



Restriction Enzyme Analysis

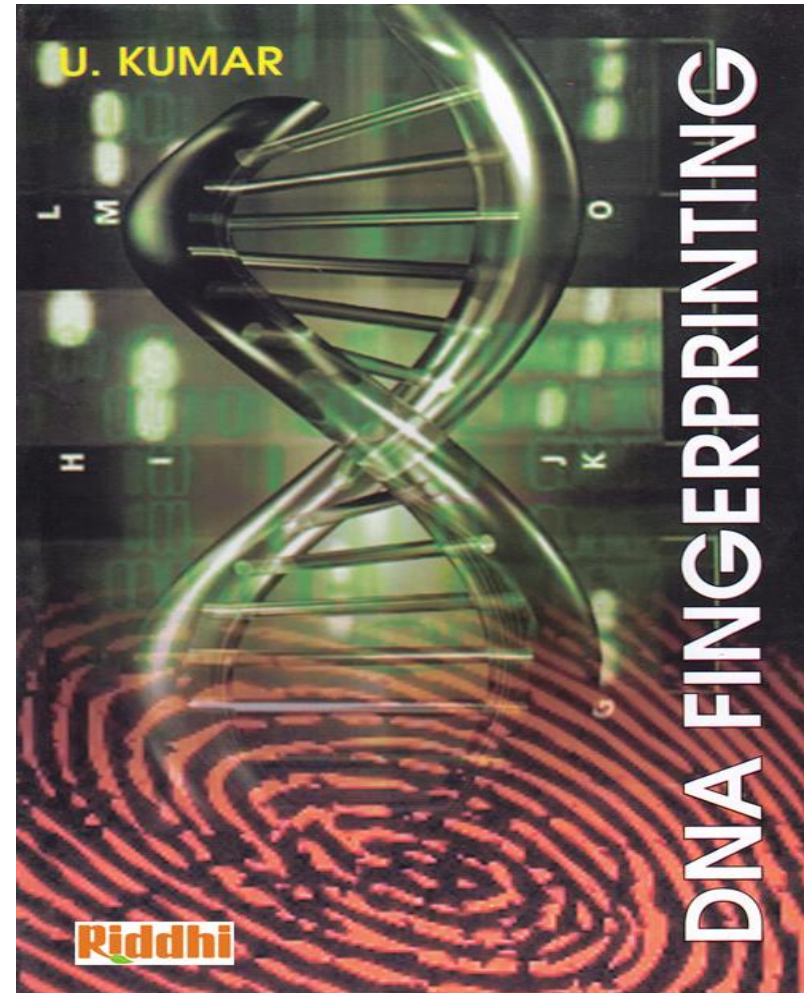
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DNA Fingerprinting

- A technique used by scientists to distinguish between individuals of the same species using only samples of their DNA

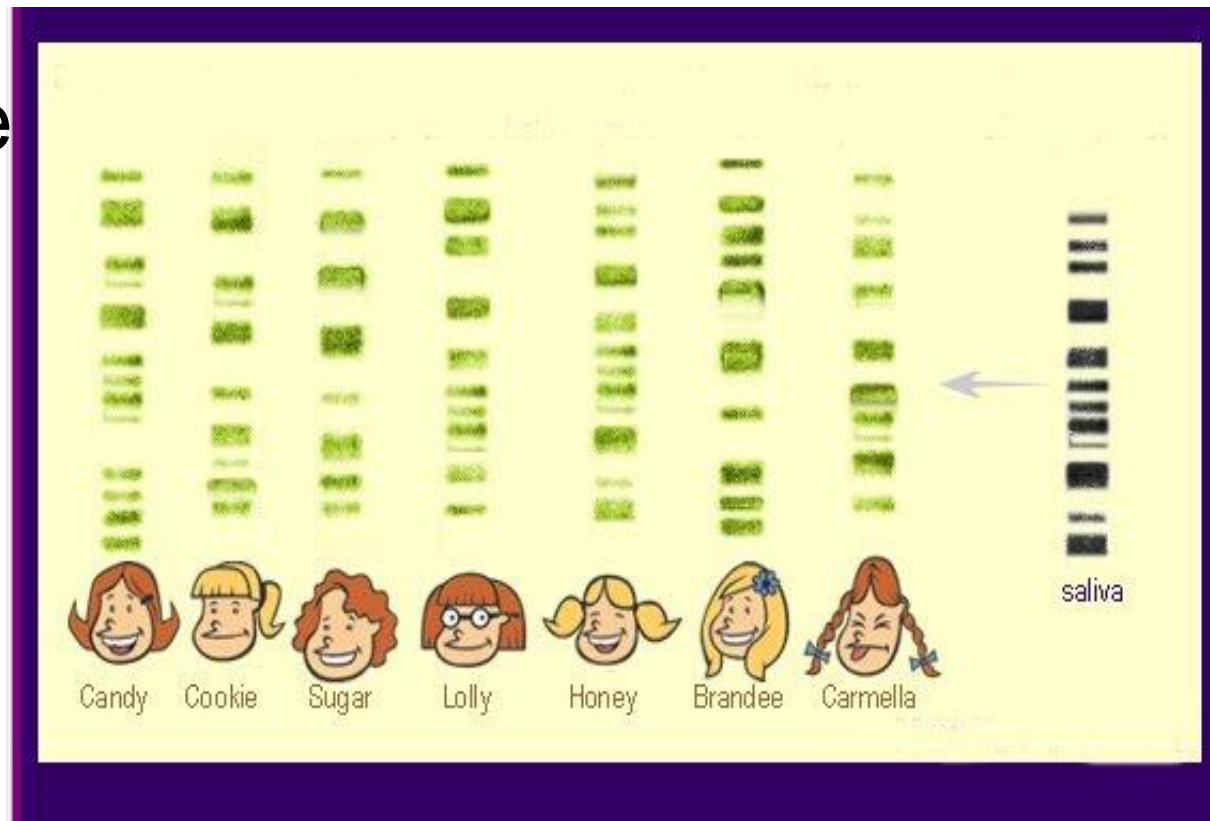


http://naturalherbalbooks.com/products/index.php?main_page=product_info&cPath=8&products_id=197

Various sample
can be used

- Any body
secretion

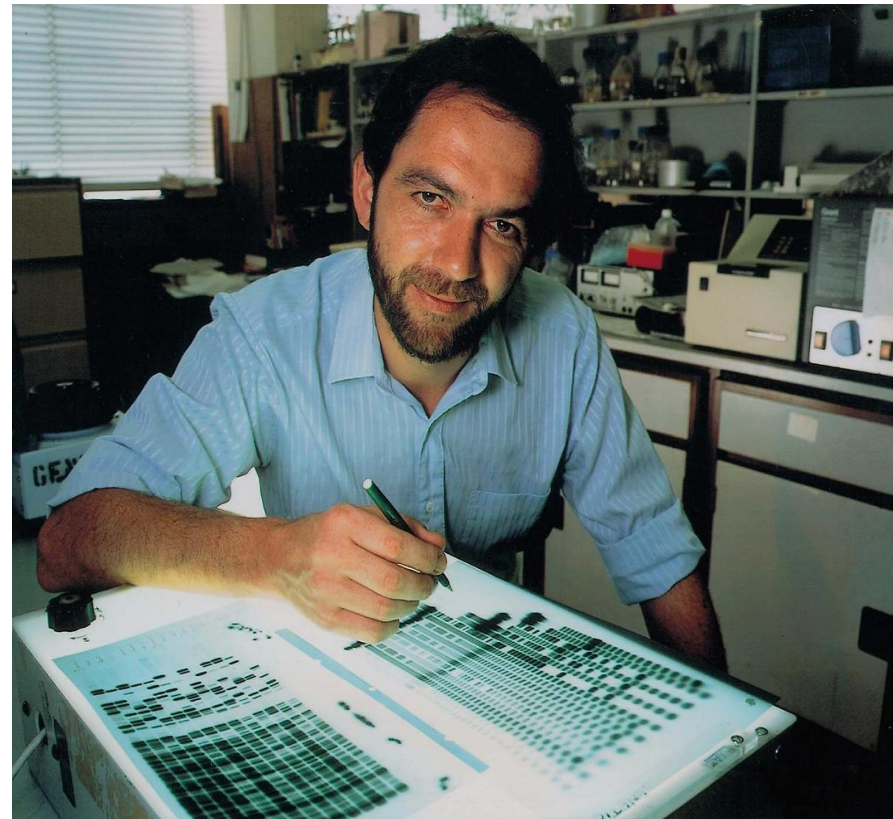
- Any body
tissue cell



http://paulnaidu.blogspot.com/2008_07_01_archive.html

Who Invented it?

- The process of DNA fingerprinting was invented by Alec Jeffreys at the University of Leicester in 1985
- He was studying the gene of myoglobin

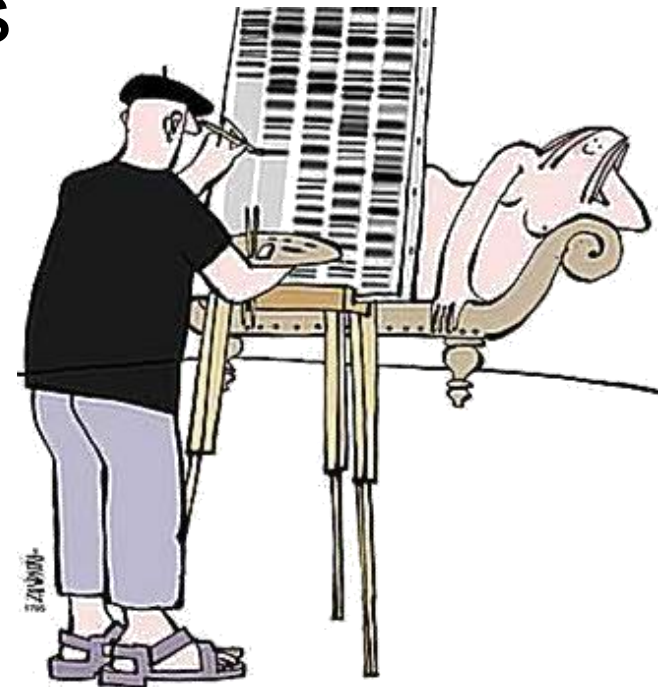


<http://www.nlm.nih.gov/visibleproofs/galleries/cases/jeffreys.html>

Types of DNA Fingerprinting

Two main type

- Restriction enzyme analysis
- PCR based methods



DNA Restriction Analysis

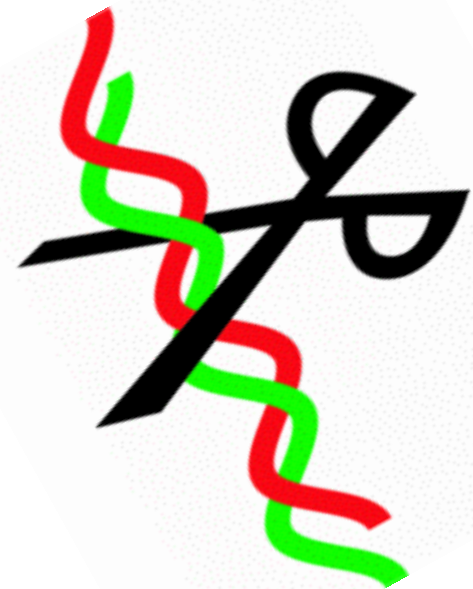
- This process makes use of special proteins called **restriction enzymes** and sections of the chromosome called **tandem repeats**.

Tandem Repeats

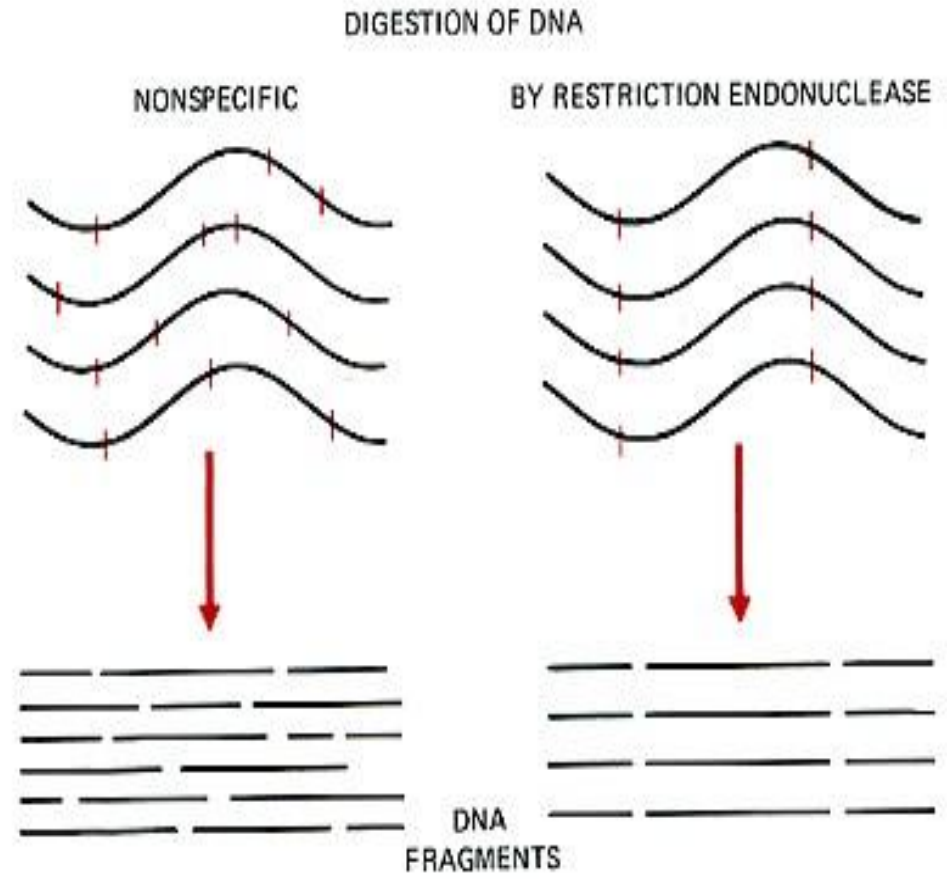
- A region of the chromosome that contains multiple copies on a core DNA sequence that are arranged in a repeating fashion
- Repeats act as fillers or spacers between coding sections of DNA
- All humans have the same type of repeats but there is tremendous variation in the number of repeats that each of us has.

Restriction Enzymes

- Restriction enzymes are **DNA-cutting enzymes** found in bacteria (and harvested from them for use)
- Because they cut within the molecule, they are often called **restriction endonucleases**.

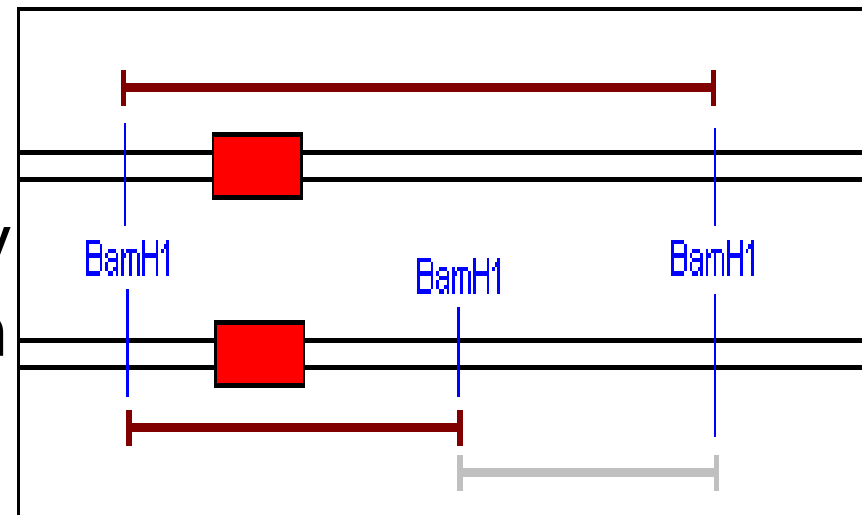


- Restriction enzymes recognize and make a cut within **specific** palindromic sequences, known as **restriction sites**, in the DNA. This is usually a 4 to 8 base pair sequence.



Restriction Fragment Length Polymorphisms (RFLPs)

- DNAs from different individuals rarely have exactly the same array of restriction sites and distances between these sites.
- Population is therefore **polymorphic** (having many forms) for these restriction fragment patterns.





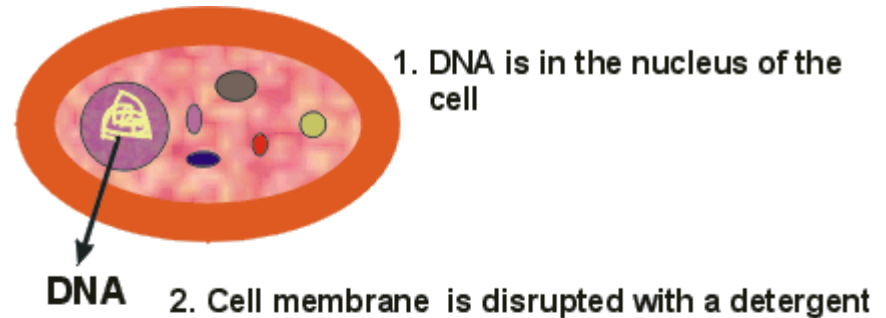
Restriction Fragment Length Polymorphisms (RFLPs)

- These differences are referred to as restriction fragment length polymorphisms (RFLPs)
- May arise through mutations.
- Determining whether or not a particular group of restriction sites exists in DNA is a very sensitive means of differentiating one individual from many others.

Steps of DNA Restriction Analysis

■ Step 1

Extraction and purification of DNA



3. Alcohol is added to the tube to separate DNA from other cell components. DNA moves to the alcohol layer



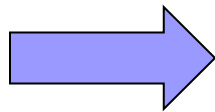
4. DNA is spooled onto a glass pipette



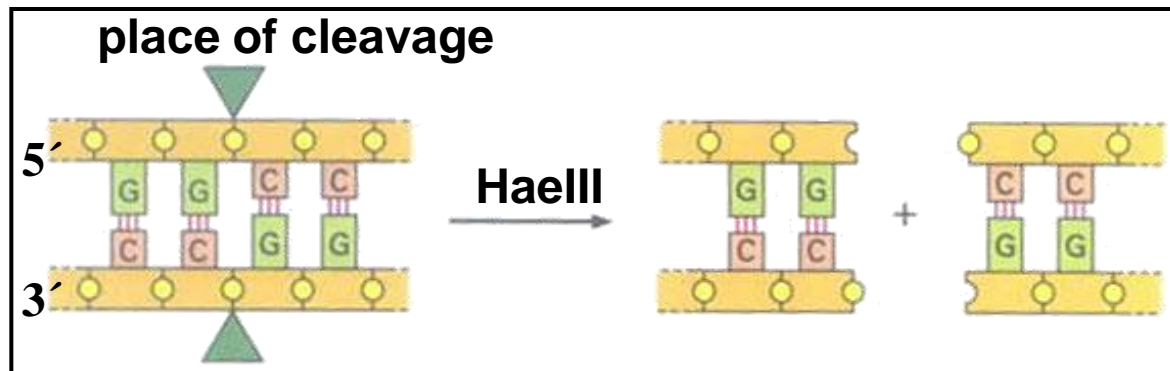
Steps of DNA Restriction Analysis

■ Step 2

Digestion with
Restriction
Enzymes



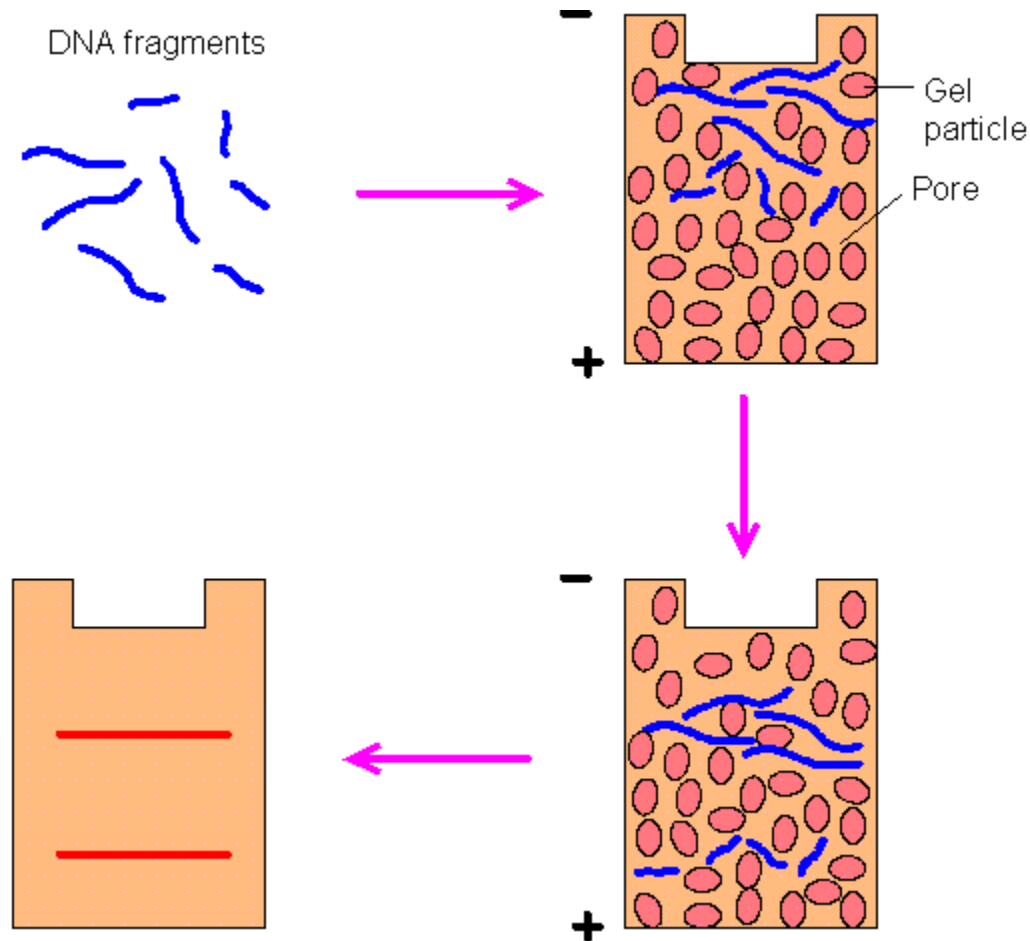
Thousands of
restriction fragments
of all different sizes



Steps of DNA Restriction Analysis

■ Step 3

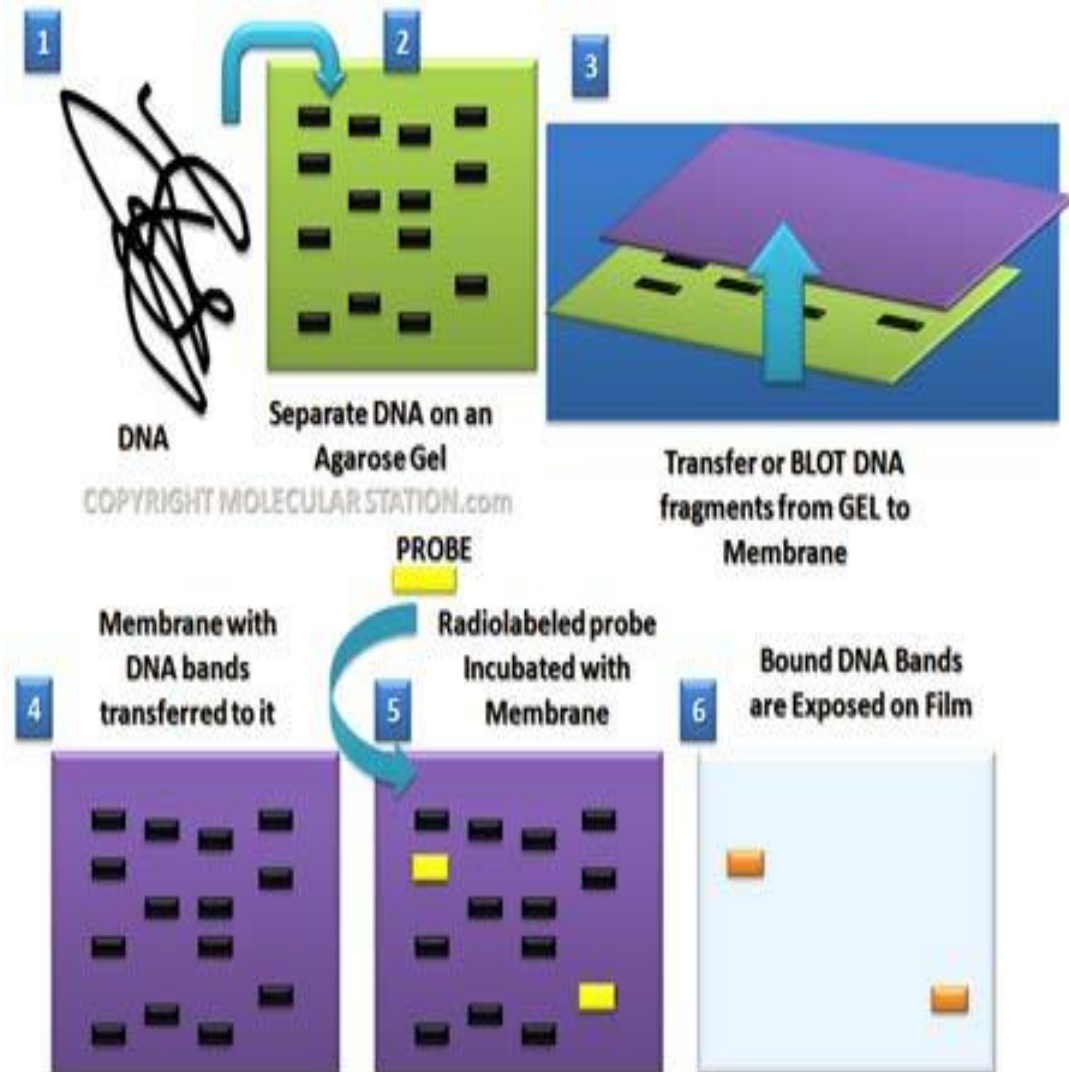
Gel Electrophoresis



Steps of DNA Restriction Analysis

■ Step 4

Southern Blot
and
hybridization
with radioactive
probe

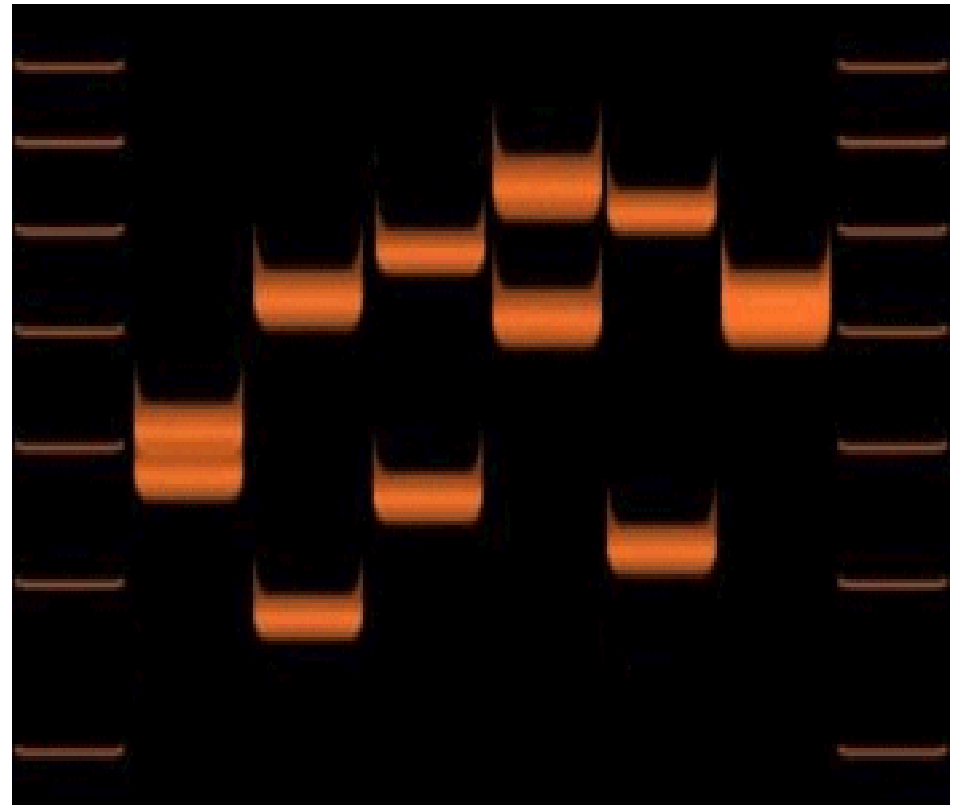


Steps of DNA Restriction Analysis

■ Step 5

Analysis of the fragment distribution

(A match may be declared if two samples have RFLP band sizes that are all within 5% of one another in size).



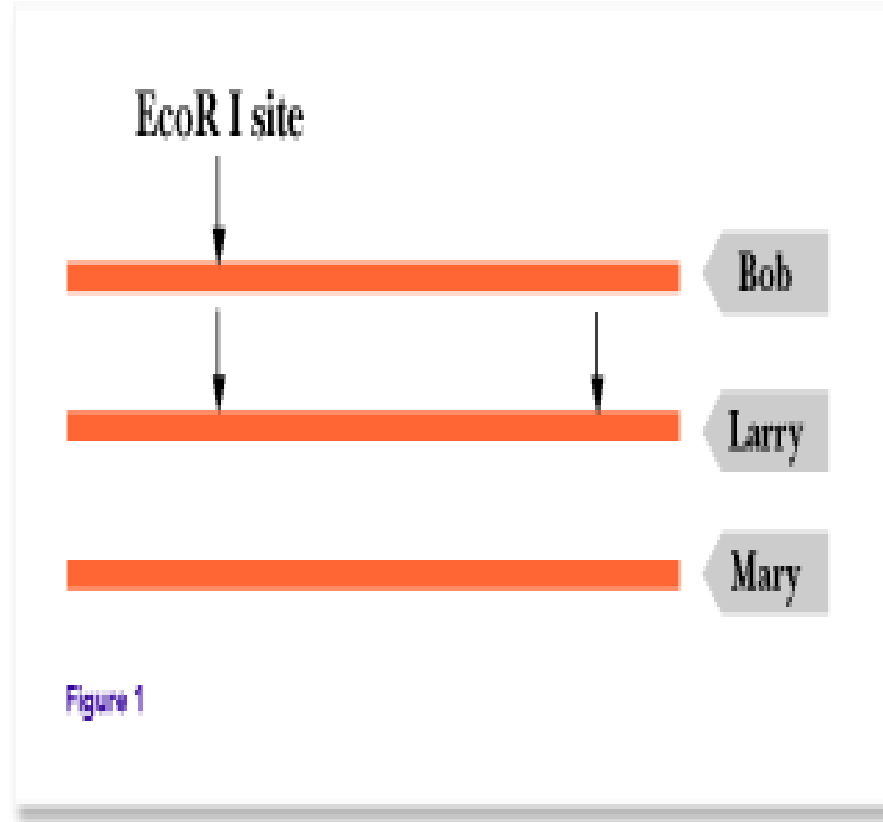
Applications of DNA Fingerprinting

- Paternity and maternity
- Criminal identification
- Diagnosis of inherited disorders

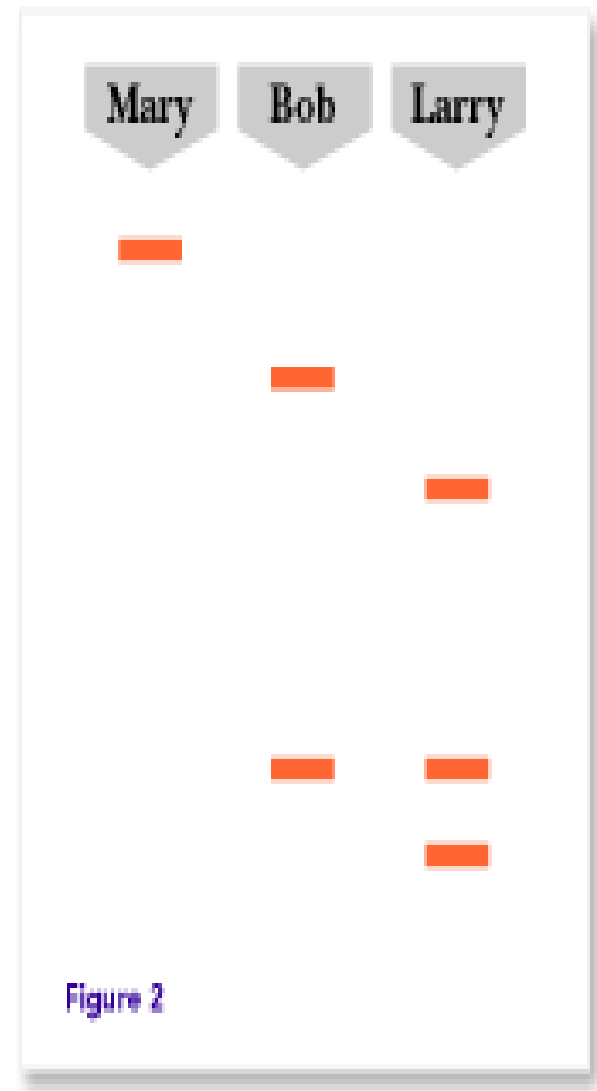


An Example Using EcoR I for a Question of Paternity

- EcoR I cuts a similar section of DNA on **Bob**, **Larry**, and **Mary**
- After the cut how many fragments Bob, Larry, and Mary have?



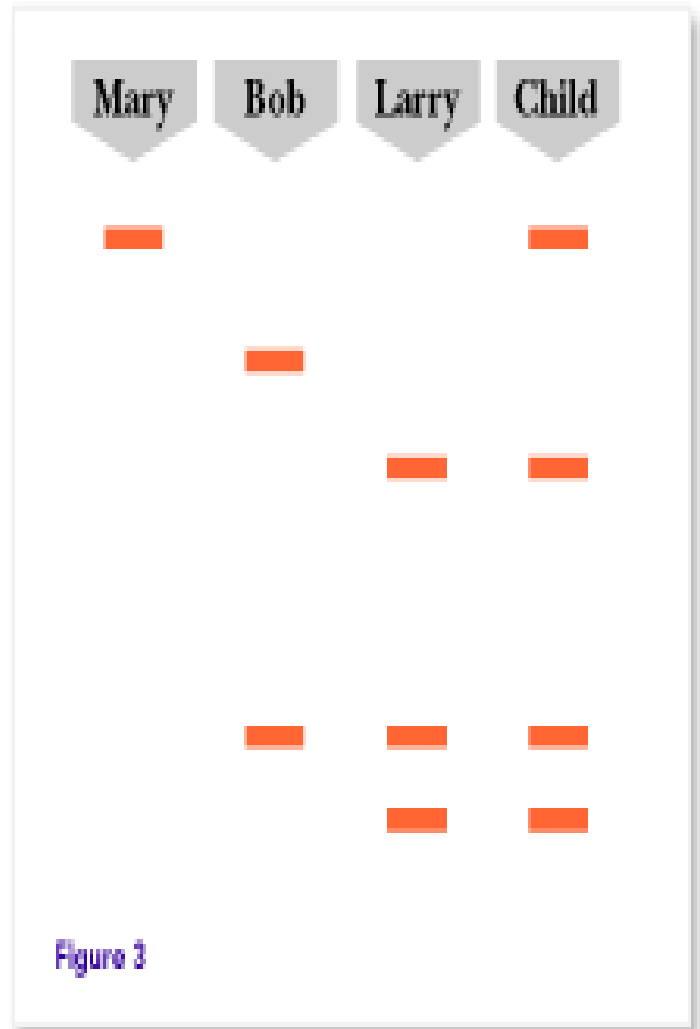
- After the DNA is cut with EcoR I, Bob's, Larry's and Mary's fragments are separated by gel electrophoresis.
- The bigger fragments are near the top



Can someone tell me who is my father?



In general, the child's DNA must be a combination of Mary's DNA and one of the men. Which man is the father?



Advantages of DNA Restriction Analysis over PCR techniques

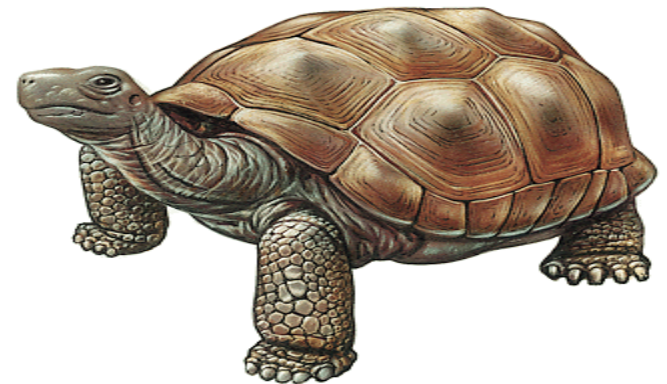
- More specific
- Reprobing with additional probes is possible



Limitations of DNA Restriction Analysis




- RFLP testing requires larger amounts of DNA.
- DNA must be undegraded
- Slower, at least one day



References

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