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FACULTY OF ENGINEERING & TECHNOLOGY DEPARTMENT OF BIOTECHNOLOGY

Dr. Simranjit Singh Assistant Professor Department of Biotechnology Rama University, Kanpur





DNA Fingerprinting





Simranjit Singh

Why Use DNA Fingerprinting?

- DNA fingerprinting is a way of telling individuals of the same species apart
- DNA sequences are variable and can therefore be used as identifying characteristics.
- DNA fingerprinting has advantages over other sources of evidence (fingerprints, blood type, etc.):
 - Highly accurate.
 - Can be gathered from trace crime scene evidence.

How do you take a DNA fingerprint?

- One way: Restriction Fragment Length Polymorphisms (huh?) → aka RFLP
- Restriction enzymes are molecules that can cut DNA into pieces --> each enzyme cuts at a very specific DNA sequence
- While all human beings share roughly the same DNA sequence, there are in fact a small number of differences → these differences can be seen by restriction enzymes

RFLP Animation







Individual 2



Individual 1 vs. Individual 2





Summary

- Essentially, once the DNA has been cut by the enzymes, we will have DNA fragments of various sizes
- Each individual's banding patterns should be different because the restriction enzymes will cut each person's DNA at different points
- Fragments of different sizes will travel different distances along a gel when an electric current is passed through it

Agarose Gel Electrophoresis



Different banding patterns from different individuals

DNA Fingerprinting and Forensic Analysis



Introduction to DNA Fingerprinting and Forensics

- Forensic science can be defined as the intersection of law and science
- First photography-then fingerprint- then, in 1985, DNA Fingerprinting

DNA Fingerprint

DNA fragments show unique patterns from one person to the next.

Used in paternity disputes and as forensic evidence.



Preparing a DNA Fingerprint

 Specimen Collection- Could be a licked envelope, dirty laundry, a cigarette butt, saliva

- Special precautions in handling specimens: gloves, disposable instruments, avoid talking and sneezing, avoid touching sample with your skin, air-dry the evidence before packaging so mold does not grow
- Enemies of evidence: sunlight, high temperatures, bacteria, moisture
- Ideal sample: 1 mL of fresh, whole blood (white blood cells) treated with EDTA



Restriction Fragment Length Polymorphism (RFLP)

 Nucleotide sequence variations in a region of DNA that generates fragment length differences according to the presence or absence of restriction enzyme recognition sites.







The RFLP fragments can be separated by gel electrophoresis.



RFLP



RFLP animation

Molecular technique where DNA is transferred onto a membrane from an agarose gel and a probe is hybridized.



The first step in preparing a Southern Blot is to cut genomic DNA and run on an agarose gel.



The next step is to blot or transfer single stranded DNA fragments on to a nylon membrane.



The next step is to hybridize a radioactively labeled DNA probe to specific sequences on the membrane.



The last step is to expose the radioactively labeled membrane to a large sheet of film.

You will only visualize bands where the probe hybridized to the DNA.





Southern Blot Animation



Variable Number Tandem Repeat (VNTR)

 sequences that are repeated multiple times and the number of repeats varies from person to person.





- VNTRs usually occur in introns
- VNTRs can be amplified by PCR and run on agarose gels to produce unique DNA fingerprints





Polymerase chain reaction (PCR)

• A lab technique used to amplify segments of DNA





Reaction requirements

- Template DNA total genomic DNA isolated from an organism that contains a target region to be amplified
- DNA primers Short pieces of single stranded DNA that flank the target
- *Taq* DNA polymerase Attaches nucleotides on the growing strand of DNA
- Nucleotides (GATC) Polymerase adds complementary nucleotides to the template







Reactions are placed in a machine called a thermal cycler. The machine cycles through three temperatures.





1. Heat samples to **94**°**C** for a minute or so to <u>denature</u> the double stranded template DNA.

R G TAR A T ARR C TAR A AT C RTART CARR ATA C C C CATRI AR C CIRTA C C RATCHA TC C RATCHTA C CATC A CHC C HAT CC CTA CATC C CACAT C

	0
9	4 C
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DNA strands are separated by heating

E CITARA RACE TAR A REALER CARE A LA CORCETAR COLLEGE

CCATCHA FCCCAFCHHA CCAFCA CHCC HATCCCTA CAHC CCACAT



2. Drop temperature to around **50 or 60**°C to allow primers to <u>anneal</u>.





3. Maintain temperature at **72**°**C** for a minute or two to allow the polymerase to <u>elongate</u> the new DNA strands.





 The thermal cycler repeats the denaturing, annealing, and elongating temperatures approximately 30 times.





 PCR amplification is logarithmic, meaning the number of copies of the target is doubled every cycle.

Cycles	5 1	2	3	4	5
	S S S S S S S S S S S S S S S S S S S	SSE	WWW WWW WWW		



PCR animation

Diagnosing Disease



Paternity Testing

Father Father Mother Child





Forensics









Genealogy



Genealogy animation

Genealogy



Thank you

