# Biotechnological Applications of Recombinant DNA

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# Genetic Fingerprints and Application in Forensic Medicine

## 1. Objectives

- Explore the methods and techniques used to create genetic profiles.
- Discuss how genetic fingerprinting is used to identify suspects.

## 2. Introduction to genetic fingerprints

DNA fingerprinting, also known as genetic fingerprinting, is a powerful technique used for identifying individuals based on their unique DNA profiles. This method relies on detecting and analyzing variations in specific regions of the genome that are known to differ significantly between individuals. By examining these regions, scientists can determine whether two DNA samples come from the same person, related individuals, or unrelated individuals, making it a critical tool in fields such as forensic medicine, paternity testing, and genealogical research*84*[84]*.

At the heart of DNA fingerprinting lies the concept of genetic variation. Human genomes share a high degree of similarity, with more than 99% of DNA being identical across all individuals. However, the remaining 1% contains genetic markers that vary from person to person, and these variations can be used as unique identifiers. DNA fingerprinting exploits these variations, focusing on regions of the DNA that are highly polymorphic, meaning they exhibit a great deal of variability within the population.

(cf. DNA Fingerprinting)

## 3. Genetic Markers in DNA Fingerprinting

Several genetic markers are commonly used in DNA fingerprinting, each offering different advantages in terms of resolution and application. The most frequently utilized markers include Short Tandem Repeats (STRs), Restriction Fragment Length Polymorphisms (RFLPs), and Single Nucleotide Polymorphisms (SNPs). These markers differ in their underlying genetic properties and the techniques required for their analysis.

### 3.1. Short Tandem Repeats (STRs)

Short Tandem Repeats (STRs) are repeating sequences of 2-6 base pairs of DNA that are found at specific loci in the genome. These repeats vary in number between individuals, making them excellent candidates for genetic identification. STRs are highly polymorphic, meaning that the number of repeats at each locus can differ significantly between individuals, and even between close relatives. By analyzing multiple STR loci simultaneously, scientists can generate a unique DNA profile for each individual*85*[85]*.

The primary advantage of STRs is their high discriminatory power. Because these loci exhibit a high degree of variability, even individuals from the same family or ethnic group can be distinguished based on their STR profiles. STRs are commonly used in forensic

science for criminal investigations, paternity testing, and identification of missing persons. The PCR (Polymerase Chain Reaction) amplification method is commonly employed to generate STR profiles, making this technique both sensitive and efficient.

***Méthode* : Short Tandem Repeats using electrophoresis**

- Collect DNA sample by obtaining from any material containing cells (saliva, hair, and blood).
- Extract DNA from the sample using chemicals (detergents) allowing to break open the cell. DNA is separated from the cell components.
- Amplify STR fragments using PCR to make copies of <13 different STR regions=**COMPLICATED**
- This is achieved by using primers which bind to the DNA on each side of the STR.
- DNA polymerase binds to the primers and synthesizes copies of the fragments= **ends with thousand of copies of STR**.
- Determine length of the STRs by separating STR fragments by gel electrophoresis. Larger fragments move through the gel slower and vis versa.
- The resulting patter of bands on the electrophoresis gel creates a DNA fingerprint referring to the DNA ladder (Blank).

***Méthode* : Short Tandem Repeats using capillary electrophoresis**

Collect DNA sample and extract them from the cell similar to the previous one.

Amplification of STR fragment involves using PCR based fluorescent primers which bind with the STR region= every copy has a fluorescent.

The capillary electrophoresis is used to separate fragment.

As each fragment passes the laser, its fluorescent tag lights up and measured by detector and recorded to produce electropherogram.

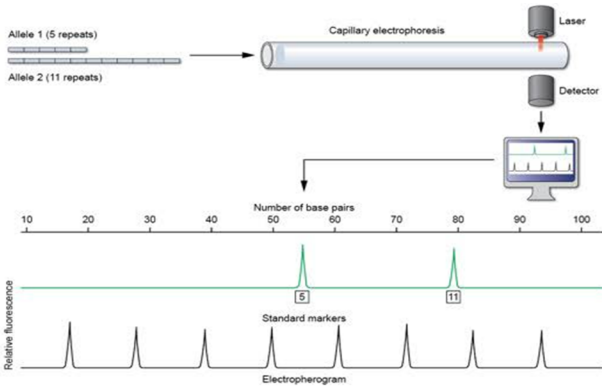



*Image 1 Short Tandem Repeats using capillary electrophoresis*

## 3.2. Restriction Fragment Length Polymorphisms (RFLPs)

Restriction Fragment Length Polymorphisms (RFLPs) involve detecting variations in DNA fragment lengths that are produced when genomic DNA is digested with specific restriction enzymes. These enzymes cut the DNA at specific sequences, and the resulting fragments can be separated and analyzed based on their size. Variations in the length of these fragments, due to differences in the presence or absence of restriction enzyme recognition sites, can be used to distinguish between individuals.

RFLPs have been widely used in genetic fingerprinting, especially in the early years of the technique's development. While RFLPs offer high-resolution genetic information, the method is relatively labor-intensive and requires large amounts of high-quality DNA. In

contrast to STRs, RFLPs are less commonly used today in forensic applications, as STRs have largely supplanted them due to their simplicity, higher throughput, and ability to generate more easily interpretable data[86].

### 3.3. Single Nucleotide Polymorphisms (SNPs)

Single Nucleotide Polymorphisms (SNPs) represent the most basic form of genetic variation. SNPs are variations in a single base pair of DNA at a specific location in the genome. Unlike STRs and RFLPs, which involve longer regions of the genome, SNPs involve changes in a single nucleotide, such as an A-to-T substitution. These variations are abundant in the human genome and are used as genetic markers for identifying individuals, especially in association with certain traits or diseases[87].

SNPs offer the advantage of being stable across generations, making them ideal for genealogical studies and ancestry tracing. However, their lower degree of variability compared to STRs limits their utility in some forensic applications, where greater discrimination is needed. Despite this, SNPs are increasingly being incorporated into DNA fingerprinting, particularly in applications where high-throughput sequencing or the analysis of large genomic datasets is required.

## 4. The use of mitochondrial genes in DNA fingerprint

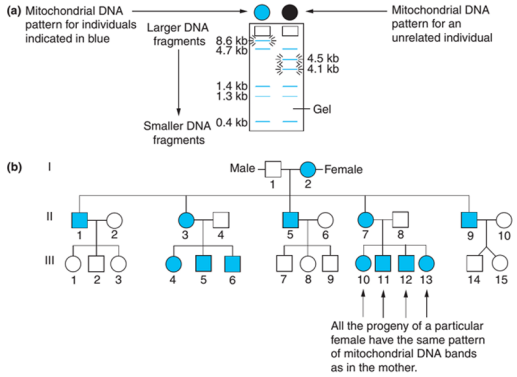
Mitochondrial DNA (mtDNA) is a unique type of DNA located within mitochondria, the membrane-bound organelles found in the cytoplasm of human cells. These organelles are primarily responsible for generating the cell's supply of adenosine triphosphate (ATP), the main energy source for cellular functions. Unlike nuclear DNA, which is inherited from both parents, mtDNA is maternally inherited, providing a distinct genetic lineage that is highly valuable in various scientific and forensic applications.

Mitochondria share many structural and functional characteristics with bacteria, which has led scientists to hypothesize that they originated as prokaryotic organisms that entered into a symbiotic relationship with early eukaryotic cells. Over evolutionary time, these prokaryotes became integral to the host cell's function, particularly in energy production. This endosymbiotic theory is supported by the fact that mitochondria have their own circular DNA, similar to bacterial genomes, and replicate independently of nuclear DNA[88].

The number of mitochondria in a cell varies depending on the cell's type and function. For instance, mature red blood cells lack mitochondria, while highly metabolic cells, such as oocytes, can contain hundreds of thousands of mitochondria. In contrast, human liver cells typically contain around 2,000 mitochondria. This variation in mitochondrial content makes mtDNA a critical target in forensic investigations, especially in cases where nuclear DNA is either absent, degraded, or available in limited amounts[89].

One of the key advantages of mtDNA in forensic science is its high copy number. While a cell only contains two copies of nuclear DNA, it can contain hundreds to thousands of copies of mtDNA. This abundance makes it more likely that usable mtDNA can be retrieved from forensic samples that are too degraded for nuclear DNA analysis, such as hair shafts, bones, and teeth. Moreover, mtDNA's maternal inheritance pattern allows for the reconstruction of maternal lineages over many generations, making it a valuable tool in cases of unidentified remains or missing persons[88].

1. Older biological samples that lack nucleated cellular material, such as hair, bones, and teeth, cannot be analyzed with STR and RFLP, but they can be analyzed with mtDNA.
2. Comparing the mtDNA profile of unidentified remains with the profile of a potential maternal relative can determine whether they share the same mtDNA profile and are related.
3. Since mtDNA remains virtually the same from generation to generation, changing only about **2% to 4%** every million years due to random mutation.
4. Consequently relationships can be traced through the unbroken maternal line, as shown in Figure bellow.
5. It has been shown that forensic scientists can amplify the HV1 and HV2 regions of the mtDNA, and then sequence each region and compare single nucleotide differences to a reference sample.
6. Also, mtDNA that is maternally inherited come from the mother and can be directly linked to maternal relatives and can be used as match references to establish family relationships through the mother's side.




*Mitochondrial genes in DNA fingerprint*

# 5. DNA fingerprinting by Y chromosome

The male-specific region of the human Y chromosome is a crucial tool in forensic DNA analysis, particularly in scenarios where conventional autosomal DNA profiling fails to provide informative results. A Y-chromosomal gene fragment can be used to determine the biological sex of an individual linked to a crime scene. Y-chromosome short tandem repeat polymorphisms (Y-STRs) are utilized to construct haplotypes that identify paternal lineages of unknown male contributors. This is especially useful in cases where both males and females have contributed to the same sample, such as in sexual assault investigations[90]*.

Y-STR haplotyping has several key applications in forensic investigations:

(i) it can exclude male suspects from involvement in a crime,

(ii) it can identify the paternal lineage of male perpetrators,

(iii) it can reveal the presence of multiple male contributors to a single sample,

(iv) it can offer investigative leads for identifying unknown male perpetrators.

Additionally, Y-STR analysis is applied in paternity disputes involving male offspring and other paternal kinship tests, including historical cases, missing person investigations, and disaster victim identification.

Y-chromosome polymorphisms are also useful in determining the paternal biogeographic ancestry of unknown individuals or missing persons when autosomal DNA profiling is inconclusive. This makes Y-chromosome analysis a valuable tool in both forensic and genealogical contexts, providing insights where other DNA methods fall short[90].

# 6. Applications of DNA Fingerprinting

The applications of DNA fingerprinting are diverse, spanning several fields of research and practice. Some of the most prominent applications include:

- **Forensic medicine**: DNA fingerprinting is a cornerstone of forensic science, used for solving crimes, identifying victims, and exonerating the innocent. By comparing DNA samples obtained from crime scenes with those from suspects, investigators can either link a suspect to a crime or rule them out as a perpetrator.
- **Paternity and kinship testing**: DNA fingerprinting is commonly used in establishing biological relationships, such as determining paternity or verifying familial connections in cases of inheritance, adoption, or immigration.
- **Genetic disease research**: Genetic fingerprinting helps identify individuals who may carry specific genetic mutations, contributing to research on the genetic basis of diseases and facilitating early diagnosis or personalized treatment strategies.
- **Wildlife conservation and biodiversity studies**: DNA fingerprinting is also employed in monitoring wildlife populations and managing biodiversity. It allows conservationists to track genetic diversity, monitor species health, and even detect poaching activities.

# 7. Exercice : DNA fingerprint

[solution n°1 p. 9]

**Which of the following is a commonly used genetic marker in DNA fingerprinting?**

Genetic marker

- ○ Long tandem repeats (LTRs)
- ○ Short tandem repeats (STRs)
- ○ Large nucleotide polymorphisms (LNPs)
- ○ MicroRNAs (miRNAs)

**Which method is used to separate DNA fragments based on size in DNA fingerprinting?**

Separate DNA fragments

- ○ PCR
- ○ DNA sequencing
- ○ Gel electrophoresis
- ○ Capillary electrophoresis

**How many STR regions are usually amplified in forensic DNA analysis?**

STR regions

**What binds DNA in PCR?**

Link DNA in PCR

**What is a maternal inheritance marker?**

DNA inherit

**Which enzyme is used to cut DNA into fragments during the DNA fingerprinting process?**

Cut DNA

- DNAase
- Restriction enzyme
- RNAase
- Helicase

# Solutions des exercices

## Solution n°1

[exercice p. 7]

**Which of the following is a commonly used genetic marker in DNA fingerprinting?**

Genetic marker

- ○ Long tandem repeats (LTRs)
- ◉ Short tandem repeats (STRs)
- ○ Large nucleotide polymorphisms (LNPs)
- ○ MicroRNAs (miRNAs)

**Which method is used to separate DNA fragments based on size in DNA fingerprinting?**

Separate DNA fragments

- ○ PCR
- ○ DNA sequencing
- ◉ Gel electrophoresis
- ○ Capillary electrophoresis

**How many STR regions are usually amplified in forensic DNA analysis?**

STR regions

13 regions

**What binds DNA in PCR?**

Link DNA in PCR

Primers bind DNA

**What is a maternal inheritance marker?**

DNA inherit

Mitochondrial DNA (mtDNA)

**Which enzyme is used to cut DNA into fragments during the DNA fingerprinting process?**

Cut DNA

- ○ DNAase
- ◉ Restriction enzyme
- ○ RNAase

- Helicase

# Glossaire

**Polymerase chain reaction (PCR)**

A method widely used to make millions to billions of copies of a specific DNA sample rapidly, allowing scientists to amplify a very small sample of DNA (or a part of it) sufficiently to enable detailed study.

# Abréviations

**DNA :** Deoxyribonucleic acid ; is the molecule that carries genetic information for the development and functioning of an organism.

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