Molecular Biology (1)

Structure of nucleic acids

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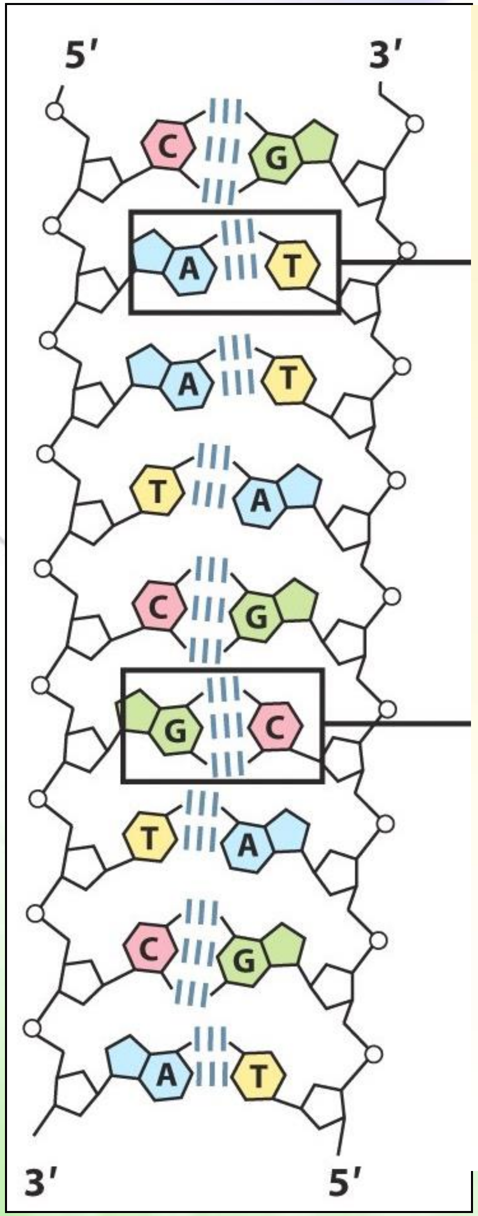
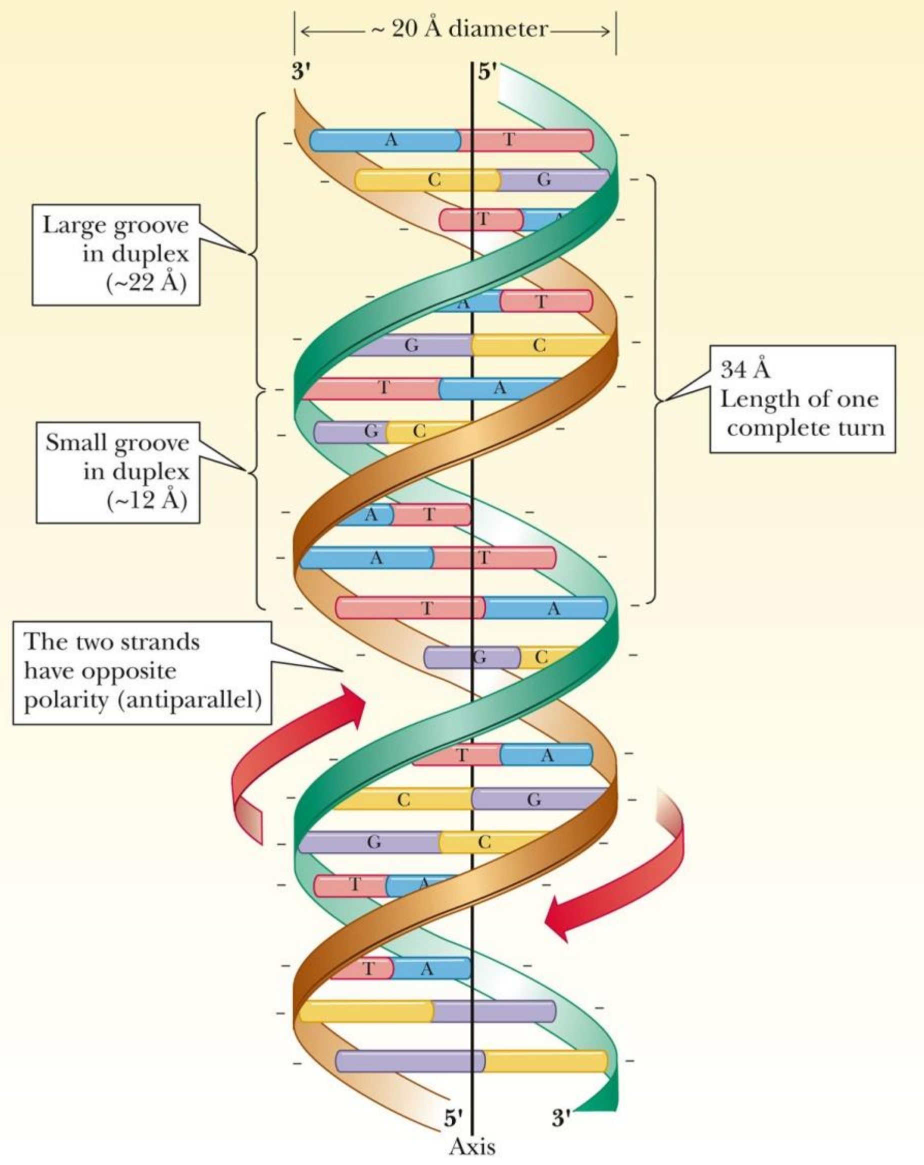
Second year, Second semester, 2024-2025

DNA structure





- The monomer
- A double helix
- Specific base-pairing
  - A = T; G = C; Pur = pyr
- Complementary
- Backbone vs. side chains
- Antiparallel
- Stability vs. flexibility
- Groovings

Writing the sequence of nucleic acids

DNA

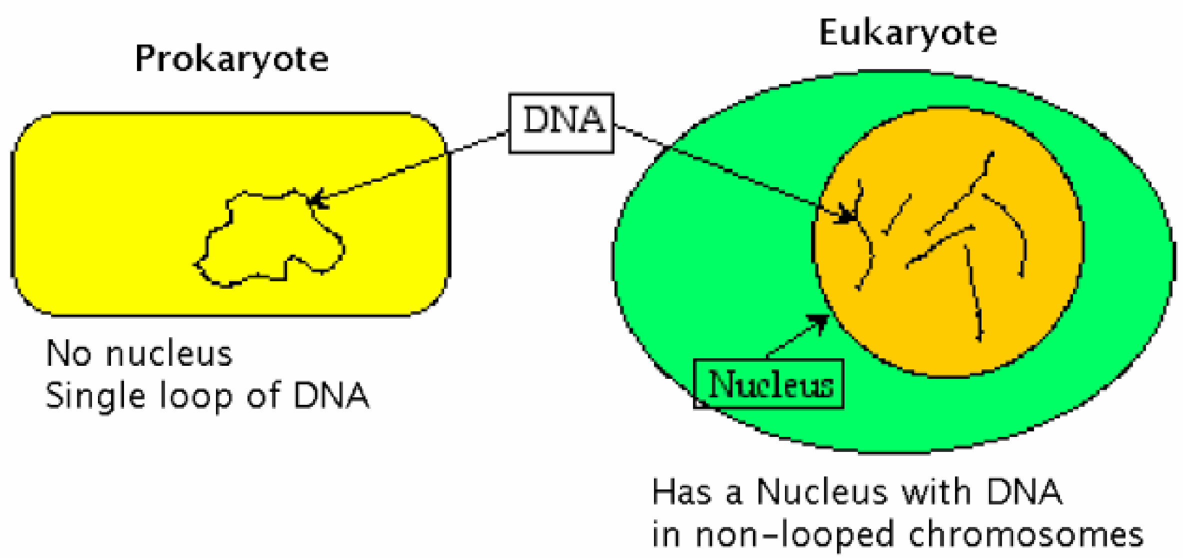
5’ ...A T G G C C T G G A C T T C A... 3’
3’ ...T A C C G G A C C T G A A G T... 5’

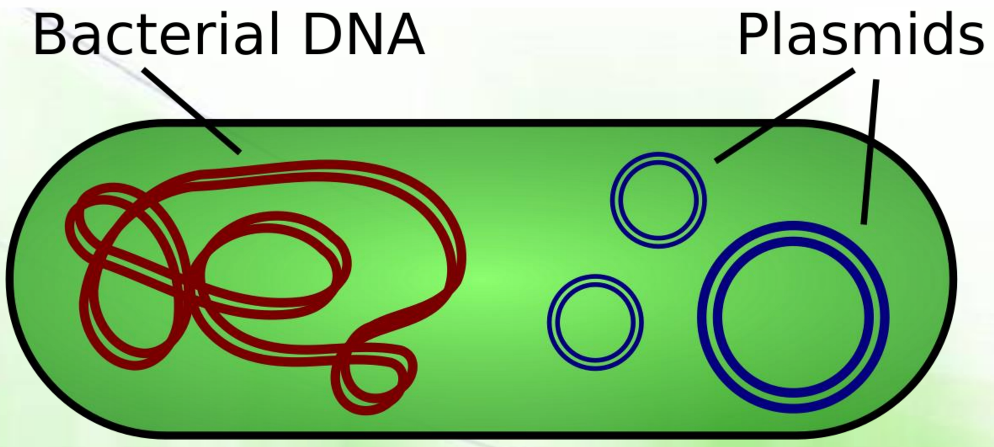
OR A T G G C C T G G A C T T C A.

RNA

5’ ...A U G G C C U G G A C U U C A... 3’

The genome of prokaryotes versus eukaryotes

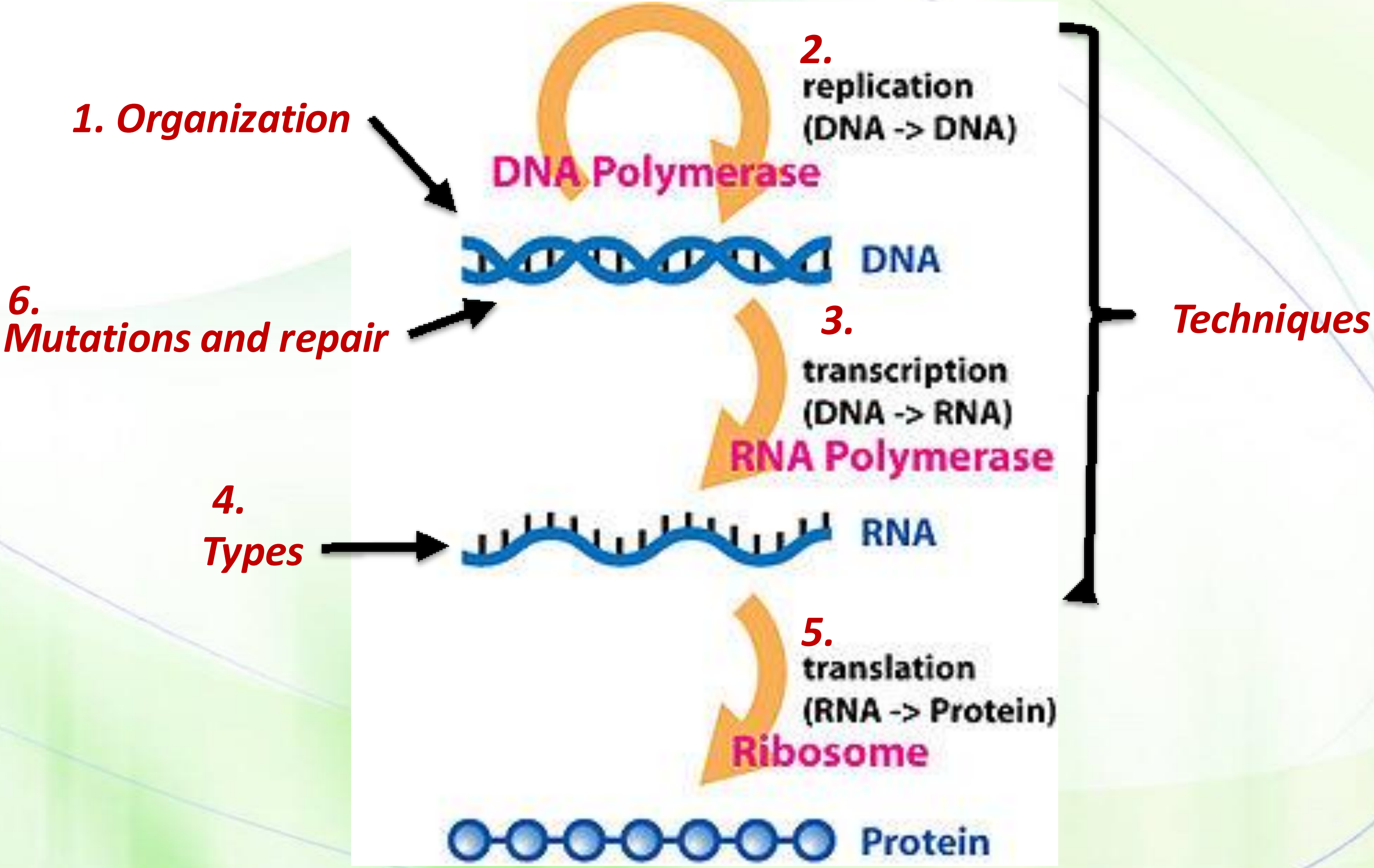




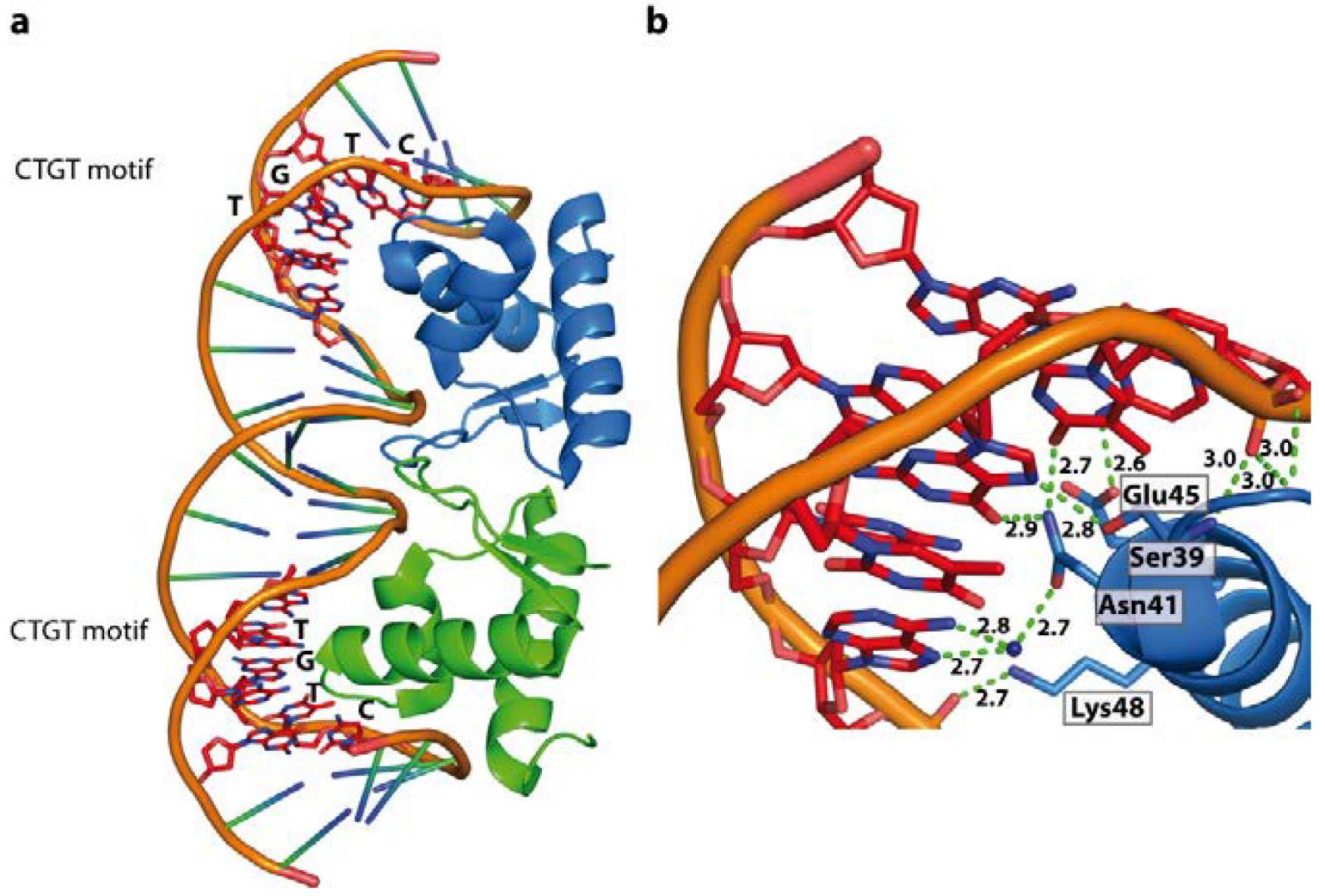
- Genome: the total genetic material of a living being (bacteria vs. human), a species (monkey vs. human) , an individual (me vs. you), or a cell (brain vs. liver), etc.
- Prokaryote: circular genome + plasmid
- Eukaryote: a linear, nuclear genome (chromosomes) + mitochondrial genome

What is molecular biology?

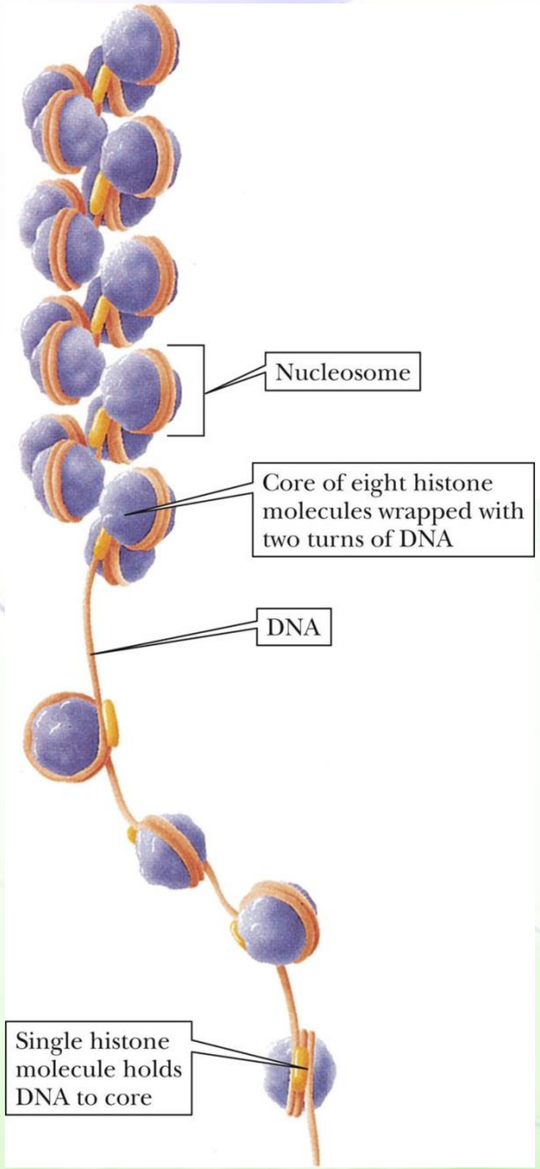
Central dogma of molecular biology



DNA-protein interaction

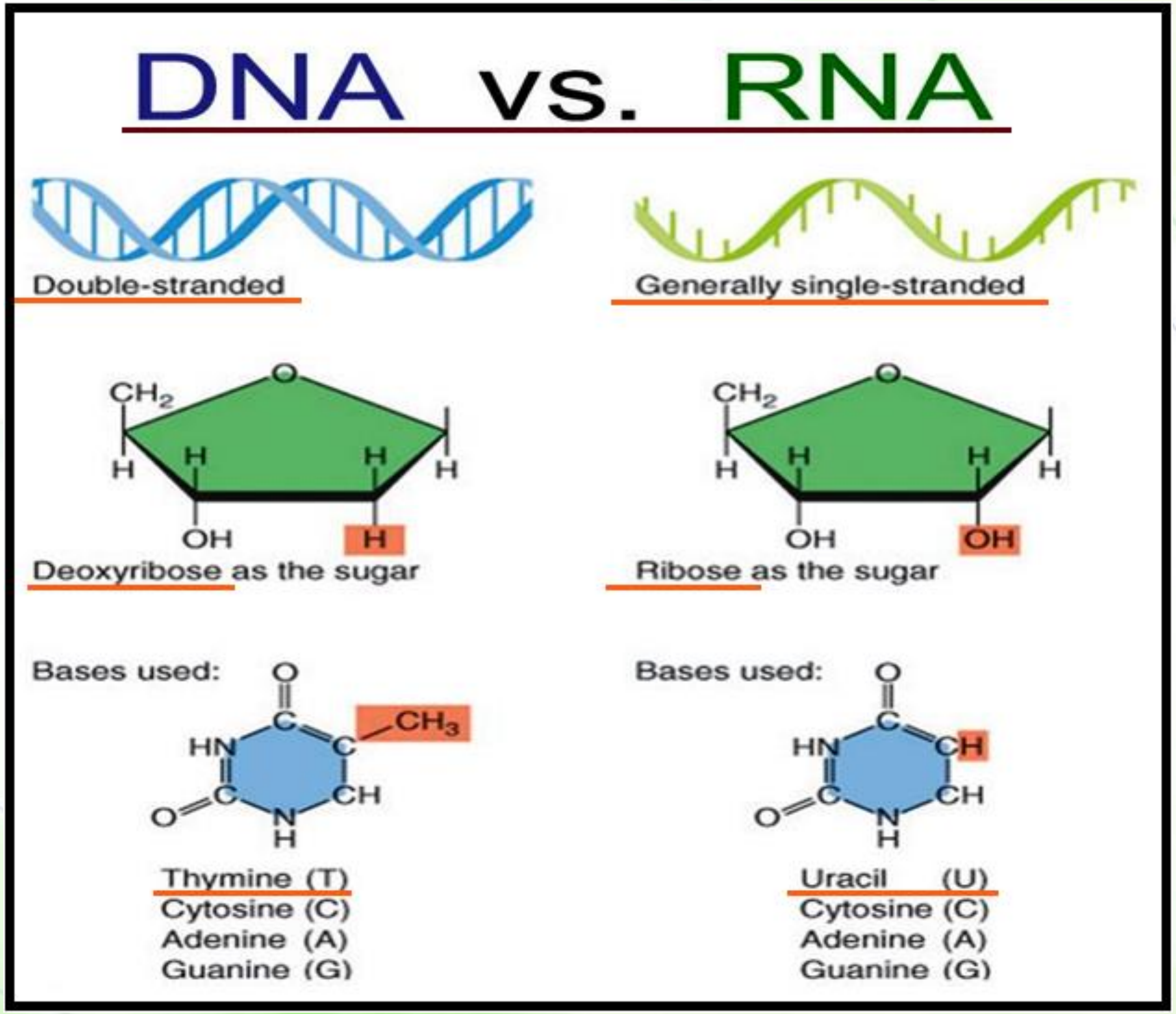


In eukaryotes...



- In eukaryotes, DNA is coiled to package the large, linear DNA.
- Eukaryotic DNA is complexed with a number of proteins, principally histones, which package DNA.
- Chromatin = DNA molecule + proteins.
- The basic structural unit of chromatin is known as a nucleosome.

In prokaryotes and eukaryotes (not viruses)



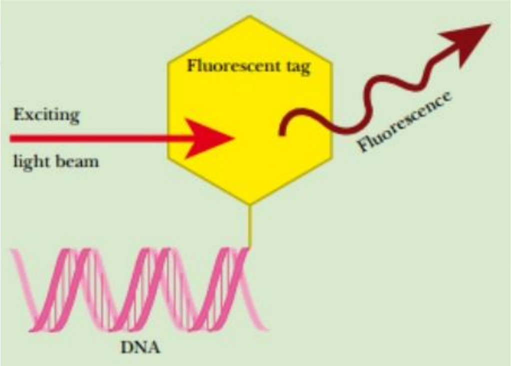
Types of RNA

RNA species	Established function(s)
mRNA	Messenger for protein production
tRNA	Translation of RNA codon to amino acid
rRNA	Enzymatic and structural part of ribosomes
snRNA	Pre-mRNA processing
snoRNA	Modification of rRNA
miRNA	Repression of translation
piRNA	Silencing of transposons
lncRNA	Regulation of transcription, pre-mRNA processing, miRNA abundance and protein function

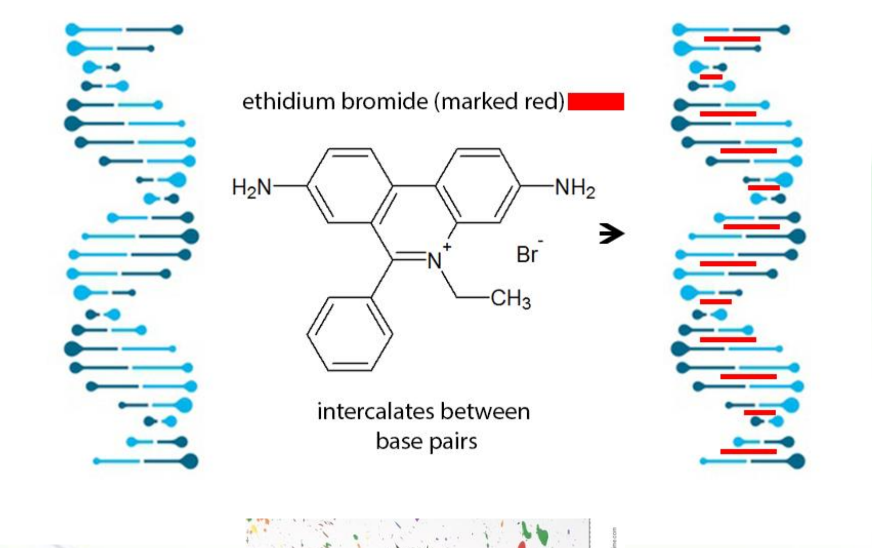
Techniques

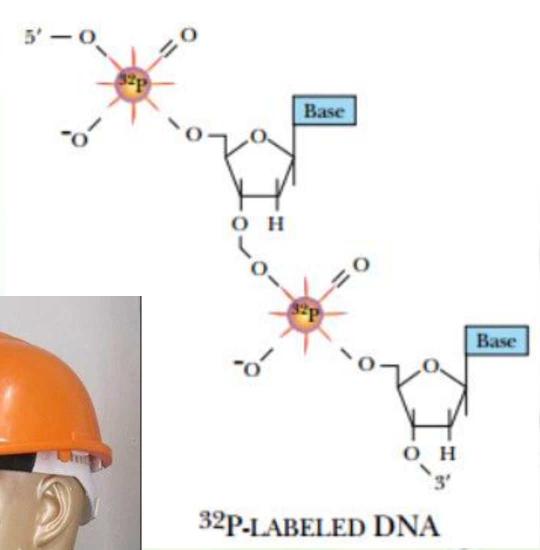
DNA labeling versus staining

DNA Labeling (more sensitive)

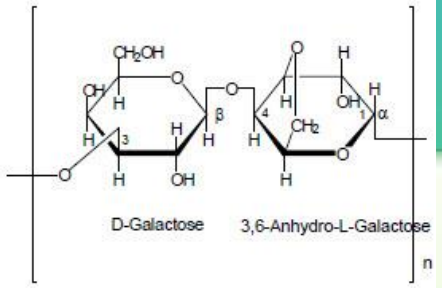


DNA staining

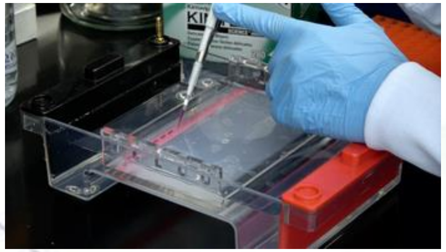


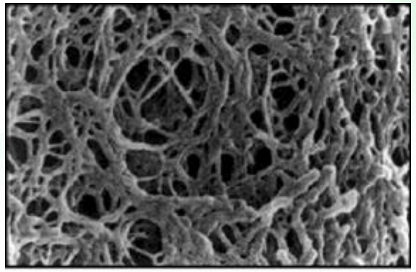


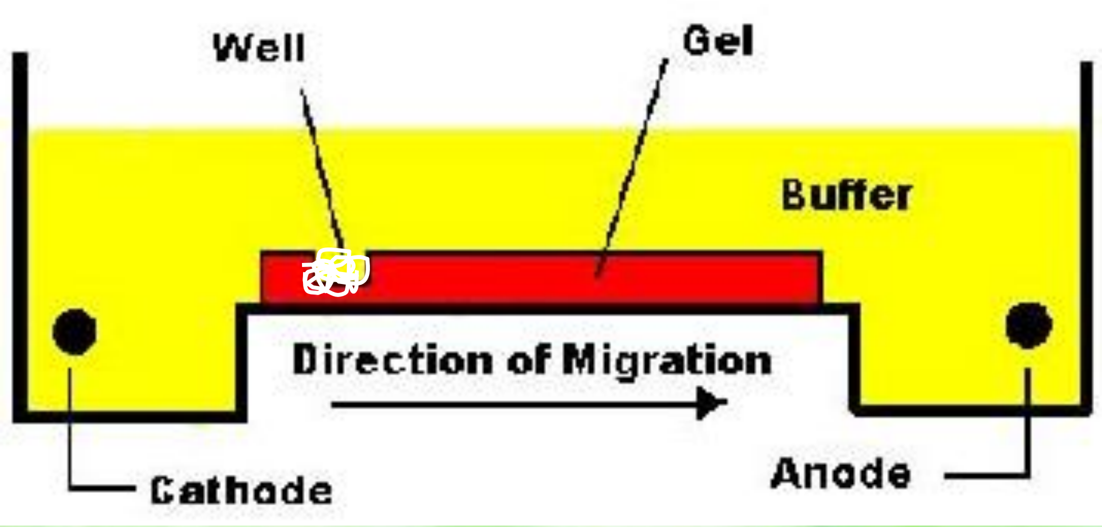
Gel electrophoresis

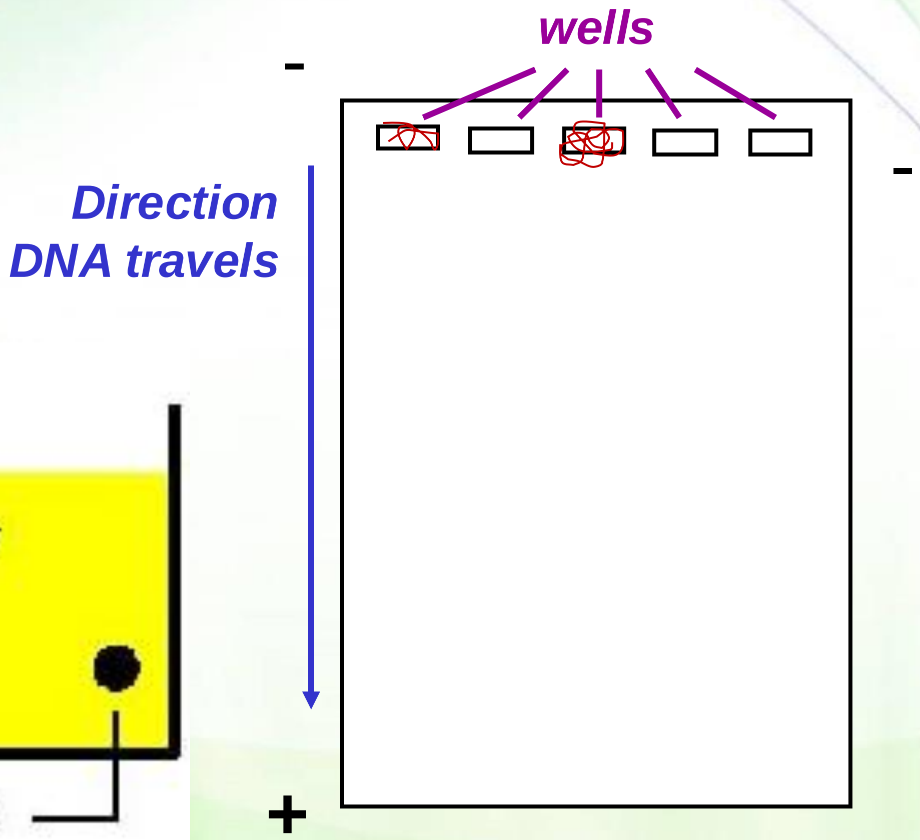


- The length and purity of DNA molecules can be accurately determined by the gel electrophoresis.



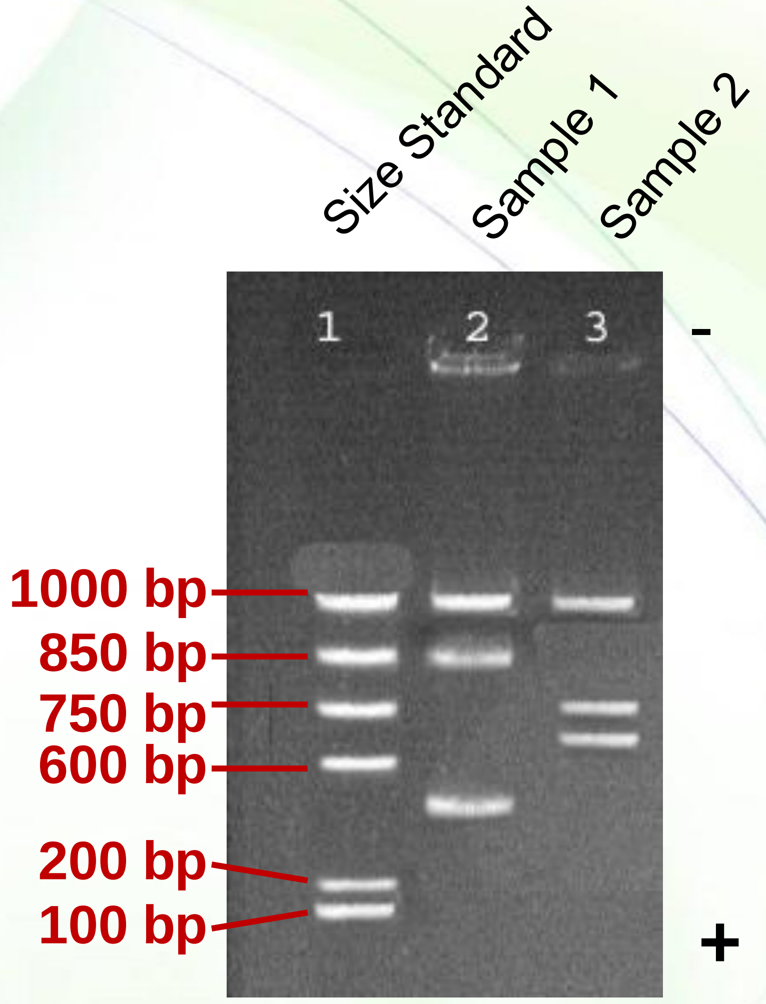






Detection

- 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 32P).
- It is common that a DNA standard is used to determine the length of the examined DNA molecule.



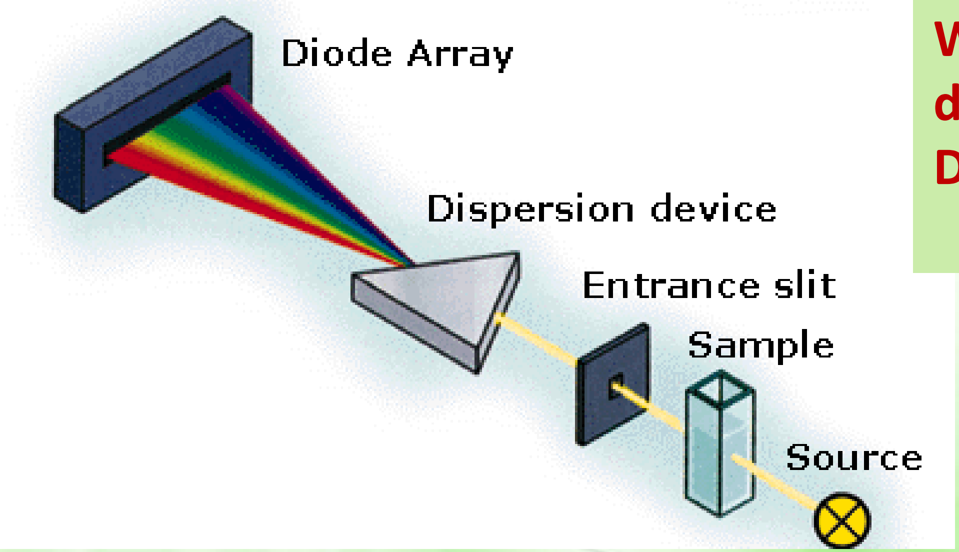
bp: base pair

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

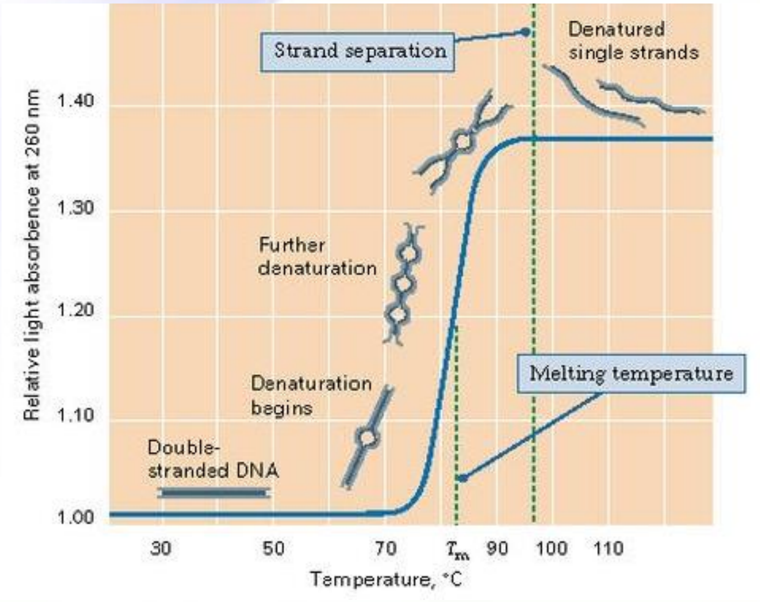


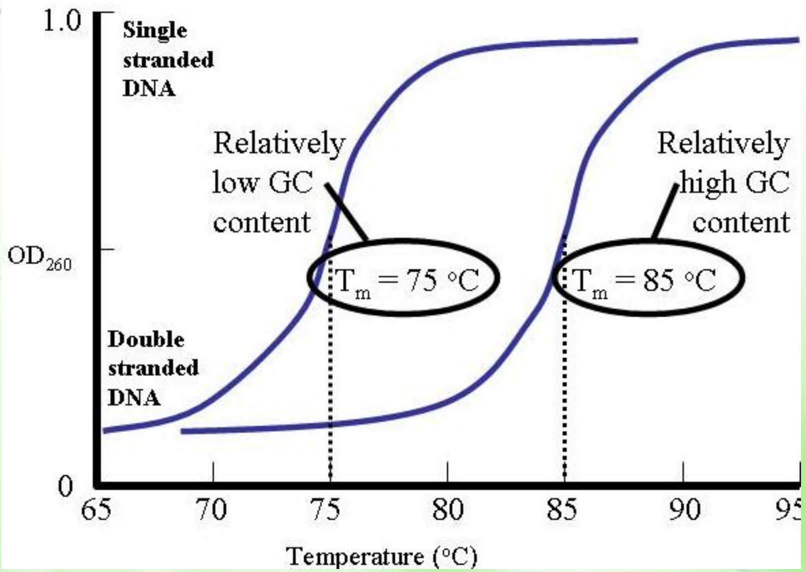
What is the concentration of a double stranded DNA sample diluted at 1:10 and the A260 is 0.1?

DNA concentration = 0.1 x 10 x 50 µg/ml

= 50 µg /ml

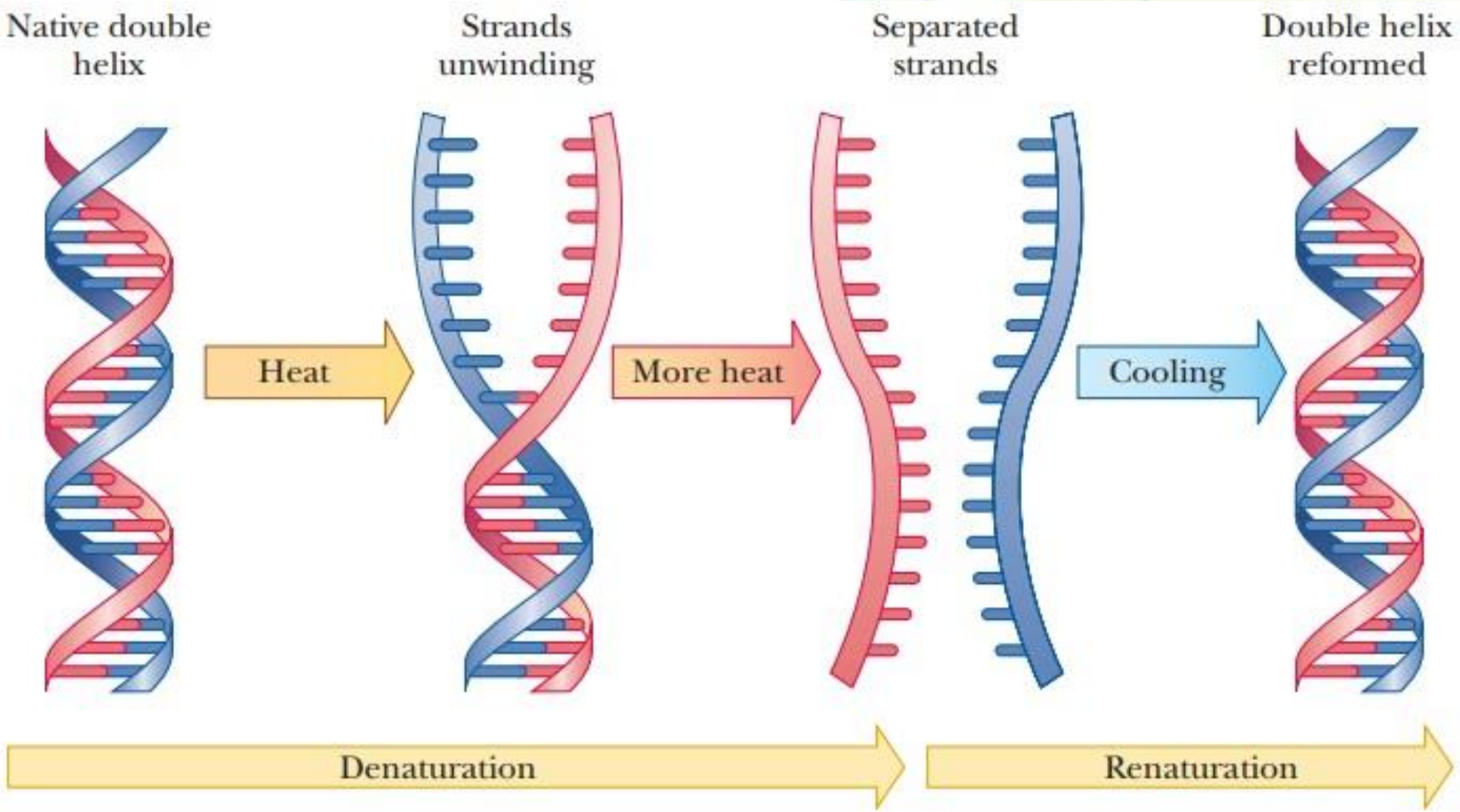
Observation of denaturation





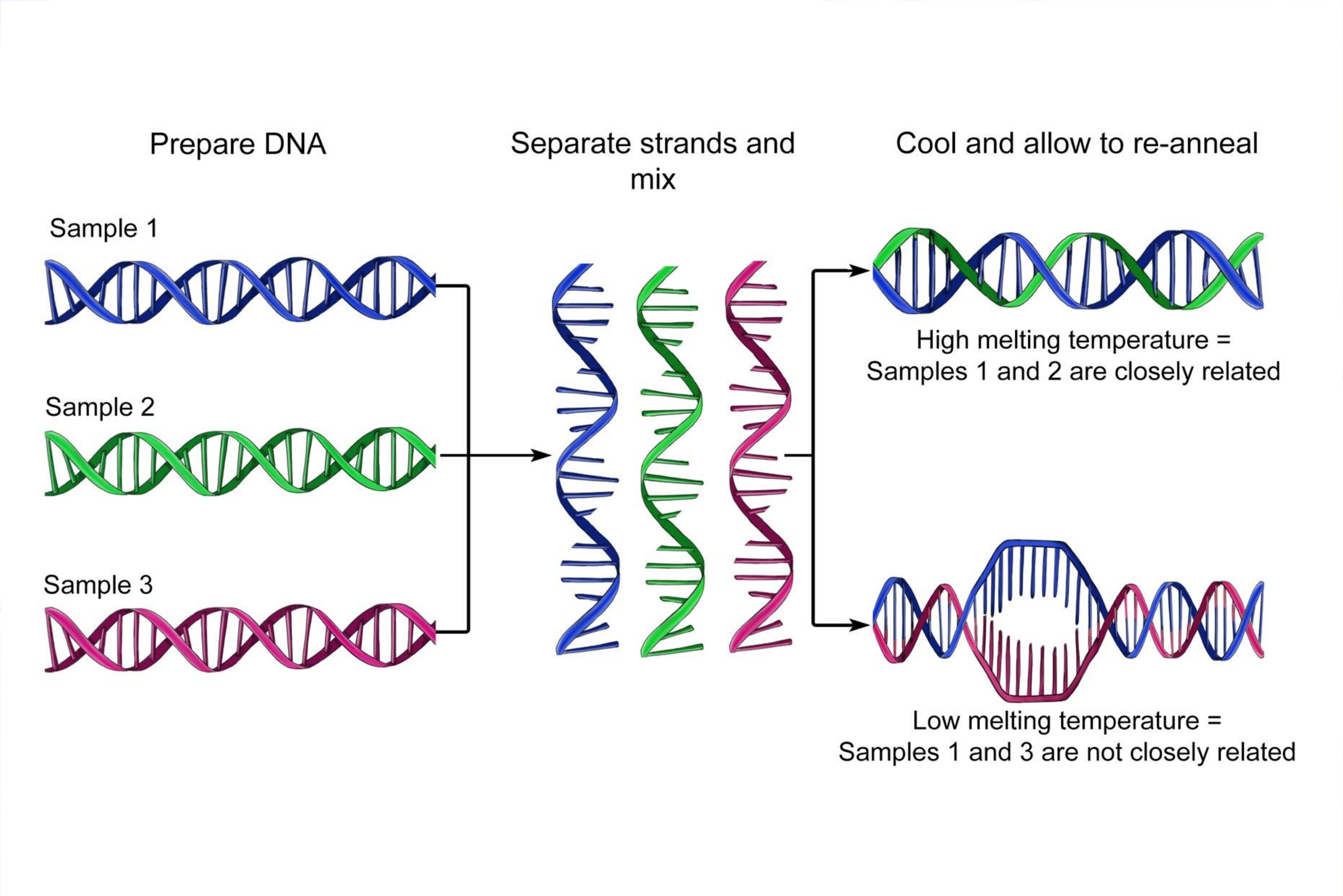
- The transition temperature or melting temperature (Tm).
- Factors influencing Tm
  - Length
  - G·C pairs
    - Hydrogen bonds
  - pH
  - Salts and ions
  - Destabilizing agents (alkaline solutions, formamide, urea)

Denaturation versus renaturation



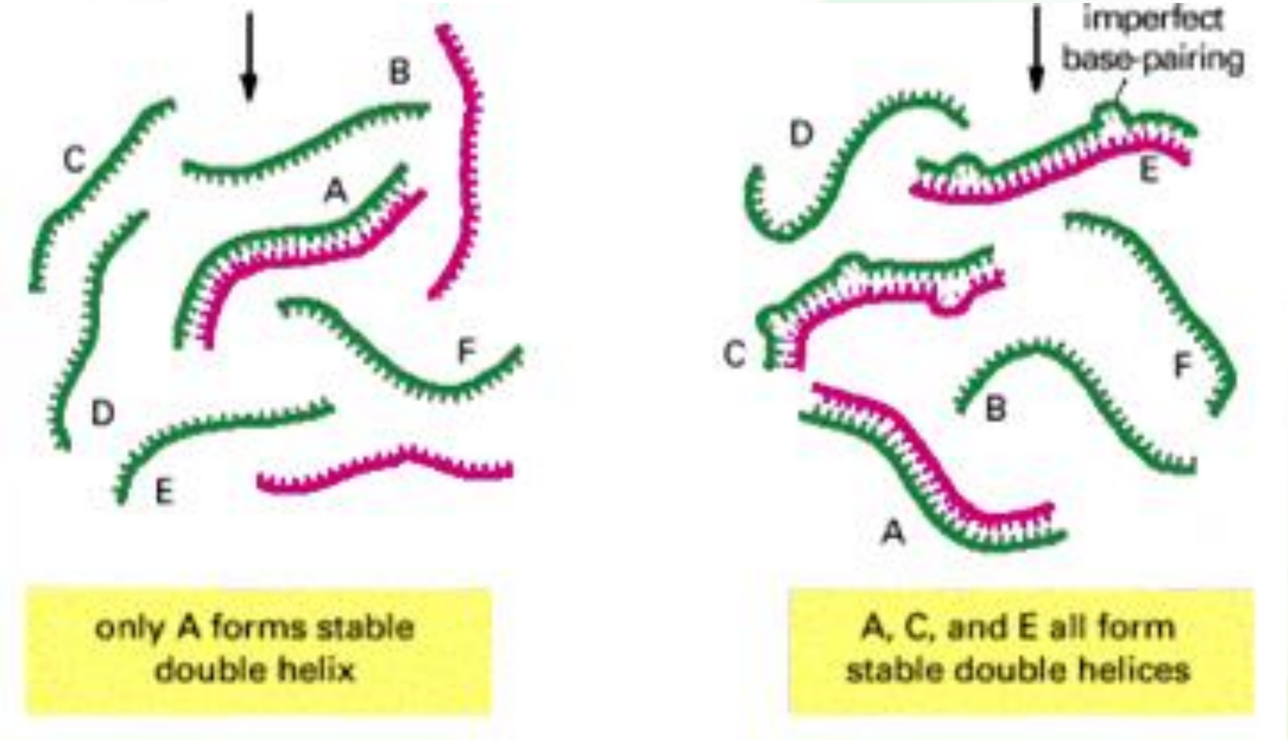
■ FIGURE 9.19 **Helix unwinding in DNA denaturation.** The double helix unwinds when DNA is denatured, with eventual separation of the strands. The double helix is re-formed on renaturation with slow cooling and annealing.

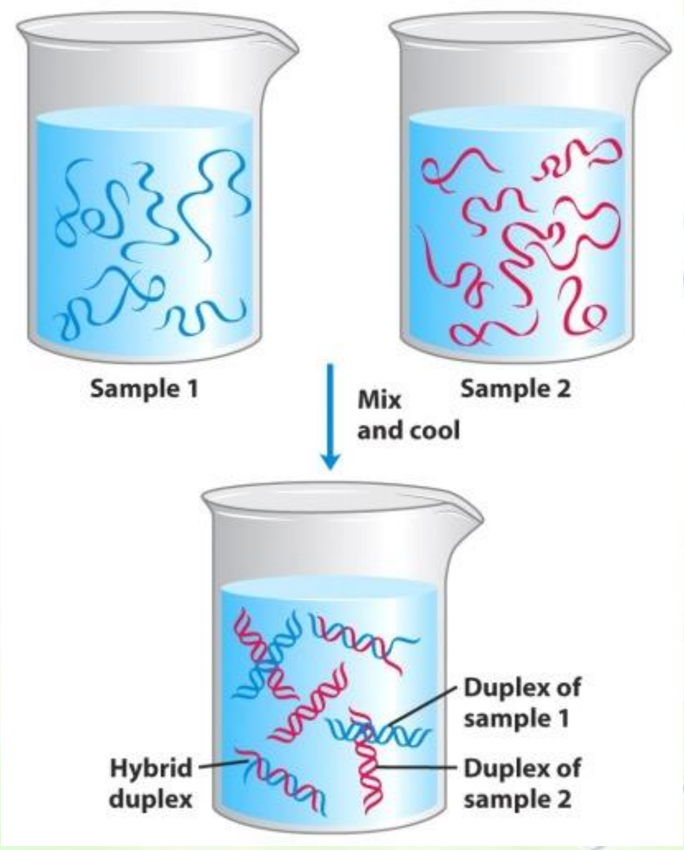
Denaturation versus hybridization



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).





Hybridization can be non-specific

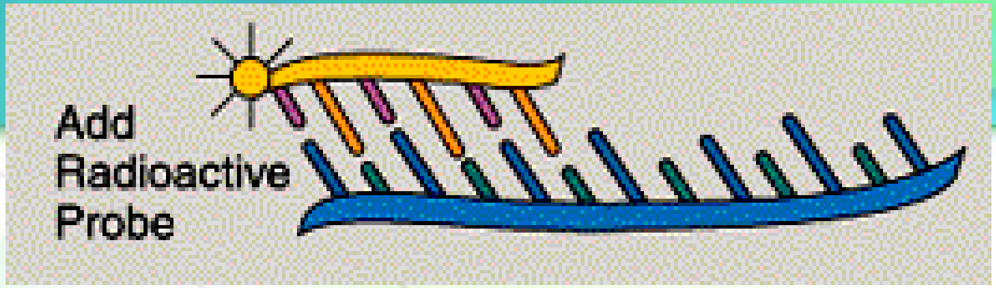
Hybridization can be controlled by changing the temperature, ionic strength of solutions, GC content, etc.

Hybridization techniques

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

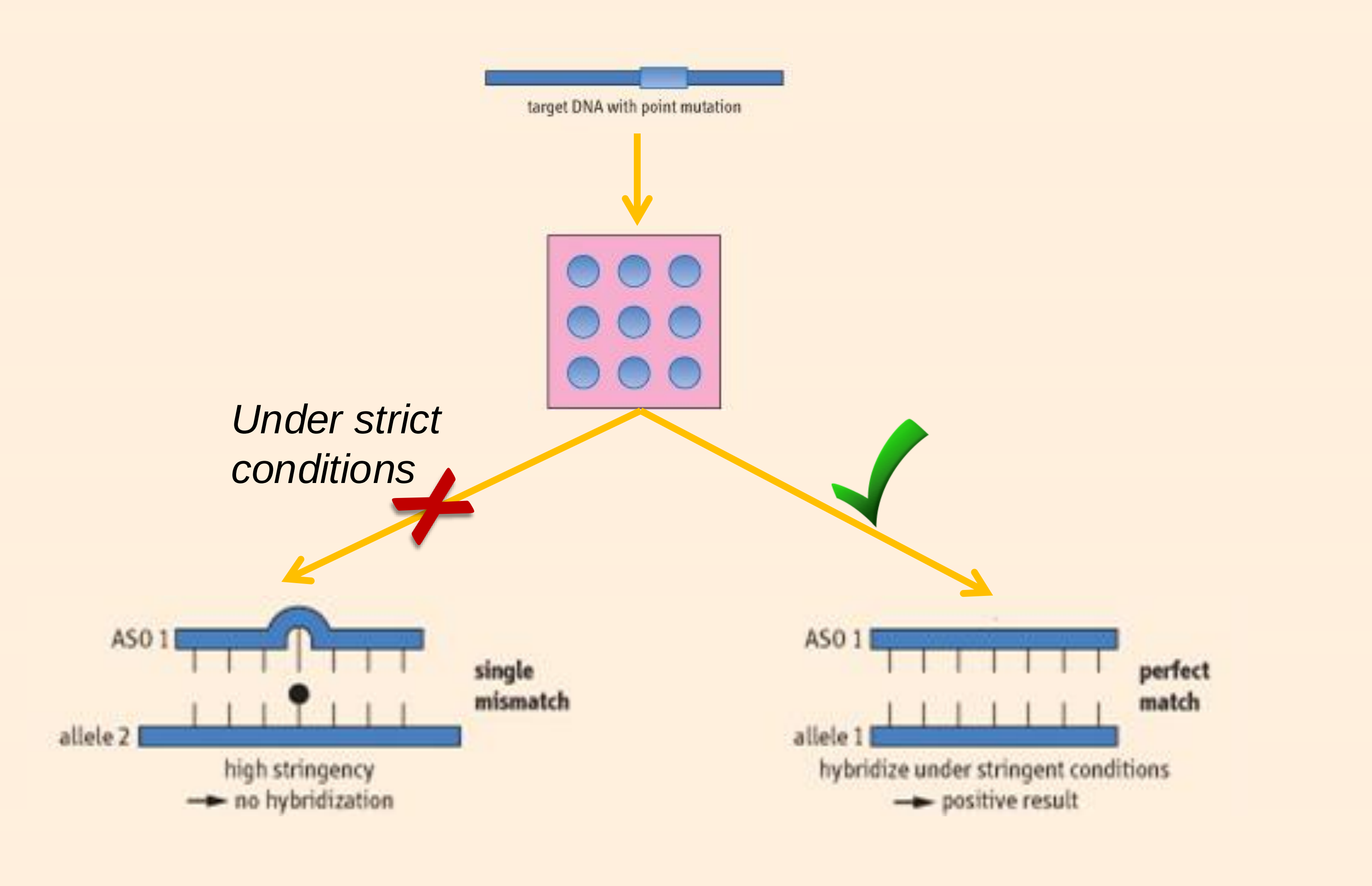
- Hybridization reactions are used to detect and characterize specific nucleotide sequences

Probes (Oligonucleotides)

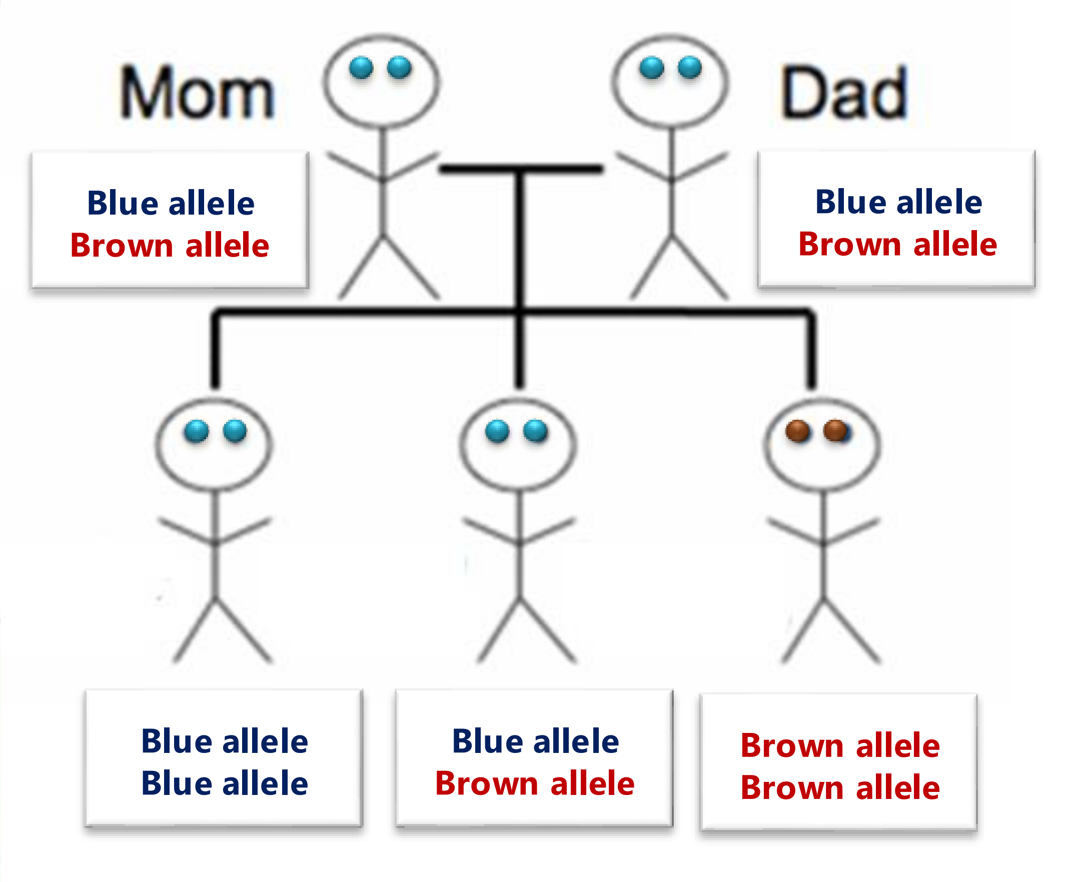


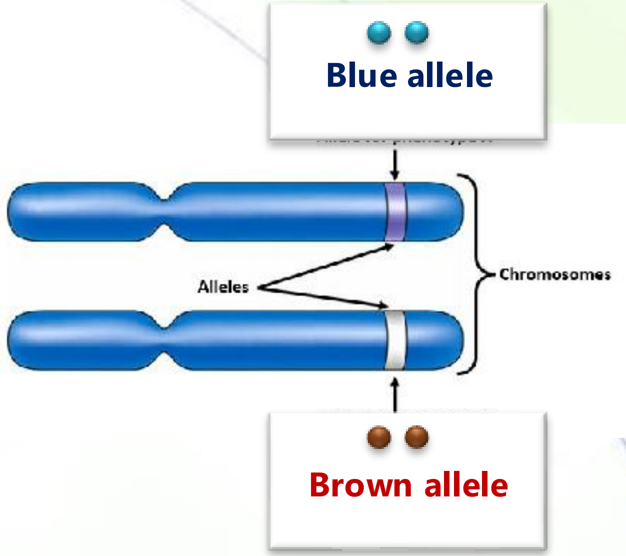
- A probes is a short sequence of single stranded DNA (an oligonucleotide) that is complementary to a small part of a larger DNA sequence.

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


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

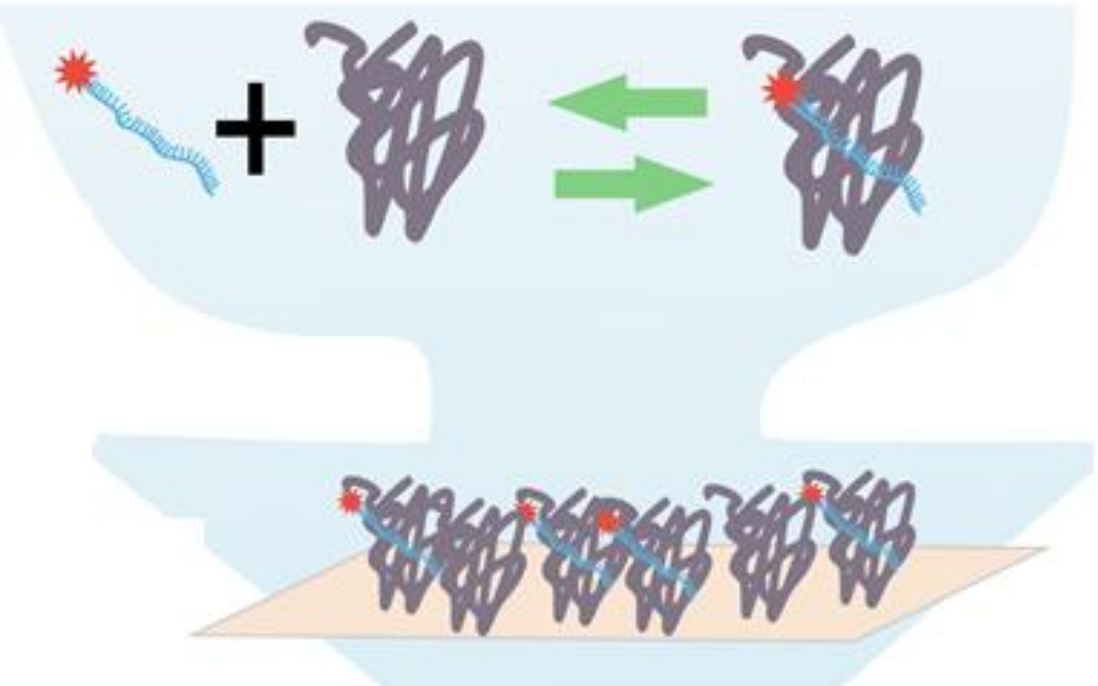
Concepts to know…

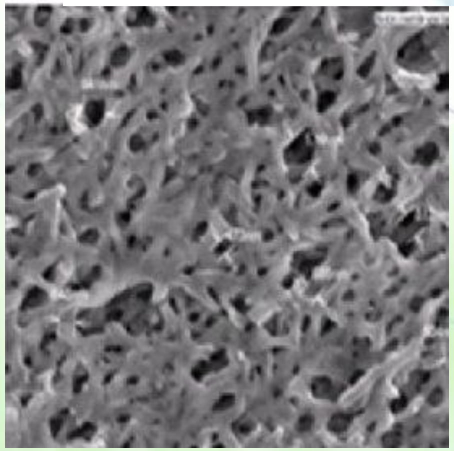




Pedigree
Allele
  Dominant vs. recessive
Homozygous vs. heterozygous

Dot blot



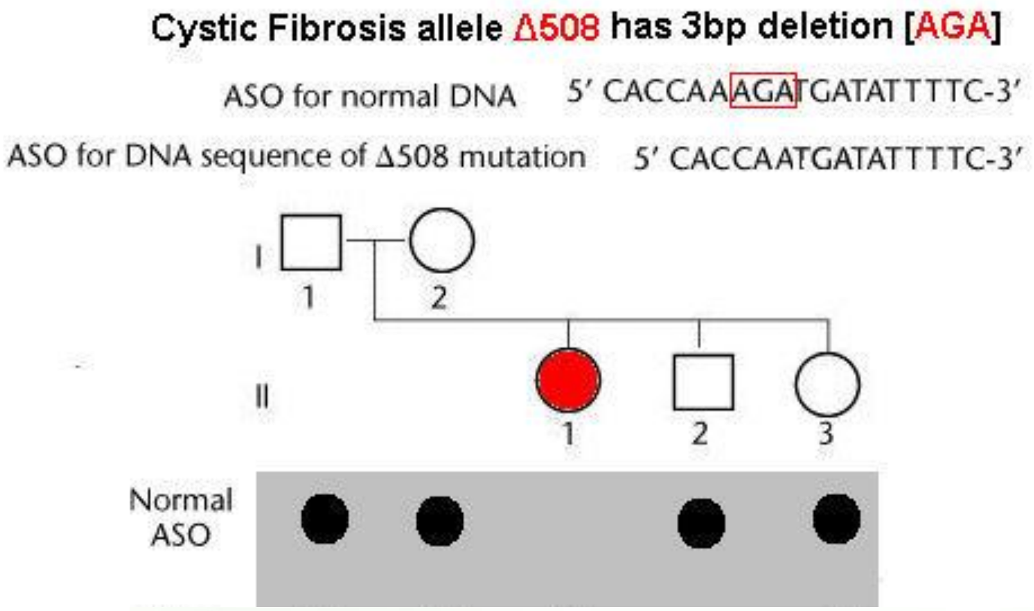


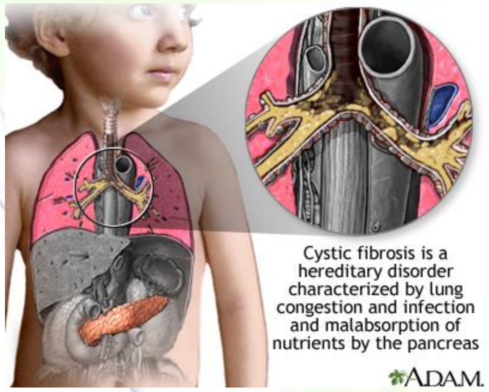
- This is a technique that informs us if a specific sequence that is complementary to a probe of a known sequence exists in a larger DNA.
- DNA is bound to a solid support and a labeled probe is added. If binding occurs, the sequence exists.

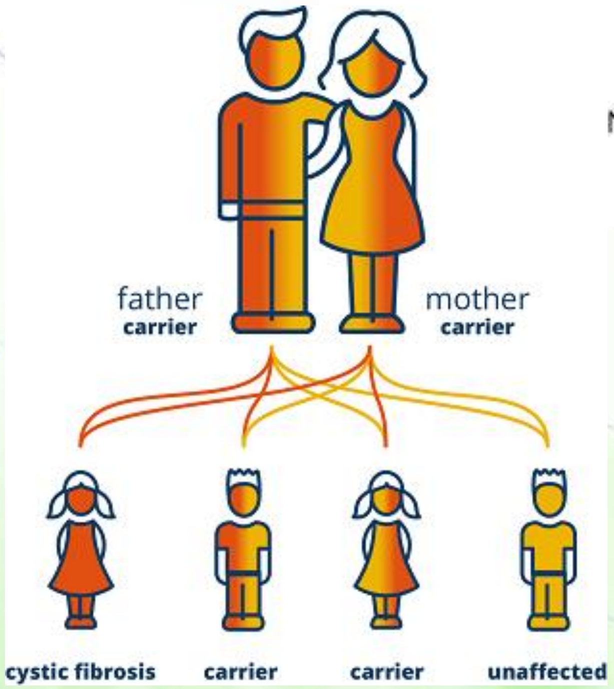
Disease detection by ASO (Cystic fibrosis)

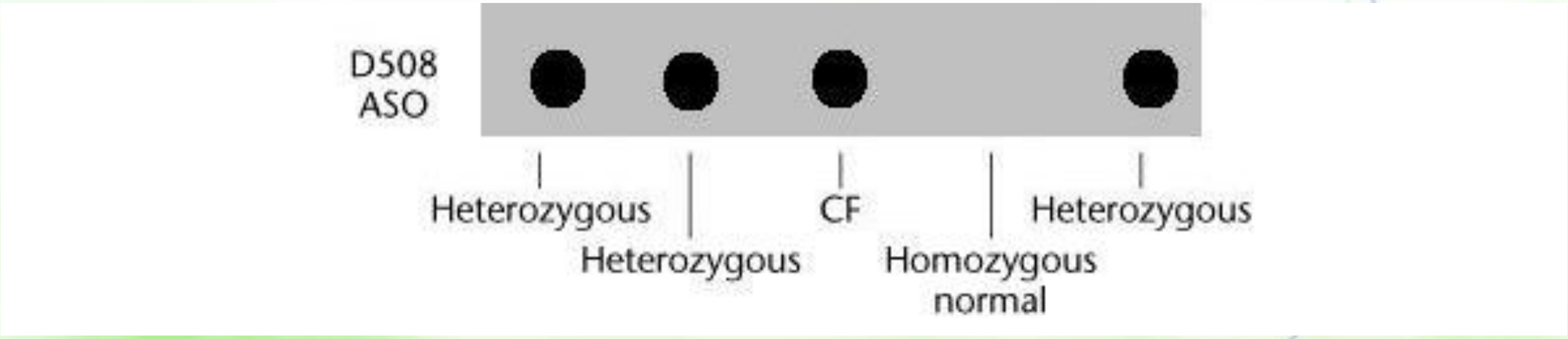
ASO: Allele-specific oligonucleotide

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









Resources

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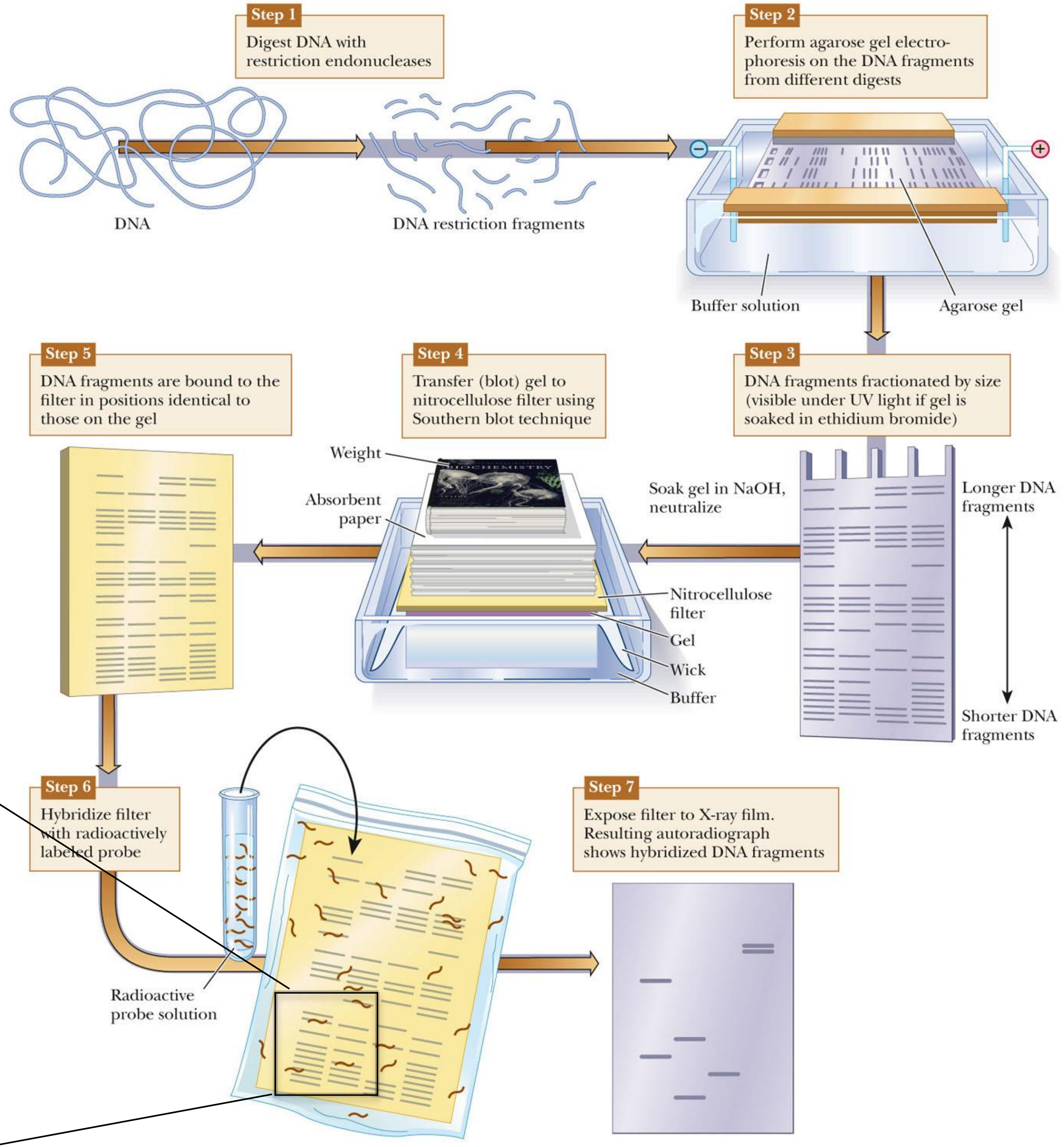
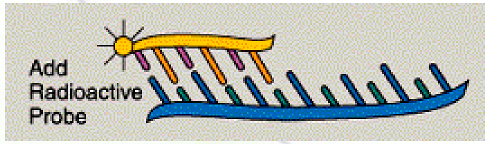
Southern blotting

- This technique is a combination of DNA gel electrophoresis and dot blotting

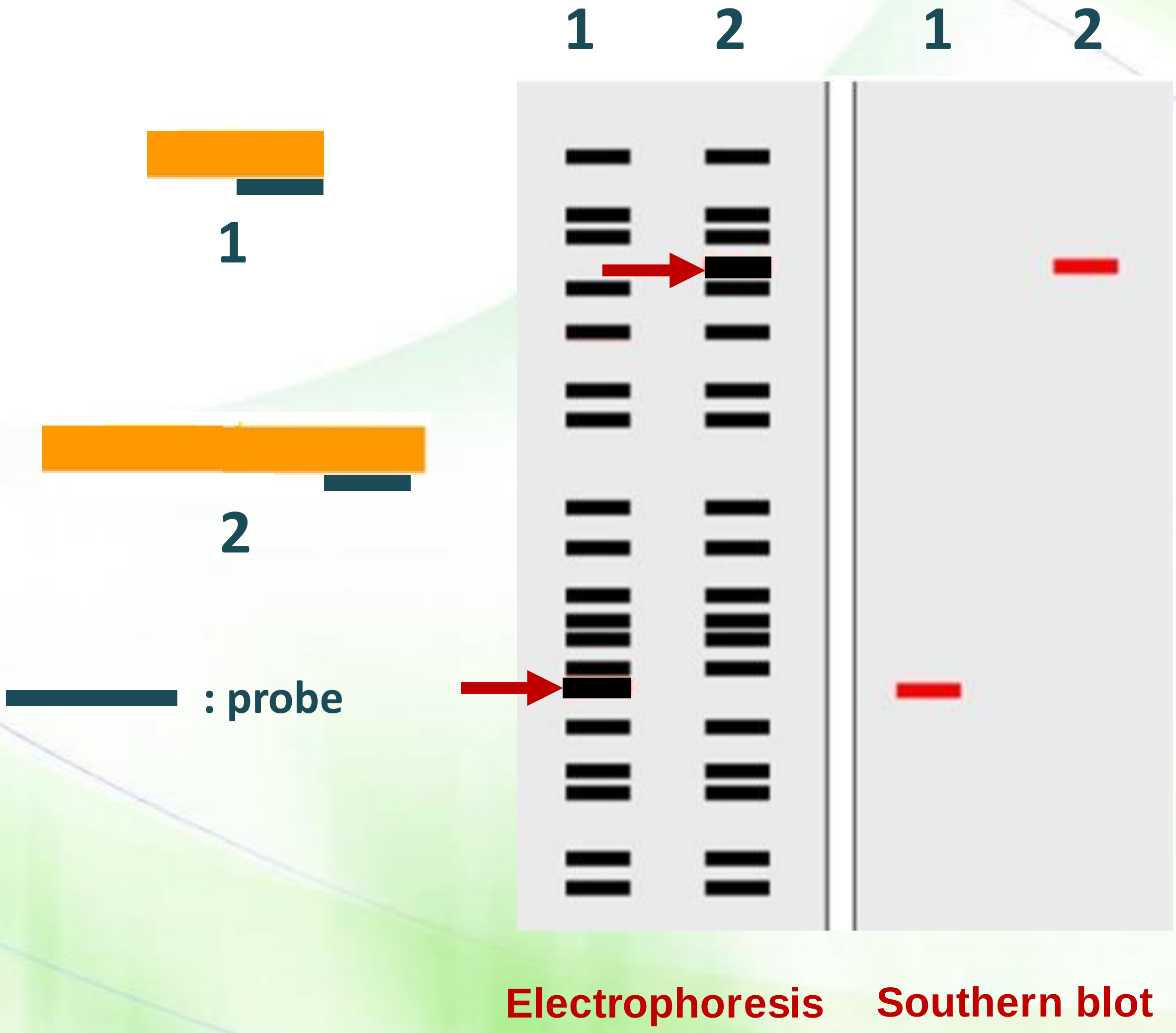
- Used to detect:
  - the presence of a DNA segment complementary to the probe
  - the size of the DNA fragment



Step 1
Digest DNA with restriction endonucleases
DNA
DNA restriction fragments
Step 2
Perform agarose gel electro-phoresis 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)
Soak gel in NaOH, neutralize
Longer DNA fragments
Shorter DNA fragments
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

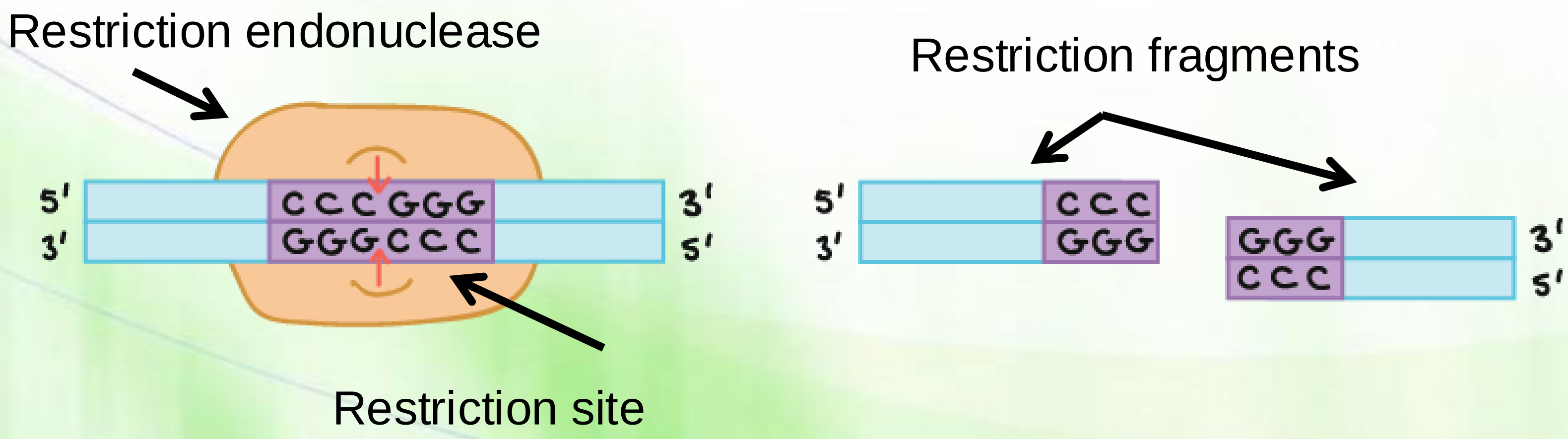


Add Radioactive Probe


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.



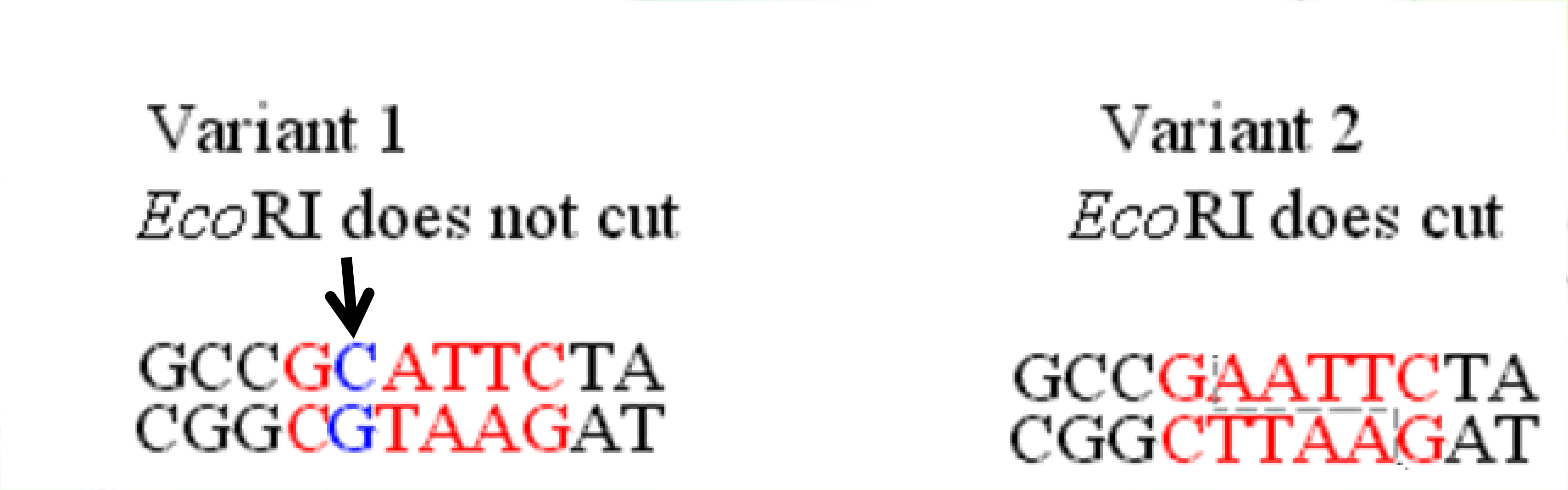
Palindromic sequences

- 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).

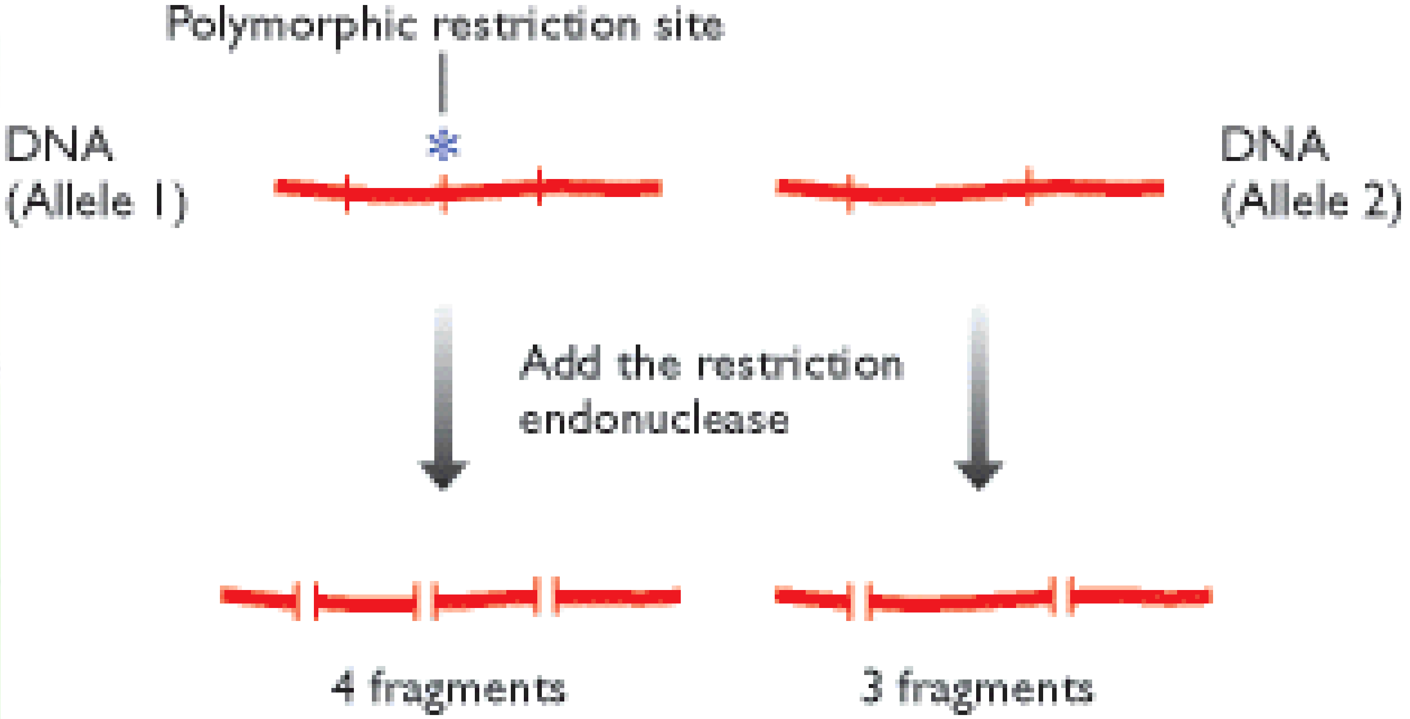


The DNA stays intact

The DNA is cut into two pieces

Cuts versus 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.

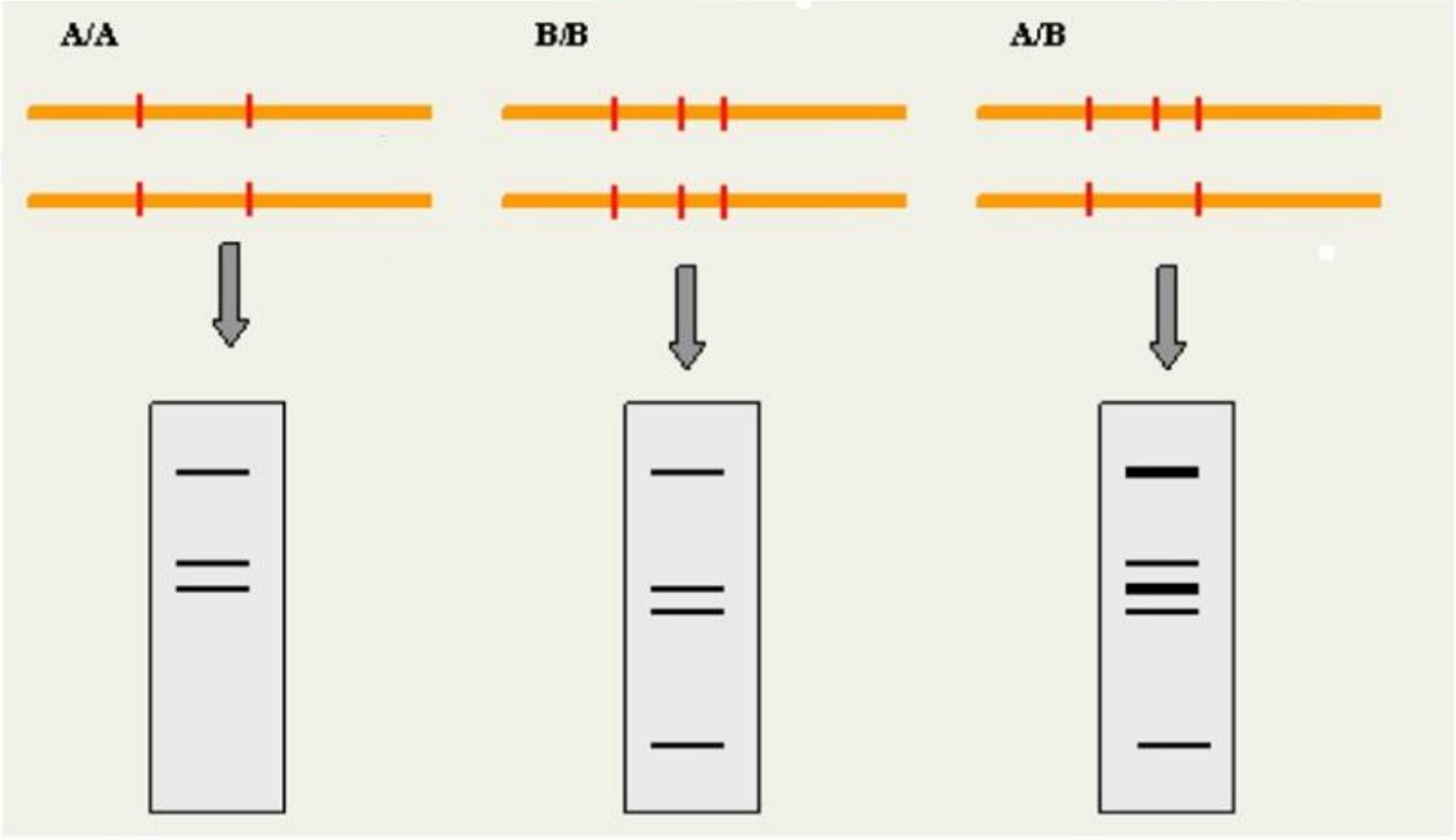
- Remember:
  - Our cells are diploid.
  - Alleles can be homozygous or heterozygous at any DNA location or sequence.

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.

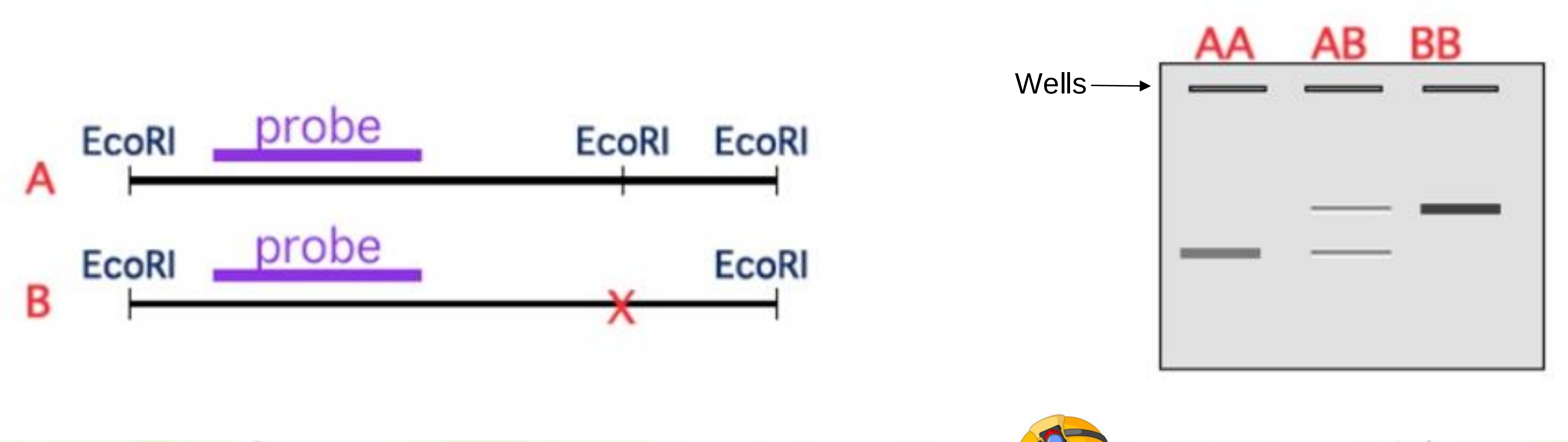
Gel electrophoresis only

Homozygous individual for A	Homozygous individual for B	Heterozygous (A/B)



Electrophoresis then blotting

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



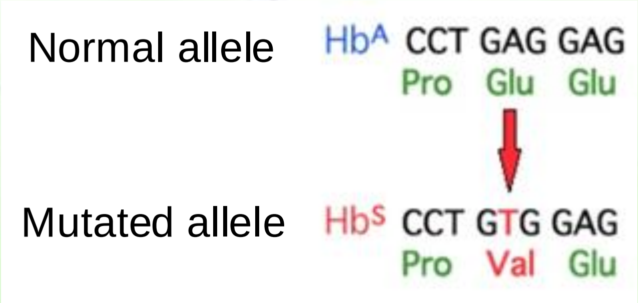
Note: the size of the detected DNA fragment reflects its size, not the size of the probe

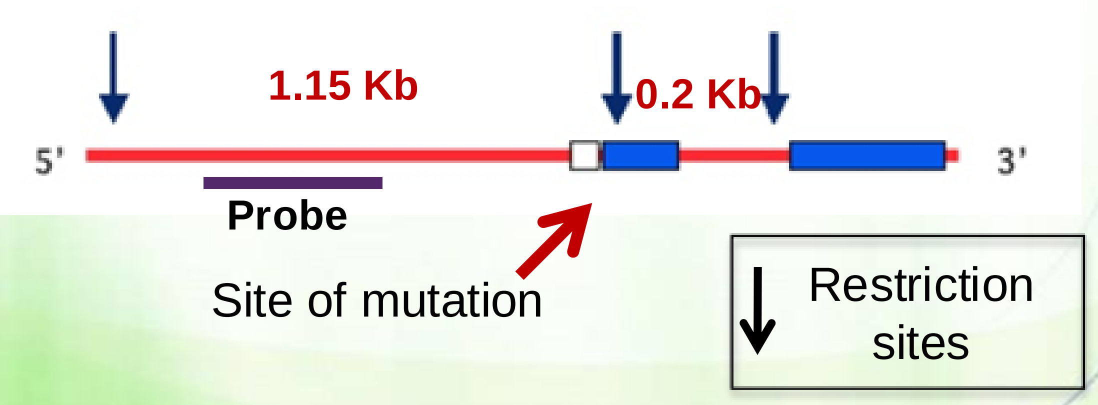
RFLP in the clinic

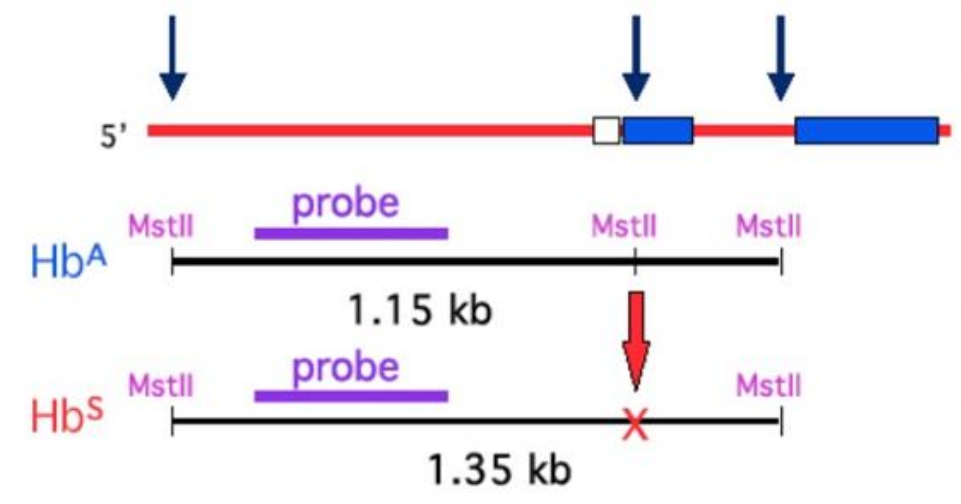
- RFLP can be used as diagnostic tools.
- For example, if a mutation that results in the development of a disease also causes the generation of distinctive RFLP fragments, then we can tell:
  - if the person is diseased as a result of this mutation
  - from which parent this allele is inherited

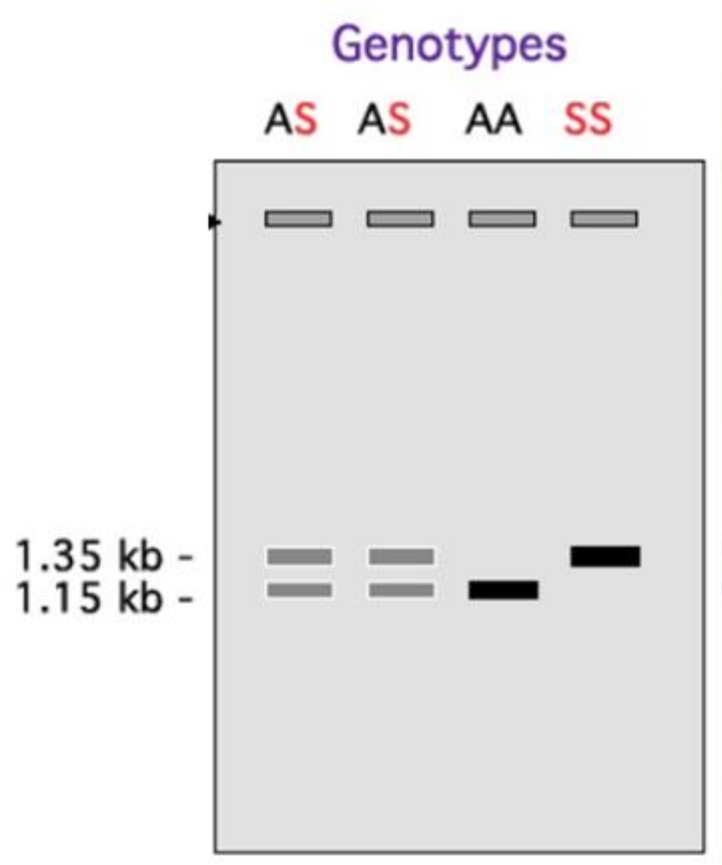
Example 1: Disease detection by RFLP (sickle cell anemia)

- 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:
  - Homozygous with two normal alleles (AA)
  - Heterozygous or carriers of one normal allele and one mutated allele (AS)
  - Homozygous for the mutated allele, or affected (SS)

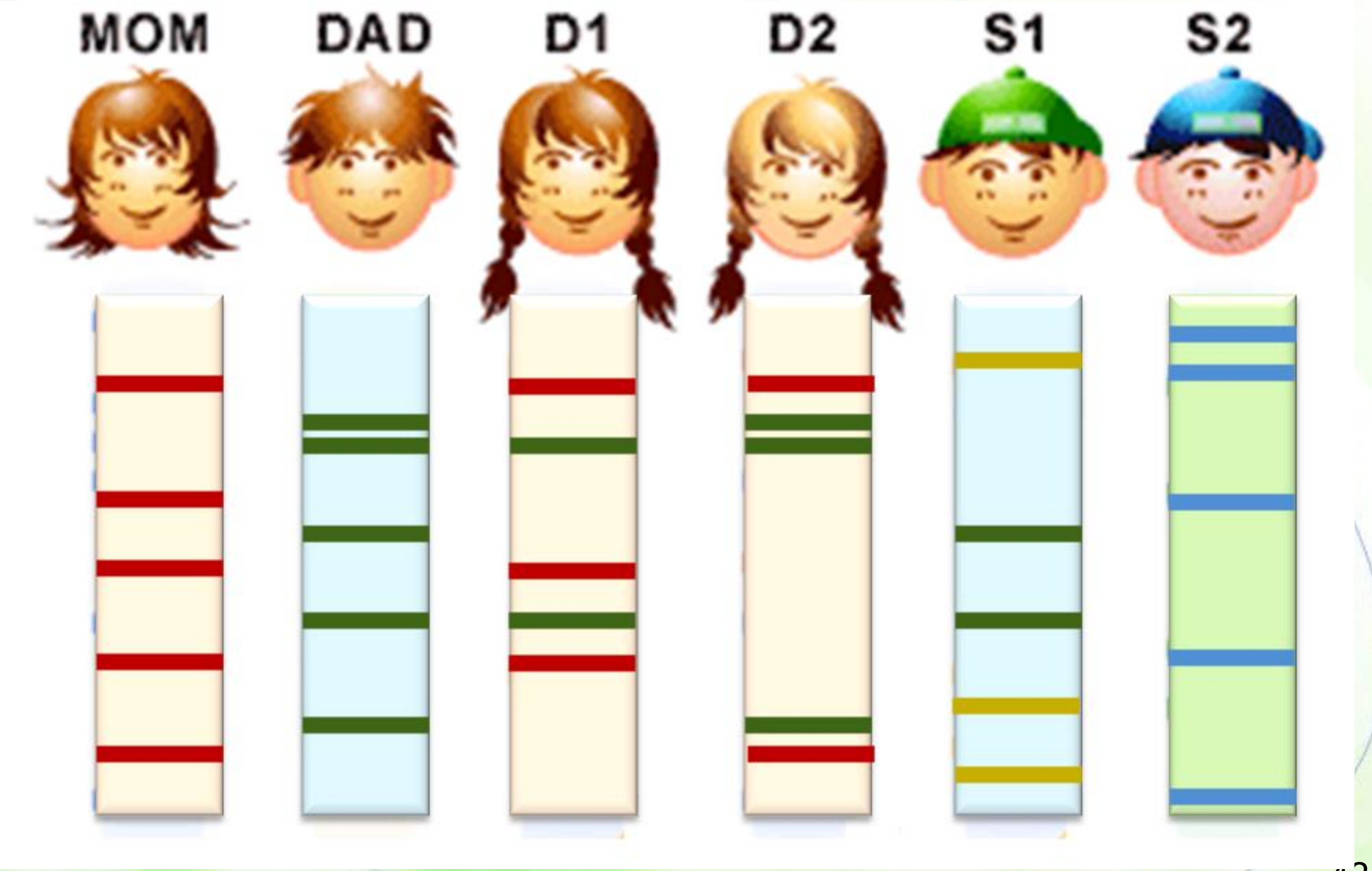




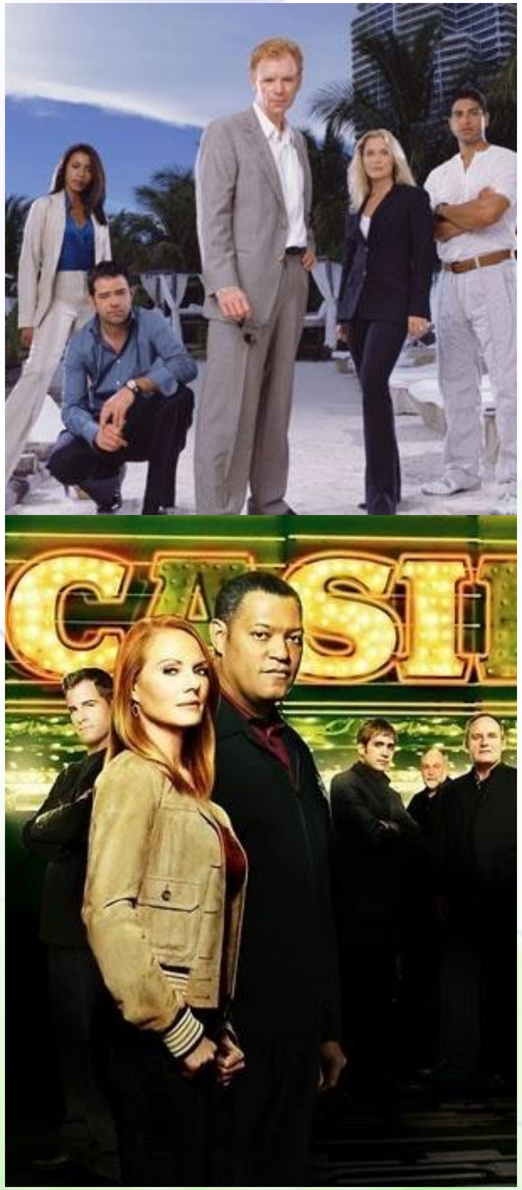

5'
probe
MstII
MstII
MstII
Hb^A
1.15 kb
probe
MstII
MstII
Hb^S
1.35 kb

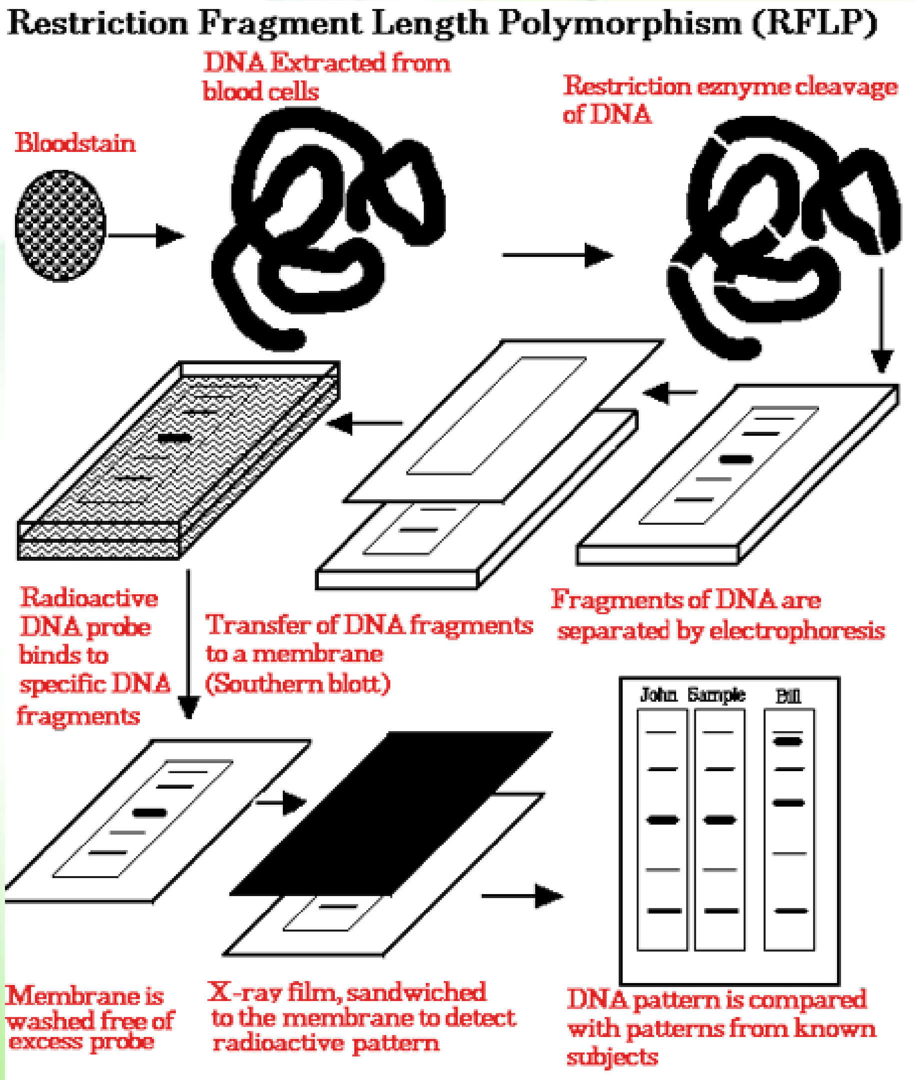

Genotypes
AS
AS
AA
SS
1.35 kb -
1.15 kb -

Example 2: Paternity testing



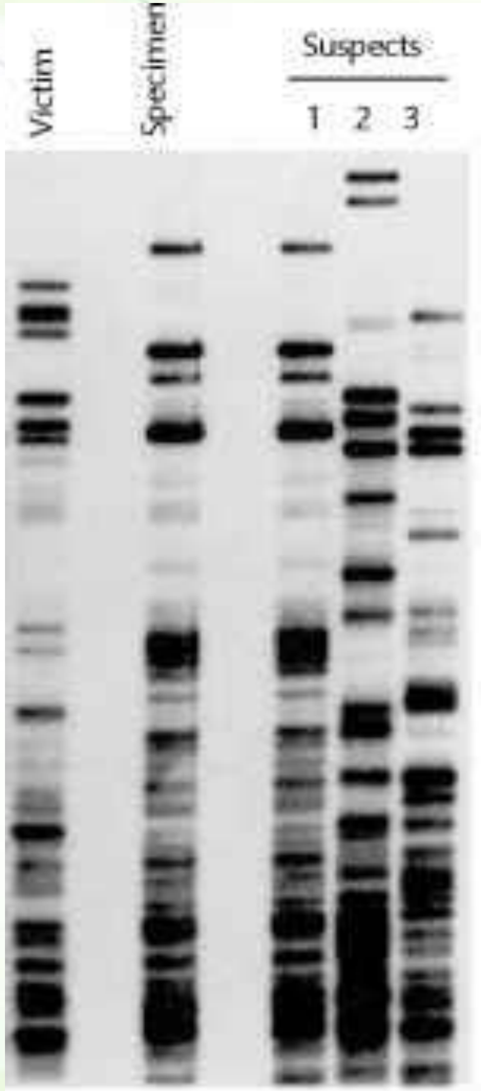
Example 3: Forensics





Real cases


Crime Scene
Victim
Suspect 1
Suspect 2


Victim
Specimen
Suspects
1
2
3