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Calina Andreea MUNTEANU

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FORENSIC DNA ANALYSIS

Călina Andreea MUNTEANU*

"DNA testing is to justice what the telescope is for the stars; not a lesson in biochemistry, not a display of the wonders of magnifying glass, but a way to see things as they really are".(Barry Scheck, Peter Neufeld, Actual Innocence)

Abstract:

DNA became a valuable forensic tool in identifying the source of physical evidence left at a crime scene. The use of restriction fragment length polymorphism analysis in the mid-1980s was replaced by polymerase chain reaction methods, which required less high-molecular-weight DNA, were more rapid and more economical. Mitochondrial DNA can be used to provide identifying information when nuclear DNA is insufficient to generate a full genetic profile.

Forensic DNA Typing charts the progres and development of DNA applied to criminal forensic, providing certain demonstration of the amazing potential of this method, not only to convict the guilty, but also to exonerate de innocent.

DNA analysis has its roots in classical genetics, biochemistry, and molecular biology and it has recently become a major player in criminal investigations and the identification of suspects. This article emphasizes the advantages and limitations of various DNA techniques used in the analysis of forensic evidence. The author presents forensic DNA issues from both a scientific and a legal perspective.

Keywords:

forensic science, DNA typing, Blood and body fluid analysis; Hair and fiber analysis; Suspect identification; Tissue analysis, mtDNA, PCR, STR.

* PhD Lecturer at the Faculty of Law from the University of „Petre Andrei" from Iaşi, barrister within Iaşi Bar, calinamunteanu@yahoo.com

MUNTEANU, C., A., (2011) FORENSIC DNA ANALYSIS, Jurnalul de Studii Juridice, Anul VI, Nr. 3-4, Decembrie, pp: 85-96

Introduction

DNA analysis has its roots in classical genetics, biochemistry, and molecular biology and it has recently become a major player in criminal investigations and the identification of suspects.

DNA is present in every nucleated cell and is therefore present in biological materials left at crime scene. DNA has been successfully isolated and analyzed from a variety of biological materials[1], but the most common materials tested in forensic laboratories are blood and semen or bloodstains and semen stains.

Since it was introduced (in the midle1980s), DNA typing has revolutionized forensic science and the ability of law enforcement to match perpetrators with crime scenes. Thousands of cases have been closed, innocent suspects freed and with guilty ones punished because of the power of a silent witness at the crime scene.

"DNA fingerprinting" or DNA typing (profiling) was first described in 1985 by an English geneticist named Alec Jeffreys (H. Margolis Nunno, (2011), : 292). Dr. Jeffreys found that certain regions of DNA contained sequences that were repeated over and over again next to each other. He also discovered that the number of repeated sections present in a sample could differ from individual to individual. By developing a technique to examine the length variation of these DNA repeat sequences, Dr. Jeffreys created the ability to perform human identity tests. (Butler, (2005),:5).

The history of the legal acceptance of DNA technology as a method to lead to the identification of the participants in a crime has been a rapid and relatively noncontroversial one. The judicial acceptance of various DNA technologies, up to and including mitochondrial DNA, has been even more rapid, to the point where judicial discussions of the scientific reliability of DNA testimony have become centered in lengthy discussions of earlier DNA cases rather than DNA technology itself.

The DNA-related progression of judicial acceptance of DNA technology has advanced from blood typing and enzyme matching to

[1] Sources of biological materials used for PCR-based DNA typing are: blood, semen, bones, teeth, hair with root, hair shaft, saliva (with nucleated cells), urine, feces, debris from fingernails, muscle tissue, cigarette butts, postage stamps, envelope sealing flaps, dandruff, fingerprints, personal items as razor blade, chewing gum, wrist watch, ear wax, toothbrush,etc.

MUNTEANU, C., A., (2011) FORENSIC DNA ANALYSIS, Jurnalul de Studii Juridice, Anul VI, Nr. 3-4, Decembrie, pp: 85-96

approval of DNA laboratory-testing methodologies categorized as RFLP, PCR, STR and mitochondrial DNA testing.

There are now available a respectable number of authoritative texts[2], articles, and Web sites addressing basic and specialized DNA subjects. There is also a rapidly developing international consensus among DNA laboratories regarding standards for laboratories and DNA technicians, as a result of major support from the FBI for working groups and conferences addressing these issues across the world, especially in Europe, through the vehicle of Interpol.[3]

DNA Methodologies:

RFLP (*Restriction fragment length polymorphism*)

DNA is the genetic code that is found in the cells of the human body. A DNA molecule is composed of more than three billion "base pairs" of four different chemicals: adenine, thymine, cytosine, and guanine. The particular pattern *185 of these base pairs dictates an individual's genetic characteristics.

Taking into consideration that most of a DNA molecule is the same from person to person, DNA profiling focuses on those parts of the DNA molecule where there is a significant variation of a base-pair pattern. The areas of significant variation are referred to as "polymorphic," and base-pair patterns in polymorphic areas are called "alleles." There are approximately 3 million distinguishable polymorphic sites between individuals. Although an examination of all of these polymorphic sites is not currently feasible, an examination of a small number of polymorphic sites can establish a DNA profile, which can be compared to that from another DNA sample.

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[2] See, generally, Saferstein: *Criminalistics: An Introduction to Forensic Science* (Prentice Hall, 6th ed., 1998), at 361 (Serology) and 403 (DNA); Robertson and Vignaux: *Interpreting Evidence: Evaluating Forensic Science in the Courtroom* (John Wiley & Sons, 1995); Eckert (ed): *Introduction to Forensic Sciences* (CRC Press, 2d ed., 1997);

[3] (*Forensic Science Communications,* available: *www.fbi.gov/hq/lab/fsc/backissu/july2000/codis2a.htm*). In November 2004, at Lyon, France, Interpol sponsored the 14th International Forensic Science Symposium. The literature reviews in each of the major areas of forensic science were published in its Review Papers, which can be viewed at http://www.interpol.int/Public/Forensic/IFSS/meeting14/abstracts.asp.

MUNTEANU, C., A., (2011) FORENSIC DNA ANALYSIS, Jurnalul de Studii Juridice, Anul VI, Nr. 3-4, Decembrie, pp: 85-96

Restriction fragment length polymorphism (RFLP) is a technique that exploits variations in homologous DNA sequences. It refers to a difference between samples of homologous DNA molecules that come from differing locations of restriction enzyme sites, and to a related laboratory technique by which these segments can be illustrated.

In RFLP analysis, the DNA sample is broken into pieces (digested) by restriction enzymes and the resulting *restriction fragments* are separated according to their lengths by gel electrophoresis.

RFLP analysis was the first DNA profiling technique inexpensive enough to see widespread application.

Restriction fragment length polymorphism is a step process which allows an analyst to physically see the results of a DNA profile in the form of bands. Because the length of polymorphic DNA fragments differs between individuals, individuals also tend to have different positioning of the bands on DNA prints. An analyst makes a visual comparison of DNA band patterns to determine whether known and unknown DNA samples came from the same source, whether the samples did not come from the same source, or whether the comparison was inconclusive. If an unknown DNA sample has not been excluded from a comparison, a computerized measurement program is used to compare the lengths of the DNA fragments. If the DNA band patterns fall within a certain range, the samples are declared a match.

For a match to be meaningful, a statistical analysis is required. The statistical analysis determines the frequency in which a match would occur in a database population.

PCR DNA Methodologies (*polymerase chain reaction*)

The **polymerase chain reaction** (**PCR**) is a scientific technique in molecular biology to amplify a single or a few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence.

Developed in 1983 by Kary Mullis, PCR is now a common and often indispensable technique used in medical and biological research labs for a variety of applications. These include DNA cloning for sequencing, DNA-based phylogeny, or functional analysis of genes; the diagnosis of hereditary diseases; the identification of genetic fingerprints (used in

MUNTEANU, C., A., (2011) FORENSIC DNA ANALYSIS, Jurnalul de Studii Juridice, Anul VI, Nr. 3-4, Decembrie, pp: 85-96

forensic sciences and paternity testing); and the detection and diagnosis of infectious diseases.

PCR allows tests to be performed on very small quantities of genetic material. In this method, the DNA is extracted from a sample of cellular material, such as blood or sperm cells. Then, depending on which genetic markers are being tested for, a particular location or set of locations on the strand of DNA is isolated and copied over and over until a sufficient quantity exists for testing.

Unlike the RFLP procedure, which is a much more accurate test used to establish a statistical match, the PCR technique is generally used as an exculpatory tool to exclude certain individuals as possible contributors to a particular sample. PCR can also be used on a much smaller sample obtained from a crime scene and may replicate samples to allow for multiple testing opportunities.

The PCR method harnesses cellular enzymes to replicate portions of the DNA so that a sufficient number of copies of the DNA can be obtained to perform testing[4].

[4] The ability of PCR testing to reach results in cases where the amount of testable material is small or partially degraded, can be illustrated by a brief summary of a 1999 Illinois Supreme Court decision. In *People v. Davis,* defendant was convicted of first-degree murder, aggravated criminal sexual assault, aggravated kidnapping, robbery, and concealment of homicidal death, and was given the death penalty. The state's evidence showed that, on Monday, August 21, 1995, Laurie Gwinn was reported missing after she failed to arrive at her job with the county health department. The next day, after 11 a.m., Gwinn's dead body was found floating in the Hennepin Canal north of Annawan, Illinois. She was nude and was missing several pieces of expensive jewelry that she always wore. A vaginal swab taken during Gwinn's autopsy contained seminal material and sperm cells. Kristin Boster, a forensic scientist and expert in deoxyribonucleic-acid (DNA) analysis, testified that she isolated the DNA taken from the swab and determined it to be too degraded for a restriction fragment DNA Analysis length polymorphism (RFLP) analysis. Elizabeth Benzinger, a molecular biologist and also an expert in DNA analysis, agreed that there was insufficient DNA to perform an RFLP analysis. She explained that the DNA was degraded because the murderer had placed Gwinn's body in the canal. Benzinger analyzed the DNA using the polymerase chain reaction (PCR) technique. She compared the DNA taken from the swab to samples taken from defendant, James Linsley, who was a close acquaintance of the victim,and the victim. Benzinger concluded that Linsley could not have contributed to the vaginal swab. Benzinger could not, however, exclude defendant as the source of the semen on the swab. According to Benzinger, the percentage of the United States population that could have contributed the DNA recovered from the swab was 2.6% of white persons and 3.6% of black persons.

MUNTEANU, C., A., (2011) FORENSIC DNA ANALYSIS, Jurnalul de Studii Juridice, Anul VI, Nr. 3-4, Decembrie, pp: 85-96

In conclusion, some of the advantages of PCR amplification for biological evidence include the following:

- Very small amounts of DNA template may be used from as little as a single cell;
- Large numbers of copies of specific DNA sequences can be amplified simultaneously with multiplex PCR reactions;
- Contaminant DNA , such as fungal and bacterial sources, will not amplify because human specific primers are used;
- Commercial kits are now available for easy PCR reaction setup and amplification. (Butler, (2005), : 80)

STR DNA Methodologies (*Short tandem repeats*)

STR analysis is a relatively new technology in the field of forensics. It is used for the genetic fingerprinting of individuals. The STRs in use today for forensic analysis are all tetra- or penta-nucleotide repeats (4 or 5 repeated nucleotides), as these give a high degree of error-free data while being robust enough to survive degradation in non-ideal conditions.[5]

There were used two methods of DNA analysis: PCR and RFLP. Each of these methods is used to identify particular characteristics of a given sample of DNA. Those characteristics are referred to as the "profile" of that DNA. Because each method of analysis, PCR and RFLP, identifies different characteristics, two different profiles are obtained by subjecting a sample of DNA to both types of analysis.

It was used the PCR method to analyze DNA found on the inhaler, carpet, femur, and box. The PCR profile of the DNA from each of these items was the same. Frank calculated that this particular DNA profile could be found in one out of 19,000 Caucasian individuals.

Using the RFLP method, which is more discriminating, it was compared the DNA in blood samples from Christopher's parents and defendant to the DNA in blood found on the box and carpet. By comparing the DNA profiles he obtained, it was determined that the blood on the box and the carpet came from a child of Mika Moulton and James Meyer, Sr., Christopher's father. It was calculated that the chance of two Caucasian parents producing a child with the same RFLP DNA profile as the DNA found on the carpet and box was one out of 3.8 million.

After preparing both a PCR and an RFLP profile for the DNA found on the box and carpet, associated with defendant's vehicle, it was proceeded to estimate the frequency of DNA with both of these profiles in the population, concluding that a person with such DNA would occur in the Caucasian population only 1 out of 419 million times.

[5] In *People v. Allen*, defendant was convicted of special circumstances murder and forcible rape. The state offered the results of laboratory DNA testing by the short tandem repeats (STR) method on a semen stain from the crime scene. The court ruled

MUNTEANU, C., A., (2011) FORENSIC DNA ANALYSIS, Jurnalul de Studii Juridice, Anul VI, Nr. 3-4, Decembrie, pp: 85-96

The analysis is performed by extracting nuclear DNA from the cells of a forensic sample of interest, then amplifying specific polymorphic regions of the extracted DNA by means of the polymerase chain reaction. Once these sequences have been amplified, they are resolved either through gel electrophoresis or capillary electrophoresis, which will allow the analyst to determine how many repeats of the STR sequence in question there are. If the DNA was resolved by gel electrophoresis, the DNA can be visualized either by silver staining , or an intercalating dye such as ethidium bromide, or as most modern forensics labs use, fluorescent dyes. These profiles are stored on a local, state and national level in DNA databanks such as CODIS. The British data base for STR

that this was competent evidence of general acceptance of testing in the scientific community.

The defendant's appellate counsel appears to suggest that STR testing is unreliable because it is too new. No specific scientific or forensic evidence or literature is offered to support that suggestion. The judge heard testimony that, in 1991, several years before the STR kit became commercially available, Cellmark, working under contract to the United States Government, used STR testing to identify the remains of soldiers killed in Operation Desert Storm, and that, by the time of the hearing, Cellmark had performed STR analysis in approximately fifty cases and had been permitted to testify as to its test results in at least five cases

STR testing is "coming into wide use," that "STR loci appear to be particularly appropriate for forensic use," and that "STRs can take their place along with variable number of tandem repeats as forensic tools." The latter comment appears to recognize that STR testing is similar in principle to the RFLP method, which has been found to be reliable.

Based on the evidence before him and his careful analysis of the subject, the judge properly concluded that the methodology underlying the PCR-based tests in this case, including the STR testing, was scientifically valid and relevant to a fact at trial.

The Allen court also noted that in 1998, in *State v. Jackson,* the Supreme Court of Nebraska affirmed a trial court's finding that the prosecution had shown STR testing was generally accepted by the relevant scientific community, emphasizing that a director of the University of Nebraska Medical Center laboratory had testified that PCR STR testing was generally accepted in the scientific community. The expert had testified that this method had "been around several years now, and there is nothing unique about PCR STR versus any PCR." The Jackson court concluded that based on this evidence, we can only conclude that the trial court was correct in determining that the PCR STR DNA test used in the instant case was generally accepted within the scientific community.

In response to Allen's argument that there was no evidence that STR testing had been validated by the time it was utilized in this case, the court stated that the issue was not when a new scientific technique is validated, but whether it is or is not valid, which was why the results generated by a scientific test once considered valid can be challenged by evidence the test has since been invalidated.

MUNTEANU, C., A., (2011) FORENSIC DNA ANALYSIS, Jurnalul de Studii Juridice, Anul VI, Nr. 3-4, Decembrie, pp: 85-96

loci identification is the UK National DNA Database (NDNAD). The British system uses 10 loci, rather than the American 13 loci.

A number of factors can introduce ambiguity into STR evidence, leaving the results open to alternative interpretations (Thompson William C. Thompson; Simon Ford; Travis Doom; Michael Raymer; Dan E. Krane, (2003) , : 16).

One of the most common complications in the analysis of DNA evidence is the presence of DNA from multiple sources. A sample that contains DNA from two or more individuals is referred to as a mixture. A single person is expected to contribute at most two alleles for each locus. If more than two peaks are visible at any locus, there is strong reason to believe that the sample is a mixture[6].

These interpretive ambiguities make it difficult, and sometimes impossible, to estimate the statistical likelihood that a randomly chosen individual will be "included" (or, could not be "excluded") as a possible contributor to a mixed sample. Defense lawyers should look carefully at the way in which laboratories compute statistical estimates in mixture cases because these estimates often are based on debatable assumptions that are unfavorable to the defendant.

As samples age, DNA like any chemical begins to degrade. This process occurs slowly if the samples are carefully preserved but can occur rapidly when the samples are exposed for even a short time to unfavorable conditions, such as warmth, moisture or sunlight[7].

[6] By their very nature mixtures are difficult to interpret. The number of contributors is often unclear. Although the presence of three or more alleles at any locus signals the presence of more than one contributor, it often is difficult to tell whether the sample originated from two, three, or even more individuals because the various contributors may share many alleles. If alleles 14, 15 and 18 are observed at a locus, they could be from two individuals, A and B, where A contributed 15 and B contributed 14, 18. Alternatively, A could have contributed 14, 15 while B contributed 15, 18, and so on. There might also be three contributors. For example A could have contributed 14, 15, while B contributed 15, 18 and C contributed 15. Many other combinations are also consistent with the findings. A study of one database of 649 individuals found over 5 million three-way combinations of individuals that would have shown four or fewer alleles across all 13 commonly tested STR loci.

[7] Degradation skews the relationship between peak heights and the quantity of DNA present. Generally, degradation produces a downward slope across the electropherograms in the height of peaks because degradation is more likely to interfere with the detection of longer sequences of repeated DNA (the alleles on the right side of the electropherogram) than shorter sequences (alleles on the left side).

MUNTEANU, C., A., (2011) FORENSIC DNA ANALYSIS, Jurnalul de Studii Juridice, Anul VI, Nr. 3-4, Decembrie, pp: 85-96

Degraded samples can be difficult to type. The process of degradation can reduce the height of some peaks, making them too low to be distinguished reliably from background "noise" in the data, or making them disappear entirely, while other peaks from the same sample can still be scored. In mixed samples, it may be impossible to determine whether the alleles of one or more contributors have become undetectable at some loci. Often analysts simply guess whether all alleles have been detected or not, which renders their conclusions speculative and leaves the results open to a variety of alternative interpretations. Further, the two or more biological samples that make up a mixture may show different levels of degradation, perhaps due to their having been deposited at different times or due to differences in the protection offered by different cell types.

Such possibilities make the interpretation of degraded mixed samples particularly prone to subjective interpretation.

Mitochondrial analysis

Mitochondrial DNA analysis (mtDNA) can be used to examine the DNA from samples that cannot be analyzed by RFLP or STR. Nuclear DNA must be extracted from samples for use in RFLP, PCR, and STR.

MtDNA analysis uses DNA extracted from another cellular organelle called a mitochondrion. While older biological samples that lack nucleated cellular material, such as hair, bones, and teeth, cannot be analyzed with STR and RFLP, they can be analyzed with mtDNA. In the investigation of cases that have gone unsolved for many years, mtDNA is extremely valuable.

All mothers have the same mitochondrial DNA as their offspring. This is because the mitochondria of each new embryo comes from the mother's egg cell. The father's sperm contributes only nuclear DNA. Comparing the mtDNA profile of unidentified remains with the profile of a potential maternal relative can be an important technique in missing-person investigations.

For highly degraded samples, it is sometimes impossible to get a complete profile of the 13 CODIS STRs. In these situations, mitochondrial DNA (mtDNA) is sometimes typed due to there being many copies of mtDNA in a cell, while there may only be 1-2 copies of the nuclear DNA. Forensic scientists amplify the HV1 and HV2 regions

MUNTEANU, C., A., (2011) FORENSIC DNA ANALYSIS, Jurnalul de Studii Juridice, Anul VI, Nr. 3-4, Decembrie, pp: 85-96

of the mtDNA, then sequence each region and compare single-nucleotide differences to a reference. Because mtDNA is maternally inherited, directly linked maternal relatives can be used as match references, such as one's maternal grandmother's daughter's son. A difference of two or more nucleotides is generally considered to be an exclusion. Heteroplasmy and poly-C differences may throw off straight sequence comparisons, so some expertise on the part of the analyst is required. MtDNA is useful in determining clear identities, such as those of missing people when a maternally linked relative can be found. MtDNA testing was used in determining that Anna Anderson was not the Russian princess she had claimed to be, Anastasia Romanov.

MtDNA can be obtained from such material as hair shafts and old bones or teeth.

III Considerations when evaluating DNA evidence

At the beginning of the use of genetic fingerprinting as criminal evidence, defense lawyers used spurious statistical arguments along these lines: given a match that had a 1 in 5 million probability of occurring by chance, the lawyer would argue that this meant that in a country of say 60 million people there were 12 people who would also match the profile. This was then translated to a 1 in 12 chance of the suspect being the guilty one. This argument is not sound unless the suspect was drawn at random from the population of the country. In fact, it should be considered how likely it is that an individual matching the genetic profile would also have been a suspect in the case for other reasons. Another spurious statistical argument is based on the false assumption that a 1 in 5 million probability of a match automatically translates into a 1 in 5 million probability of innocence.

Recent studies have quoted relatively high error rates which may be cause for concern. In the early days of genetic fingerprinting, the necessary population data to accurately compute a match probability was sometimes unavailable. Between 1992 and 1996, arbitrary low ceilings were controversially put on match probabilities used in RFLP analysis rather than the higher theoretically computed ones. Today, RFLP has become widely disused due to the advent of more discriminating, sensitive and easier technologies.

MUNTEANU, C., A., (2011) FORENSIC DNA ANALYSIS, Jurnalul de Studii Juridice, Anul VI, Nr. 3-4, Decembrie, pp: 85-96

Since 1998 the DNA profiling system supported by The National DNA Database in the UK is the SGM+ DNA profiling system which includes 10 STR regions and a sex indicating test. STRs do not suffer from such subjectivity and provide similar power of discrimination. It should be noted that figures of this magnitude are not considered to be statistically supportable by scientists in the UK, for unrelated individuals with full matching DNA profiles a match probability of 1 in a billion is considered statistically supportable. However, with any DNA technique, a man should not be convicted on genetic fingerprint evidence alone if other factors raise doubt. Contamination with other evidence is a key source of incorrect DNA profiles and raising doubts as to whether a sample has been adulterated is a favorite defense technique.

IV Conclusions

DNA became a valuable forensic tool in identifying the source of physical evidence left at a crime scene. The use of restriction fragment length polymorphism analysis in the mid-1980s was replaced by polymerase chain reaction methods, which required less high-molecular-weight DNA, were more rapid and more economical. Mitochondrial DNA can be used to provide identifying information when nuclear DNA is insufficient to generate a full genetic profile.

Forensic DNA Typing charts the progres and development of DNA applied to criminal forensic, providing powerful demonstration of the amazing potential of this method, not only to convict the guilty, but also to exonerate de innocent.

DNA profiling has become a standard technique in criminal investigations because results can be obtained from any source of biological material provided it contains nucleated cells with genomic DNA. Due to the rapid progress in the field during the last ten years, parallel developments of methods and typing systems have been made in the laboratories involved in forensic DNA profiling. This has resulted in heterogeneity of typing procedures and genetic systems used for forensic casework within the European Union. However, intercomparison of DNA typing results becomes not only desirable, but absolutely necessary within Europe as mobile serial offenders will not be detected by DNA profiling unless methods are standardized.

MUNTEANU, C., A., (2011) FORENSIC DNA ANALYSIS, Jurnalul de Studii Juridice, Anul VI, Nr. 3-4, Decembrie, pp: 85-96

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MUNTEANU, C., A., (2011) FORENSIC DNA ANALYSIS, Jurnalul de Studii Juridice, Anul VI, Nr. 3-4, Decembrie, pp: 85-96