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Siniša Franjić
Independent Researcher,
Osijek, Croatia

DNA profiling is an important forensic technique

Siniša Franjić

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Abstract

DNA profiling is a forensic technique in criminal investigations, comparing the profile of crime suspects to DNA evidence to assess the likelihood of their involvement in a crime. It is also used in determining parenthood and in genealogical and medical research. DNA profiling is also used in the study of animal and plant populations in the fields of zoology, botany, and agriculture.

Keywords: DNA, PCR, genome, crime scene, forensics

Introduction

The DNA (Deoxyribonucleic acid) analyst, in order to decide which alleles are present in a sample, uses a graph called the electropherogram (or electrophoretogram, epg), usually referred to simply as the epg^[1]. The epg represents the separated DNA molecules that are produced after the amplification process of the original sample. It is not necessary here to detail all of the issues affecting the production of the epg as it most rarely materially affects the profile result. However, it is important to understand the general manner in which an epg is produced and how steps can be taken to verify a profiling result.

DNA analysis is one of the greatest technical achievements for criminal investigation since the discovery of fingerprints^[2]. Methods of DNA profiling are firmly grounded in molecular technology. When profiling is done with appropriate care, the results are highly reproducible. In particular, the methods are almost certain to exclude an innocent suspect. One of the most widely used techniques involves VNTRs (variable number tandem repeat). These loci are extremely variable, but individual alleles cannot be distinguished, because of intrinsic measurement variability, and the analysis requires statistical procedures. The laboratory procedure involves radioactivity and requires a month or more for full analysis. PCR-based methods are prompt, require only a small amount of material, and can yield unambiguous identification of individual alleles.

The state of the profiling technology and the methods for estimating frequencies and related statistics have progressed to the point where the admissibility of properly collected and analyzed DNA data should not be in doubt. We expect continued development of new and better methods and hope for their prompt validation, so that they can quickly be brought into use.

Whether the DNA laboratory generates DNA profiles “in house” or they are generated by a vendor (outside) laboratory, the resulting electropherograms must be evaluated for quality and accuracy prior to their use in the identification process^[3]. Acceptable profile parameters can be based on the laboratory’s validation studies (internal testing) demonstrating that the profiles can be properly interpreted. These parameters include characteristics such as minimum peak height, maximum peak height, maximum allowable stutter, allowable background, and peak balance between and across loci. When the DNA operation must review thousands of profiles, often produced by a number of different laboratories, the data presentation parameters should be set high enough to ensure very little data interpretation needs to be performed during the data review process. By setting the data acceptance parameters high enough, the DNA operations will not have to send questionable samples back for retesting because the allele calls generated from the electropherograms are questionable or subject to interpretation. This practice can be more costly for the laboratories generating the profiles but will result in clean and clear data that is unambiguous and efficient to review.

Corresponding Author:
Siniša Franjić
Independent Researcher,
Osijek, Croatia

Development

DNA profiling as we know it today, was developed, thanks to two independent breakthroughs in molecular biology that occurred at the same time on different sides of the Atlantic [4]. In the United States, the polymerase chain reaction (PCR) was developed by Kary Mullis of Cetus Corporation. Almost simultaneously, the individual-specific banding patterns observed after restriction fragment-length polymorphism (RFLP) analysis of repeated DNA sequences were discovered by Professor Sir Alec Jeffreys at the University of Leicester. In its earliest incarnation, this technique termed as DNA fingerprinting by its creators was performed by restriction of 0.5–10 µg of extracted DNA using the restriction enzyme *HinFI*, followed by Southern blotting hybridization with probes termed 33.5, 33.6, and 33.15, designed to bind to multiple “minisatellites” present in the restricted DNA. This multilocus probing (MLP) technique would result in the binding of probes to multiple independent DNA fragments at the same time, giving rise to the traditional “bar code” pattern that is often visualized, discussing DNA profiling even today. Differences in the number of times the probe sequence is repeated in each DNA fragment form the basis of the individual patterns observed on the autoradiogram image.

The first report concerning the use of DNA profiling in a criminal investigation was published in 1987. This investigation used two unpublished SLPs to link semen stain samples collected from two rape and murder cases that had occurred 3 years apart in 1983 and 1986 in Leicestershire, United Kingdom. The probability of this match occurring by chance was calculated as 5.8×10^{-8} . This result not only linked the two crimes but also exonerated an innocent man implicated in the murders and led to the first mass screening project undertaken for DNA profiling in the world.

The potential of DNA analysis for forensic science had now been demonstrated; the technology now required statistical validation by analysis of population frequencies and application to casework samples before it could progress. Early evaluation studies on MLP 33.15 provided optimistic support for the use of DNA for the personal identification and the identification of male rapists from a mixed male/female sample. It does, however, also begin to uncover the limitations of this method. A mean success rate of only 62% for the DNA fingerprinting of donated vaginal swabs was observed and no typing was possible for blood or semen stains that had been stored for 4 years at room temperature, and difficulty in directly comparing related samples run on different gels was also cited as a potential problem. Similar studies and European collaborations were undertaken on SLPs such as YNH24 and MS43a. Difficulties were again observed when interpreting gel images, with only 77.9% of 70 samples distributed between nine laboratories producing matching results when a 2.8% “window” for size differences between gel runs and laboratories was used. It was recognized that subtle differences between laboratory protocols were responsible for some of the observed discrepancies, leading to a requirement for the standardization of laboratory methodology and DNA profile interpretation.

Such standardizations could improve the reproducibility of DNA typing results for MLP and SLP (single locus probes) marker systems, but in order to be applicable to forensic investigation, DNA systems must be robust and must be applicable to samples of a less than pristine nature or that

which consists of only a few cells. PCR was first applied to forensic DNA profiling for the investigation of the HLA-DQ- α 1 gene, a polymorphic gene that encodes a human leukocyte antigen cell surface protein located in the major histocompatibility complex (MHC) class II region on chromosome 6.

PCR

Forensic science and DNA-typing laboratories have greatly benefited from the discovery of a technique known as the polymerase chain reaction or PCR [5]. First described in 1985 by Kary Mullis and members of the Human Genetics group at the Cetus Corporation (now Roche Molecular Systems), PCR has revolutionized molecular biology through the ability to make hundreds of millions of copies of a specific sequence of DNA in a matter of only a few hours. The impact of PCR has been such that its inventor, Kary Mullis, received the Nobel Prize in Chemistry in 1993—less than 10 years after it was first described.

Without the ability to make copies of DNA molecules, many forensic samples would be impossible to analyze. DNA from crime scenes is often limited in both quantity and quality and obtaining a cleaner, more concentrated sample is normally out of the question (most perpetrators of crimes are, not surprisingly, unwilling to donate more evidence material to aid their prosecution). The PCR DNA amplification technology is well suited to analysis of forensic DNA samples because it is sensitive, rapid, and not as limited by the quality of the DNA as the original restriction fragment length polymorphism (RFLP) methods were when they were used.

A PCR sample is prepared by mixing several individual components and then adding deionized water to achieve the desired volume and concentration of each of the components. Commercial kits with pre-mixed components may also be used for PCR. These kits have greatly simplified the use of PCR in forensic DNA laboratories.

The most important components of a PCR sample are the two primers, which are short DNA sequences that precede or “flank” the region to be copied. A primer acts to identify or “target” the portion of the DNA template to be copied. It is a chemically synthesized oligonucleotide that is added in a high concentration relative to the DNA template to drive the PCR sample. Considerable knowledge of the DNA sequence to be copied is required in order to select appropriate primer sequences.

The other components of a PCR sample consist of template DNA that will be copied, deoxynucleotide triphosphate (dNTP) building blocks that supply each of the four nucleotides, and a DNA polymerase that adds the building blocks in the proper order based on the template DNA sequence. Thermally stable polymerases that do not fall apart during the nearboiling denaturation temperature steps have been important to the success of PCR. The most commonly used thermally stable polymerase is *Taq*, which comes from a bacterium named *Thermus aquaticus* that inhabits hot springs.

Genome

The DNA genome contains human genes that encode proteins, but it also contains a lot more than just the genes [6]. In fact, only about 2% of the human DNA sequence encodes protein-coding genes; the other 98% of the sequences do not code for proteins. The protein-coding

sequence of a gene contains a specific DNA sequence that codes for a specific protein. Changes in the DNA sequence coding for the protein will change the amino acid sequence of the protein, making a mutant product. When a mutant gene produces a mutant protein, that does not work properly, then the individual might inherit a genetic disorder or genetic disease.

The large amount of non-protein-coding DNA (noncoding DNA) in the human genomes does not produce proteins, but some noncoding DNA regions do have functions in the cell. However, changes in the noncoding DNA is usually silent in terms of traits; even drastic changes in the sequence of the noncoding DNA might not affect the individual. These stretches of DNA do not encode proteins, so the differences in the noncoding DNA sequence might not cause differences in the individual's development or health. However, it would be incorrect to think that the noncoding DNA in the human genome is entirely without function or does not carry genetic information. Scientists know much less about the characteristics of the noncoding human DNA, and relatively little research has been focused on noncoding DNA sequences compared to the research efforts on the regions that code for proteins.

Forensic analysts use the polymerase chain reaction (PCR) to obtain a DNA profile using field evidence or a suspect's reference sample. Even tiny amounts of DNA can be amplified by the PCR method to produce millions of copies of a predetermined DNA sequence specified by the analyst. In the case of an STR test, the analyst chooses specific DNA primers that allow the amplification of the tetranucleotide repeat DNA plus some of the sequence flanking it on either side. Because the sequence flanking the repeat does not vary from one person to another, the size of the PCR DNA product will directly reflect the number of repetitions of the repeated sequence that exist at that particular locus. For example, if a specific allele containing 10 repetitions of a tetranucleotide repeat yields a PCR product that is 200 base pairs in size, a different version of that allele containing 15 repetitions of the 4 bp sequence will yield a PCR product that is 220 bp in size because it contains an extra 20 nucleotides (5 more repetitions of a 4 bp sequence). By determining the lengths of the PCR DNA products obtained from a sample, the forensic analyst can figure out how many repeated sequences exist in each of the individual's alleles for each of the markers tested. This is part of the information that the forensic analyst uses to create an individual's DNA profile.

Crime Scene

Many techniques have been developed to determine the type of biological material present at a crime scene or on items pertaining to a criminal investigation [7]. While DNA profiling is commonly perceived as the gold standard in associating a stain to an individual, it can be just as important to establish the biological source of the material to assist in the recreation of the events surrounding the commission of a crime. Expertise is required to locate possible biological material, select the most probative items for further examinations, and select those that are likely to yield DNA profiles although there is much to learn about the last. Preliminary testing at scenes may further help in the screening, triaging, and prioritizing of items to be submitted for full biological examinations in a laboratory. Frequently, this means identifying semen or saliva in sexual assault

cases and blood in assaults and homicides, but may also include other biological materials that are sometimes found at crime scenes.

It is the standard practice of the forensic biologist to first conduct a visual examination of the items or scenes of interest. Various light sources and techniques including ambient and white light, oblique lighting, ultraviolet (UV) light, alternate light sources such as multiwavelength Polilight™ examination, and lasers as well as microscopy are commonly used to help locate potential biological material. Chemical and biochemical detection methods may also be used.

Broadly speaking, chemical or biochemical detection methods can be categorized as either "presumptive" or "confirmatory tests. As a general rule, a presumptive test is very sensitive but not specific while a confirmatory test is less sensitive but more specific for the stain it is designed to detect. Because of this, a positive result from a presumptive test cannot be used to demonstrate that a particular stain is composed of a certain body fluid only that it might be. A presumptive test narrows the range of materials that a stain could be comprised of; it is not proof that a stain is a specific material. Conversely, a negative result from a confirmatory test cannot be taken as definitive that a stain is not of a certain type.

Forensics

A criminal always leaves something behind at the scene of a crime, some kind of evidence that connects criminal and crime [8]. Various forms of evidence include blood, semen, fingerprints, skin, hair, pieces of clothing, and bits of DNA. The fundamental challenge for the criminologist is to connect a piece of evidence with some specific individual—the perpetrator of the crime. That challenge consists of two parts: (1) finding, collecting, analyzing, interpreting, and preserving the evidence; and (2) locating and confirming the identity of the person with whom that evidence is associated. Today, criminologists have a host of techniques by which to achieve these objectives. They include serology (the study of blood samples), toxicology (the study of poisons and drugs), document analysis (the study of letters, forms, and other written materials), toolmarks (marks found on tools), and handwriting analysis. These tools make up the arsenal of the forensic scientist. Forensic science is the application of scientific knowledge and techniques to legal issues.

Some of these forensic tools have been in use for centuries; others have been developed only recently. Two of the oldest forensic technologies are anthropometry and fingerprint analysis. Both of these techniques are based on the same assumption: In order to connect a suspect with a crime, the criminologist must find at least one specific characteristic (or set of characteristics) that match both the evidence and the suspect, but that match no other individual anywhere in the world. For example, a forensic serologist attempts to show that the blood found at the scene of a crime could have come from only one specific person and not from anyone else.

Testing

DNA testing remains at the forefront of the forensic disciplines as a standard bearer of reliable testing procedures both in the scientific community and the legal system [9]. This level of integrity as a testing standard is

founded on the many stringent quality assurance policies and practices that DNA testing has adopted over time. The discipline is subject to many quality assurance components including guidelines and recommendations established by scientific working groups (SWG's), annual audit process, proficiency testing, in depth peer review of casework, testing controls, validations, and established interpretation criteria based on observed empirical data.

In its brief existence, DNA testing has flourished and established itself as a crucial and commonly utilized method of individualization in criminal investigations. As is the nature of science, there is a constant state of evolution and progressive development as technologies advance, novel methods are pioneered and limits of detection are pushed to ever increasing sensitivities.

As a result, today's crime laboratory is expected to keep pace with these changes while maintaining a strict commitment to quality. Critical to the establishment of new technology or approaches to DNA testing is the validation process to demonstrate the integrity of a method before utilization in case work.

Validation is achieved through documented testing and can be viewed as a critical aspect of any laboratory's quality assurance program. With regards to forensic short tandem repeat STR (short tandem repeat) analysis, delineation of boundaries and thresholds from validation data contribute to sound interpretation. Validation is necessary to create confidence in testing results, operating procedure, and assurance in quality, all of which play an important part in reassuring the reliability and impartiality required of DNA testing.

The DNA testing methodologies include RFLP, PCR, STR, RAPD and mtDNA (mitochondrial) [10]. Touch DNA (t-DNA) technique has given a new impetus as few cells only can be amplified into several copies of DNA using polymerase chain reaction (PCR) technique. Human beings have 99.9% of their DNA in common pool. The key features of DNA profiling include sequences of code known as short tandem repeats (STRs), which differ widely among individuals and provide nearly infallible means of connecting a genetic sample to its donor source.

DNA forensic provides invaluable assistance in analysing, evaluating and solving issues like (i) linkage to crime, (ii) identification of the culprit or the victim and (iii) correct parentage in paternity disputes. The matrix of DNA mainly addresses human identification based on analysis of biological sample and has established credibility in both civil and criminal legal fraternity. In criminal justice realm, DNA has successfully been used in investigating sexual offences and other violent body offences especially in cracking cold cases where conventional methodologies of investigation were found lacking. Non-human DNA also plays a vital role in forensic analysis of evidence.

Conclusion

The DNA profiling process begins with an individual sample commonly referred to as a "reference sample". Reference samples are usually collected with a buccal swab. When this is not available, other methods may be needed to take a blood sample, saliva, semen or sample from an item for personal use. Samples obtained from blood relatives may indicate the profile of the individual as well as previous profiled human remains. The reference sample is then analyzed to create an individual's DNA profile. The DNA

profile is then compared with other samples to determine if there is a genetic match.

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