and accuracy, as the method of choice in individual identification and biometric verification.

As early as at the beginning of 1990s the technological concept, called Lab-on-a-Chip (LOC) was introduced, which combined the function of several research processes in one place, on the surface a few inches square only. Rapid development of this type of technology explored the possibilities to produce miniature devices aimed also for genetic purpose. Development of this type of equipment is caused by many advantages such as saving time (the result of biochemical analysis samples can be obtained within a few minutes), costs and space reduction as well as minimizing possibility of making mistakes, because analysis would be performed automatically. Advantages presented above play an important role in work with biological material, where quite often only small amount of material is available. In this kind of technology thin capillary ducts play the crucial role as a reaction environment, reducing the quantity of reagents needed to perform the process [27]. The idea of the Lab-on-a-Chip is used at present in the diagnostic procedures as well as in an analytical chemistry. Using this technology could remove the main obstacle which disturb the use of DNA in biometry – long time processing, but it is still the matter of future.

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