



Molecular Biology (2)

Basic Applications:

Electrophoresis, denaturation, hybridization, dot blot, Southern blotting, and RFLP

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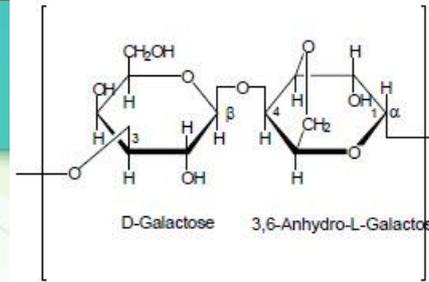
Second semester, 2019-2020

Resources

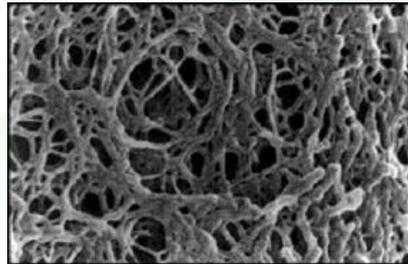
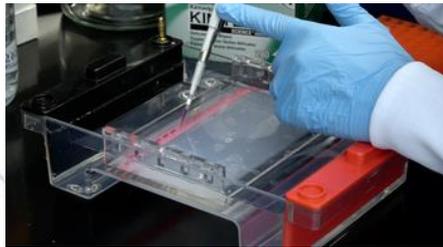


- This lecture
- Cooper, Ch. 4, pp.127, 137-138

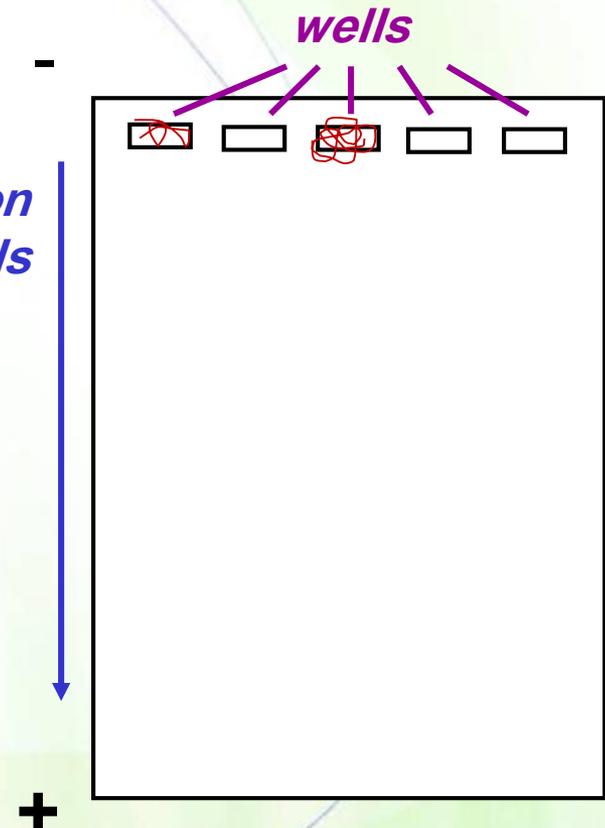
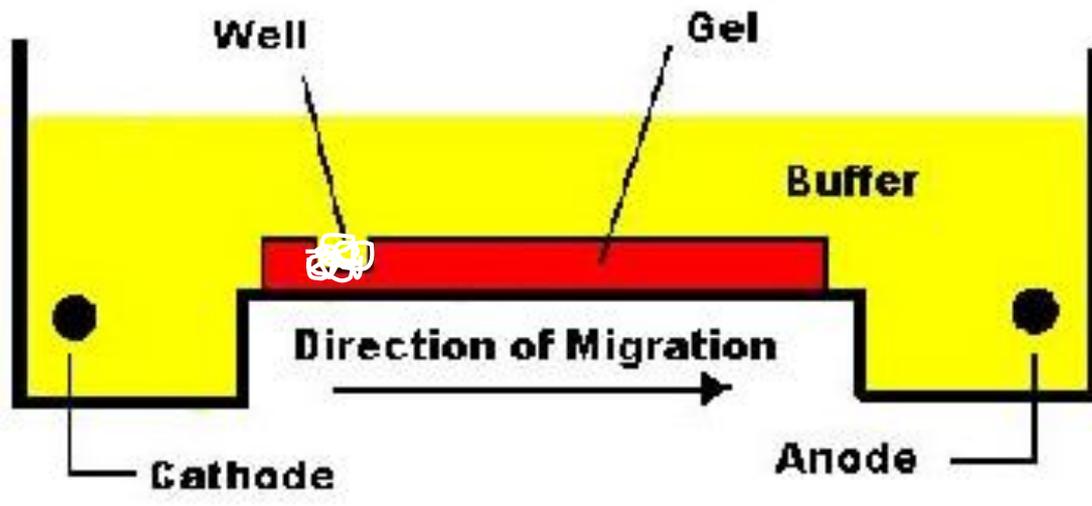
Gel electrophoresis



- The length and purity of DNA molecules can be accurately determined by the gel electrophoresis.

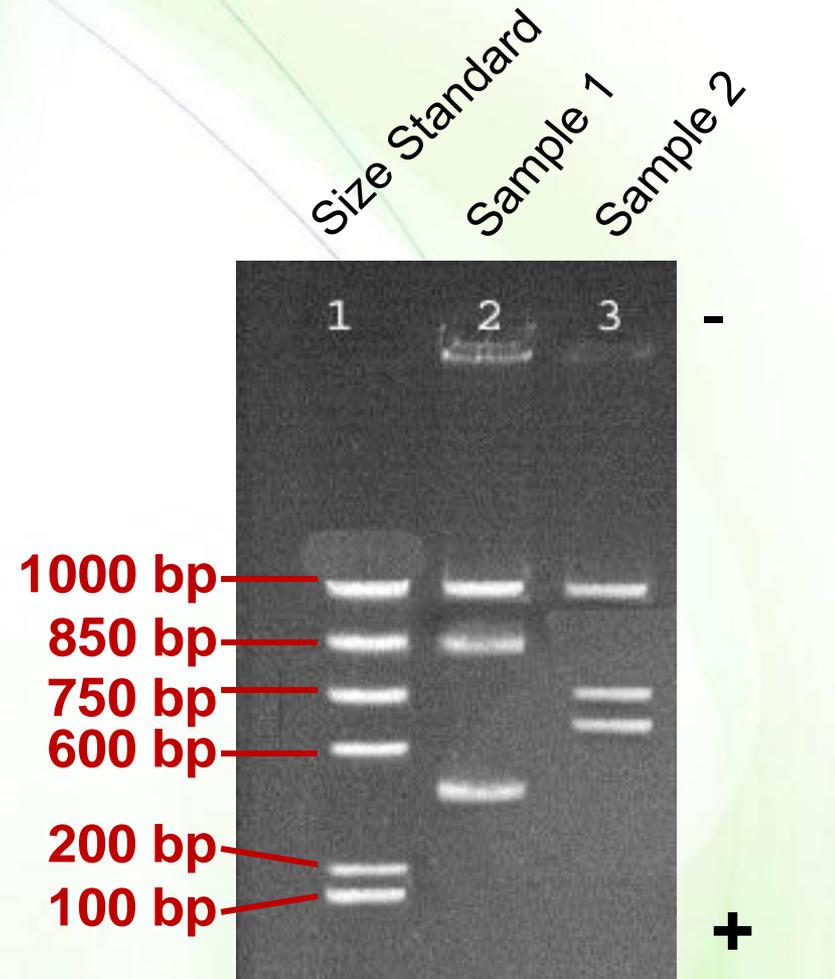


*Direction
DNA travels*



Detection

- The DNA molecules of different lengths will run as "bands".
- **Each bands contains thousands to millions of copies of DNA fragments of the same length. They can be of same or different type (not one DNA molecule).**
- DNA is **stained** (that is, colored) with a dye (ethidium bromide) or radioactively **labeled** (^{32}P).
- It is common that a DNA standard is used to determine the length of the examined DNA molecule.



bp: base pair

Resources



- <http://www.sumanasinc.com/webcontent/animations/content/gelelectrophoresis.html>

Watch this....very important

Light absorbance of nucleic acids



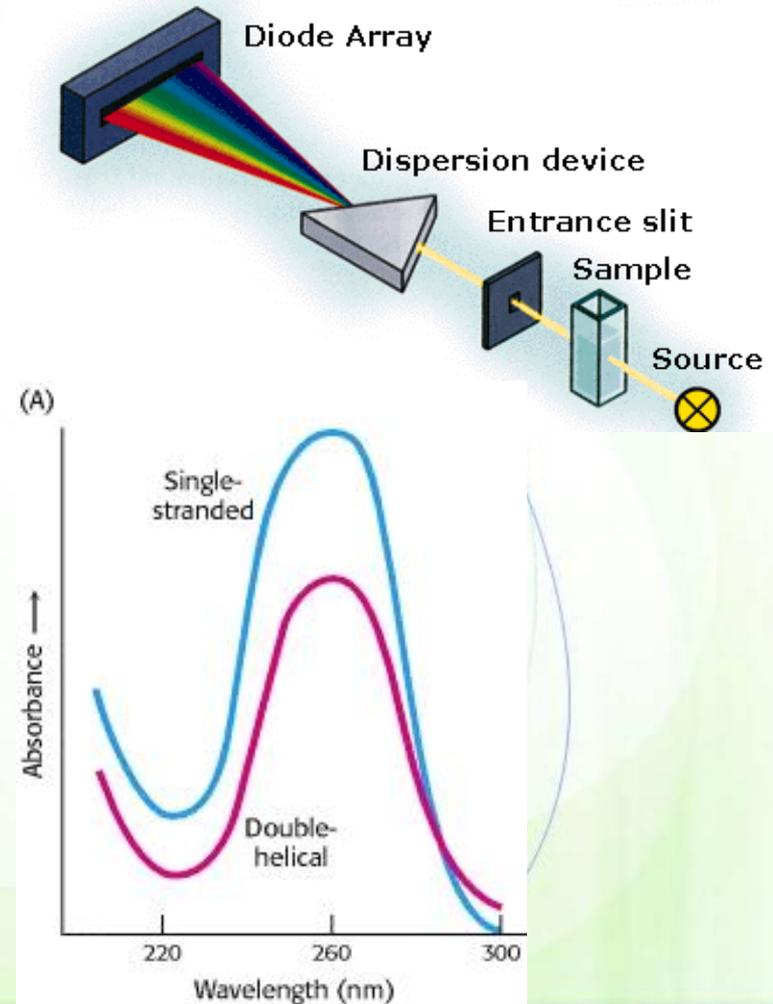
- Aromatic pyrimidines and purines can absorb UV light
- The peak absorbance is at 260 nm wavelength
- The absorbance of nucleic acids at 260 nm (A_{260}) is constant
 - dsDNA: A_{260} of 1.0 = 50 $\mu\text{g}/\text{ml}$

Reason for ss vs. ds absorbance:

- Unstacked bases vs. stacked bases

What is the concentration of a double stranded DNA sample diluted at 1:10 and the A_{260} is 0.1?

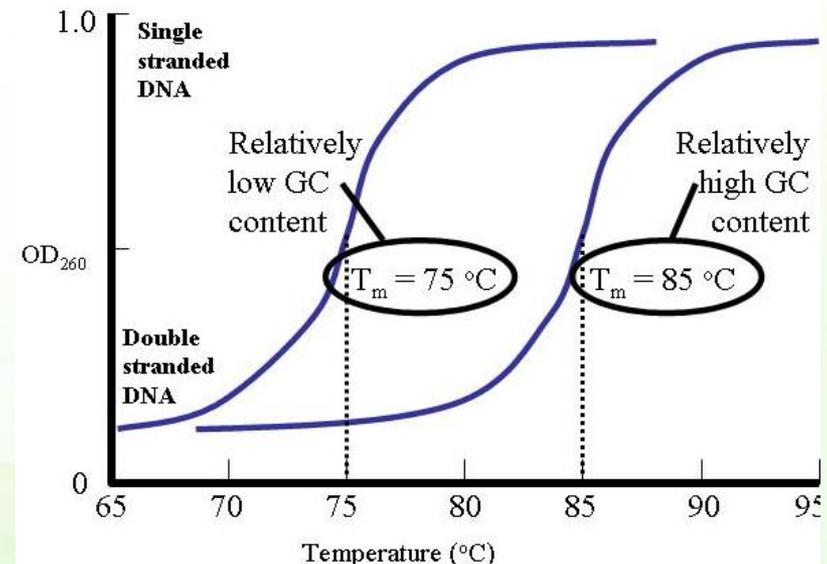
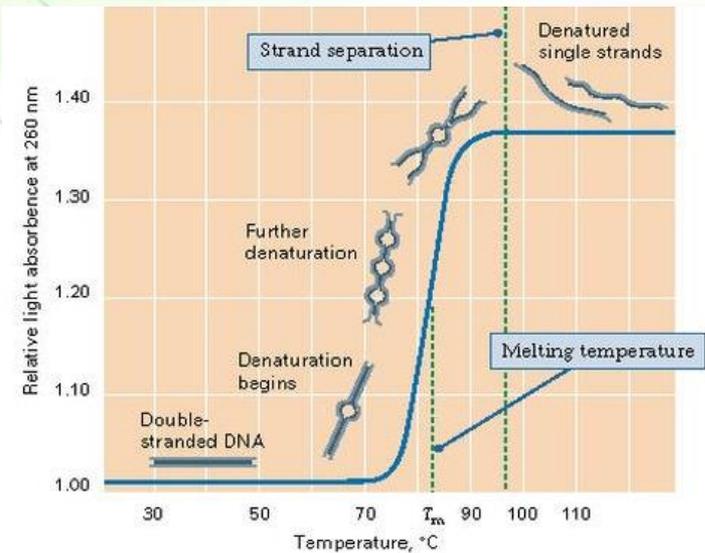
$$\begin{aligned}\text{DNA concentration} &= 0.1 \times 10 \times 50 \mu\text{g}/\text{ml} \\ &= 50 \mu\text{g}/\text{ml}\end{aligned}$$



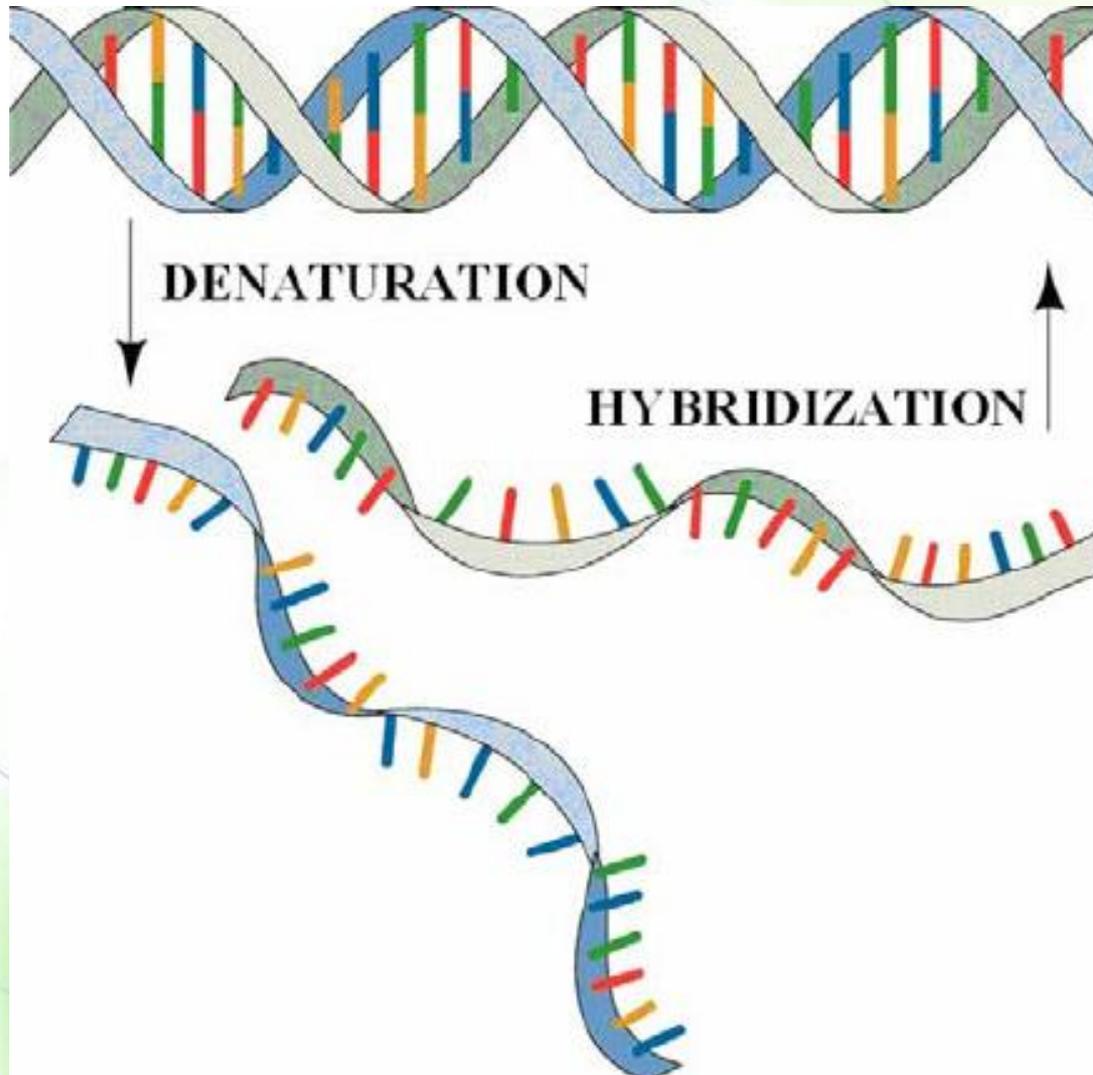
Observation of denaturation



- The transition temperature, or melting temperature (T_m).
- Factors influencing T_m
 - Length
 - G·C pairs
 - Hydrogen bonds
 - Base stacking
 - pH
 - Salts and ions
 - Destabilizing agents (alkaline solutions, formamide, urea)



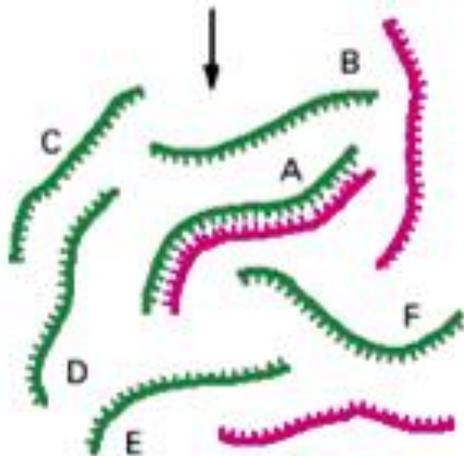
Denaturation versus renaturation (hybridization)



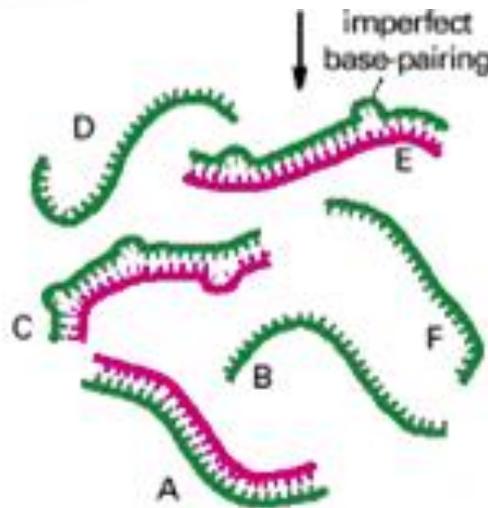
Hybridization



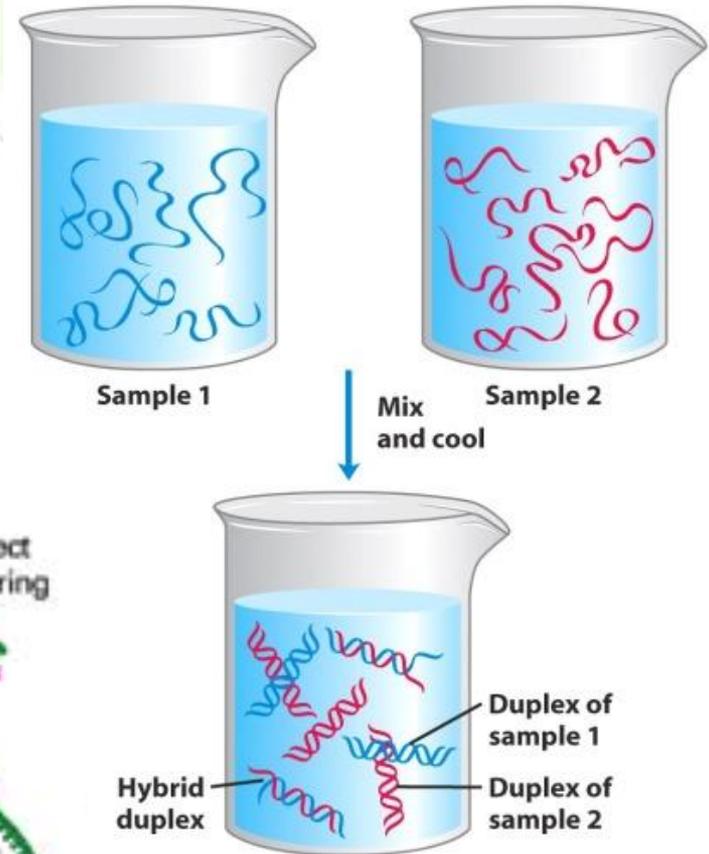
- DNA from different sources can form double helix as long as their sequences are compatible (hybrid DNA).
- Hybridization can be imperfect (*when temperature is low, salt concentration is high, etc*).



only A forms stable double helix



A, C, and E all form stable double helices



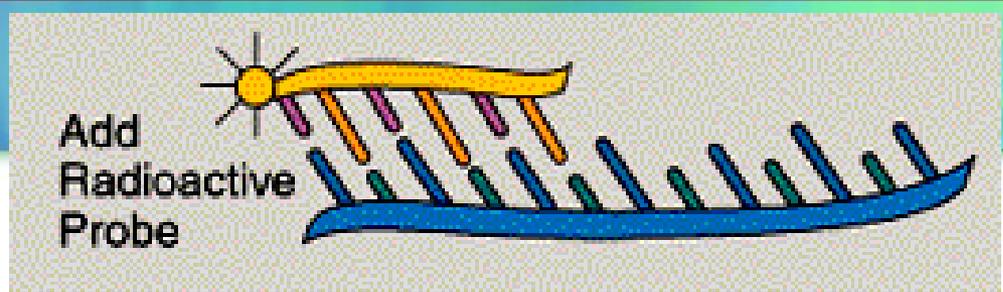
Hybridization techniques



- Hybridization reactions can occur between any two single-stranded nucleic acid chains provided that they have complementary nucleotide sequences
- Hybridization reactions are used to detect and characterize specific nucleotide sequences

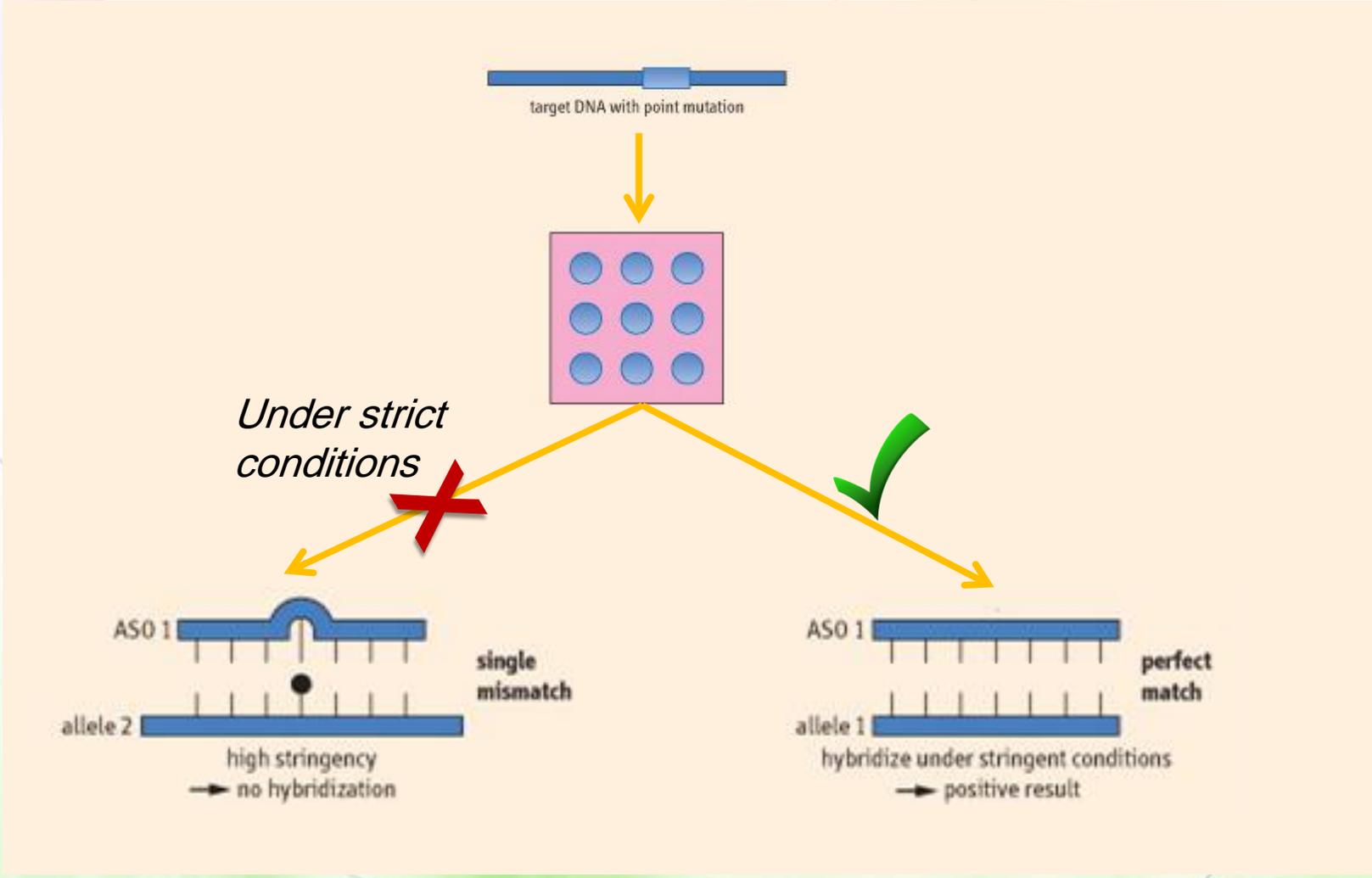
Probes

(Oligonucleotides)

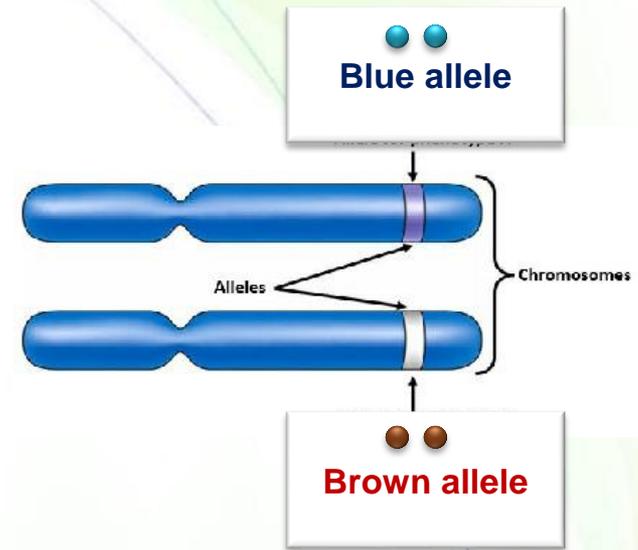
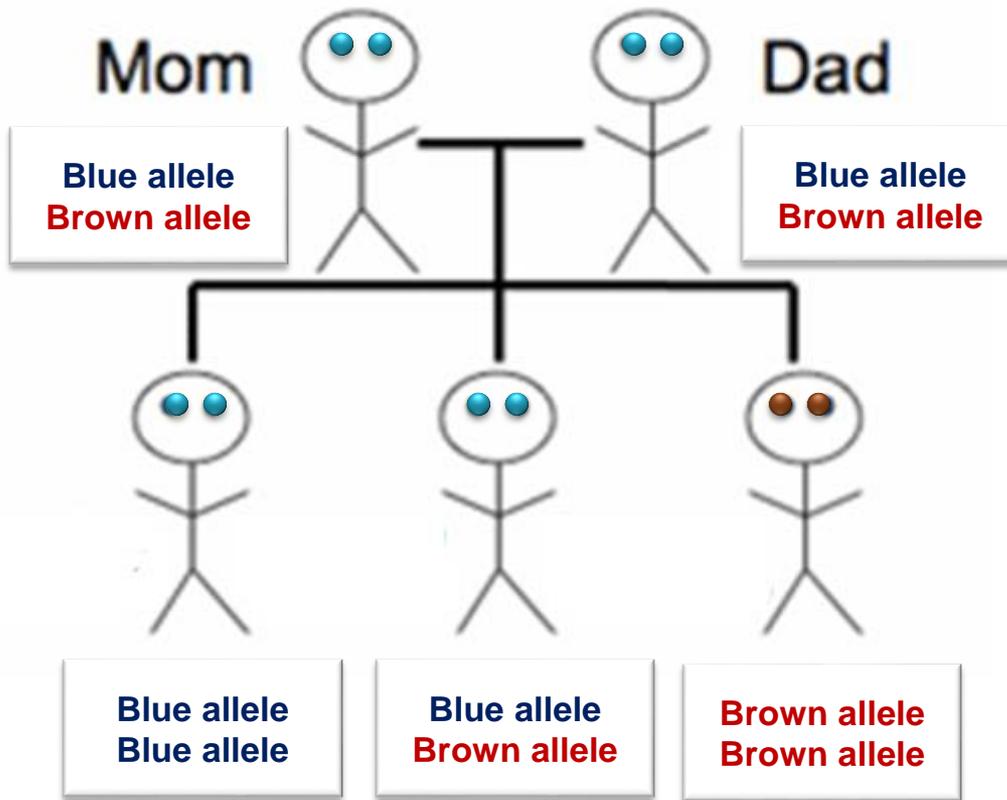


- A probe is a short sequence of single stranded DNA (an oligonucleotide) that is complementary to a small part of a larger DNA sequence.
- Hybridization reactions use labeled DNA probes to detect larger DNA fragments.





Concepts to know...



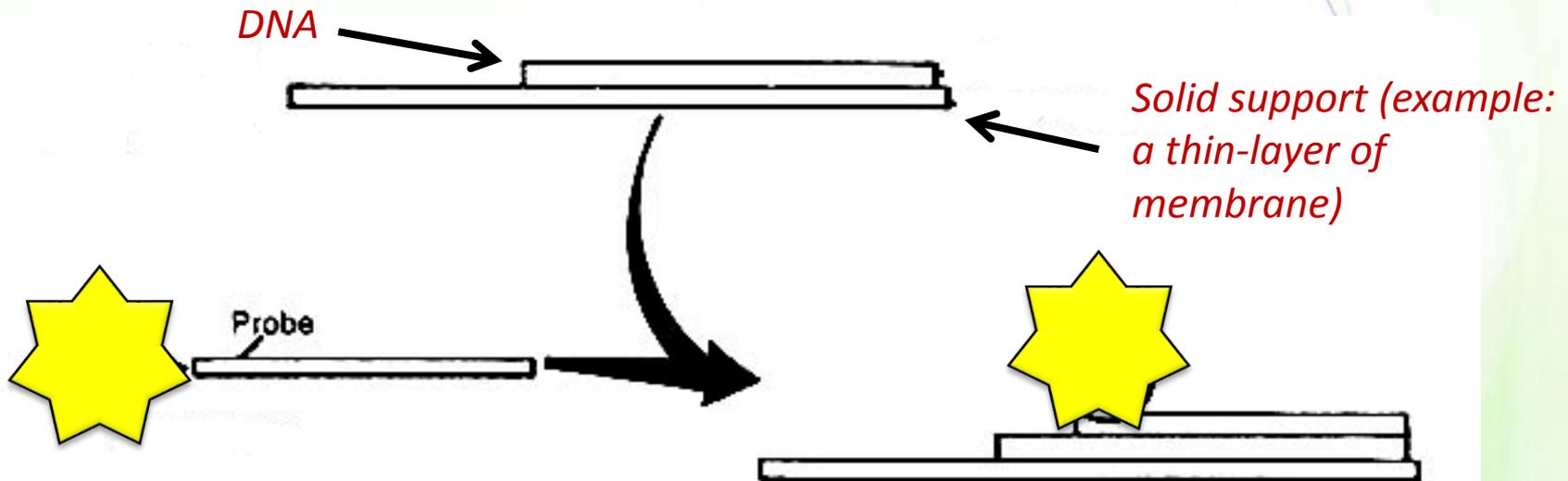
Pedigree
Allele

Dominant vs. recessive

Dot blot



- This is a technique that informs us if a specific sequence that is complementary to a probe of a known sequence exists in a larger DNA.
- DNA is bound to a solid support and a labeled probe is added. If binding occurs, the sequence exists.



Disease detection by ASO (Cystic fibrosis)



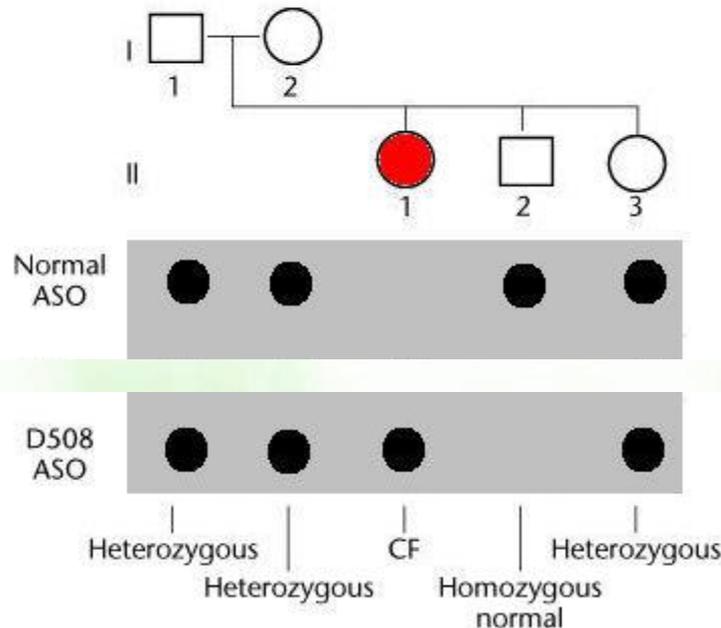
ASO: Allele-specific oligonucleotide

The whole genomic DNA is spotted on a solid support (like a nylon membrane) and hybridized with two ASO's, one at a time.

Cystic Fibrosis allele $\Delta 508$ has 3bp deletion [AGA]

ASO for normal DNA 5' CACCAA[AGA]GATATTTTC-3'

ASO for DNA sequence of $\Delta 508$ mutation 5' CACCAATGATATTTTC-3'



Resources



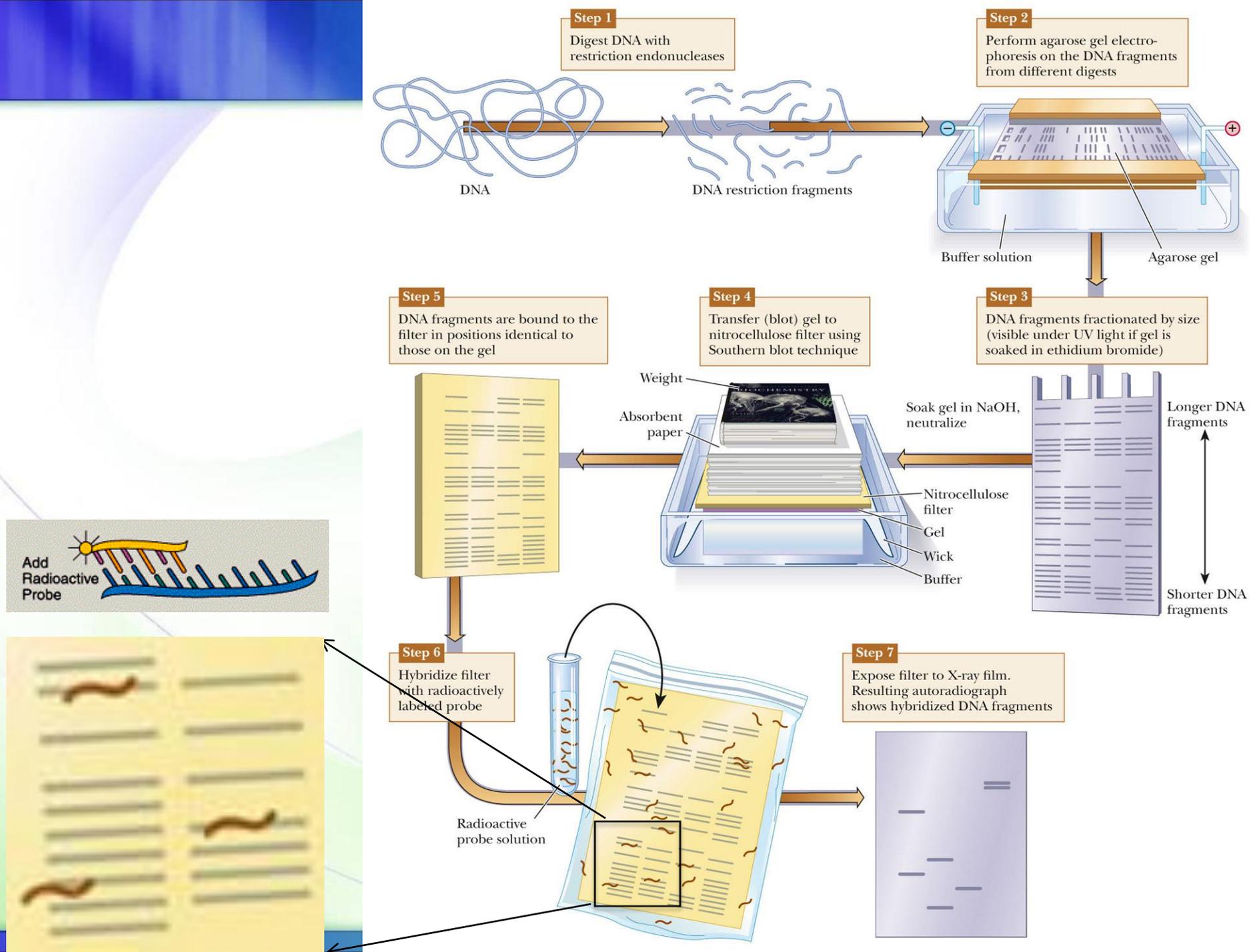
- <http://www.sumanasinc.com/webcontent/animations/content/gelelectrophoresis.html>

Watch this....very important

Southern blotting



- This technique is a combination of DNA gel electrophoresis and hybridization
- Used to detect:
 - the presence of a DNA segment complementary to the probe
 - the size of the DNA fragment

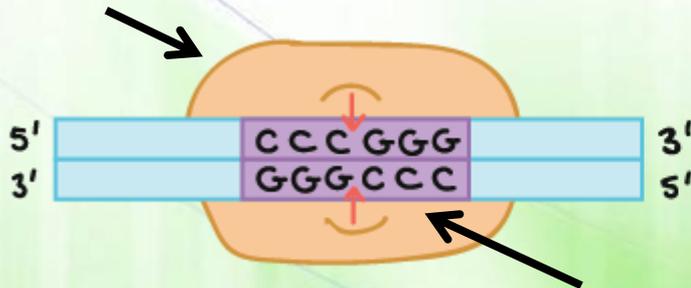


Restriction endonucleases



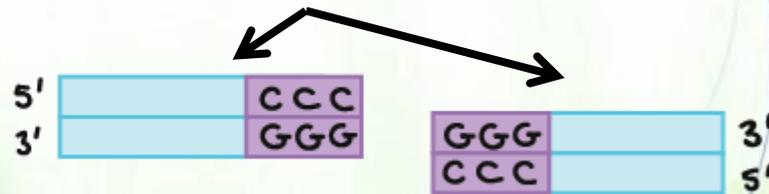
- Endonucleases are enzymes that degrade DNA within the molecule.
- Restriction endonucleases: Bacterial enzymes that recognize and cut (break) the **phosphodiester bond** between nucleotides at *specific* sequences (4- to 8-bp **restriction sites**) generating **restriction fragments**.

Restriction endonuclease



Restriction site

Restriction fragments



They recognize specific sequences



- The enzyme *Eco*RI recognizes and cuts within the sequence (GAATTC).

Variant 1

*Eco*RI does not cut



GCCGCATTC TA
CGGCGTAAGAT

The DNA stays intact

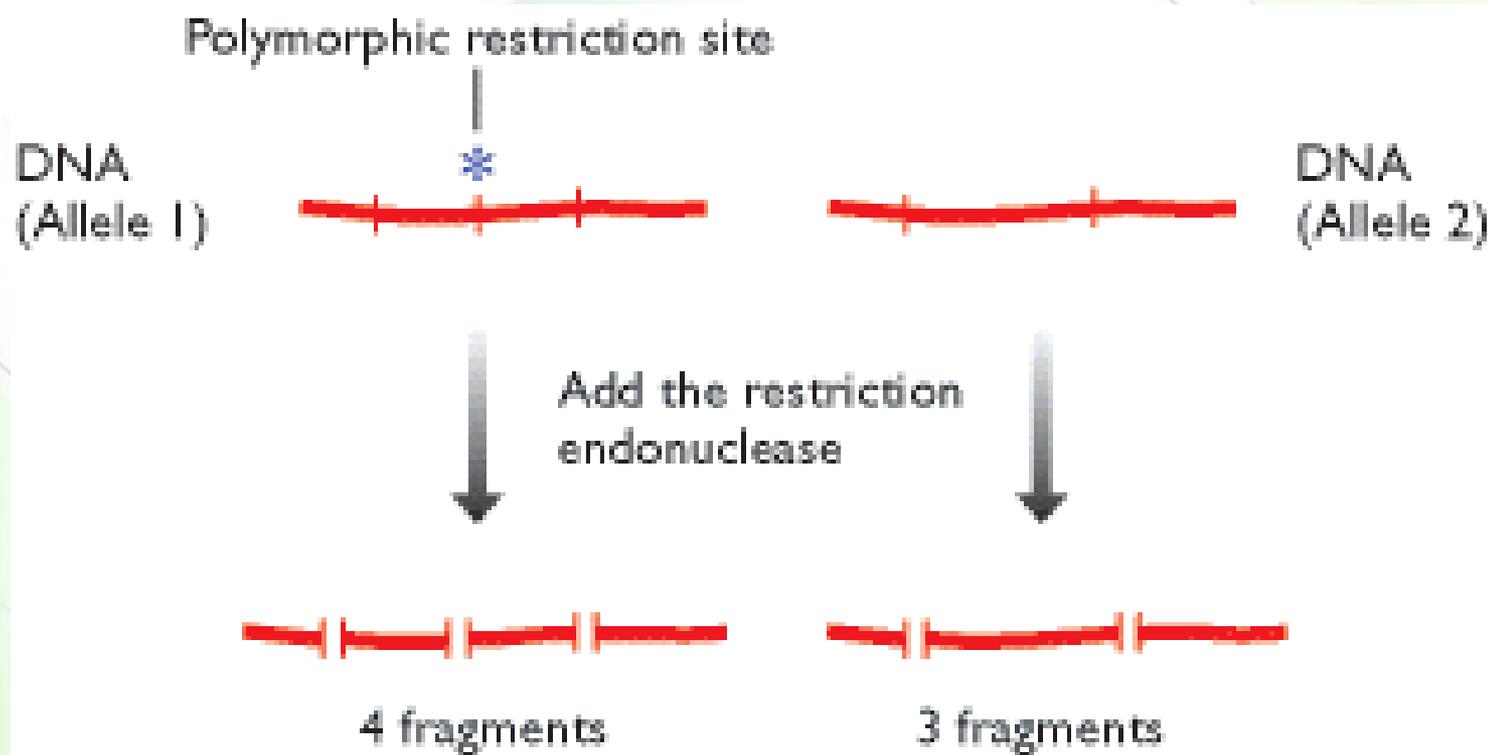
Variant 2

*Eco*RI does cut

GCCGAATTC TA
CGGCTTAAGAT

**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



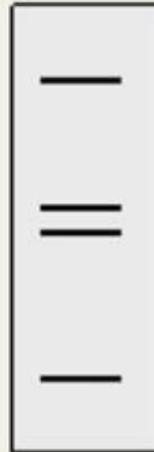
**Homozygous
individual for A**

A/A



**Homozygous
individual for B**

B/B



**Heterozygous
(A/B)**

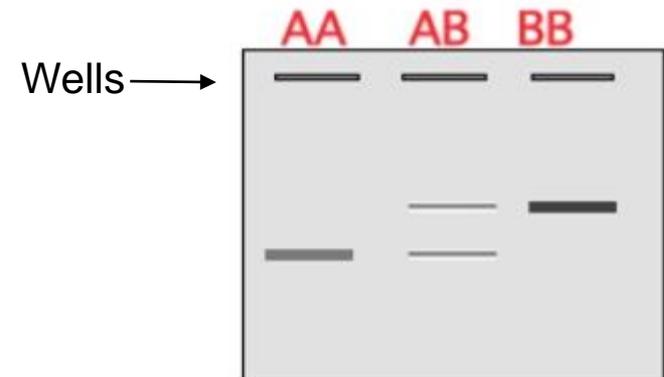
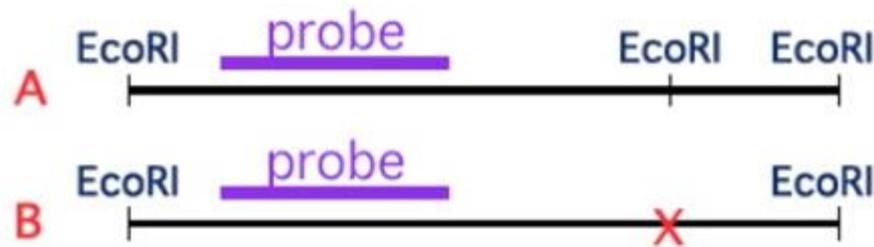
A/B



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



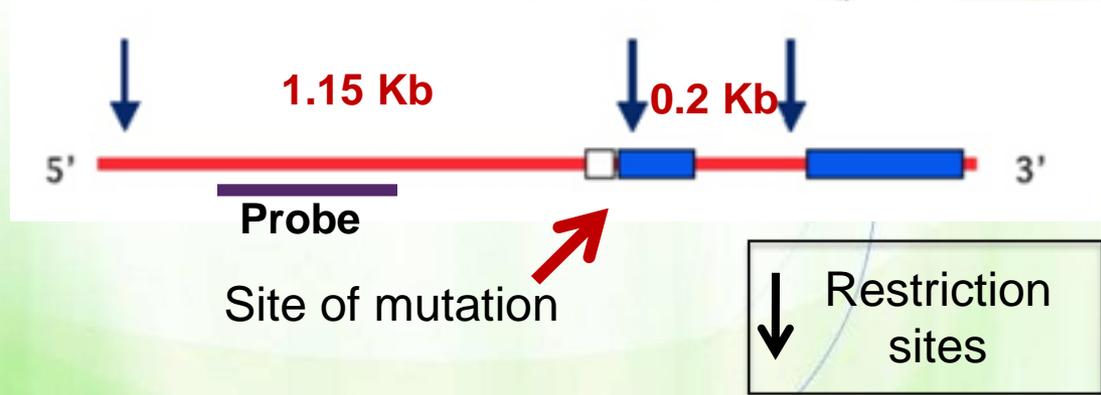
- RFLP can be used as diagnostic tools.
- For example, if a mutation that results in the development of a disease also causes the generation of distinctive RFLP fragments, then we can tell:
 - if the person is diseased as a result of this mutation
 - from which parent this allele is inherited

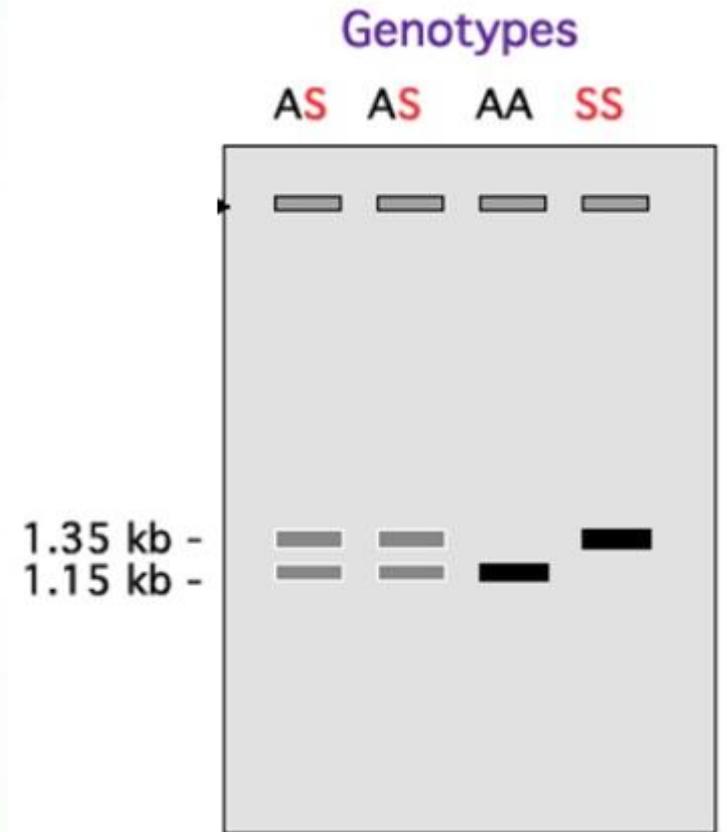
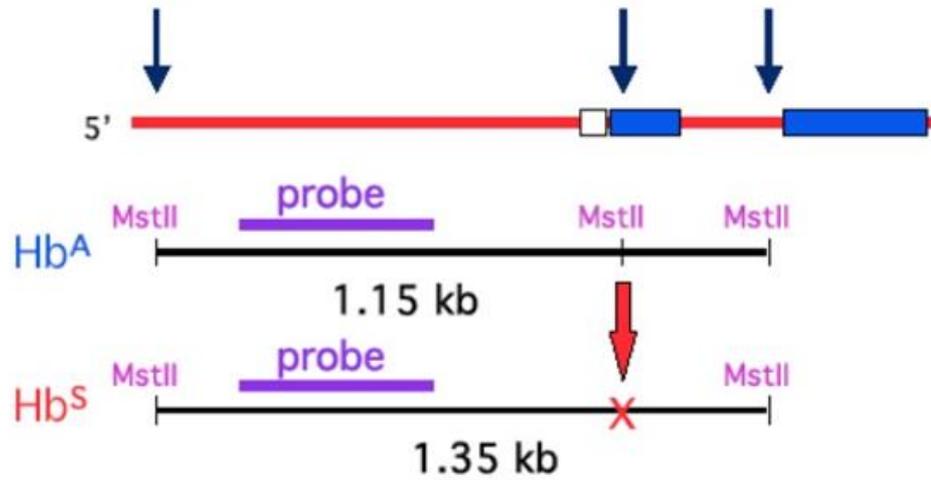


Example 1: Disease detection by RFLP

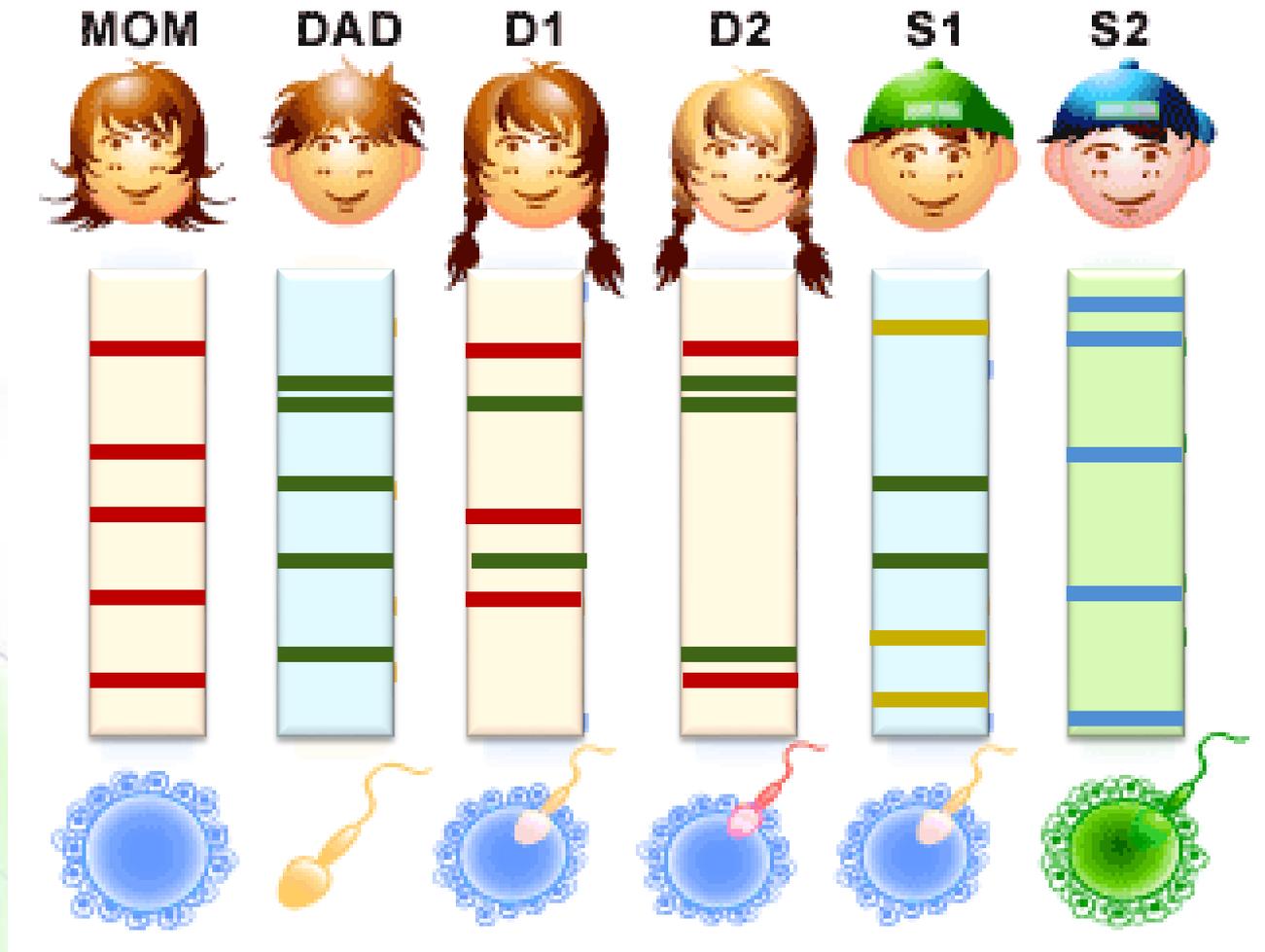
(sickle cell anemia)

- 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)





Example 2: Paternity testing



Real cases

