
# Molecular Biology (2)

## Basic Applications:

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

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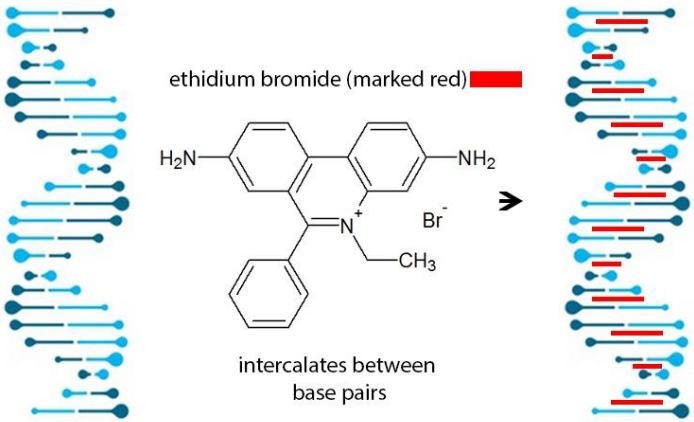
Second semester, 2020-2021

# Resources

- This lecture
- Cooper, Ch. 4, pp. 127-128, 137-138

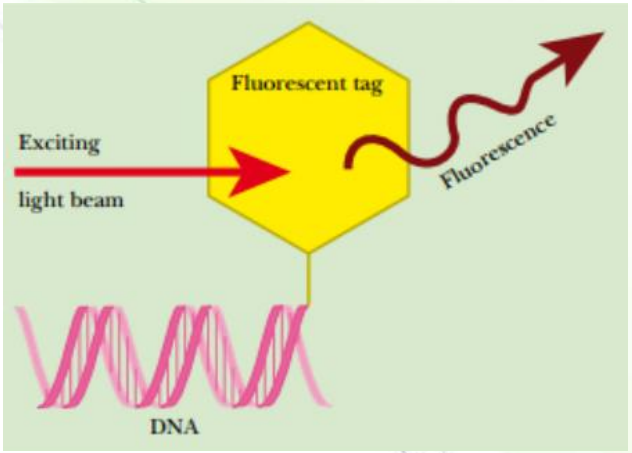
# DNA labeling versus staining

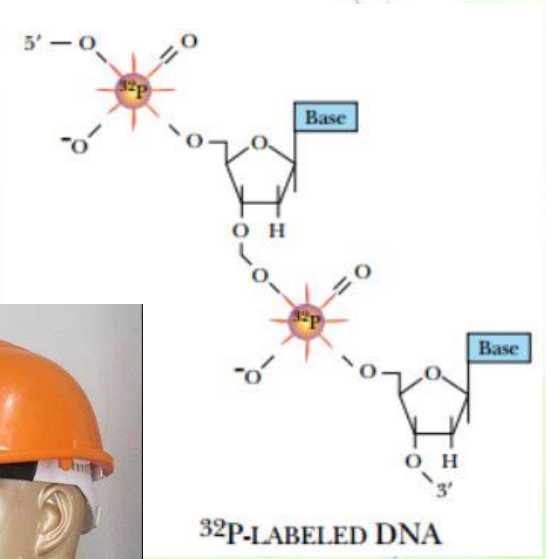
## DNA staining



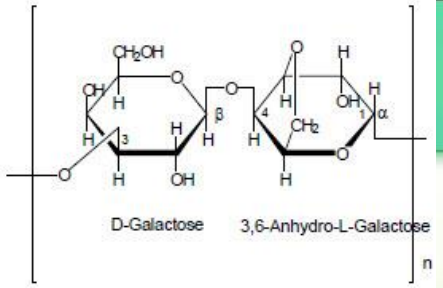


## DNA Labeling (more sensitive)

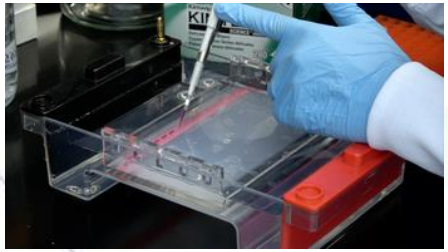


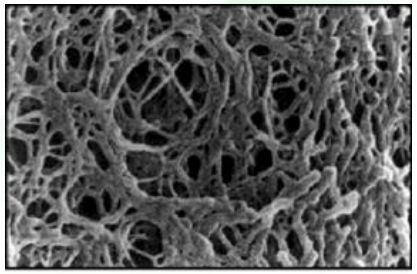




# Gel electrophoresis

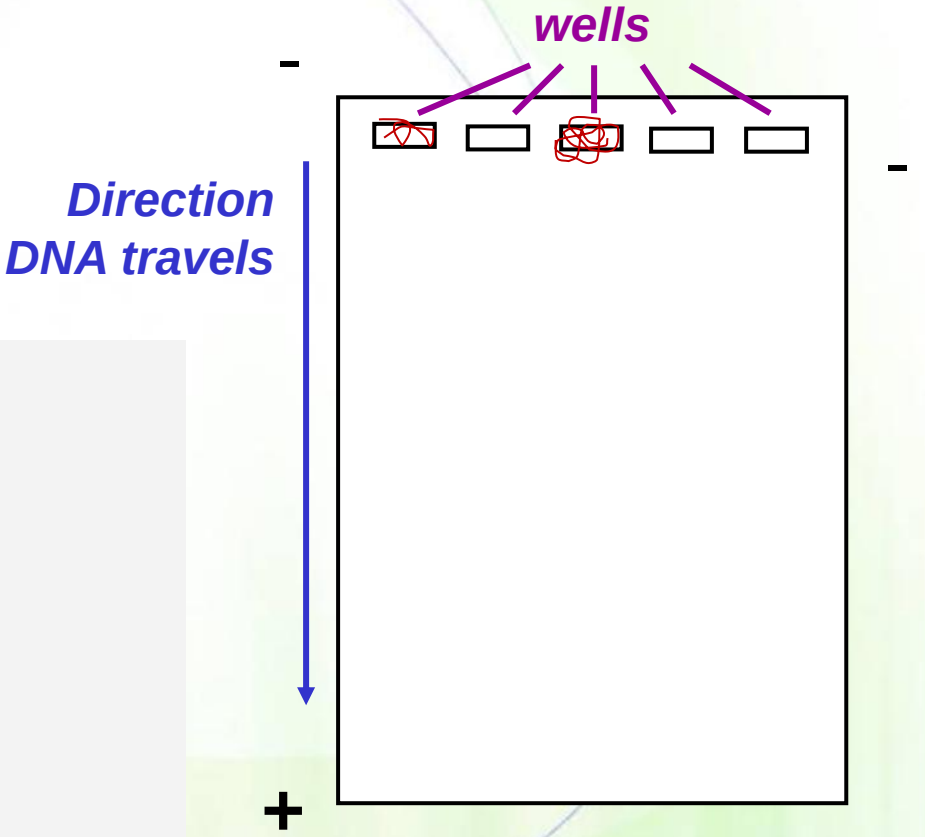



- The length and purity of DNA molecules can be accurately determined by the gel electrophoresis.



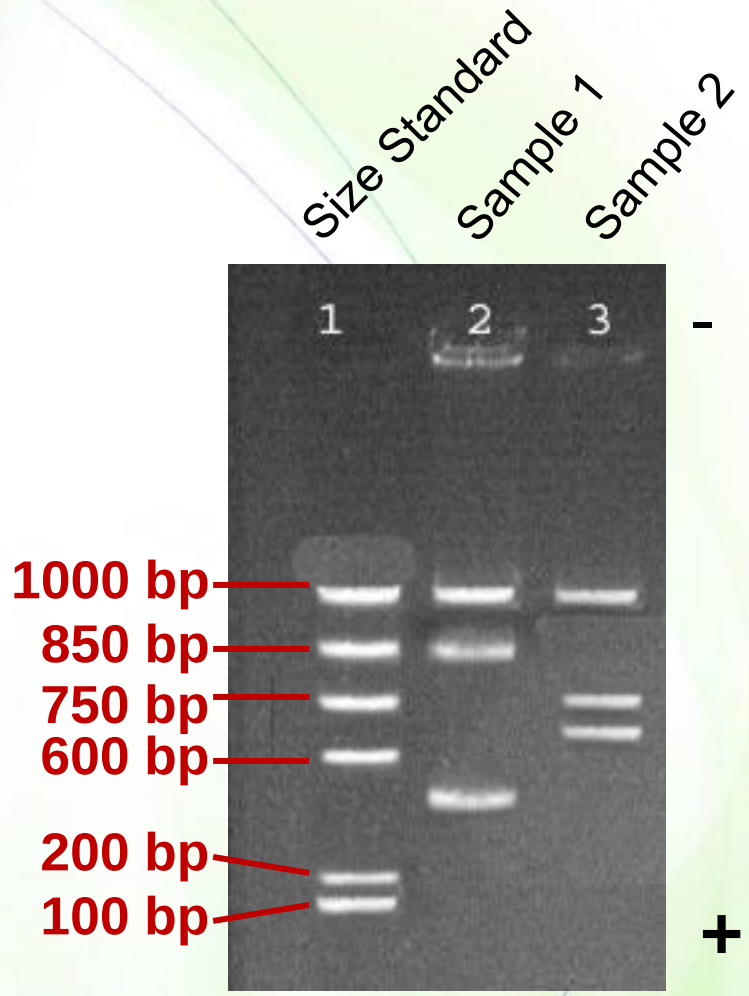






# Detection

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




*bp: base pair*

# Resources

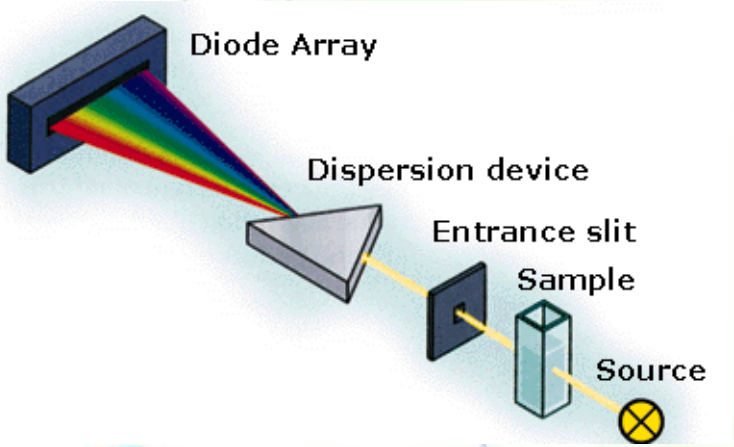
- http://www.sumanasinc.com/webcontent/animations/content/gelelectrophoresis.html

**Watch this….very important**

# Light absorbance of nucleic acids

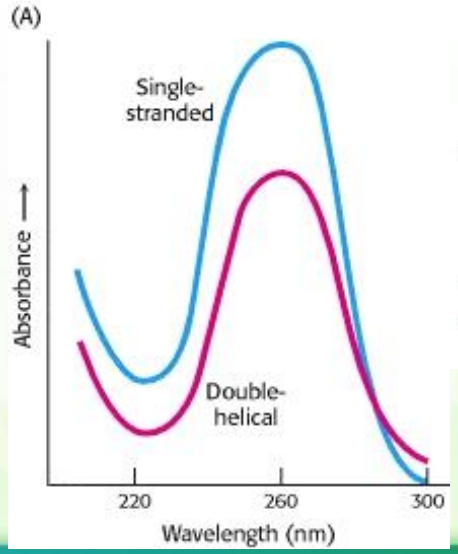
- Aromatic pyrimidines and purines can absorb UV light.
- Using spectrophotometry, the peak absorbance can be measured at 260 nm wavelength.

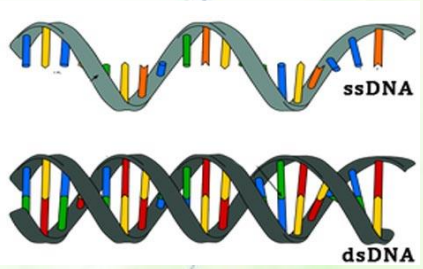
- The absorbance of nucleic acids at 260 nm (A260) is constant
  - dsDNA: A260 of 1.0 = 50 ug/ml




Reason for ss vs. ds absorbance:

- Unstacked bases vs. stacked bases



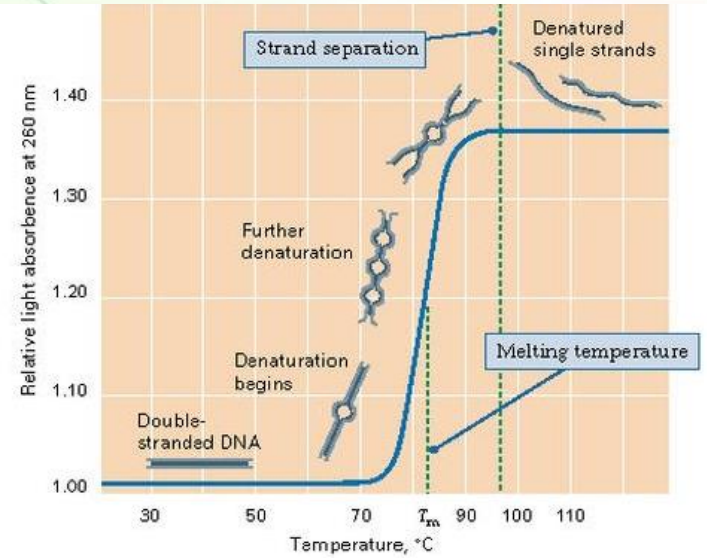

What is the concentration of a double stranded DNA sample diluted at 1:10 and the A260 is 0.1?

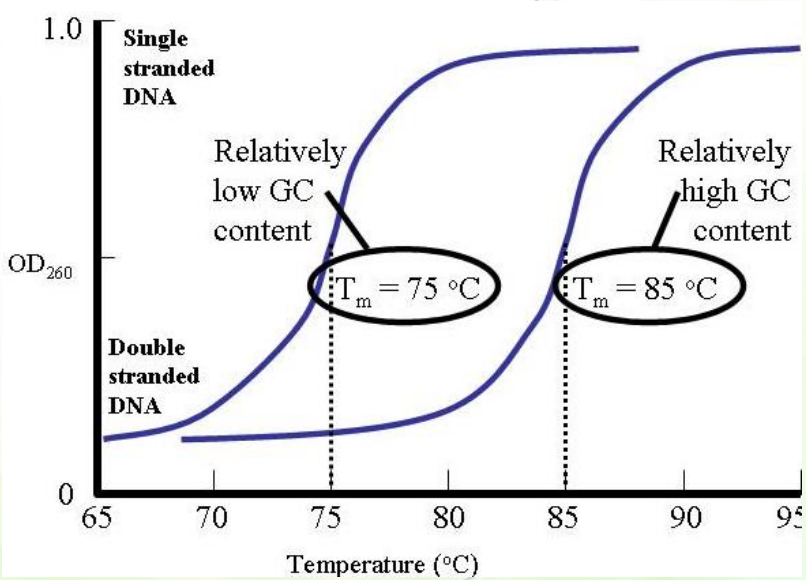
DNA concentration = 0.1 x 10 x 50 µg/ml

= 50 µg /ml

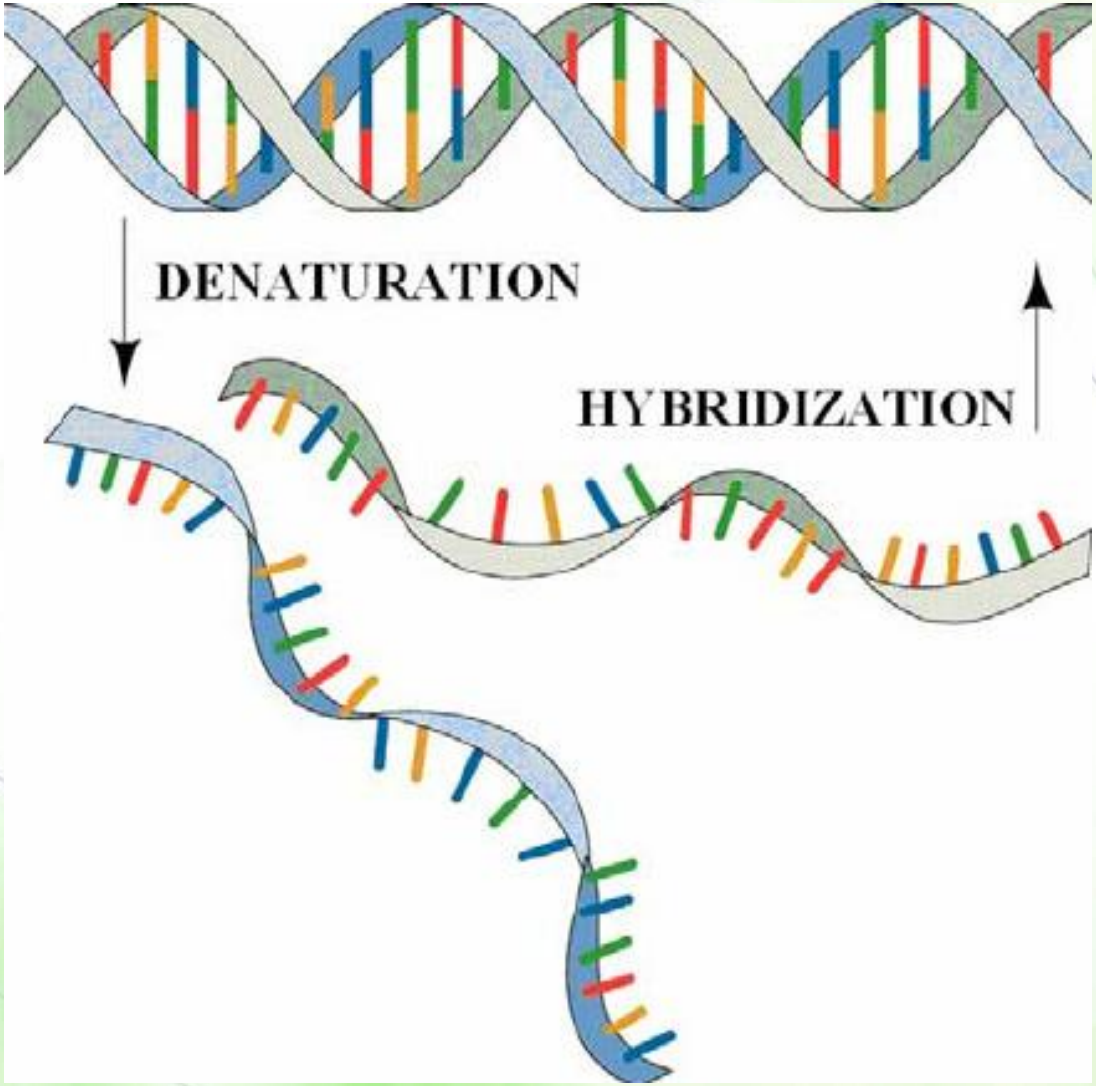
# Observation of denaturation

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



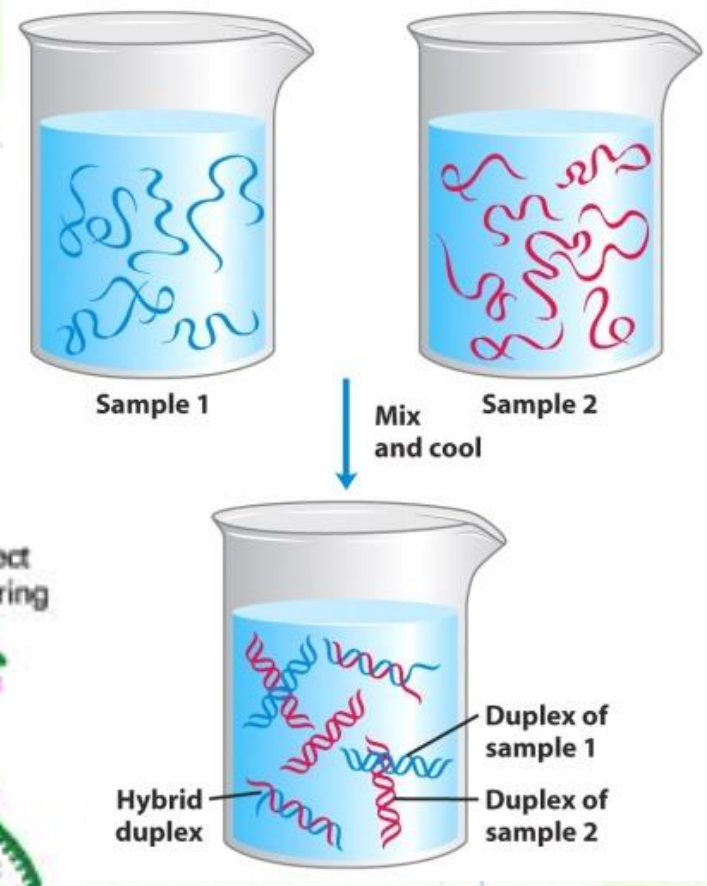



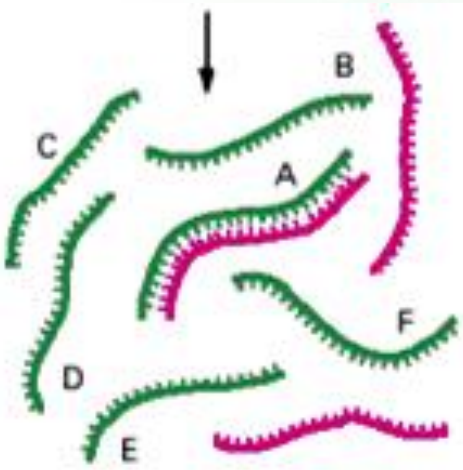
# Denaturation versus renaturation (hybridization)



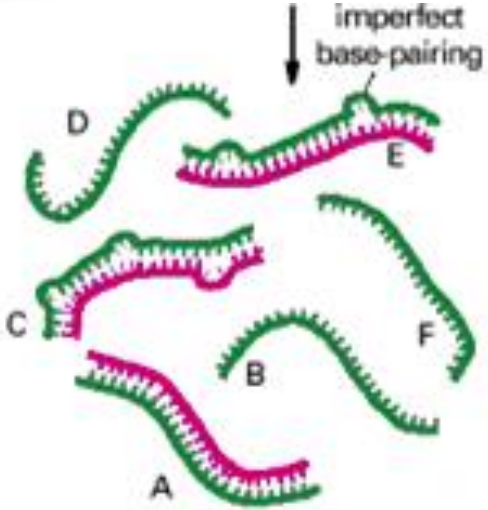
# Hybridization

- 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



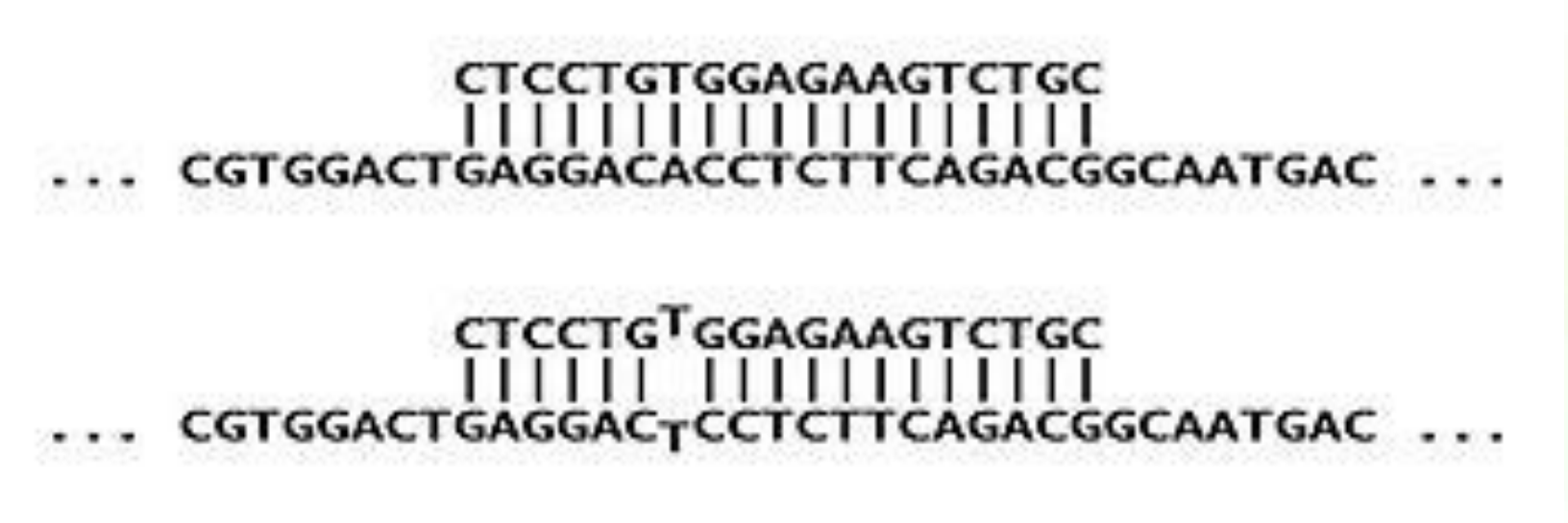

A, C, and E all form stable double helices

# Hybridization techniques

- Hybridization reactions can occur between any two single-stranded nucleic acid chains provided that they have complementary nucleotide sequences

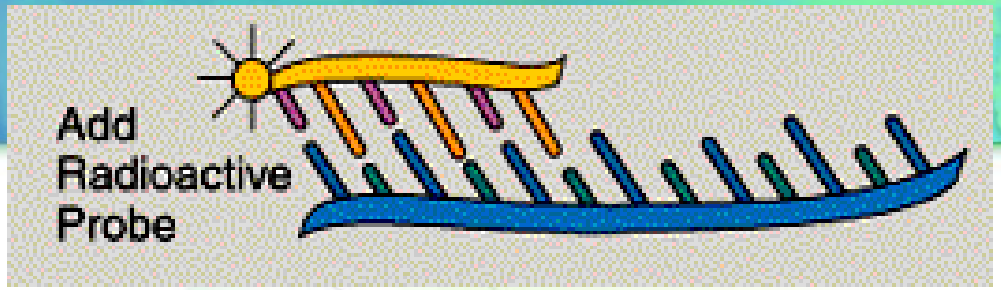
- Hybridization reactions are used to detect and characterize specific nucleotide sequences

# Hybridization can be non-specific



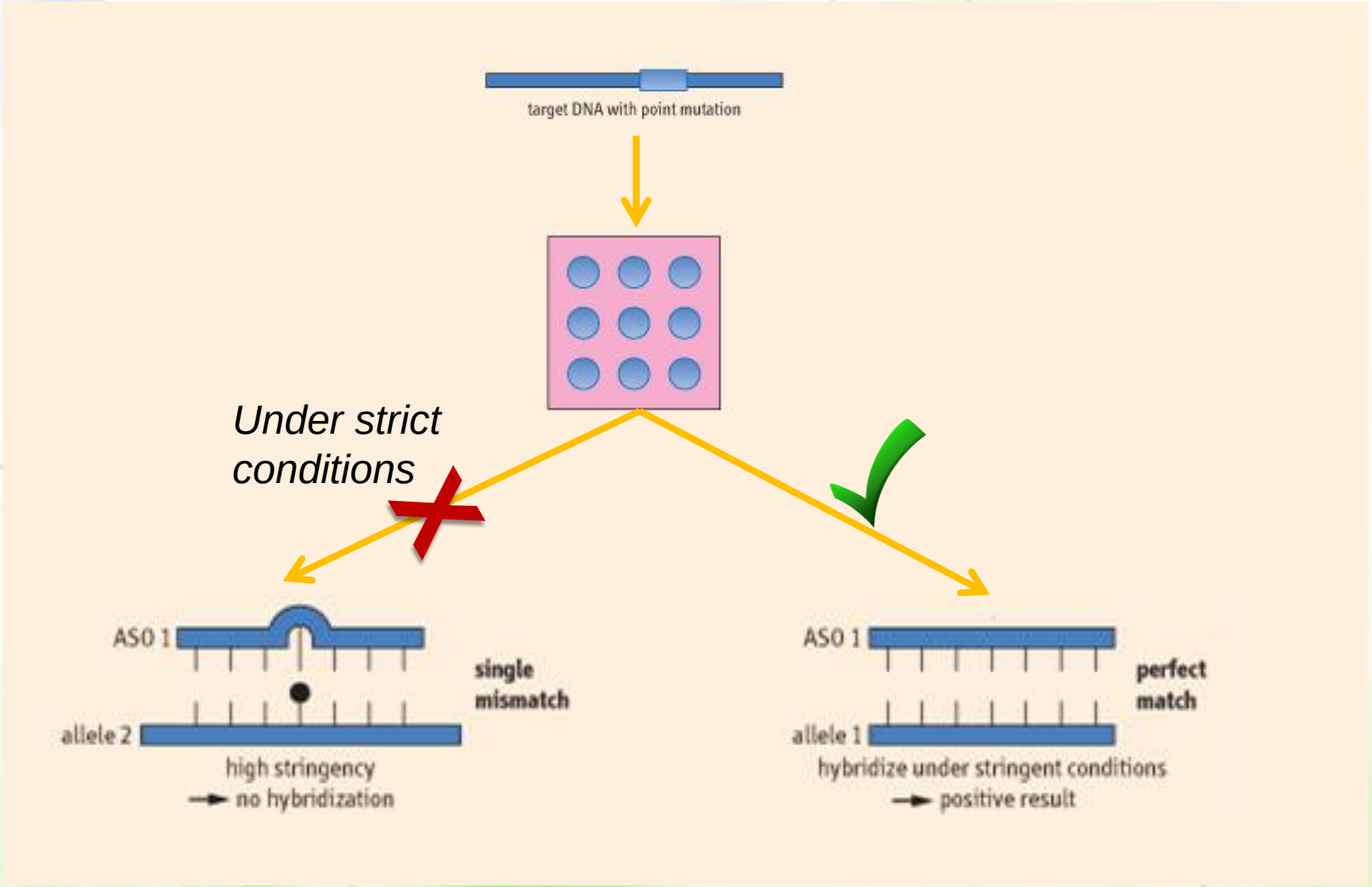

**Hybridization can be controlled by changing the temperature, ionic strength of solutions, GC content, etc.**

# Probes
## (Oligonucleotides)

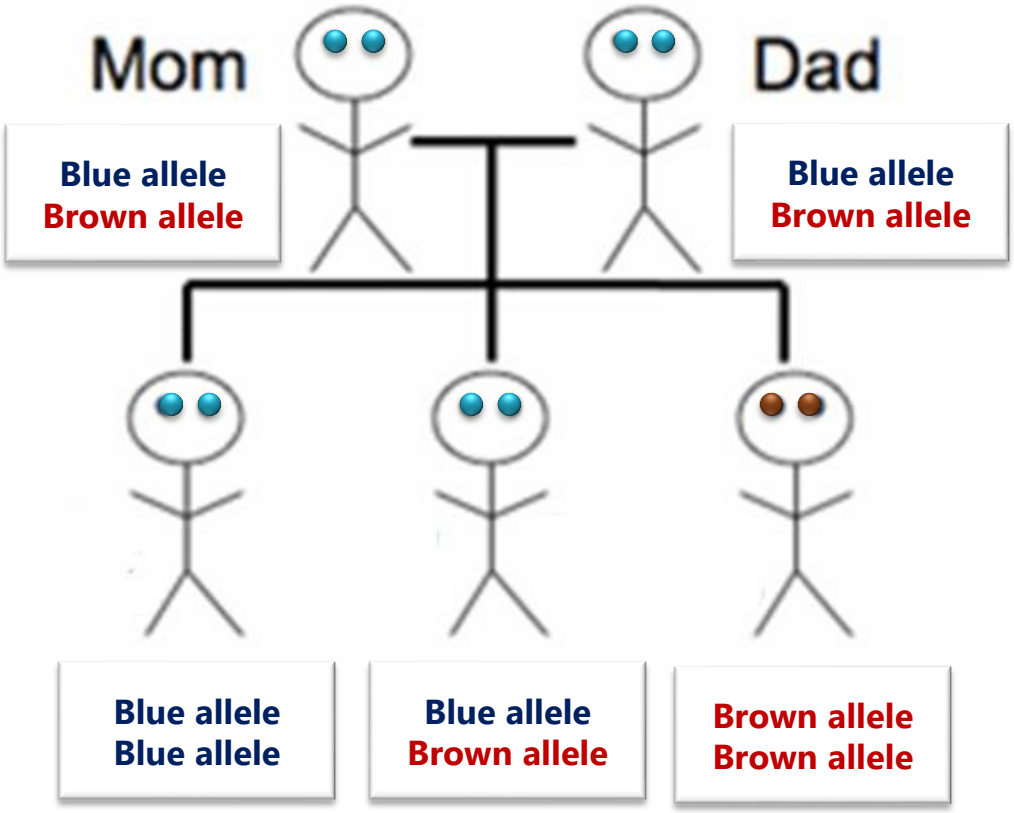


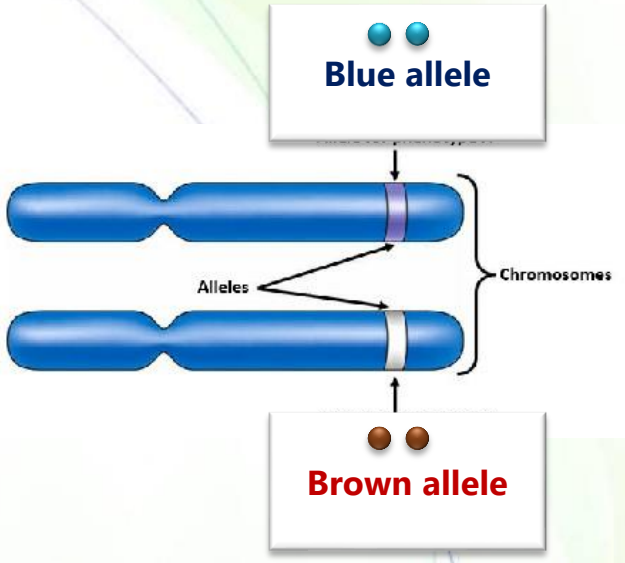

- A probes is a short sequence of single stranded DNA (an oligonucleotide) that is complementary to a small part of a larger DNA sequence.

- Hybridization reactions use labeled DNA probes to detect larger DNA fragments.


target DNA with point mutation
Under strict conditions
ASO 1
single mismatch
allele 2
high stringency
no hybridization
ASO 1
perfect match
allele 1
hybridize under stringent conditions
positive result

# Concepts to know…

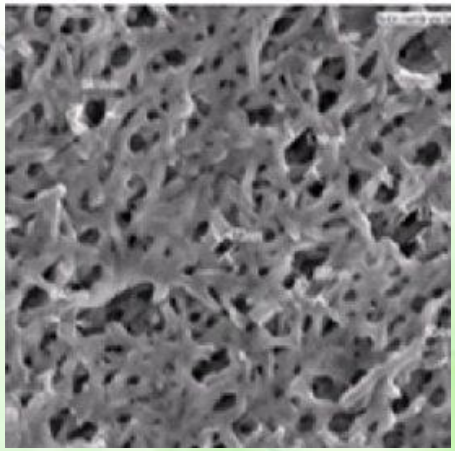


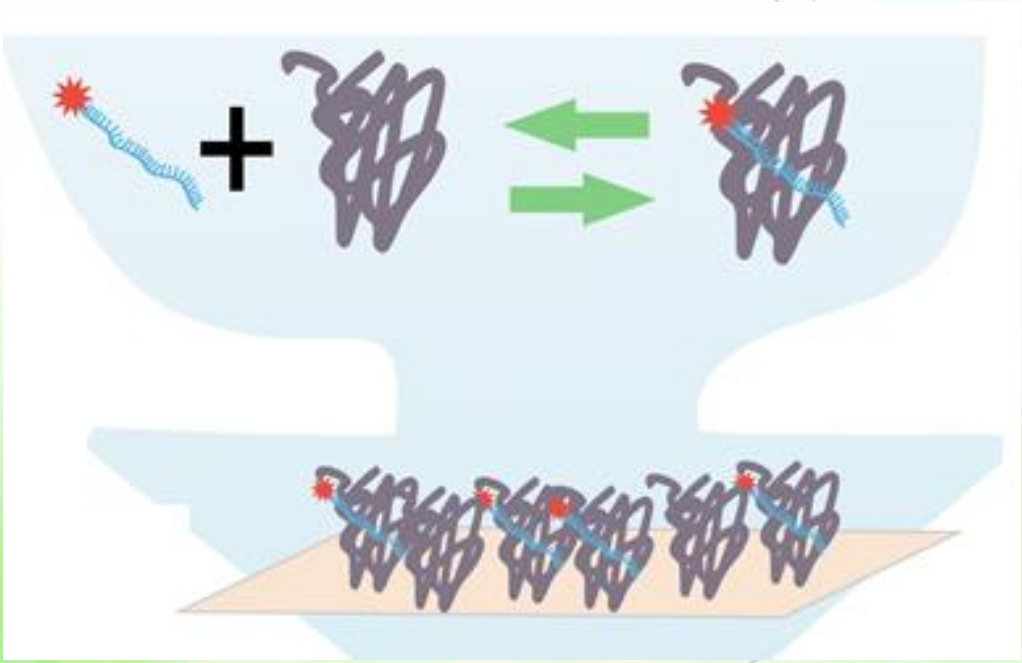




Pedigree
Allele
  Dominant vs. recessive
Homozygous vs. heterozygous

# 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)

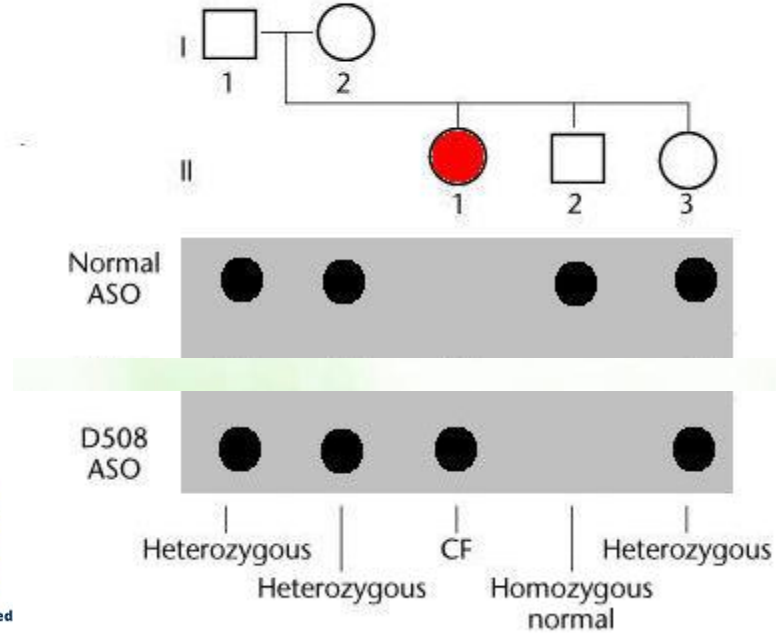
***ASO: Allele-specific oligonucleotide***

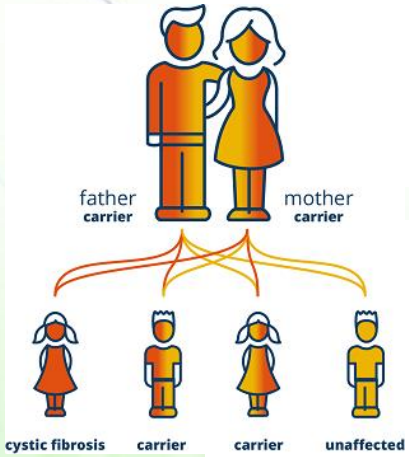
**The whole genomic DNA is spotted on a solid support (a membrane) and hybridized with two ASO's, one at a time.**

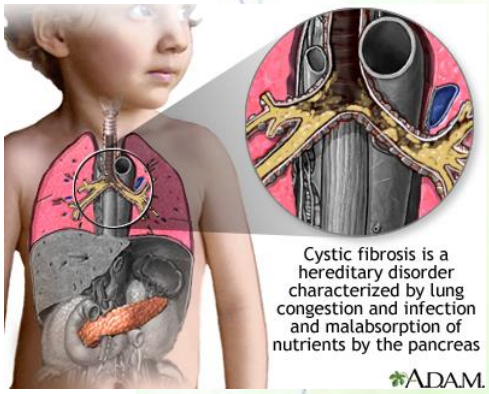
**Cystic Fibrosis allele Δ508 has 3bp deletion [AGA]**

ASO for normal DNA 5′ CACCAAAGATGATATTTTC-3′

ASO for DNA sequence of Δ508 mutation 5′ CACCAATGATATTTTC-3′



# Resources

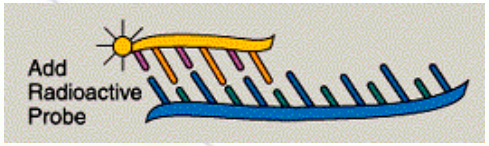
- http://www.sumanasinc.com/webcontent/animations/content/gelelectrophoresis.html

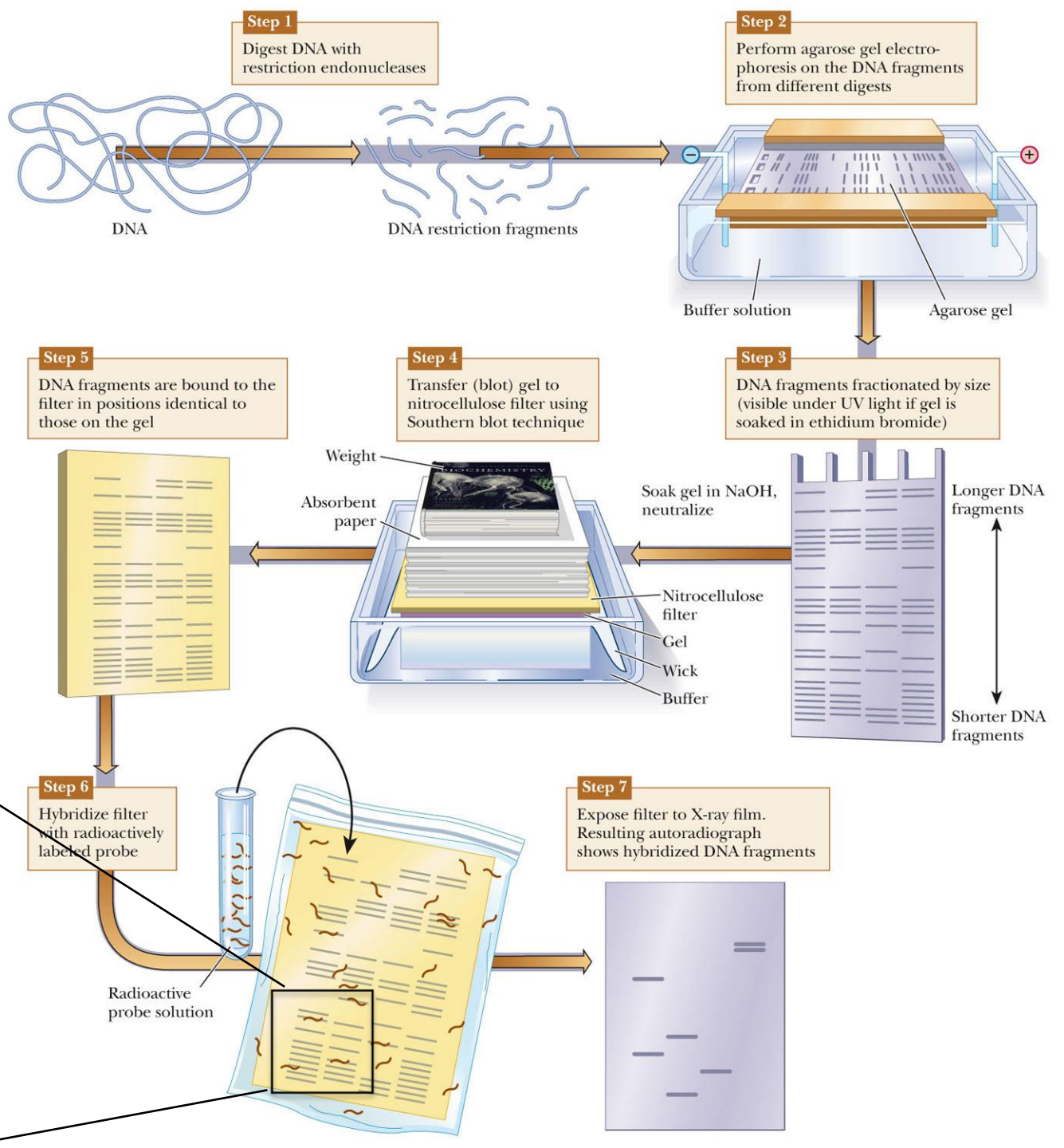
**Watch this….very important**

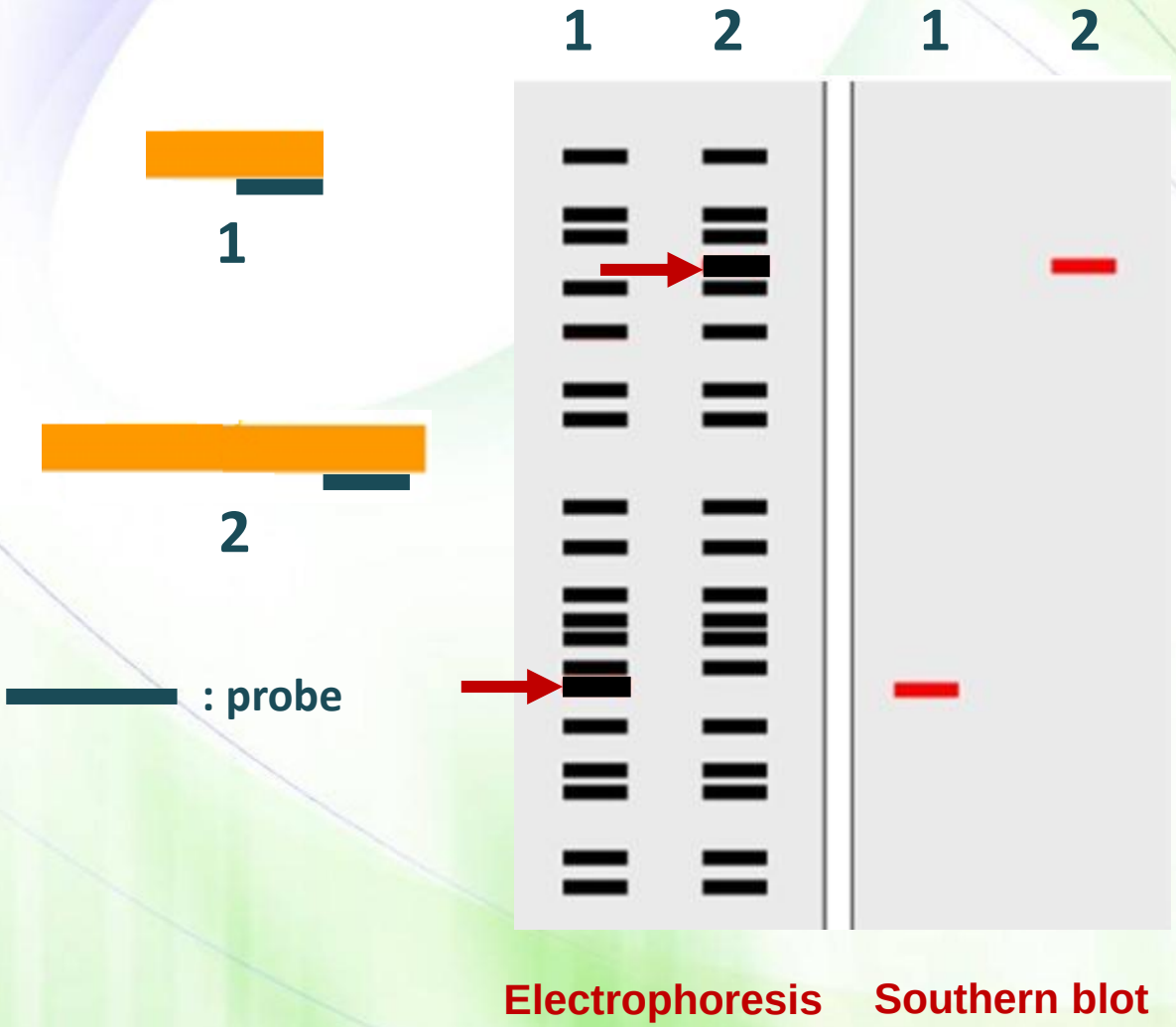
# Southern blotting

- 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

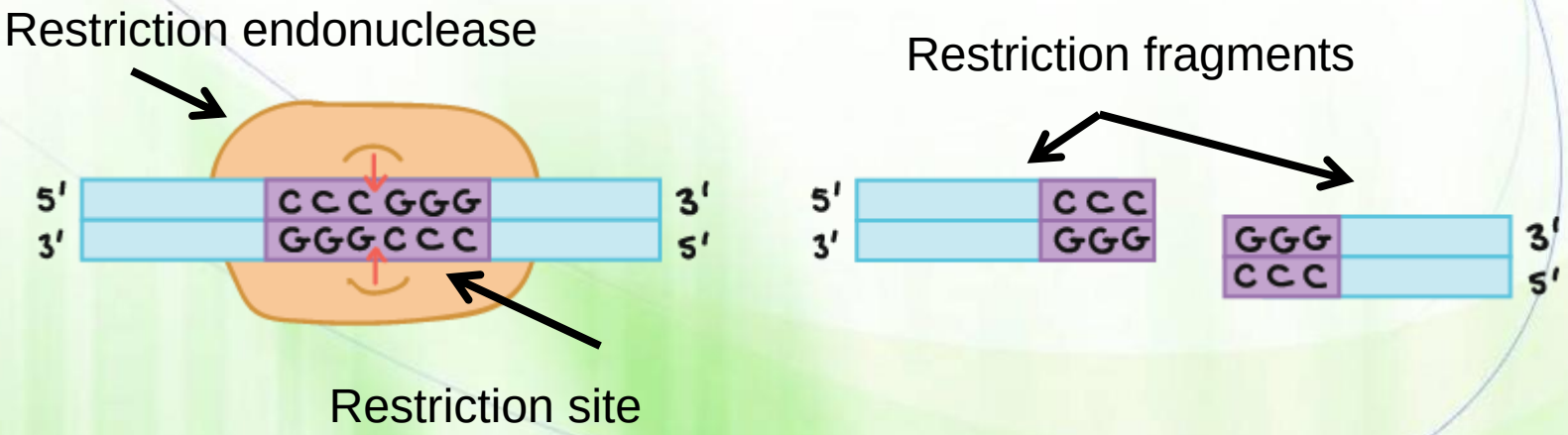

Add
Radioactive
Probe


Step 1
Digest DNA with restriction endonucleases
DNA
DNA restriction fragments
Step 2
Perform agarose gel electrophoresis on the DNA fragments from different digests
Buffer solution
Agarose gel
Step 3
DNA fragments fractionated by size (visible under UV light if gel is soaked in ethidium bromide)
Longer DNA fragments
Shorter DNA fragments
Soak gel in NaOH, neutralize
Step 4
Transfer (blot) gel to nitrocellulose filter using Southern blot technique
Weight
Absorbent paper
Nitrocellulose filter
Gel
Wick
Buffer
Step 5
DNA fragments are bound to the filter in positions identical to those on the gel
Step 6
Hybridize filter with radioactively labeled probe
Radioactive probe solution
Step 7
Expose filter to X-ray film. Resulting autoradiograph shows hybridized DNA fragments


1
2
: probe
1
2
1
2
Electrophoresis
Southern blot

# 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.



# Palindromic sequences

- The sequences recognized by restriction endonucleases—their sites of action—read the same from left to right as they do from right to left (on the complementary strand).

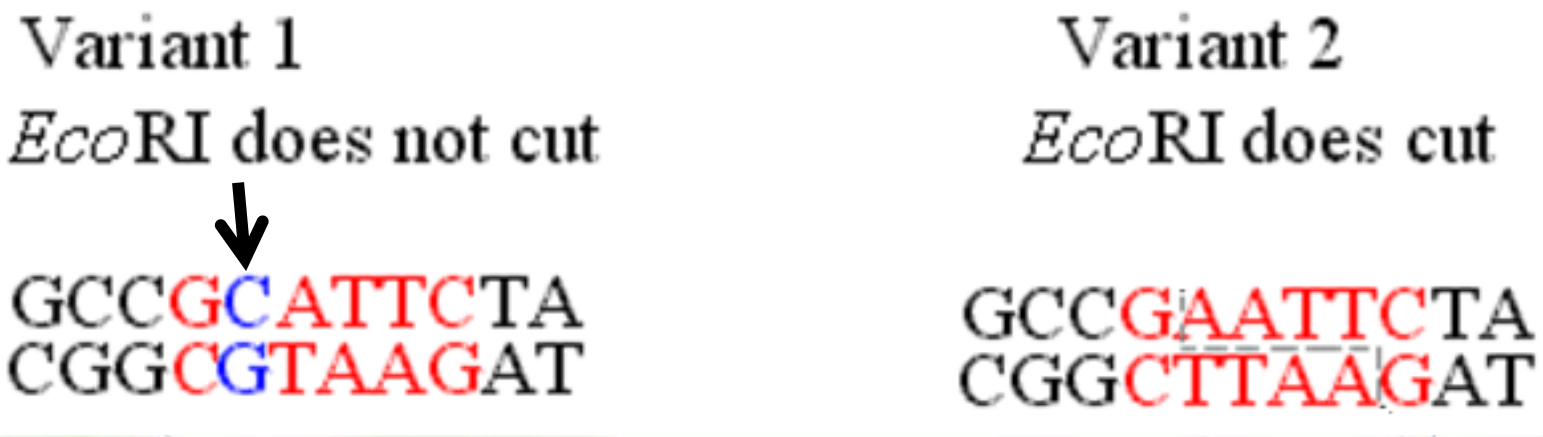
```
EcoRI       5'  GAATTC  3'
            3'  CTTAAG  5'

HindIII     5'  AAGCTT  3'
            3'  TTCGAA  5'

SmaI        5'  CCCGGG  3'
            3'  GGGCCC  5'
```

# They recognize specific sequences

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

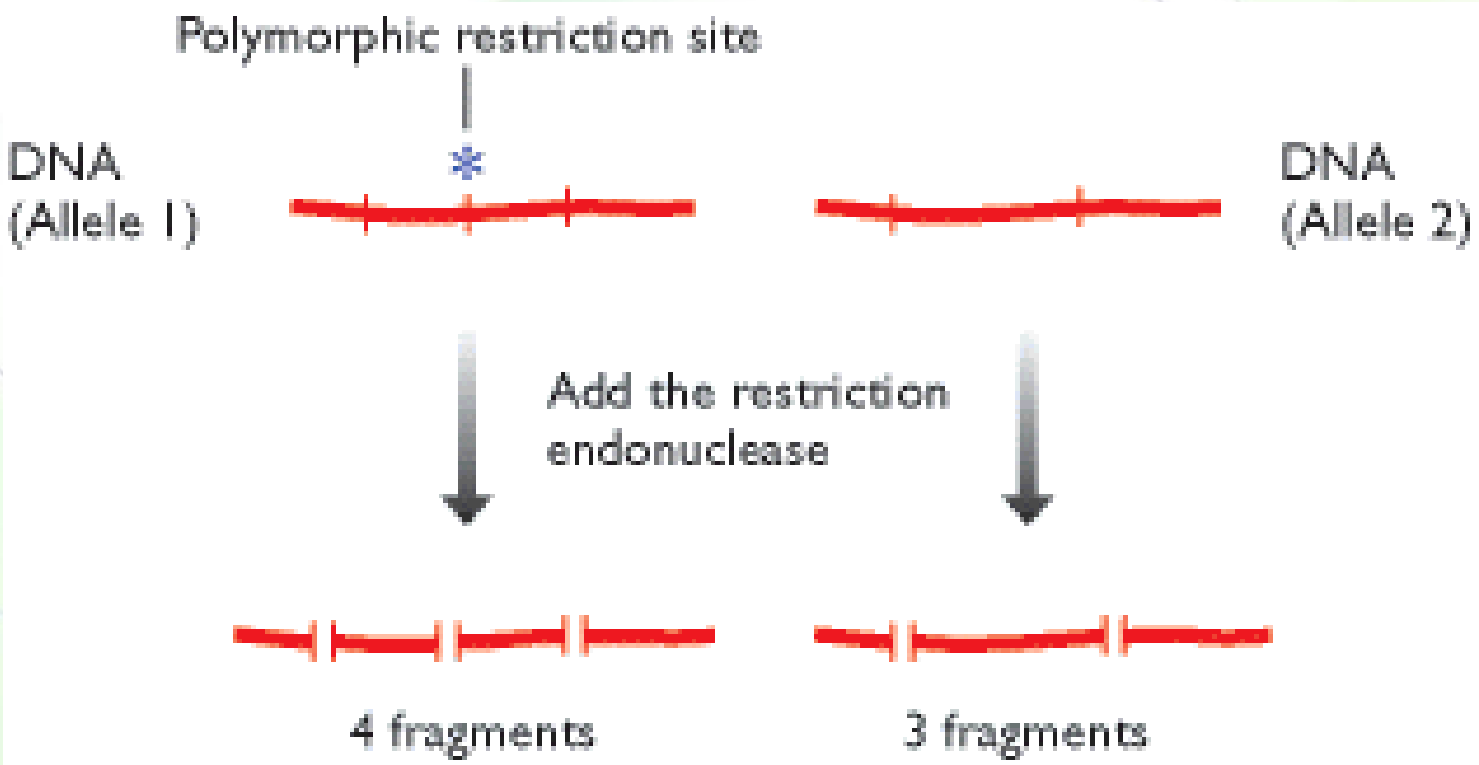



**The DNA stays intact**

**The DNA is cut into two pieces**

# Cuts and number of fragments

- 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

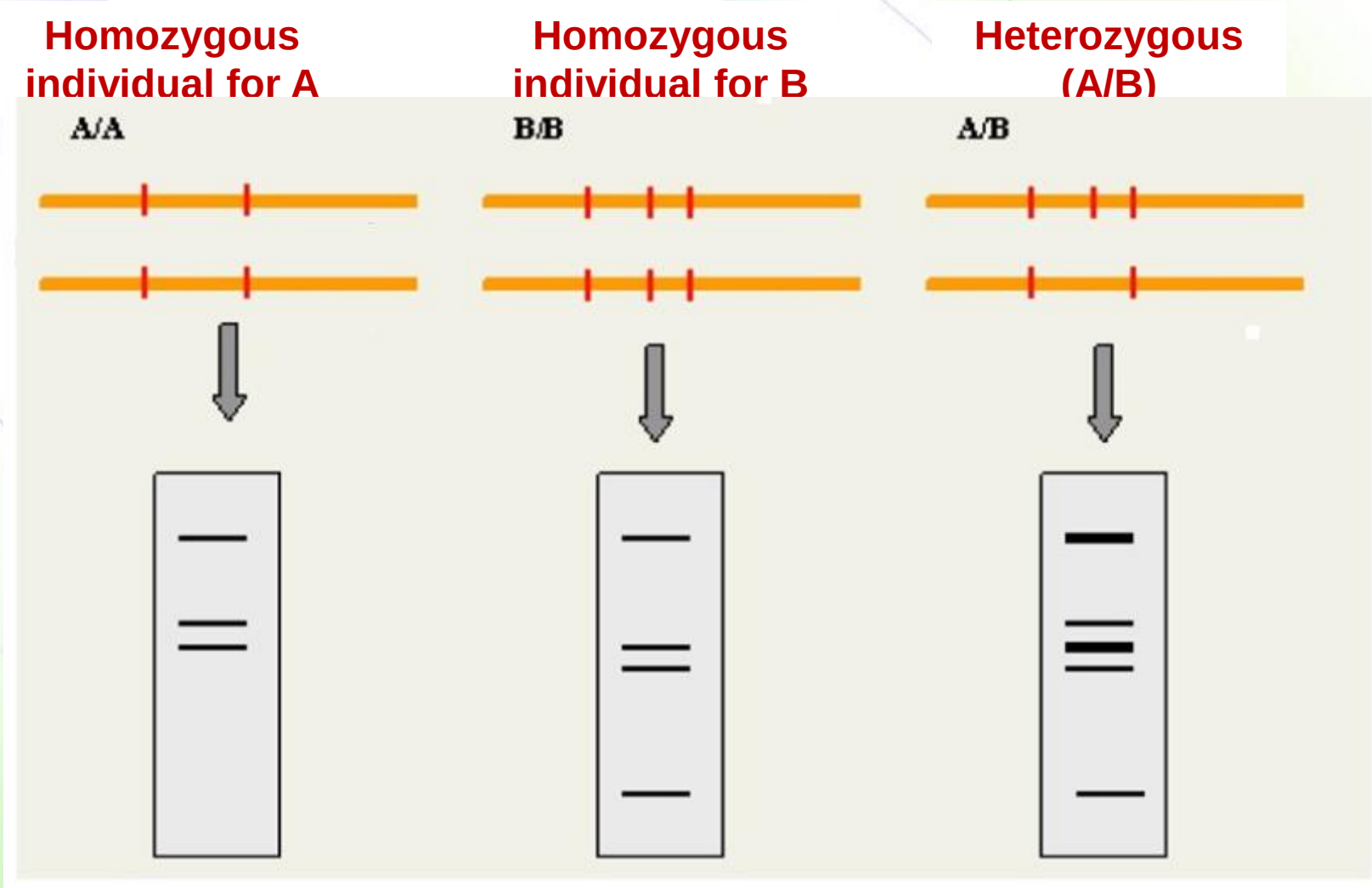
- Individual variations in DNA sequence (*genetic variants*) may create or remove restriction-enzyme recognition sites generating different restriction fragments.

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

# Restriction fragment length polymorphism

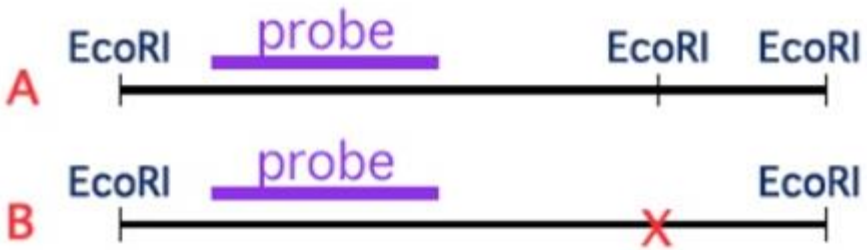
- The presence of different DNA forms in individuals generates a restriction fragment length polymorphism, or RFLP.
- Individuals can generate restriction fragments of variable lengths. This is known as molecular fingerprinting.
- These can be detected by gel electrophoresis by itself or along with Southern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

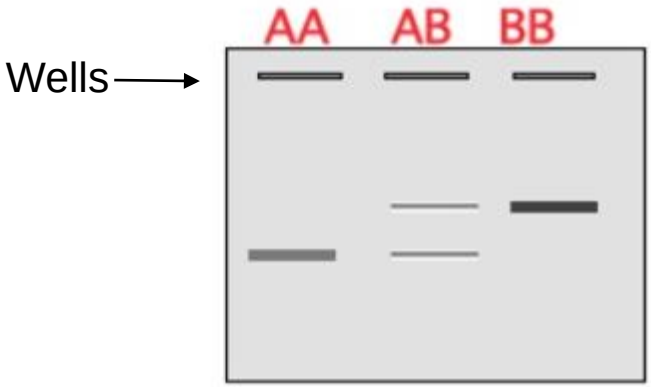
# Gel electrophoresis only



# Electrophoresis then Southern blotting

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




***Note: the size of the DNA detected DNA fragment reflects its size, not the size of the probe***

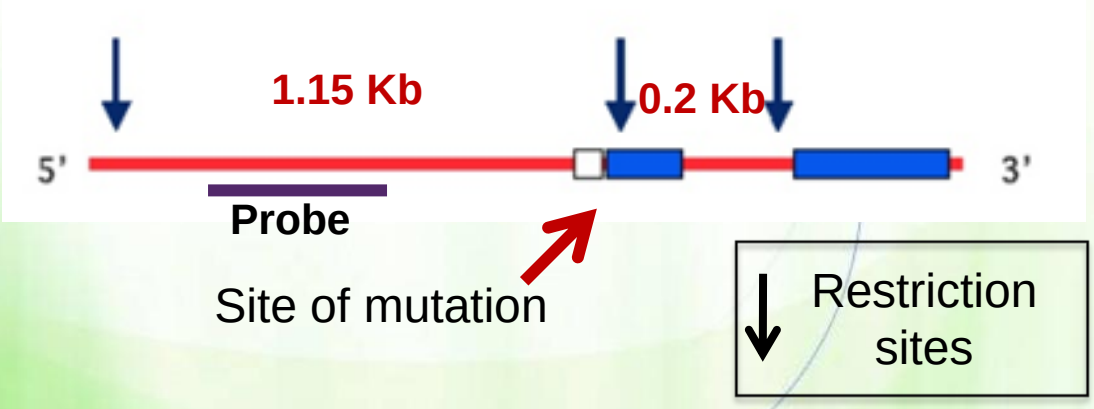
# RFLP in the clinic

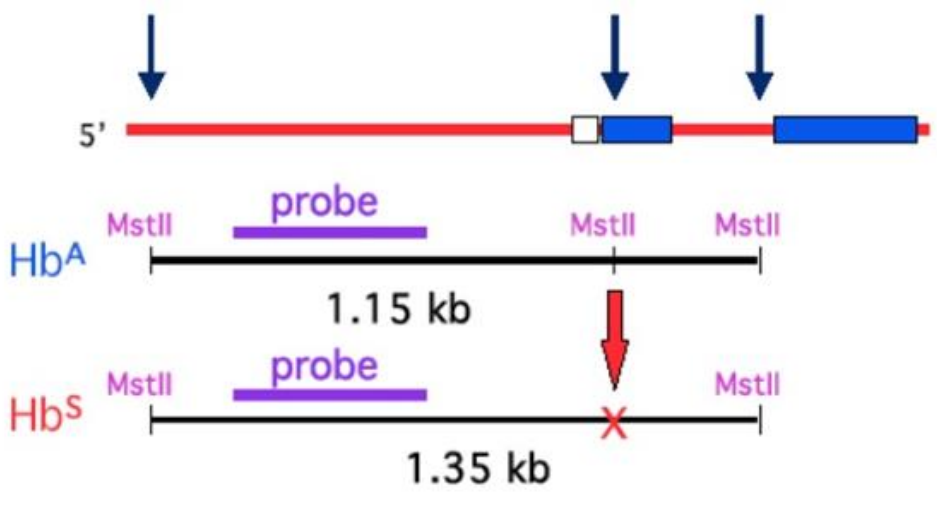
- RFLP can be used as diagnostic tools.

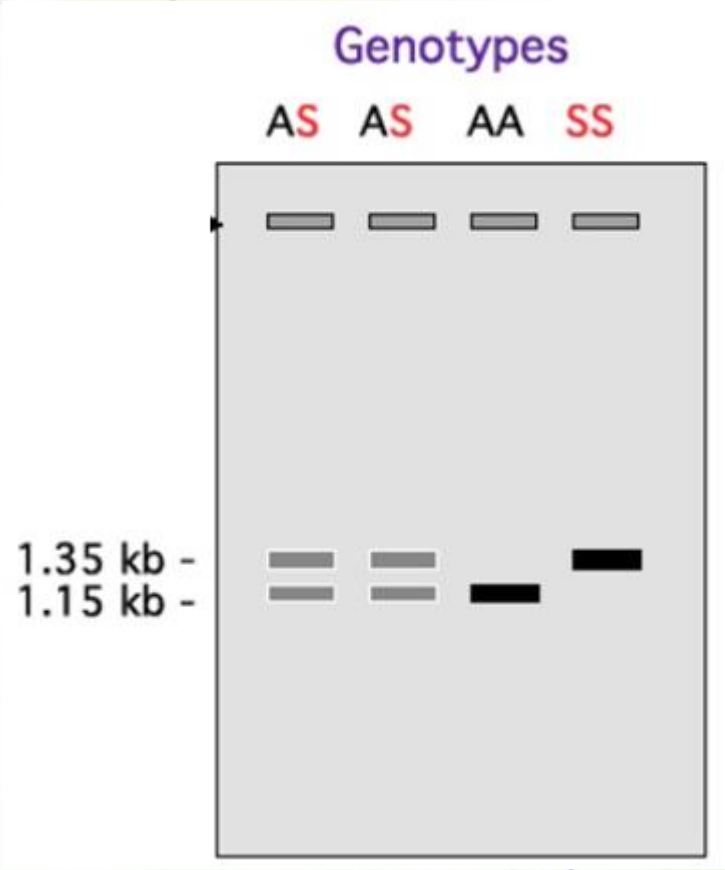
- For example, if a mutation that results in the development of a disease also causes the generation of distinctive RFLP fragments, then we can tell:
  - if the person is diseased as a result of this mutation
  - from which parent this allele is inherited

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

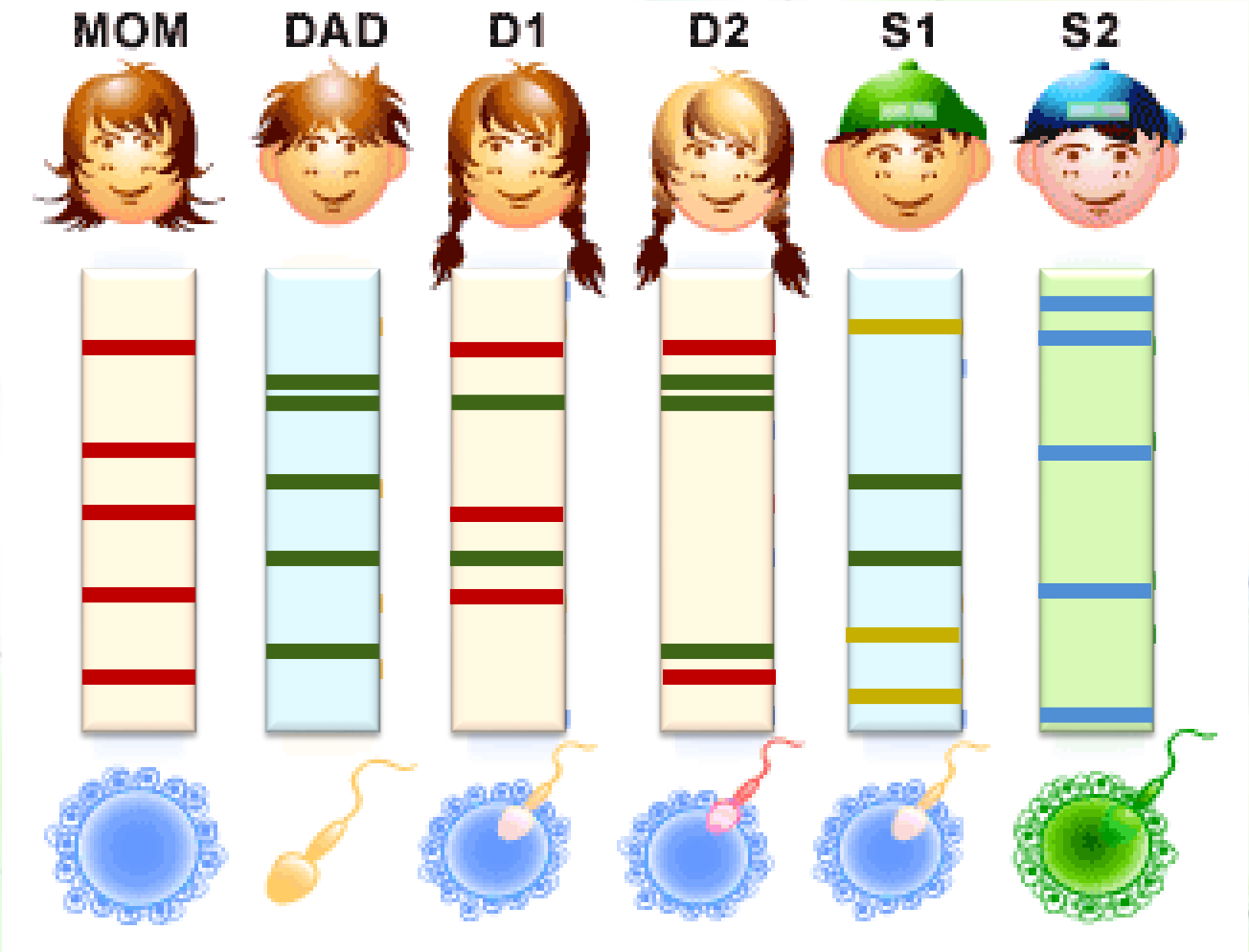
- Sickle cell anemia is caused by a mutation in one nucleotide (base) in the globin gene that is responsible for making hemoglobin.
- The position of this nucleotide happens to be within a restriction site.
- Individuals can be have
  - Homozygous with two normal alleles (designated as A)
  - Heterozygous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

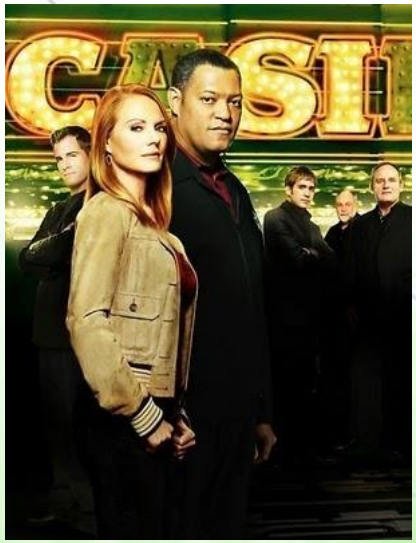

5'
probe
MstII
MstII
MstII
HbA
1.15 kb
probe
MstII
MstII
HbS
1.35 kb


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

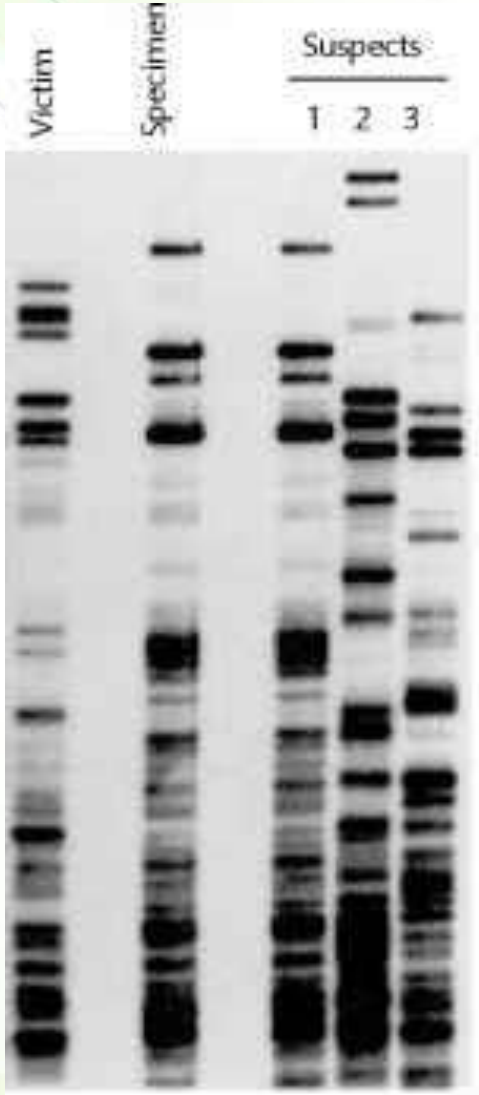
Membrane is washed free of excess probe

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