NOTICIAS Y OPINIONES / NEWS AND VIEWS

DNA fingerprinting
Huella Genética

David Garcia[1] and Karla Miño[1]

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ABSTRACT

DNA is the hereditary material presents in all the cells of the body. This molecule presents some characterizes, as VNTR, unique present in different individual. This is a key in the development of some techniques, in this case DNA fingerprinting. This procedure has simple steps that we will review in this work. DNA fingerprinting technique has become an important tool for scientific research, we will review some applications in fields like forensic investigations and parentage testing, moreover how this technique has revolutionized and evolved in areas as Anthropological genetics, botany and zoology.

Keywords: DNA fingerprinting, forensic, parentage testing, botany, zoology.

RESUMEN

El ADN es el material hereditario presente en todas las células de nuestro cuerpo. Esta molécula posee algunas características como los VNTR, que son repeticiones de secuencias únicas para cada individuo. Esta característica ha sido la clave para el desarrollo de algunas técnicas de identificación como la huella genética. Este procedimiento tiene pasos simples que revisaremos en este trabajo. La huella genética se ha convertido en una herramienta útil para las investigaciones científicas y también ha sido usada en varios campos como las investigaciones forenses, pruebas de paternidad, genética antropológica, botánica y zoología. En este trabajo revisaremos como la huella genética ha revolucionado y evolucionado en las áreas mencionadas.

Palabras claves: huella genética, forense, prueba de paternidad, botánica, zoología.

Introduction

DNA, or deoxyribonucleic acid, is a complex molecule that contains all the information necessary to build and maintain an organism. It is the hereditary material. Every cell in the human body has the same DNA. The information of DNA is stored as a code constituted by four nitrogenous bases: Adenine (A), Thymine (T), Cytosine (C) and Guanine (G). The order or sequence of these bases determines the information available for building and maintaining an organism[1]. The human genome size is about 3,107 megabases (Mb) but only about 1.2 percent of the total genome encodes for proteins, this is around 20,000 genes, while 98.8 percent is noncoding DNA[2,3], which means that do not encode proteins. Within this group we have, for example, a variable number of tandem repeats (VNTR), which are repeated sequences of 9 to 100 base pairs (bp), that play a key role in the elaboration of DNA fingerprinting. Knowing the main DNA characteristics, specificity is the key to the emergence of DNA analysis. Numerous other techniques used to determine biological markers, such as HLA and blood group substances, have been successfully applied for identification purposes. All are based on exclusion, where markers are tested until a difference is found. Other factors favoring DNA analysis include the small sample requirement, the ability to rapidly replicate a sequence a millionfold or more in vitro, and the relative stability of DNA. The point is that DNA analysis alone can be a definitive test. Once the technique becomes routine, there is little doubt that, provided a suitable specimen can be obtained, DNA fingerprinting will be the single best test for excluding a falsely associated individual[4].

A brief history of DNA fingerprinting

In 1980, Wyman and White laid the foundations for the concept based on the observation of a polymorphic DNA locus characterized by a number of variable-length restriction fragments called restriction fragment length polymorphisms (RFLPs), which are specific sequences where restriction enzymes cleave the DNA. However, the history of DNA dates back to 1985 with the paper "Hypervariable Minisatellite Regions In Human DNA" written by Alec Jeffreys. Jeffreys and his coworkers were analyzing the human myoglobin gene when they discovered a region consisting of a 33-base-pair sequence repeated four times. This tandem repeat was referred to as a minisatellite and similar regions as hypervariable because the number of tandem repeats is variable both within a locus and between loci. In 1987, Nakamura coined the term variable number of tandem repeats (VNTR) to describe individual loci where alleles are composed of tandem repeats that vary in the number of core units. When DNA is isolated, cleaved with a specific enzyme, and hybridized under low-stringency conditions with a probe consisting of the core repeat, a complex ladder of DNA fragments is detected. This profile appears to be unique to each individual. Different core repeats were later isolated and used to produce a number of different probes useful for fingerprinting[4]. For that time, this technique was unknown but its potential was evident. DNA fingerprinting had its first application in 1985 in a case of parentage

[1]Universidad de Investigación de Tecnología Experimental Yachay Tech
Autor de correspondencia: david.garcia@yachaytech.edu.ec

testing, actually a maternity test, with paternal DNA unavailable. In this unusual case, a mother with her little 13 years old son were arrested in the airport when they arrived in England from Ghana because the authorities thought that he was not her son. A DNA fingerprinting applied to both demonstrated that, effectively, they told the truth. The first application of DNA fingerprinting in forensic identification happened later that same year, in a case that beautifully exemplifies the power of DNA evidence to link crime-scenes, to exclude suspects, and to support convictions. A suspect was arrested for allegedly committing a double rape and suicide to 2 minors[5]. A DNA fingerprinting using a sample of semen left in the crime scene demonstrated that a man had been responsible for both crimes but it was not the arrested suspect. He was released and the real culprit was arrested. Nowadays, this technique is still used to create DNA profile of each individual in order to clarify some crimes or parentage testing[6].

What is DNA fingerprinting?

In simple words, DNA Fingerprinting is the technology which is used to identify individuals on the basis of the molecular characteristics of the DNA7. More specific, this method uses VNRT because the number of bases and repeats within a locus is unique to each individual. For example, an individual can have in his genome the sequence gatagata and this repeats 10 times and another can have the same sequence but only repeats 5 times. The technique is used, as we have seen before, in parentage testing and forensic cases but it can be used for anthropological genetics, zoology, and botany among others disciplines. Importantly, the technique of DNA Fingerprinting is very sensitive, which means that it can also generate data even from half (partially) decomposed biological material[7].

Procedure to create a DNA fingerprinting.

The steps involve others techniques used in Molecular Biology, such as polymerase chain reaction (PCR) and electrophoresis among others. The following are the steps to generate a DNA fingerprinting.

1. The DNA is extracted from the nuclei of any cell in the body.
2. The DNA molecules are broken with the help of enzyme restriction endonuclease (called chemical knife) that cuts them into fragments. The fragments of DNA also contain the VNTRs.
3. The fragments are separated according to size by gel electrophoresis in agarose gel.
4. The separated fragments of single-stranded DNA are transferred onto a nylon membrane. Radioactive DNA probes having repeated base sequences complementary to possible VNTRs are poured over the nylon membrane. Some of them will bind to the of single-stranded VNTRs. The method of hybridization of DNA with probes is called Southern Blotting.
5. The nylon membrane is washed to remove extra probes.
6. An X-ray film is exposed to the nylon membrane to mark the places where the radioactive DNA probes have bound to the DNA fragments. These places are marked as dark bands when X-ray film is developed. This is known as autoradiography.
7. The dark bands on X-ray film represent the DNA fingerprints (DNA profiles)[5].

These steps are shown better in figure 1.

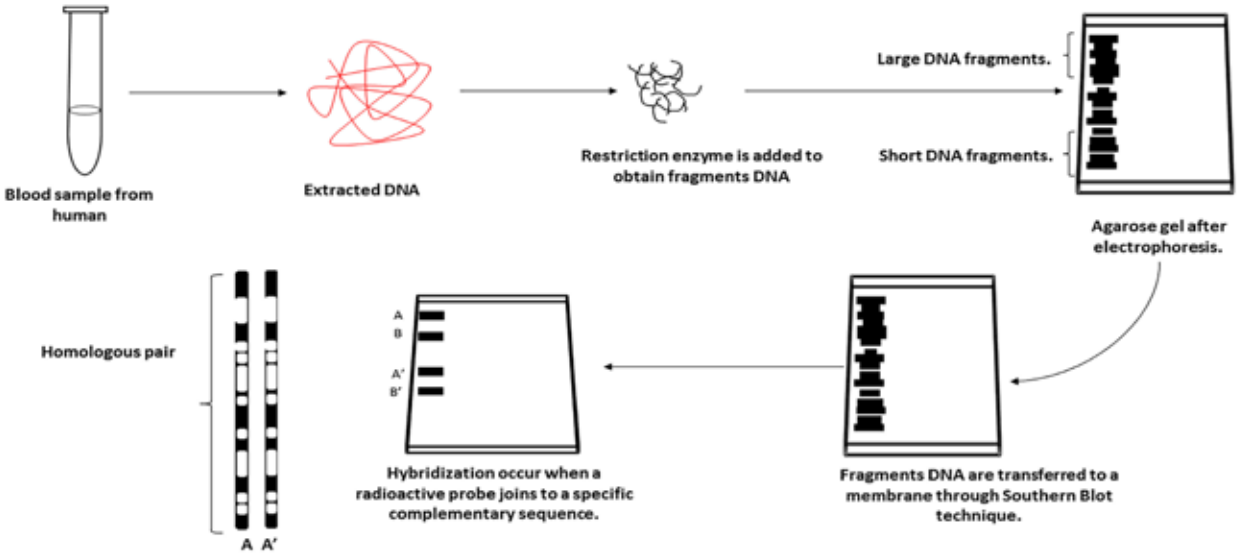

Figure 1. Schematic illustration of procedure in DNA fingerprinting

DNA Fingerprinting applications

Since Alec Jeffreys developed the DNA fingerprinting technique, it has been used in different scientific fields. In forensic investigations has helped to send to prison criminals, and identify victims of crimes, natural disaster, wars. Paternity disputes have been resolved thanks to this method. Moreover, disciplines as anthropological genetics, zoology, and botany among others have driven profiling research in order to interpret the origin and behavior of some species. In the next lines, we are going to describes how the technique has been applied and evolved in the areas mentioned above.

Forensic Investigations

Famous Crime T.V shows as CSI, Bones and others have popularized this technology. To summarize the methodology, genetic material like blood, semen, saliva, hair and skin found at the crime scene are processed, and afterward the samples are compared with the DNA of the suspects, in order to determine guilt or innocence of the accused.

DNA fingerprinting markers have evolved since 1984. In the beginning, sets of minisatellites or oligonucleotides stretches were used, also called multi-locus probes (MLP) which detected sets of 15 to 20 variable fragments per individual ranging from 3.5 to 20 kb in size. Minisatellites were replaced because they needed a large amount of molecular weight of DNA, usually not found at the crime scene and errors in the linkage between loci. For this reason was changed by single locus probe (SLP) which recognized single hypervariable locus, using high stringency hybridization and just 10 ng of DNA8.

Multilocus and Single Locus probes were part of the so-called restriction fragment length polymorphism (RFLP)-based methods were still limited by the available quality and quantity of the DNA. Those procedures were replaced by PCR-based methods because they improved sensitivity, speed, and genotyping precision. PCR-based methods use microsatellites as markers instead of minisatellites; microsatellites as short tandem repeats (STRs) are more sensitive and less prone to allelic dropout than VNTR (variable number of tandem repeat) systems8.

In cases, when there exist a low proportion of nuclear DNA samples, lineage marker is used which are obtained from mitochondrial and Y DNA, and they are very useful to reconstruct the paternal and maternal relationship and historical reconstruction in unidentified remains typically skeletonized, hair shafts without roots, or very old specimens where only heavily

degraded DNA is available likewise samples of sexual assault without ejaculation, sexual assault by a vasectomized male, male DNA under the fingernails of a victim, male 'touch' DNA on the skin8.

Parentage testing

DNA fingerprinting is an advantageous technique in cases, such as, ~~of~~ establishing the paternity of disputed offspring or ~~cases of~~ baby swapping. This method replaced ABO blood antigen systems which cannot establish paternity but can conclusively exclude an alleged father from being a candidate. Disputed paternity originates because of affiliation orders, divorce proceedings and questioned the legitimacy, also is used to discover paternity in cases of inheritance, guardianship, maintenance, legitimacy, adultery or fornication9.

In Parentage testing, a DNA comparison is performed between progeny against potential parents. Children inherit half of their alleles from each parent and thus should possess an alleles combination of their parents.

Anthropological genetics

In anthropological genetics, markers have been used as ancestry-informative markers to reconstruct the human diaspora and to interpret the evolutionary history of human populations to inquire population origins, migration, admixture and adaptation to different environments, as well as susceptibility and resistance to disease[10].

The main markers used by anthropological genetic are variable-number tandem repeats (VNTRs), short tandem repeats (STRs), mitochondrial DNA haplo groups, Y-specific non-recombining region (NRY) haplotypes, and single nucleotide polymorphisms (SNPs).

In the medical field, researchers have made possible the mapping quantitative trait loci involved in biological pathways of diseases such as diabetes mellitus, cancers, obesity, osteoporosis, and coronary heart disease. In the studies of population, markers allow identifying the presence, absence, or high frequency in some populations and low frequencies in others, of certain genetic traits that characterize some specific population[11].

Botany

DNA fingerprinting is an essential tool for genotype identification in both wild plant and cultivated species. DNA profiling is used for protection of biodiversity, identifying markers for traits, identification of gene diversity and variation[12].

Identification in plants always been an issue for botanists because of the large variability of the composition and relative amount of chemicals in particular species of the plant varies with growing condition, harvesting period, post-harvesting period and storage conditions.

Due to large variability, DNA fingerprinting technique uses several types of markers for example, Inter Simple Sequence Repeat (ISSR), Random Amplification Polymorphic DNA (RAPD)/Arbitrary Primed PCR, Amplified Fragment Length Polymorphism (AFLP), DNA Amplification Fingerprinting (DAF), Simple Sequence Repeats (SSR), Sequence Characterized Amplified Region (SCAR), Cleaved Amplified Polymorphic Sequence (CAPS) and Single Nucleotide Polymorphism (SNP)[12].

DNA markers help to study fundamental evolutionary influences of natural selection, mutation, gene flow and genetic drift on wild plant populations and identify groups are characterized by highly variable ploidy levels, often even within the same species. Moreover, the method detects both ancient and ongoing hybridization between crops and wild species[12].

Zoology

In Zoology, DNA fingerprinting determine the genetic identity of individuals and measure genetic variation in natural populations, allowing true genetic relationships among individuals to be determined, rather than them being inferred from field observations. Furthermore, it helps to test predictions of kin selection models in a realistically way, and detect hybrids species[13].

The DNA marker clarified mating system in reproductive ecology for example ~~in~~ vertebrates that give birth to more than one offspring has revealed concurrent multiple paternities. This kind of behavior has been observed in a wide range of organisms, particularly in reptiles.

DNA microsatellites have been useful tools describing population connectivity, isolation, and the particulars of interpopulation gene flow, also now they are being used to document levels of genetic variation in rare and endangered species and thus better inform conservation management actions[13].

Conclusions

DNA fingerprinting technique has become an important tool for scientific research, because it allows identifying patterns in the known coding region of genetic material that makes every individual unique, for that reason, areas as forensic investigations and parentage testing have found an instrument to convict criminals, identify victims, and solved parentage disputes.

Since Alec Jeffreys developed DNA fingerprinting technique in 1984, the technique has gone through for many adjustments, from southern blot to PCR methods, from minisatellites to micro satellites and new markers have been developed according to the needs of research fields for example in Anthropological genetics, botany, and zoology.

In the near future, we will be able to learn more about the dynamics of the history of the populations in humans and animals, discover new or hybrid species in plants and animal, besides knowing more about their genetic information.

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