

DNA and Forensic Science

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I. INTRODUCTION

This paper represents a discussion of the fundamental principles of DNA technology as it applies to forensic testing. A brief overview of DNA science will be given to clarify the terminology and techniques described for forensic and basic analysis. A historical as well as scientific discussion of the molecular biological tools used to determine DNA profiles and database analysis following such application will also be covered. Finally, specific questions raised during the Symposium will be addressed.

II. DNA AND ITS USES

Deoxyribonucleic acid, or DNA, is an organic polymer which is found within every cell of every organism. The polymer is composed of three specific parts: (1) the phosphate backbone, (2) the deoxyribose sugar, and (3) the nitrogenous base. The first two components remain constant across all individuals, while the third is what distinguishes each constituent of the polymer and thus aids in distinguishing between individuals. This base is responsible for the nucleotides which can contain one of four structures: adenine (A), cytosine (C), guanine (G); or thymine (T). It is the combination of these four bases which determines the precise function and coding capacity of the DNA. It is important to note that DNA is double stranded, meaning that two identical copies of DNA are present in every cell. The double stranded nature is based on complementarity of the bases, where G pairs with C and A pairs with T. This complementarity is critical for various DNA testing techniques and the basic principles of DNA chemistry. When put together in a unique way, the string of A, C, T, or G can serve as a template for messenger RNA (ribonucleic acid), which in turn codes for proteins. These proteins ultimately form the structure and function for every process within the cell and within an organism. Therefore, sequences of DNA coding for proteins enable the construction and maintenance of the cell, which is ultimately responsible for all living processes.

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Now that it is clear how important DNA is to generating and maintaining life, we will discuss the application of DNA to forensic testing. As discussed, DNA is composed of a string of nucleotides. Within each human cell, this string is approximately three billion bases long. A fortunate consequence of such a long string is that variation arises and small changes, which may not affect the function of the DNA or the proteins for which it codes, can allow for distinction between samples. Thus, very small differences in human DNA sequences can be detected and used to differentiate between individuals. This observation led to the first forensic DNA testing in 1984.

Initially, the technique used for this analysis was based on Restriction Fragment Length Polymorphisms (RFLP). In this technique, a restriction enzyme, which is an enzyme that cuts DNA at a specific sequence, is used to break DNA into small pieces. The size of the pieces is distinct based on the location within the DNA of the specific sequences. Thus, a restriction profile¹ for a particular person's DNA can be generated. Since each person's DNA contains small differences, a change in one of these restriction sites would result in a different profile of the restriction fragments.² Therefore, the technique analyzes polymorphisms, or differences within multiple samples, of restriction fragments.

This technique was the standard in DNA forensics for a long time because it offered a high degree of discrimination³ and in some circumstances it is still used. However, it usually requires large amounts of non-degraded DNA which is difficult to find outside of the human body.

In 1986, Kary Mullis developed a technique called the Polymerase Chain Reaction (PCR). This technique revolutionized not only forensic DNA science, but all of molecular biology. PCR is a method which allows the cell's normal machinery for replicating DNA to amplify DNA within a sample,⁴ which may contain as little as one nanogram⁵ of DNA,

1. The restriction profile is the pattern of different sized DNA fragments observed after cutting with a specific restriction enzyme (as analyzed on a gel separating these fragments by size).

2. A restriction fragment is the piece of DNA which has been cut by a restriction enzyme (i.e. if you have a 2000 base piece of DNA and there are two restriction sites at position 50 and position 500 then you will have 50, 450, and 1500 base restriction fragments. When you run this on a gel separating the fragments by size you can get the restriction profile described above).

3. Discrimination refers to the ability to discriminate between samples fairly efficiently (i.e. for prior techniques, one may have had to do several rounds of a particular assay to be confident in the differences between samples, whereas with RFLP a relatively high level of confidence in discriminating between samples was easier to come by).

4. Essentially, amplification is valuable because it allows for scientists to draw more DNA out of a smaller piece of DNA evidence than would be possible

in order to analyze it. This sensitivity gives PCR a major advantage over other techniques.

For example, a sample of DNA from a crime scene can be used as a template for amplification. A specific region of the DNA is selected based on known sequences and small pieces of DNA, twenty to thirty bases in length, called primers, are generated on either side of the selected region. These primers are added to the DNA sample along with a DNA polymerase, which is an enzyme that polymerizes⁶ DNA monomers,⁷ and free monomers. The mix is heated to a temperature that causes the two strands of DNA to melt apart. Then, the mix is cooled to a temperature where DNA strands will anneal.⁸ At this point, the two primers will bind to the complementary site on the DNA as chosen based on their sequence. A final heating step to an ideal temperature for the DNA polymerase to function enables the production of two copies of the region that was initially chosen for analysis. This cycle of heating and cooling is then repeated many times until an exponential rate of replication occurs and a very large amount of DNA containing the specific region is obtained. This enables the investigators to further manipulate or analyze this region of DNA which otherwise would have been undetectable.

Subsequently, techniques involving small changes in DNA sequence as mentioned above would be used. These small changes in sequence as determined by PCR are known as Single Nucleotide Polymorphisms (SNPs). A similar analysis follows for SNPs as for RFLPs. The advantages to PCR include its speed, sensitivity, and also the ability to process many samples at once. Intriguingly, PCR makes it possible to lift a DNA profile from a tiny blood droplet, hair, bones, teeth, saliva, or semen.⁹

A new forensic DNA approach that is being more broadly applied is mitochondrial DNA (mtDNA) sequencing. Mitochondria are structures within cells that contain DNA that is distinct from the DNA in the nucleus. Mitochondrial sequencing is used extensively in FBI labs, as well as state and private crime labs. This technique, which takes into account small differences between people's mtDNA, is very useful for minute samples or ancient or degraded samples. This is because cells are

without PCR.

5. One nanogram is equal to one-billionth of a gram.

6. Polymerize means to make a polymer from a monomer (so for DNA, take bases and lengthen the chain).

7. A monomer is a single unit of something (for DNA it is the bases).

8. Anneal means to stick together (in this case the melted/separated DNA is annealing back together, but with primers instead of the original other strand).

9. For more discussion on DNA, see KEITH INMAN & NORAH RUDIN, AN INTRODUCTION TO FORENSIC DNA ANALYSIS 1 (1997).

loaded with mitochondria, so even in highly degraded samples or old hair, there is often enough mtDNA to obtain a sequence.

The target sequence in mtDNA is a hypervariable region¹⁰ which has a five to ten times greater mutation rate than nuclear DNA, so the variation arises through mutation. Usually there is 1-2% variance of mtDNA sequence between unrelated individuals, or 12 in 100 bases. Mutations are random and preserved through maternal inheritance, so there is only a remote chance that any two individuals will show the exact same mutation, unless they are from the same recent maternal lineage.

The legal power of forensic DNA profiling is in exclusion. These techniques cannot typically prove that a person committed a particular crime, but they can prove whether he/she was there and left DNA evidence. Also, DNA evidence can be used to overturn previous serologically¹¹ based guilty verdicts because of its higher discriminatory power.¹² Based on statistical results from allele¹³ frequency databases, a DNA investigator can generate a likelihood that a suspect's DNA profile will randomly match an evidentiary sample. Using the 13 CODIS (Combined Offender DNA Indexing System) loci, one can typically obtain a frequency on the order of one-in-a-billion to one-in-a-trillion that the profiles will match at random, given non-related subjects. The CODIS loci are currently being used to build a national database of violent crime offenders, which will serve to link unsolved crimes with profiled criminals.

III. COMMONLY ASKED QUESTIONS

The following are some commonly asked questions which arose during the Symposium:

Q: Is there enough DNA in a single cell for forensic testing, and what concentration of DNA in a solution is sufficient to complete forensic testing?

A: Theoretically yes, but realistically one needs an extracted DNA concentration of 0.05-0.125 nanograms per microliter of sample. As techniques become more sensitive, however, this threshold will be pushed lower.

10. Hypervariable region means that the actual sequence of DNA in that region varies greatly from individual to individual.

11. Serology is the use of people's serum (antibodies in the serum) to detect differences in reactivity against certain antigens.

12. For example, the re-examination of a fifteen-year-old's blood mixture on a hammer, or old semen stains.

13. An allele is one of two copies of a gene (for example, in genetics where L = dominant and l = recessive, you could have Ll, ll, LL, or LL . . . the L would represent a gene).

Q: Do all cells from the same person contain the same DNA?

A: Yes, all cells from the same person contain the same DNA, however, there are specialized cells of the immune system, for example, which contain specific mutations in certain DNA sequences that are important for immune system functions and render different DNA in different cells. This example would not account for a sufficient amount of variability from cell to cell within the same person to be unable to distinguish one person's DNA from a another person's DNA.

Q: What sort of environmental factors can degrade DNA?

A: DNA degradation can occur over time due to changes in temperature (usually increases), humidity (which may cause bacterial contamination), UV light exposure, oxidants, and various other potential chemical modifications that would render the DNA unstable.

III. CONCLUSION

As techniques for manipulating and analyzing DNA become more efficient and more durable, forensic DNA testing will improve. Currently, DNA can be used to very specifically discriminate between individuals, using a wide variety of techniques. Depending on the amount of sample and the level of degradation, several techniques can be applied to narrow the likelihood that a particular individual was present at a crime scene. An additional advantage of DNA testing is the ability to review previous cases that were decided primarily based on older technology. In these instances, DNA techniques can be used to reanalyze material that may allow for previously convicted individuals to be acquitted. It is clear that DNA technology will advance as will the database technology for analyzing forensics data. Thus, the power of DNA technology should be incredibly useful to lawyers in the years to come. However, the limitations of these technologies should always be kept in mind. DNA can never be used to prove that an individual committed a crime, but simply that they were present at the scene.