Molecular Biology (1S) Supplementary

Applications of Southern blotting

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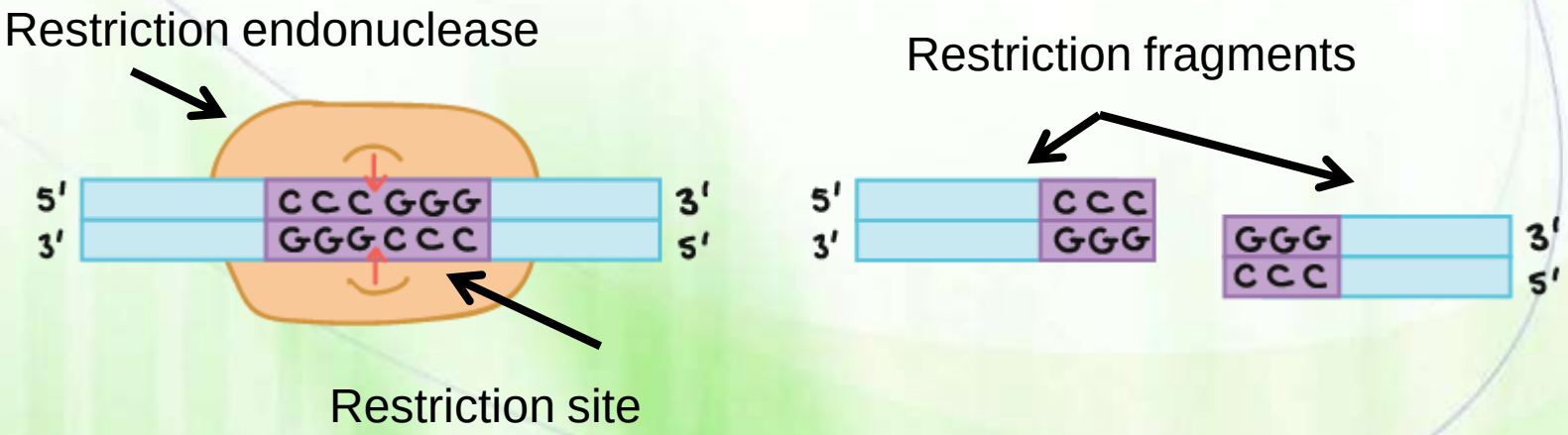
Resources

- This lecture
- Cooper, pp 120-124

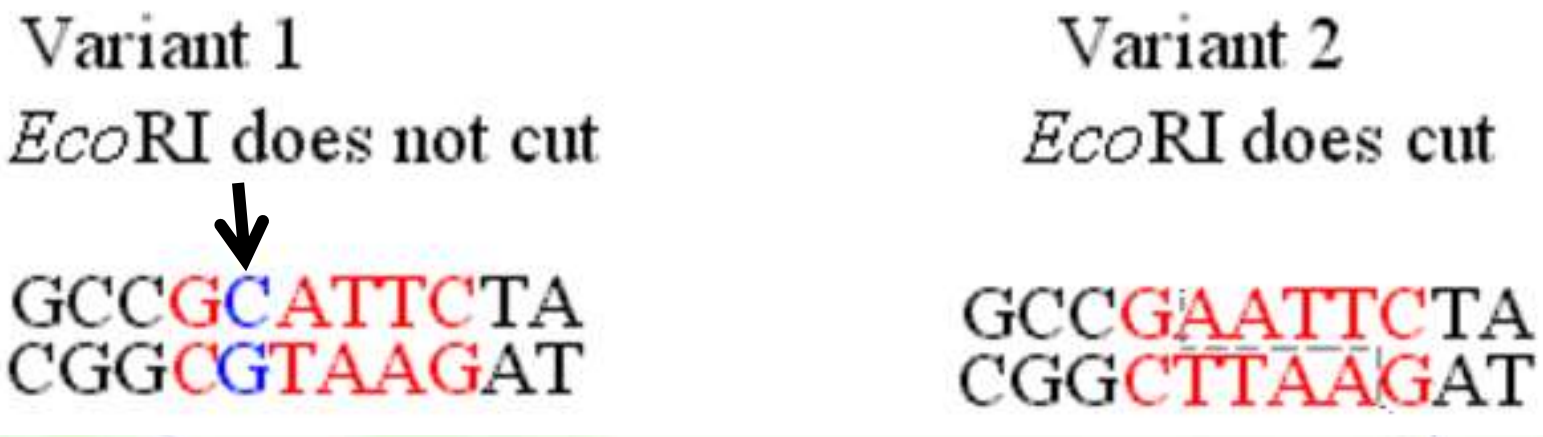
Restriction endonucleases

- Endonucleass are ezymes that degrade DNA within the molecule.
- Restriction endonucleases: Bacterial enzymes that recognize and cut (break) the phosphodiester bond between nucleotides at *specific* sequences (4- to 8-bp restriction sites) generating restriction fragments.



They recognize specific sequences

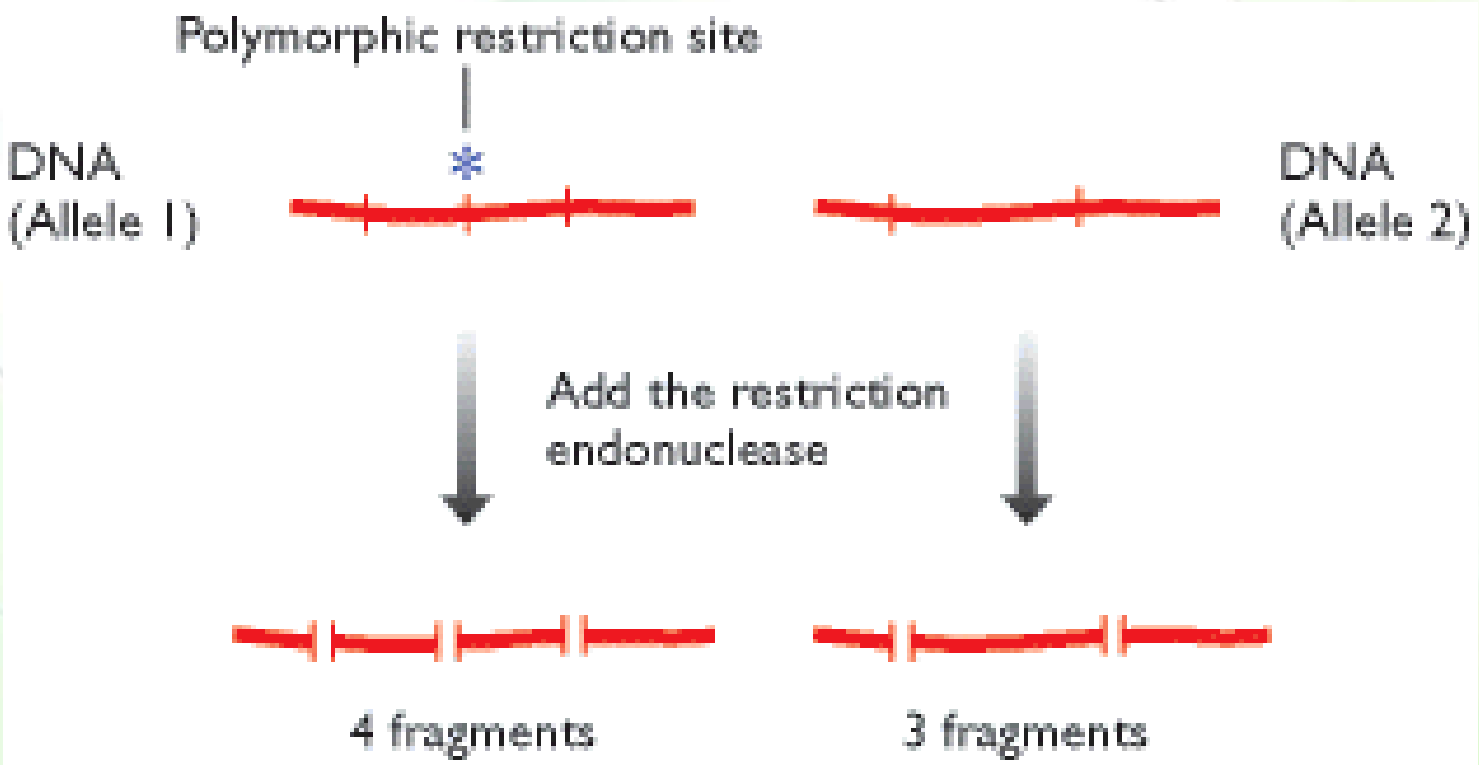
- The enzyme EcoRI recognizes and cuts within the sequence (GAATTC).



The DNA stays intact

The DNA is cut into two pieces

- Restriction endonucleases can cut the same DNA strand at several locations generating multiple restriction fragments of different lengths.
- What if a location on one strand is not recognized?



DNA polymorphisms

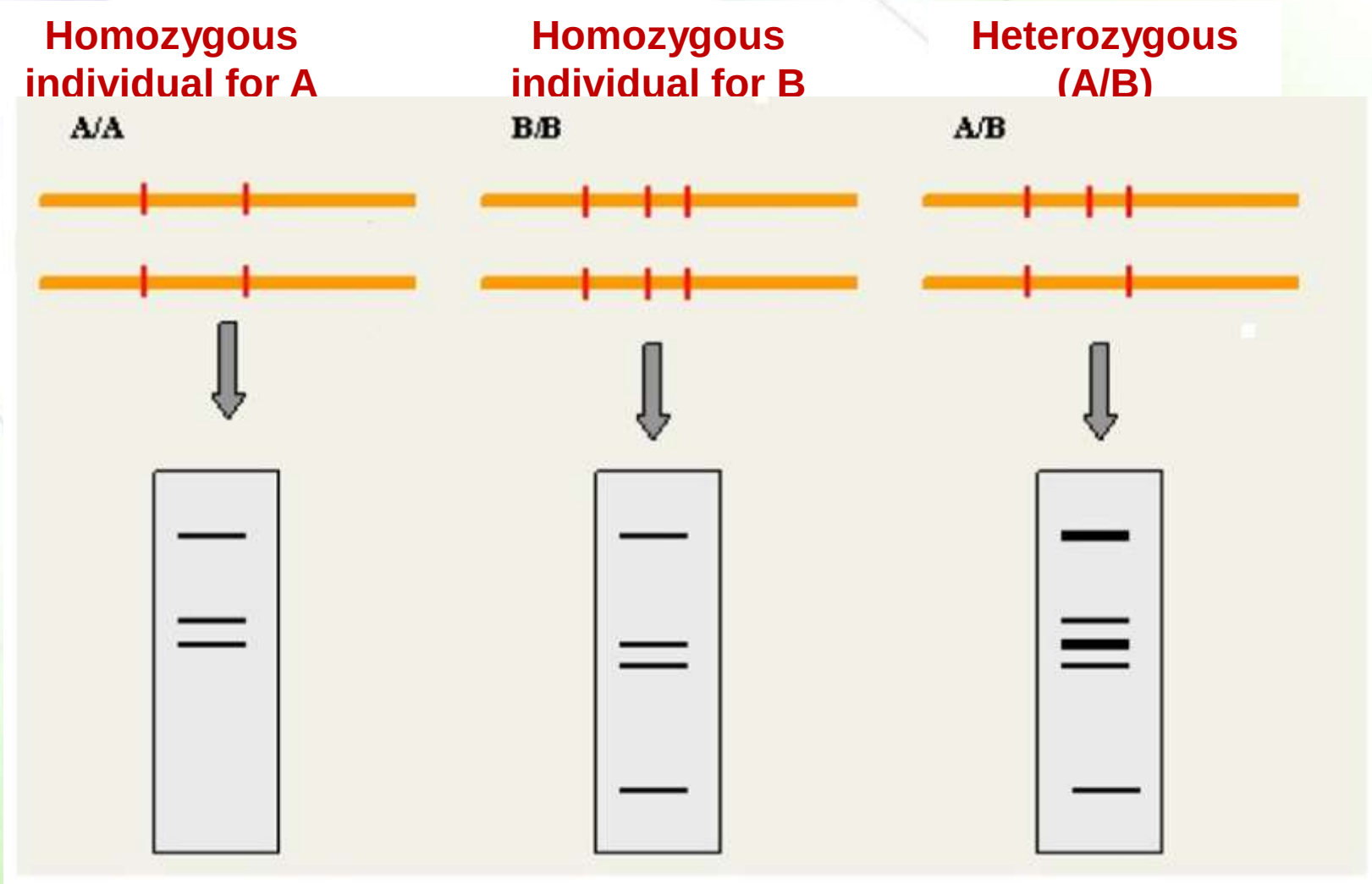
- Individual variations in DNA sequence (*genetic variants*) may create or remove restriction-enzyme recognition sites generating different restriction fragments.

- Remember:
  - Our cells are diploid.
  - Alleles can be homozygous or heterozygous at any DNA location or sequence.

Restriction fragment length polymorphism

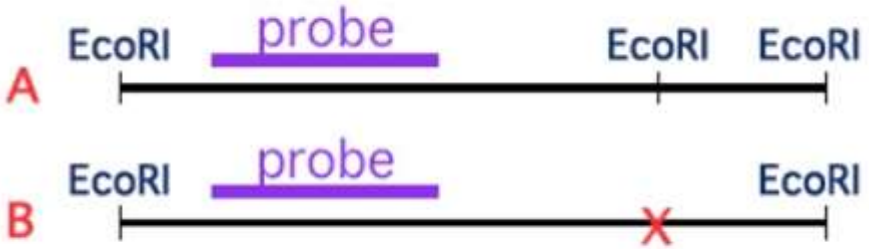
- The presence of different DNA forms in individuals generates a restriction fragment length polymorphism, or RFLP.
- Individuals can generate restriction fragments of variable lengths. This is known as molecular fingerprinting.
- These can be detected by gel electrophoresis by itself or along with Southern blotting.

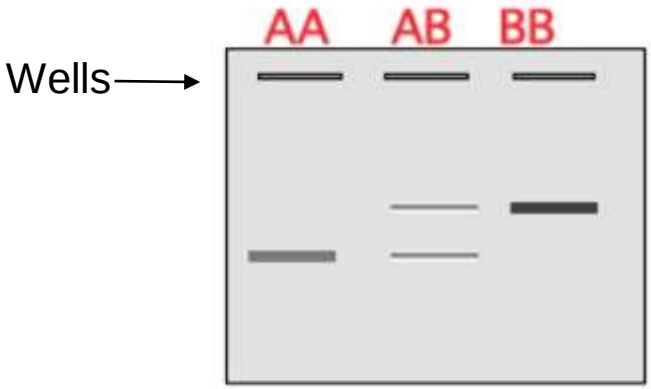
Gel electrophoresis only



Electrophoresis then Southern blotting

- Only DNA fragments that hybridize to the probe are detected.





Note: only the fragment that the probe hybridizes to is detected.

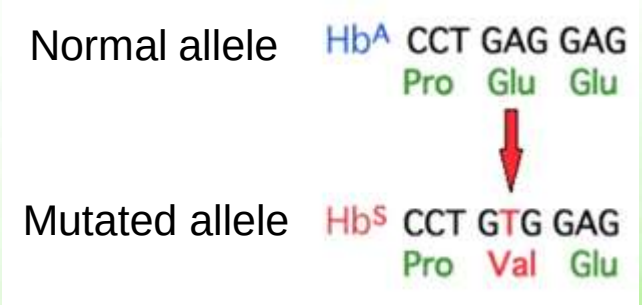
RFLP in the clinic

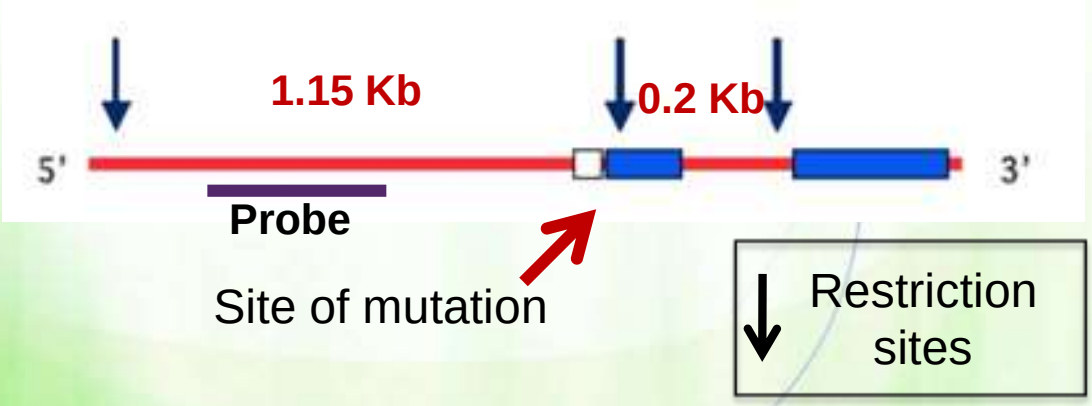
- RFLP can be used as diagnostic tools.

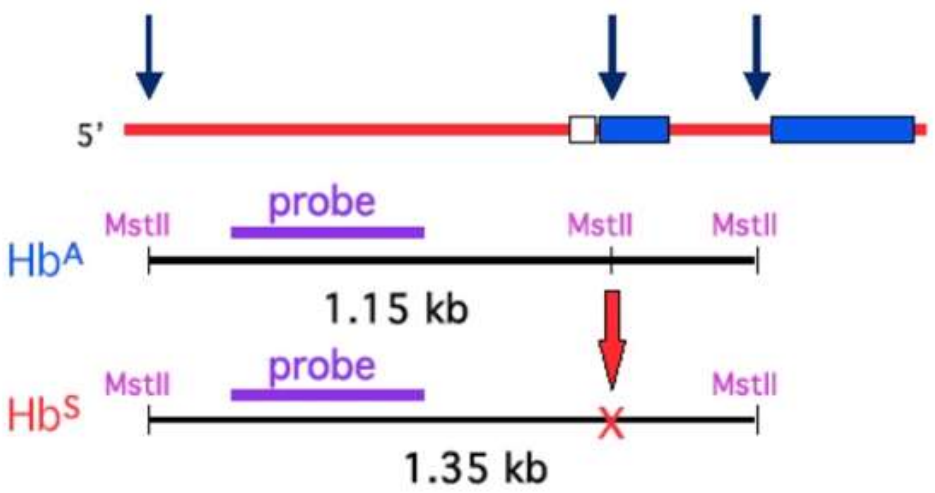
- For example, if a mutation that results in the development of a disease also causes the generation of distinctive RFLP fragments, then we can tell:
  - if the person is diseased as a result of this mutation
  - from which parent this allele is inherited

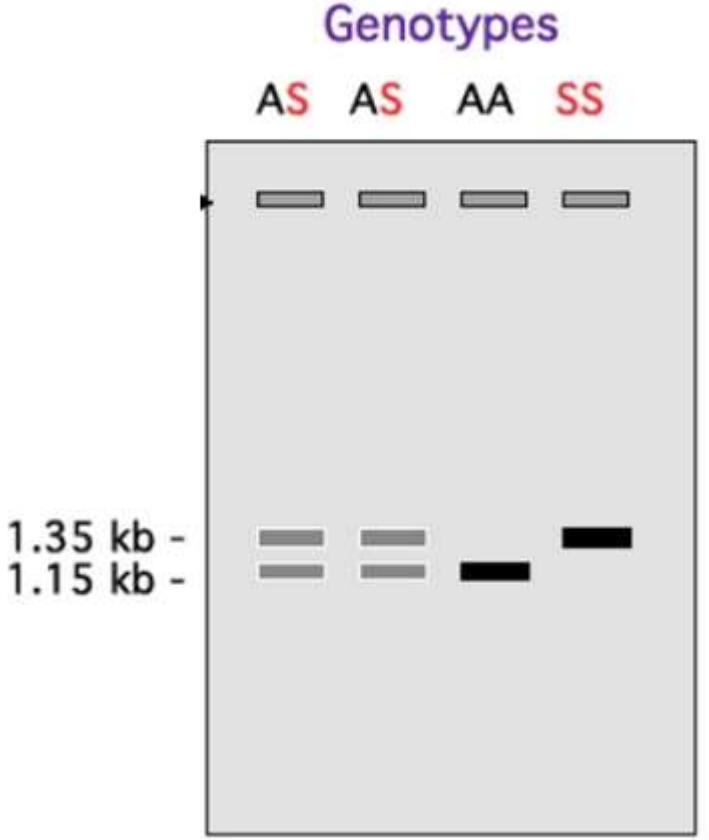
Example 1: Disease detection by RFLP (sickle cell anemia)

- Sickle cell anemia is caused by a mutation in one nucleotide (base) in the globin gene that is responsible for making hemoglobin.
- The position of this nucleotide happens to be within a restriction site.
- Individuals can be have
  - Homozygous with two normal alleles (designated as A)
  - Heterozygous or carriers of one normal allele and one mutated allele (designated as AS)
  - Homozygous for the mutated allele, or affected (designated as S)

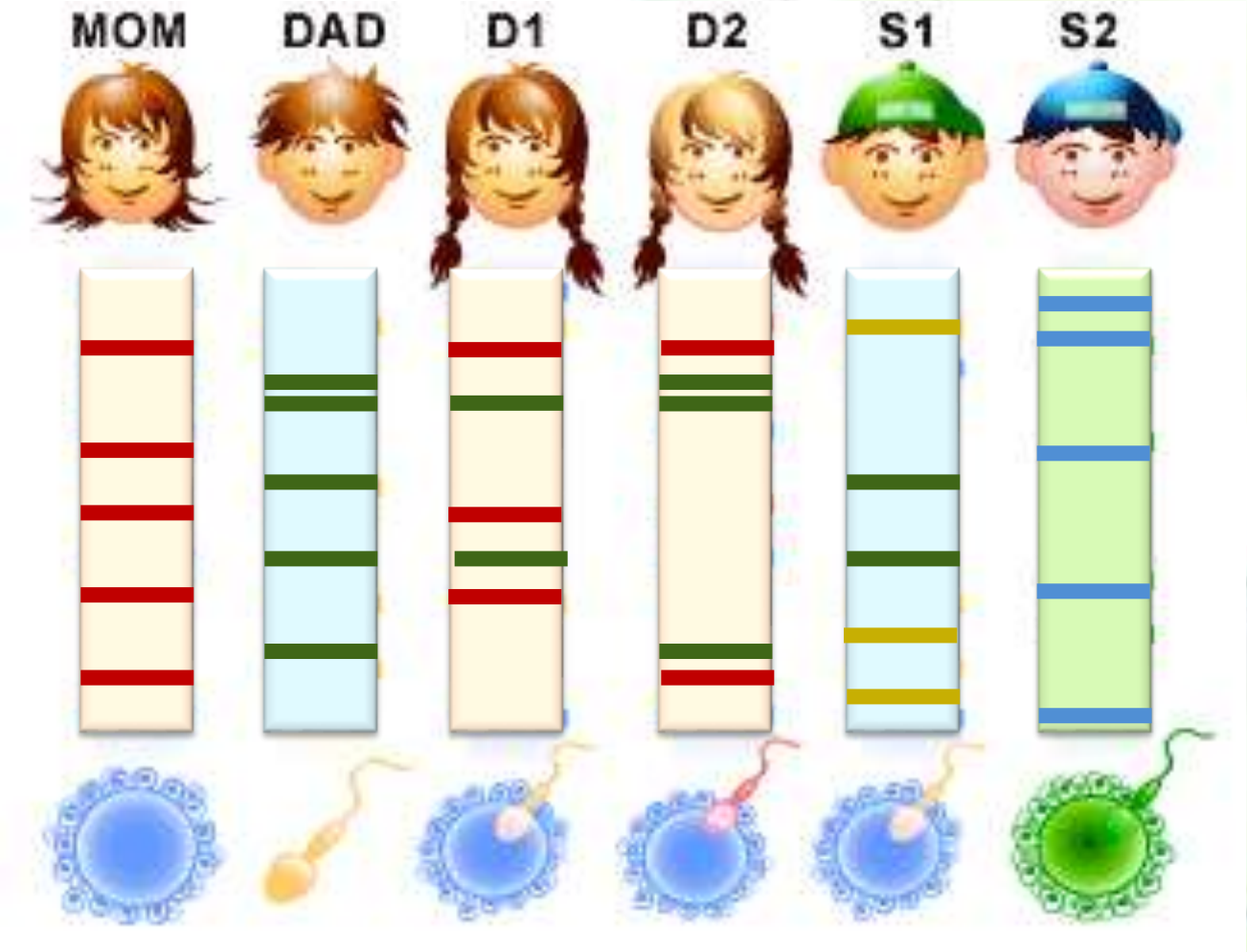




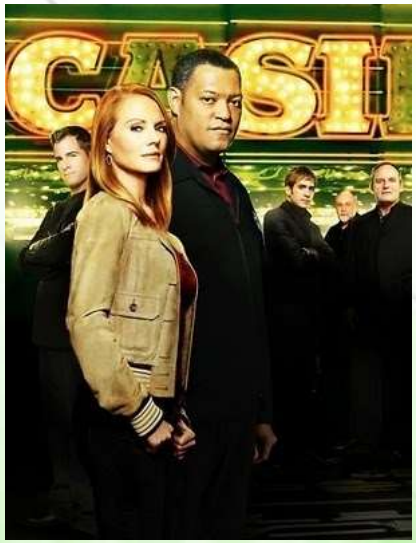

5'
probe
MstII
MstII
MstII
Hb^A
1.15 kb
probe
MstII
MstII
Hb^S
1.35 kb


Genotypes
AS
AS
AA
SS
1.35 kb -
1.15 kb -

Example 2: Paternity testing



Example 3: Forensics



Restriction Fragment Length Polymorphism (RFLP)

DNA Extracted from blood cells

Restriction eznyme cleavage of DNA

Bloodstain

Radioactive DNA probe binds to specific DNA fragments

Transfer of DNA fragments to a membrane (Southern blott)

Fragments of DNA are separated by electrophoresis

John Sample Bill

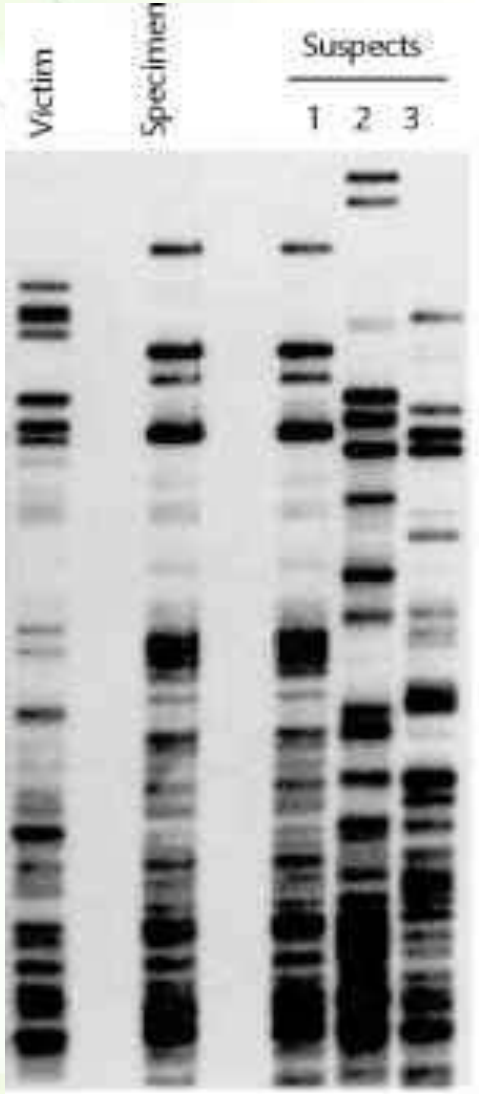
Membrane is washed free of excess probe

X-ray film, sandwiched to the membrane to detect radioactive pattern

DNA pattern is compared with patterns from known subjects

Real cases


Crime Scene
Victim
Suspect 1
Suspect 2


Victim
Specimen
Suspects
1
2
3