DNA FINGERPRINTING

By
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Introduction

- DNA profiling (also called DNA testing, DNA typing, or genetic fingerprinting) is a technique employed by forensic scientists to assist in the identification of individuals by their respective DNA profiles.
- Although 99.9% of human DNA sequences are the same in every person, enough of the DNA is different to distinguish one individual from another.
- DNA profiling uses repetitive ("repeat") sequences that are highly variable, called variable number tandem repeats (VNTRs), and short tandem repeats (STRs). VNTR loci are very similar between closely related humans, but so variable that unrelated individuals are extremely unlikely to have the same VNTRs.

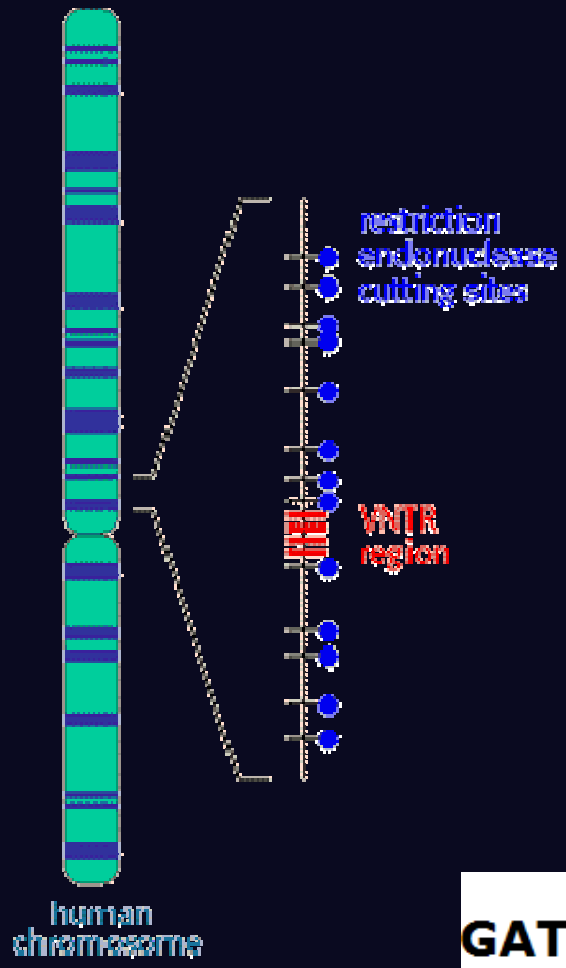
History

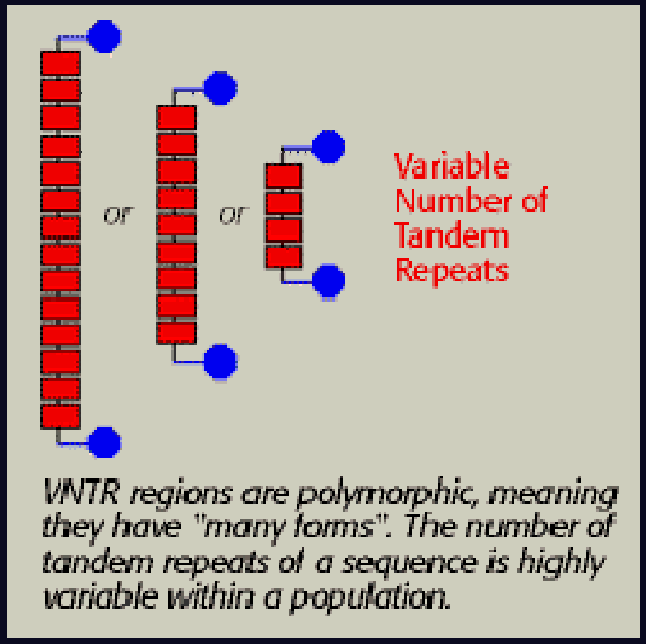
- The DNA profiling technique was first reported in 1984 by Sir Alec Jeffreys at the University of Leicester in England.

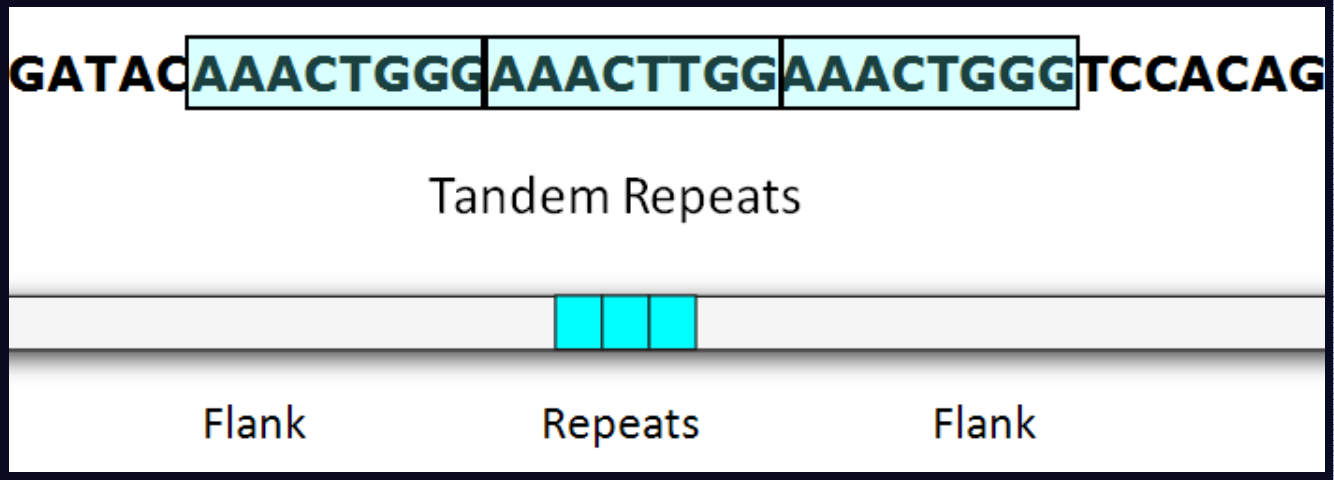
- Dr. Jeffreys's genetic fingerprinting was made commercially available in 1987.

Principle

- The entire genetic information of an individual is called genome. Genome contains the DNA sequence, which has both coding and non coding genes.
- The DNA sequences of humans are 99.9% similar in every individual. However, the other 0.1% is what makes each one of us unique. This 0.1% sequence mainly has specific codes that repeat itself throughout the sequence.
- These are short and varied sequences, and are known as VNTRs (Variable Number of Tandem Repeats). The frequency and position of these repeats vary greatly from one individual to the other. DNA fingerprinting uses such VNTRs from an unknown DNA sample to compare and match with the known.


restriction endonuclease cutting sites
VNTR region
human chromosome

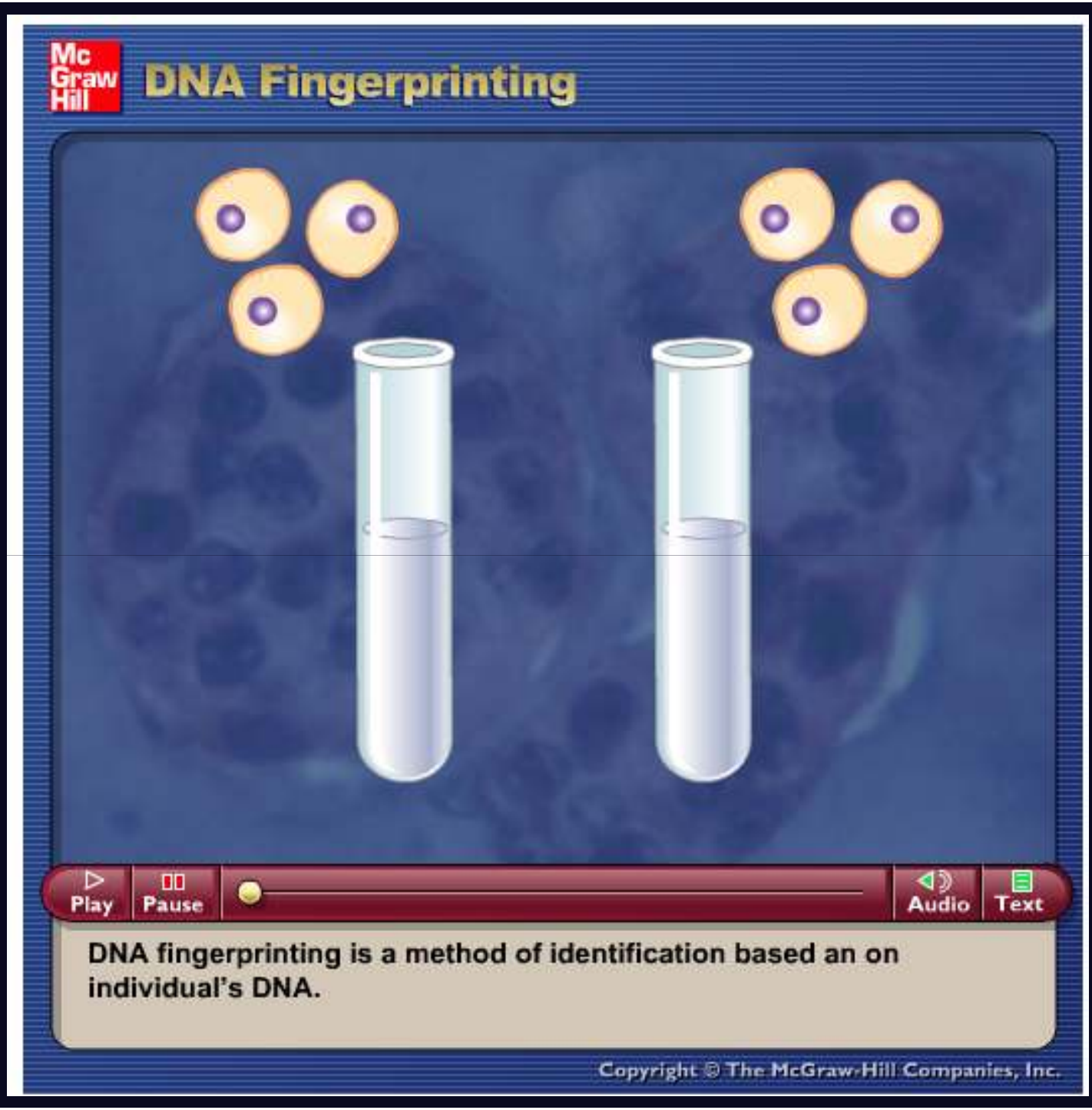

or
or
Variable Number of Tandem Repeats
VNTR regions are polymorphic, meaning they have "many forms". The number of tandem repeats of a sequence is highly variable within a population.


GATACAAACTGGGAAACTTGGAAACTGGGTCCACAG
Tandem Repeats
Flank
Repeats
Flank

- Step 1: To begin with, one should have a source of DNA sample. This source could be a single strand of hair, semen, blood (a single drop will work too!), saliva, cheek cell, etc. During criminal investigations the hair, semen, or blood of the suspect is collected for further analysis.
- Step 2: The next step is to extract the DNA sample from its source. The extraction process is devised in a way to break down the cell membrane and release the DNA to its outer environment. Detergents are used for this purpose. They tend to break open the cell membrane by forming micelles with protein and lipid entities of the membrane.

- Step 3: Once the DNA is extracted, it is subjected to digestion by restriction endonucleases. These are enzymes that cut a DNA fragment at specific sites which it recognizes. It means that each restriction enzyme (R.E.) recognizes a specific DNA sequence and cuts at a specific site.
- Step 4: These fragments are then separated by difference in their length using gel electrophoresis technique. DNA pieces are separated according to their length.

- Step 5: The next step is the Southern blotting technique.
- Blotting the DNA gel on to a suitable membrane. The most commonly used is the nitrocellulose membrane.
- Due to this, DNA fragments are pulled and transferred on the membrane. The membrane is now a replica of the original DNA pattern on the gel.
- This step is followed by hybridization with a suitable DNA probe. This DNA probe having a sequence complementary to the desired sequence.
- Before these probes are used, they are tagged with a radioactive substance or a color-inducing (chromogenic) or fluorescent dye. This helps in detecting the matched DNA visually.
- Resulting membrane is visualized using autoradiography. The X-ray film shows the hybridization pattern. The DNA sample thus hybridized with the probe can be seen and studied. This hybridization pattern is called DNA fingerprint, having a sequence complementary to the probe.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DNA Fingerprinting
Play
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Audio
Text
DNA fingerprinting is a method of identification based an on individual's DNA.
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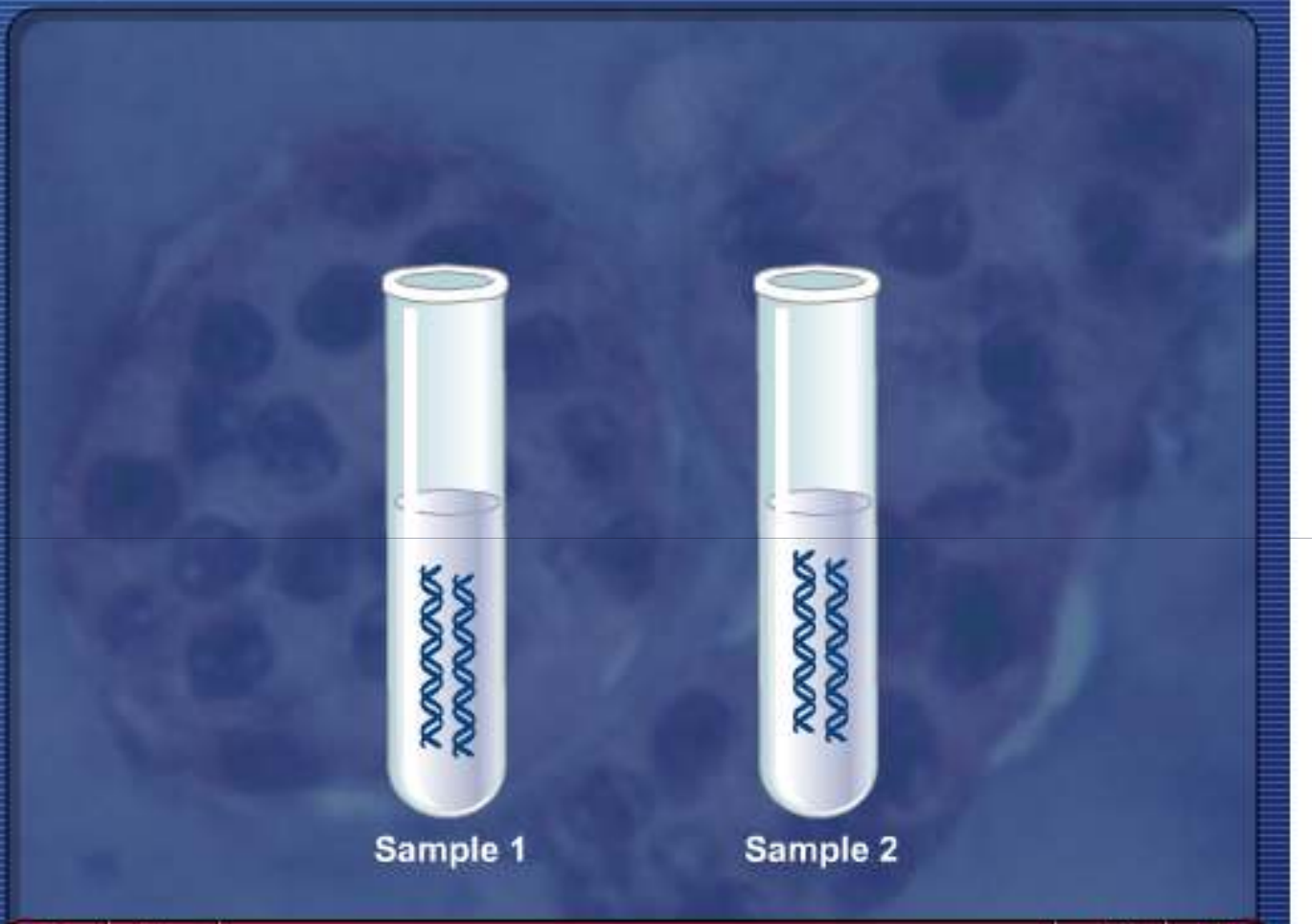
DNA Fingerprinting



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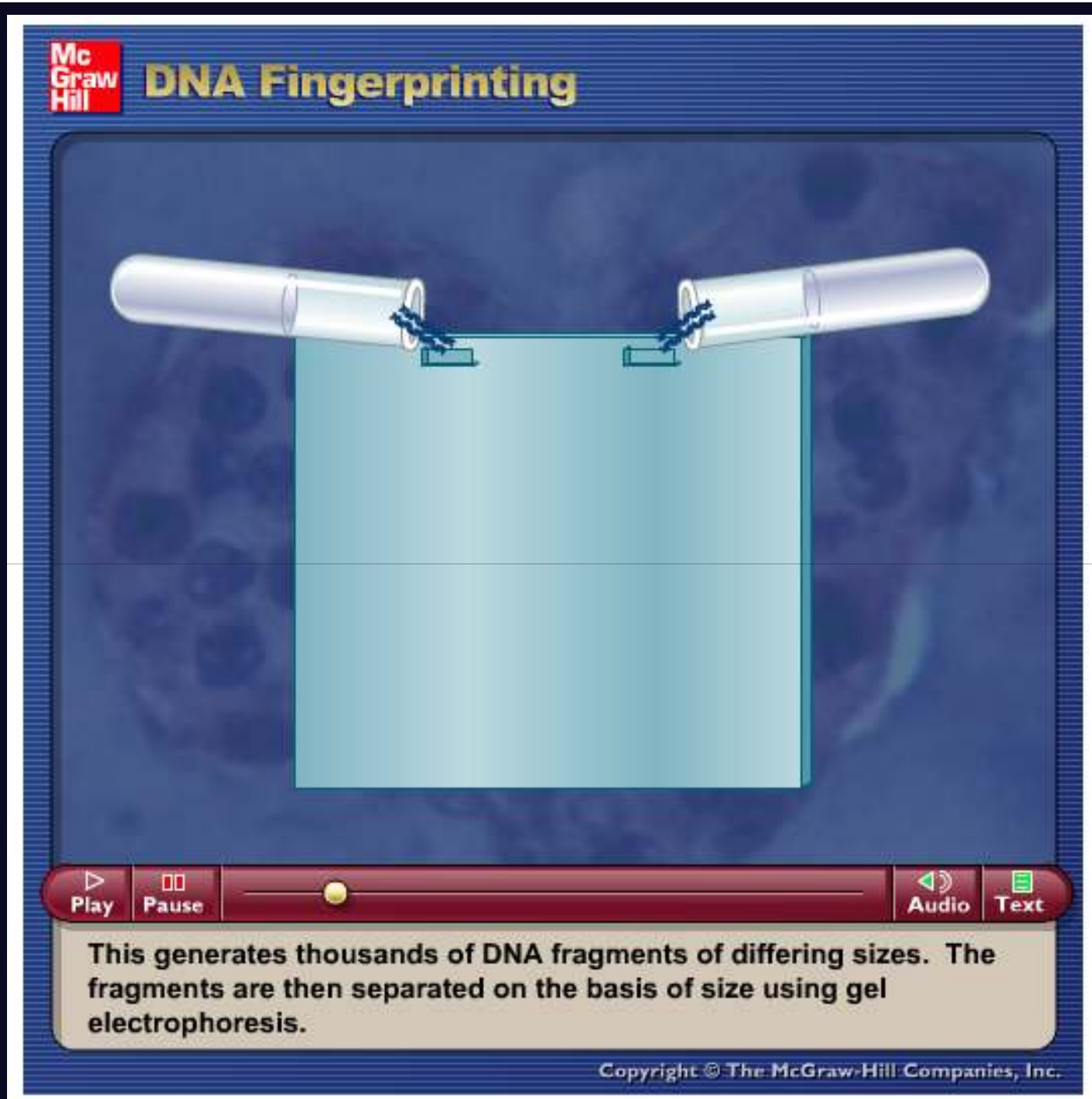
In traditional DNA fingerprinting, the DNA is collected from cells, such as a blood sample, and cut into small pieces using a restriction enzyme.


Mc Graw Hill
DNA Fingerprinting
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This generates thousands of DNA fragments of differing sizes. The fragments are then separated on the basis of size using gel electrophoresis.
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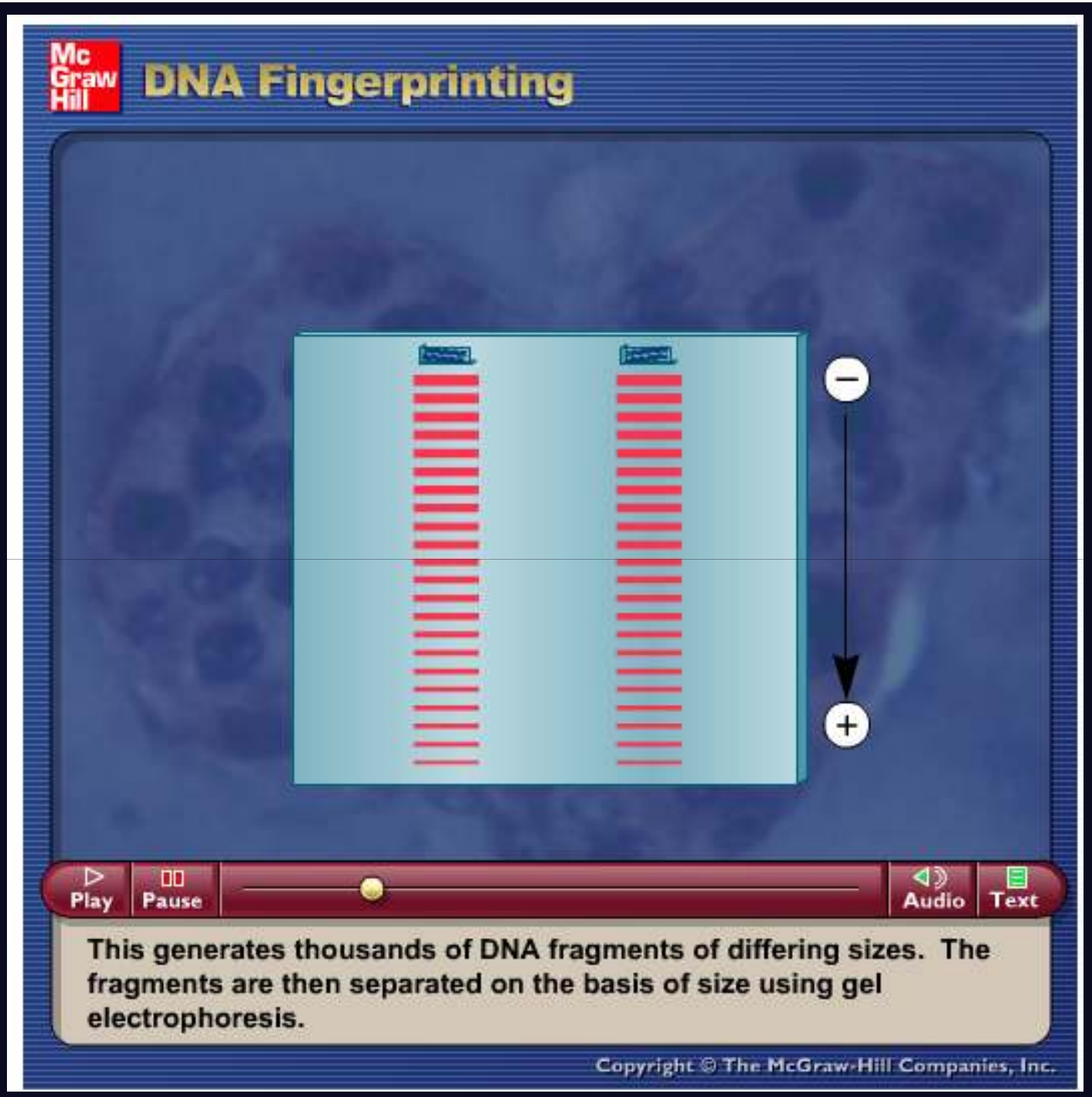
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DNA Fingerprinting
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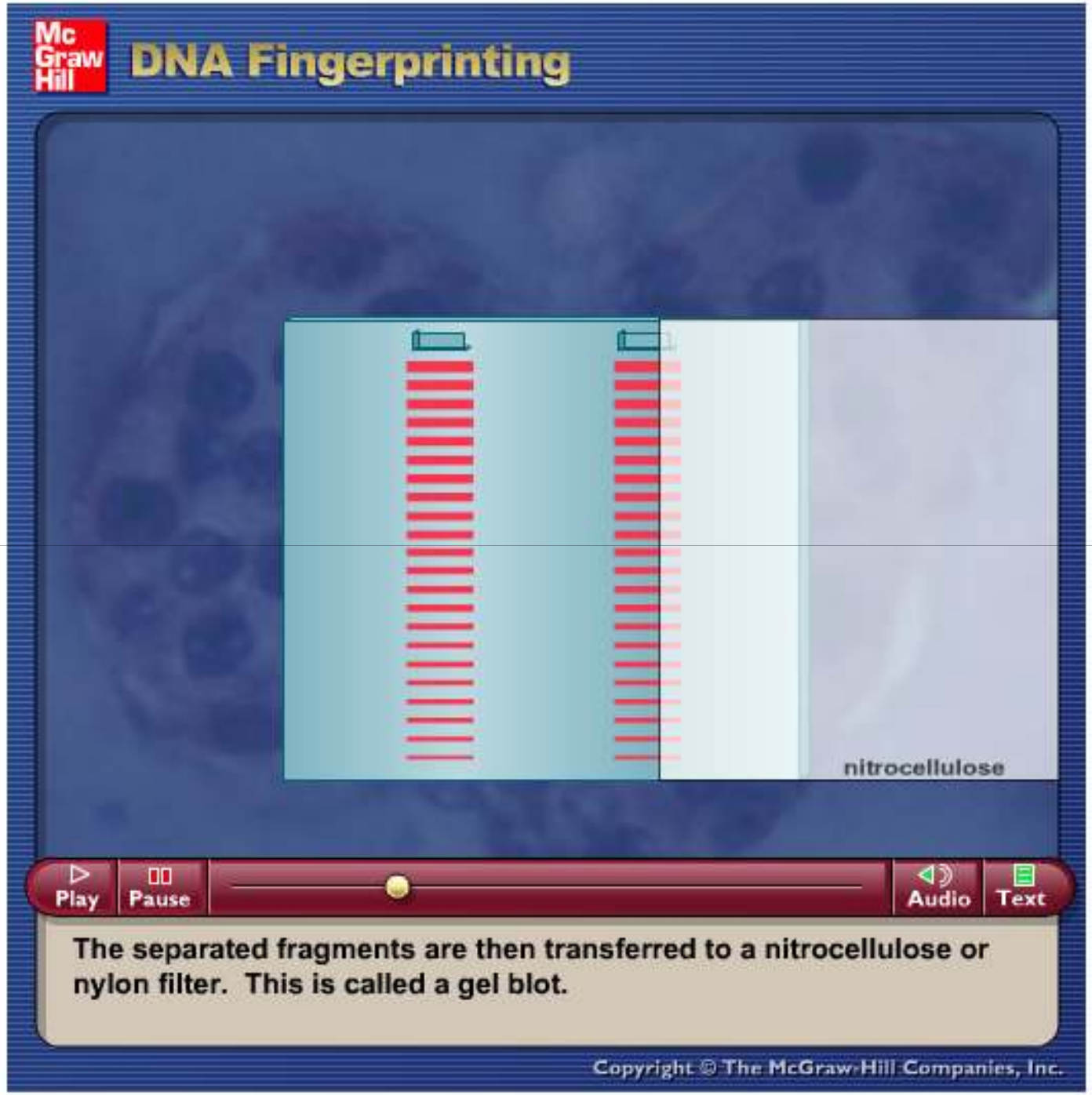
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DNA Fingerprinting
nitrocellulose
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The separated fragments are then transferred to a nitrocellulose or nylon filter. This is called a gel blot.
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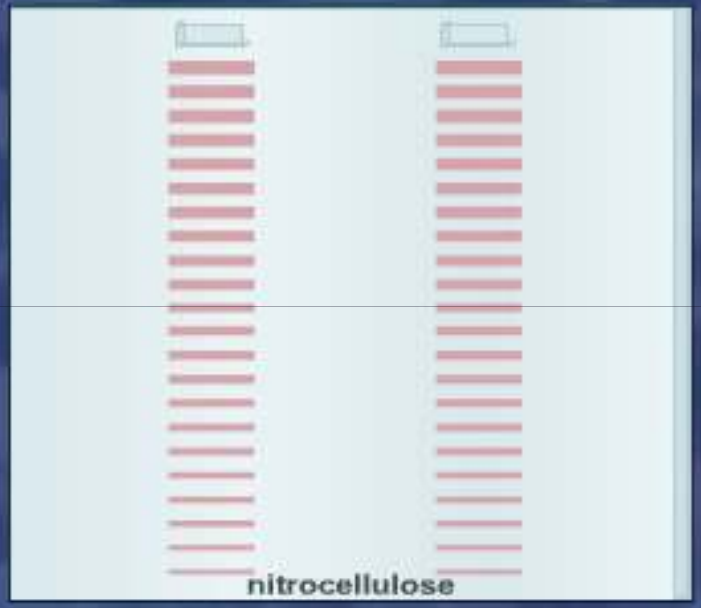
DNA Fingerprinting



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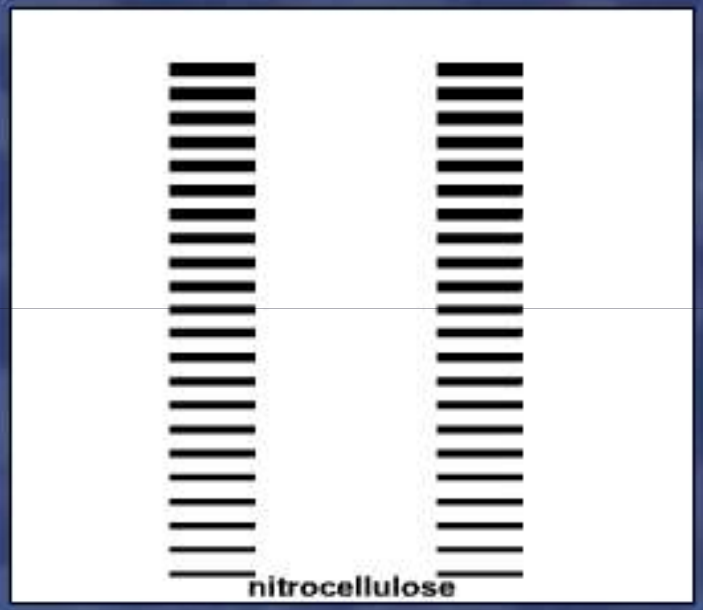
DNA Fingerprinting



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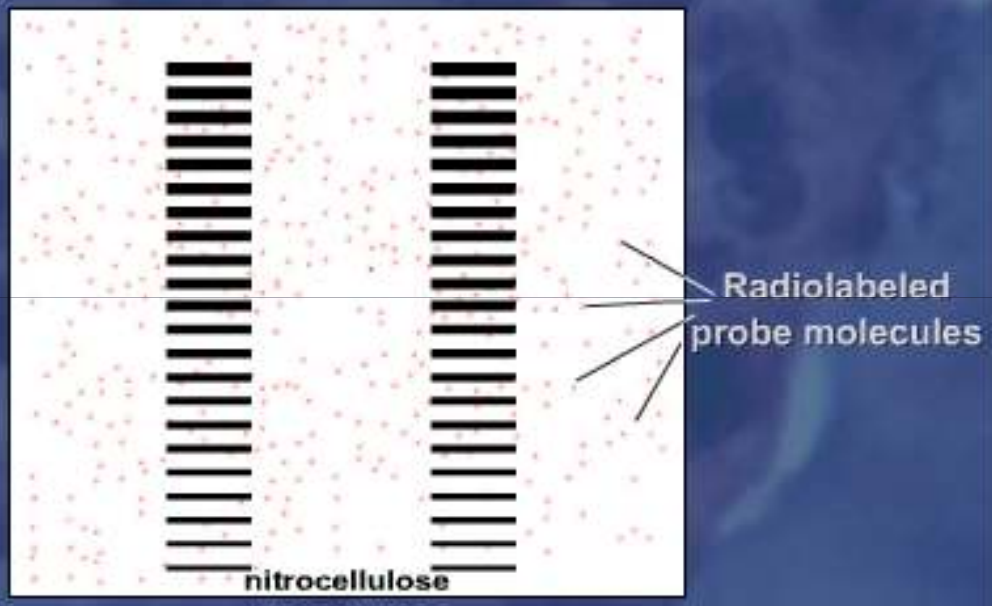
The DNA fragments within the blot are permanently fixed to the filter, and the DNA strands are denatured.

DNA Fingerprinting



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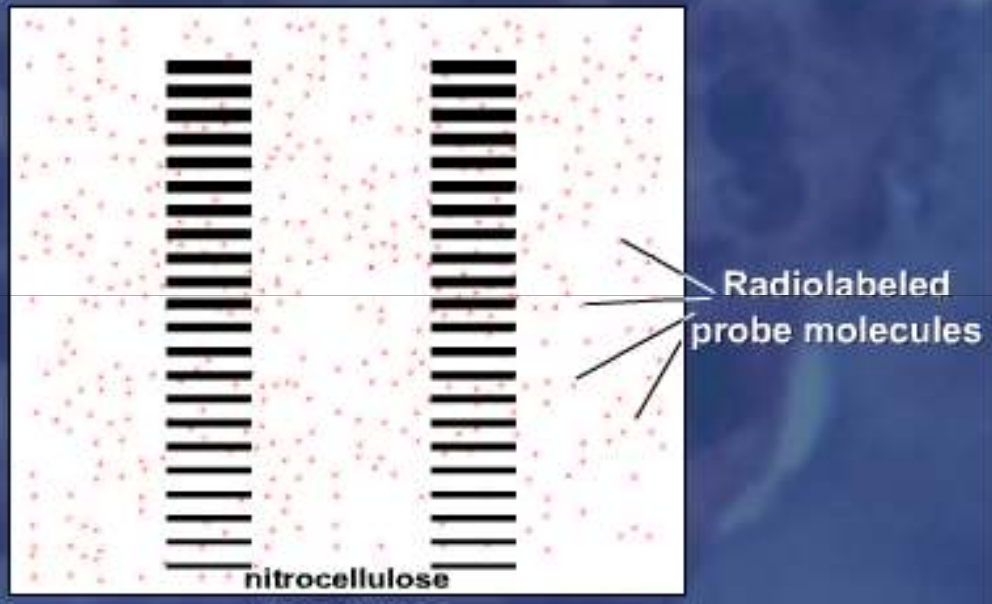
Radiolabeled probe molecules are then added that are complementary to sequences in the genome that contain repeat sequences.

DNA Fingerprinting



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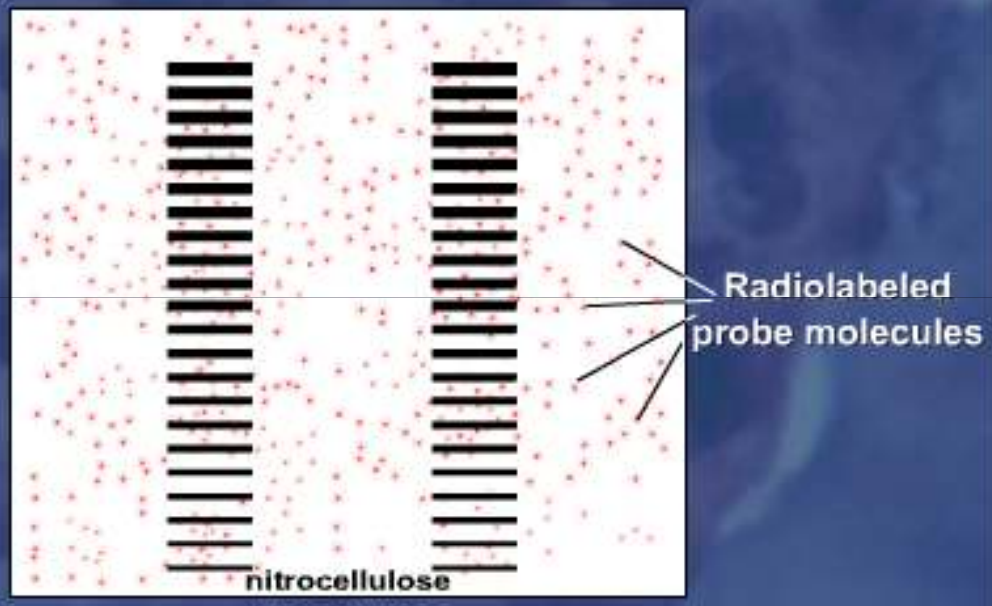
These repeat sequences tend to vary in their length among different individuals and are called variable number of tandem repeat sequences or VNTRs.

DNA Fingerprinting



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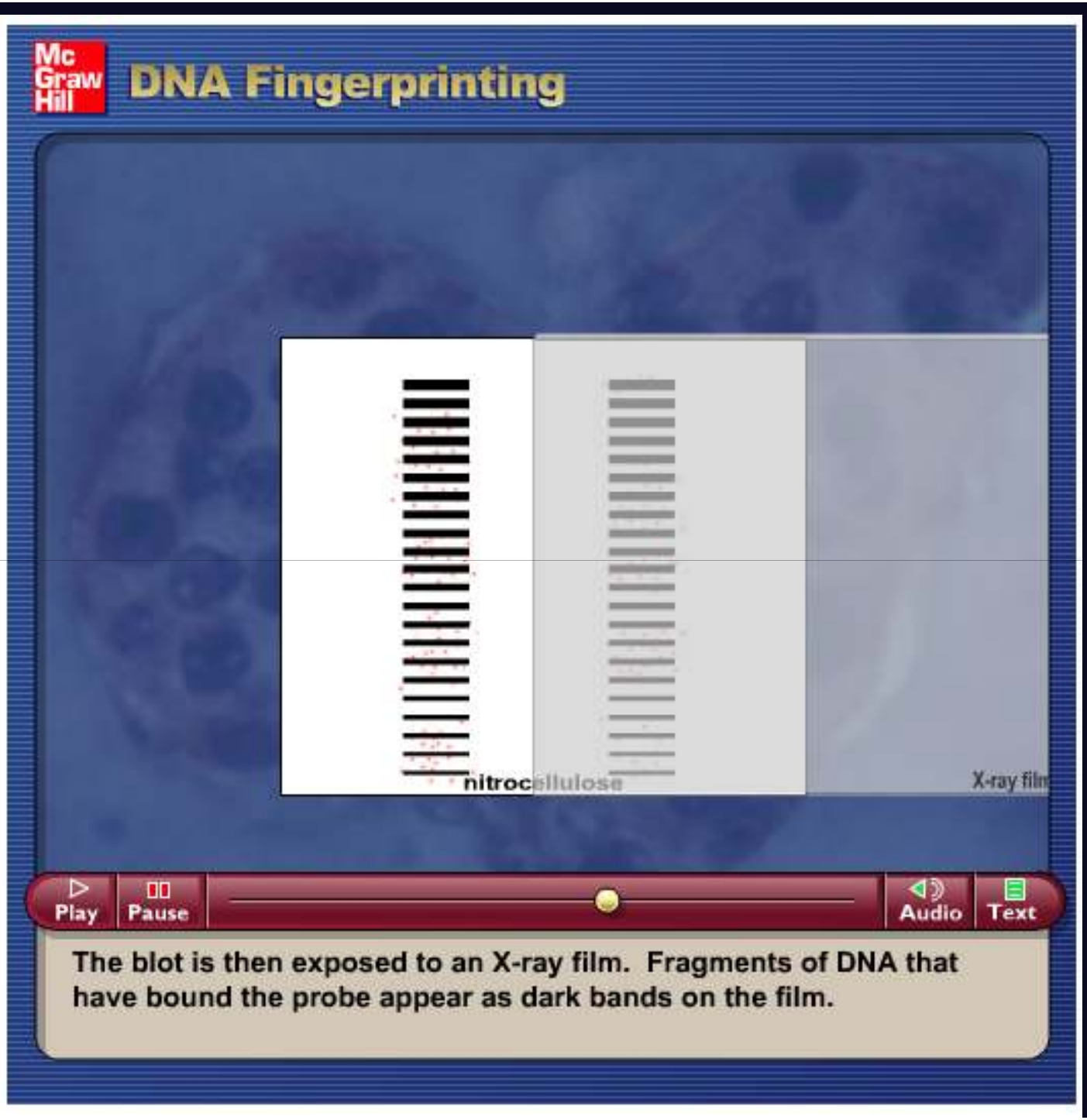
The probe molecules hybridize to DNA fragments containing the repeat sequence and excess probe molecules are washed away.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DNA Fingerprinting
nitrocellulose
X-ray film
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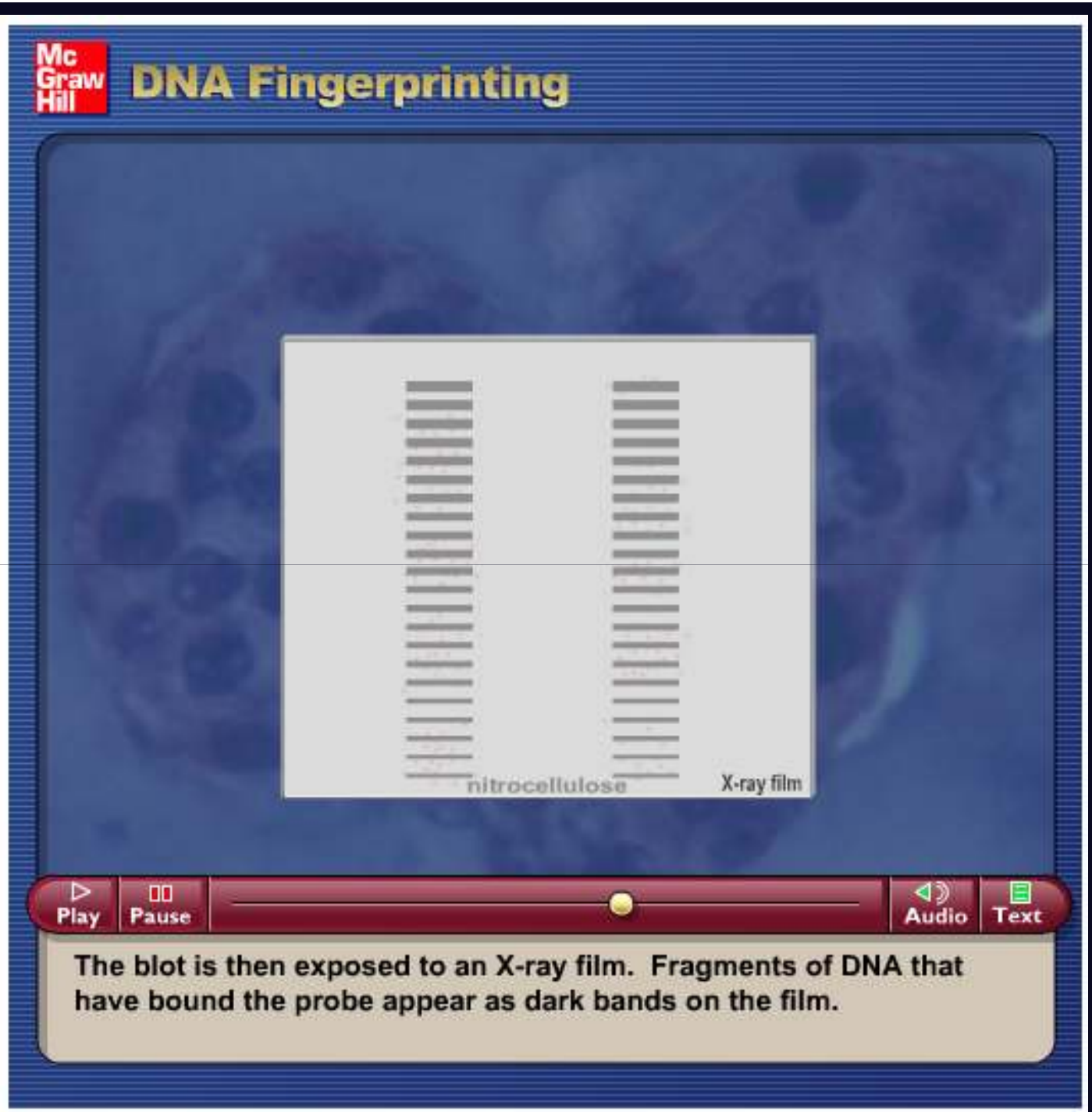
Text
The blot is then exposed to an X-ray film. Fragments of DNA that have bound the probe appear as dark bands on the film.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DNA Fingerprinting
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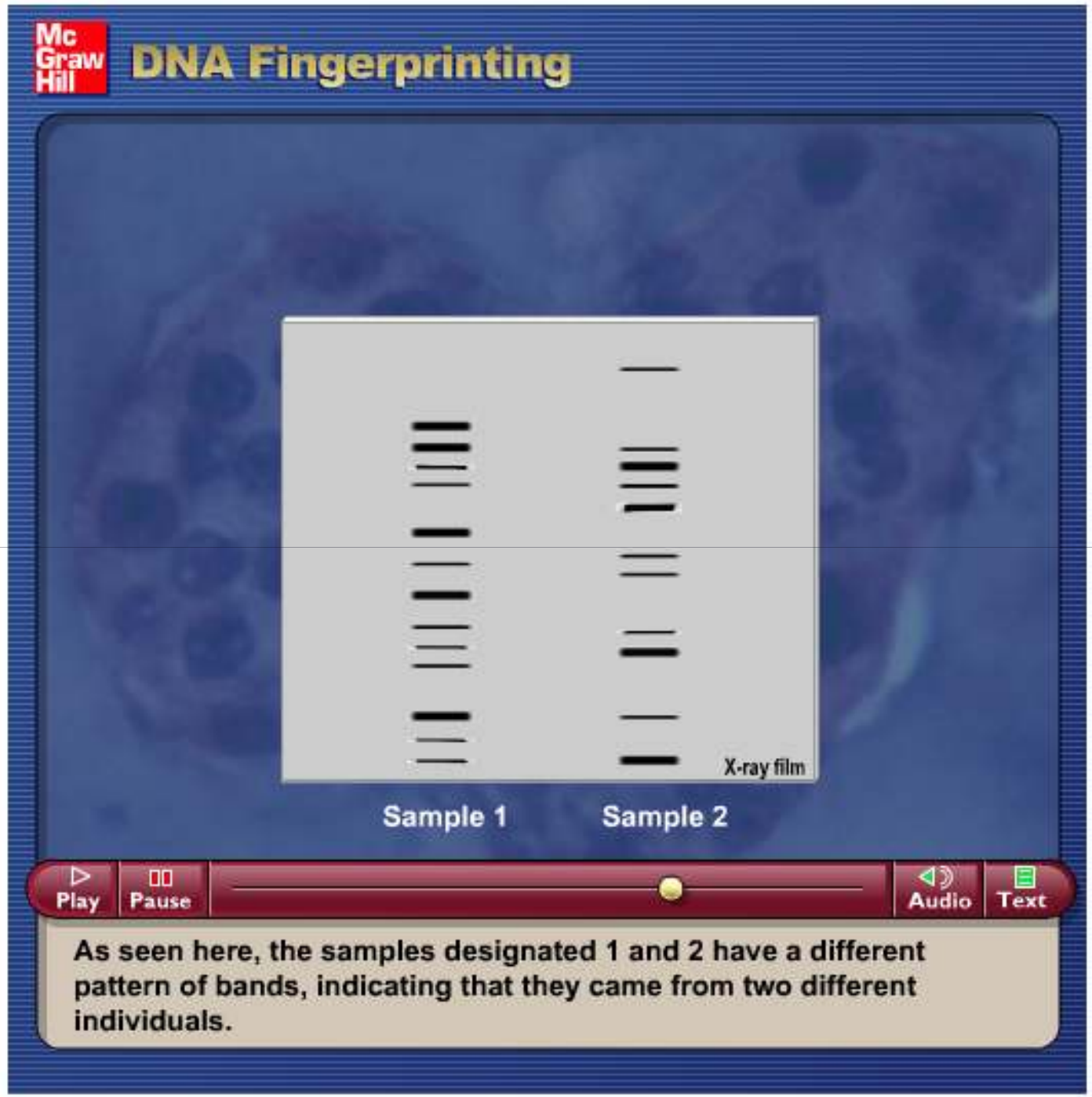
Mc Graw Hill
DNA Fingerprinting
X-ray film
Sample 1
Sample 2
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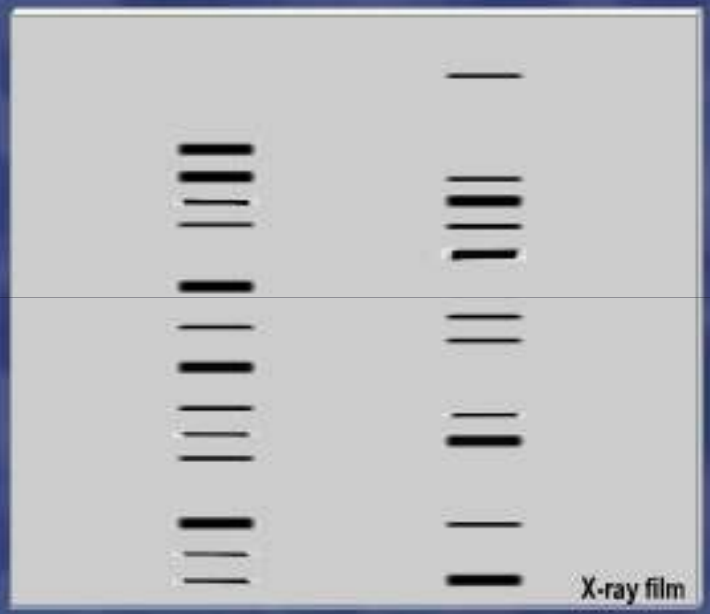
As seen here, the samples designated 1 and 2 have a different pattern of bands, indicating that they came from two different individuals.

DNA Fingerprinting



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Traditional DNA fingerprinting has been superceded by an automated procedure in which microsatellites, which also contain variable repeat sequences, are amplified by PCR.

STR

- STRs (Short Tandem Repeats) and SSRs (Simple Sequence Repeats) are 2-6 base pair long repeating sequences. These are unique for every individual and are shorter than VNTRs. Hence they produce accurate DNA fingerprints.

DNA fingerprinting in India

- Singh and his colleagues developed a probe called Bkm-derived probe for DNA fingerprinting in CCMB. Since then this probe is being extensively used for forensic investigation, paternity determination etc. For the first time DNA fingerprinting evidence was presented in the court of law in India. The Kerala High Court upheld the verdict and since then this indigenous technique has been used as evidence in more than 300 cases including the assassination of the late Prime Minister of India Shri Rajiv Gandhi. This fetched him and his group the CSIR Technology Award for the year 1992 for biological sciences. The direct result of this work was the formation of an autonomous institution, Centre for DNA Fingerprinting and Diagnostics (CDFD) in 1995.

Dr. Lalji Singh, VC BHU, Varanasi
Former Director – CCMB Hyderabad

Applications of DNA fingerprinting

- **1. Paternity and Maternity**
  Because a person inherits his or her VNTRs from his or her parents, VNTR patterns can be used to establish paternity and maternity. The patterns are so specific that a parental VNTR pattern can be reconstructed even if only the children's VNTR patterns are known.

- **2. Criminal Identification and Forensics**
  DNA isolated from blood, hair, skin cells, or other genetic evidence left at the scene of a crime can be compared, through VNTR patterns, with the DNA of a criminal suspect to determine guilt or innocence.

- **3. Personal Identification**
- Social security numbers, picture ID, and other methods are much more likely to remain the prevalent ways to establish personal identification.

- **4. Diagnosis and Cures for Inherited Diseases**
- DNA fingerprinting can also be used to detect and cure genetically inherited diseases. Using a DNA fingerprinting one can detect genetic diseases like cystic fibrosis, hemophilia, Huntington's disease and many others. If the disease is detected at an early age it can be treated and there is a greater chance that it can be defeated.

Thank you