Sheet no. 3

Molecular biology

Writer: Ahmad Allaham

Corrector: Noor Abu Hantash

Doctor: Mamoun Ahram

Hybridization techniques

-first of all know that these techniques are not used extensively as they used to.

-• 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

-we will talk about two techniques:

1 Dot blotting

2 Southern blotting

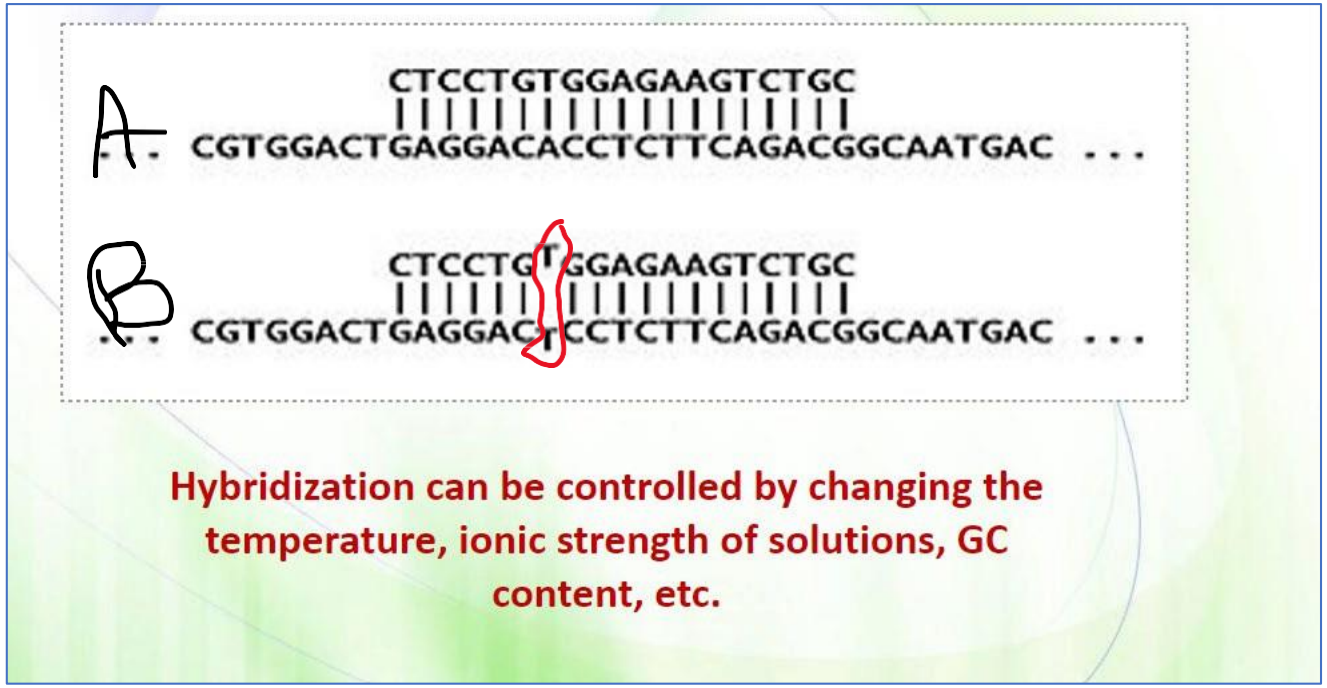
-these techniques are based on hybridization we take a DNA strand from a foreign source and allow it to combine with another DNA strand

-also know that imperfect hybridization can occur under specific conditions that is if enough hydrogen bonds

Are formed. The doctor said that enough is irrelevant for us in this course so just know that we need enough bonds no more no less.

- One more thing to keep in mind is that if a lot of T-A pairs were presented, hybridization may not occur because T-A bonds are 2 hydrogen bonds not 3 like C-G

Hybridization can be non specific



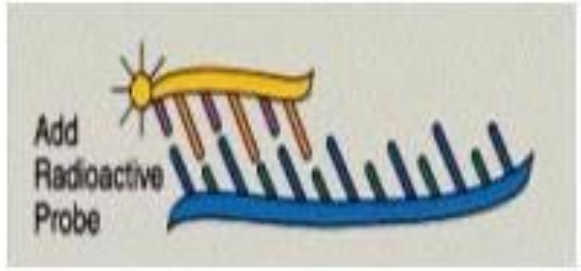
-hybridization can be non specific you can have the hybridization between a small and a large DNA fragment

-Figure A shows perfect hybridization 100% compatible.

-Figure B shows imperfect hybridization not 100% compatible.

- Pay attention to the circled pair which shows a mismatch no hydrogen bonds between these two which caused B to be imperfect and it will require less temperature to denature because it is less stable less hydrogen bonds.
- Important: Higher T-A pairs means less possible hybridization (because we will have less hydrogen bonds).

-Probes(Oligonucleotides)



- Oligo: means short

•A probe is a short sequence(up to 20 nucleotides) 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.

- We use the probes to look for a DNA sequence that is complementary to the Probe(short nucleic acid).
- We label the probe by either making it radioactive or attaching a phosphorus tag to it so we can observe it.

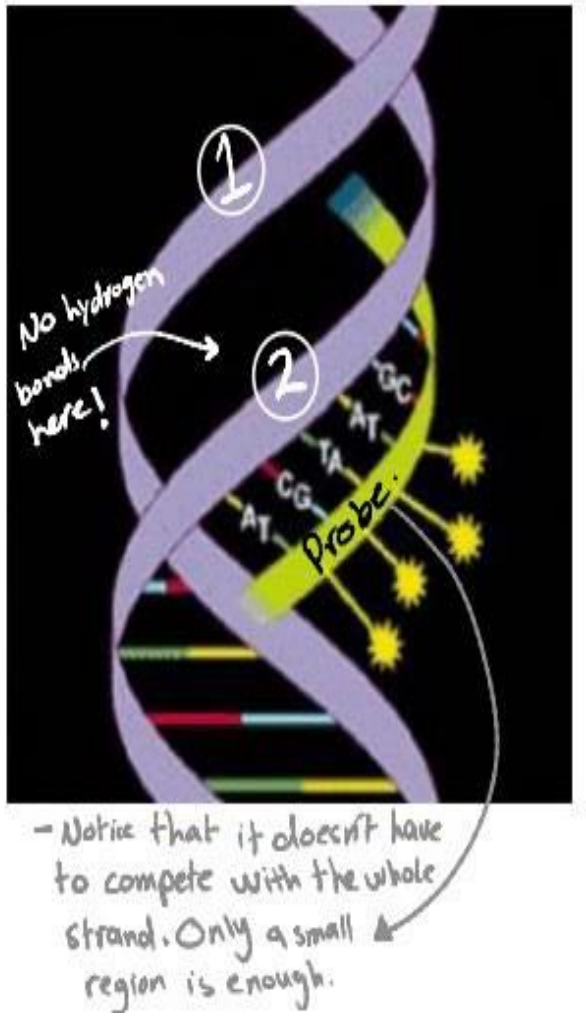
•We usually use labeled probes (with radioactive phosphorus 32P).

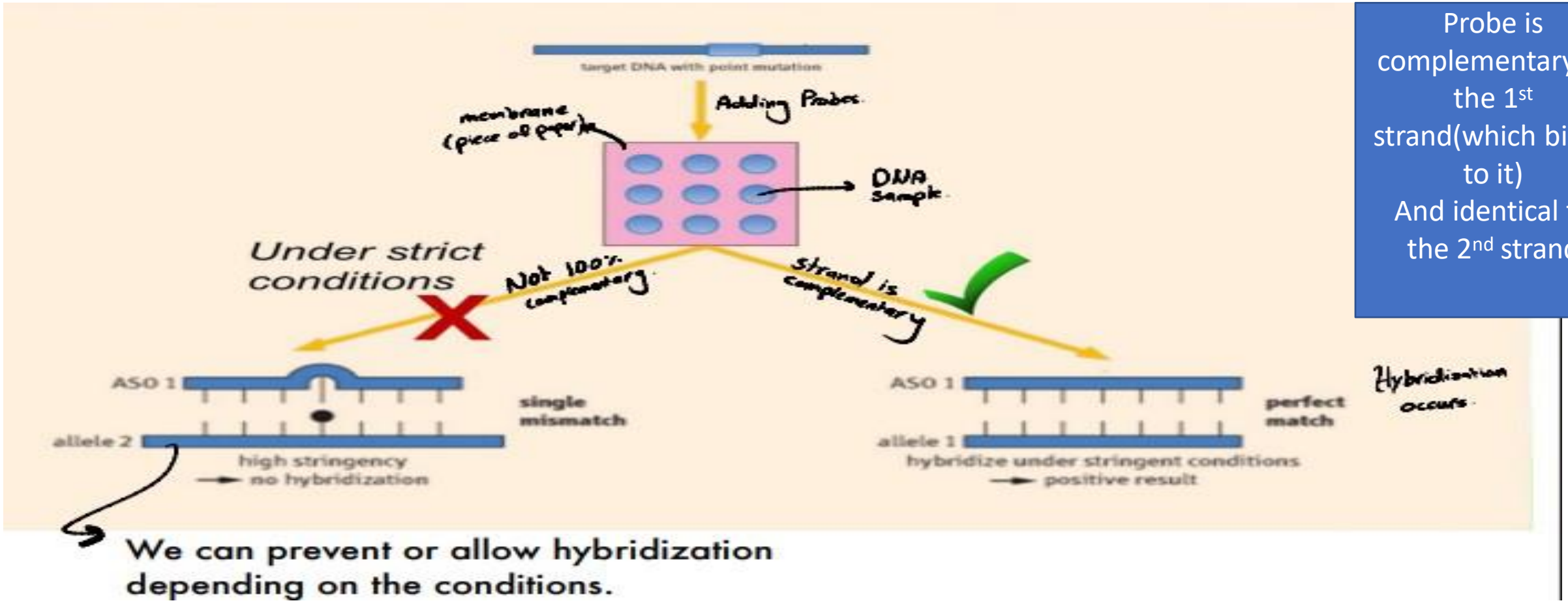
•When annealing (hybridization) happens, the probe produces signal.

• the probe doesn't have to be 100% complementary.

•When a probe is added to dsDNA (ds:double strand), it competes with the complementary (1) strand. Because hydrogen bonds between the DNA strands are noncovalent (weak interactions) and that means it is reversible.

•Probes normally win the competition because we add it with large(abundant) quantities, and hydrogen bonds form with its complementary strand (2) and break between (1&2).





Probe is complementary to the 1st strand(which binds to it)
And identical to the 2nd strand

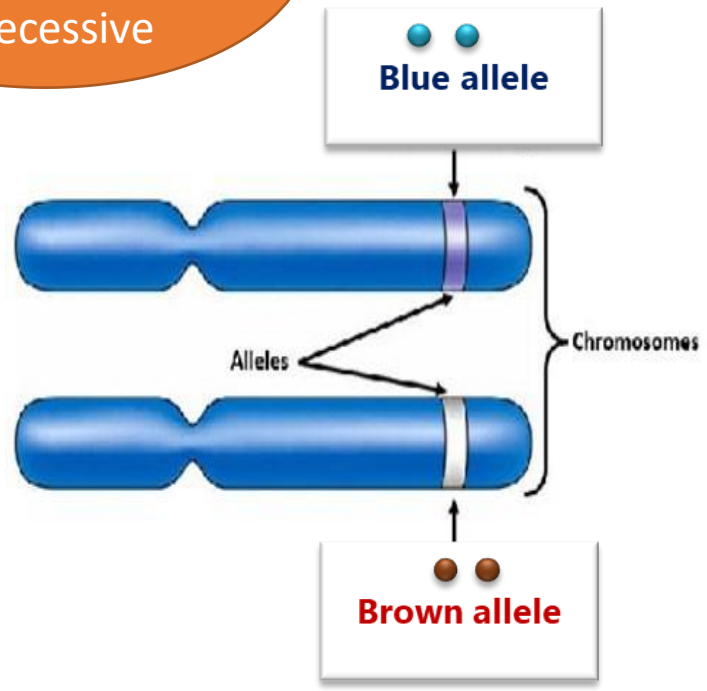
- When adding the probe if it is not 100% complementary and we put it under strict conditions(high temperature, low ion salt concentration) hybridization may not occur and we won't have an imperfect hybridized sample.

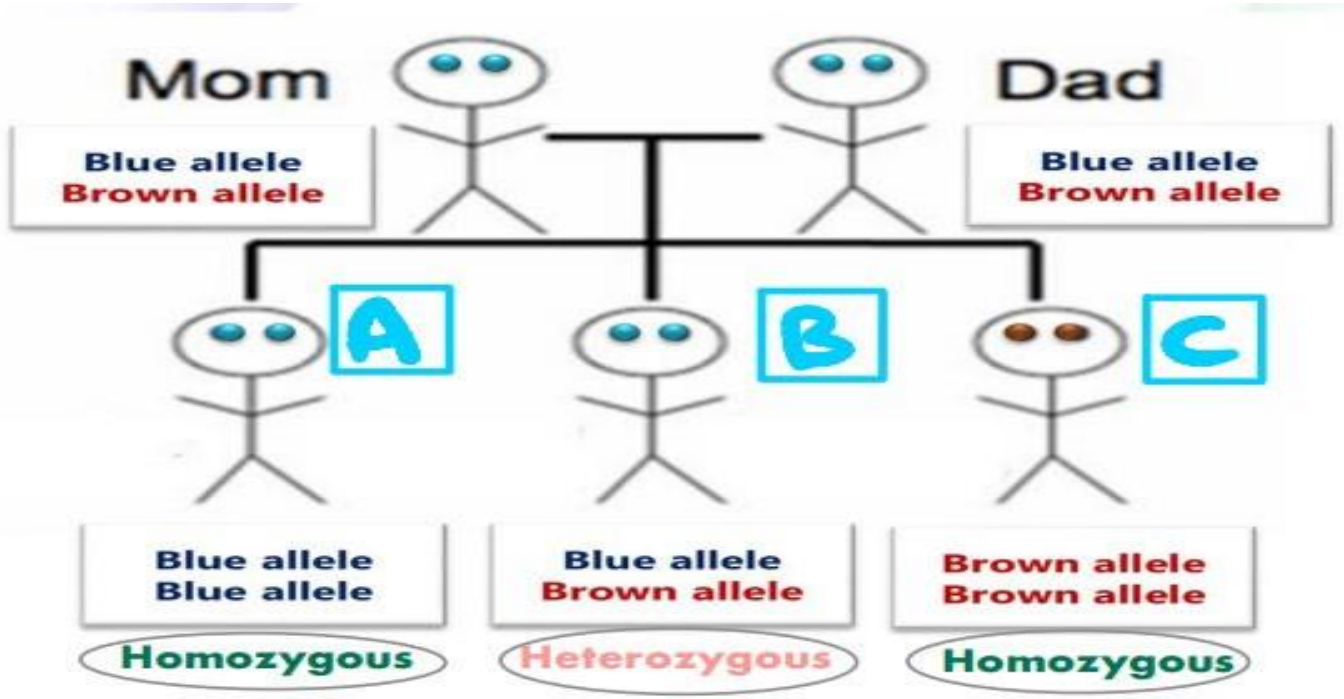
Concepts to know.

- Allele: a form of a gene
- Homozygous: both alleles are the same.
- Heterozygous: two different alleles.
- An allele can be dominant(سائد)or recessive (متنحي).
- Phenotype: the set of observable characteristics of an individual resulting from the interaction of its genotype with the environment. (طراز شكلي)
- Genotype: the genetic composition of a person.(طراز جيني)
- Dominant: if you have two alleles one would prevail dominate the other so that the phenotype of the other allele will not appear and the dominant one will be the one to affect the phenotype(appearance).
- Recessive: the allele that won't affect the phenotype when combined with a dominant allele meaning that alleles need to be homozygous for the recessive alleles to cause affect on the phenotype.
- Pedigree: drawing of the distribution of a phenotype or genotype.
- Genome: the total collection of DNA in a cell or total collection of DNA in an individual we say human genome or cell genome.

Blue eye gene is dominant
Brown eye gene is recessive

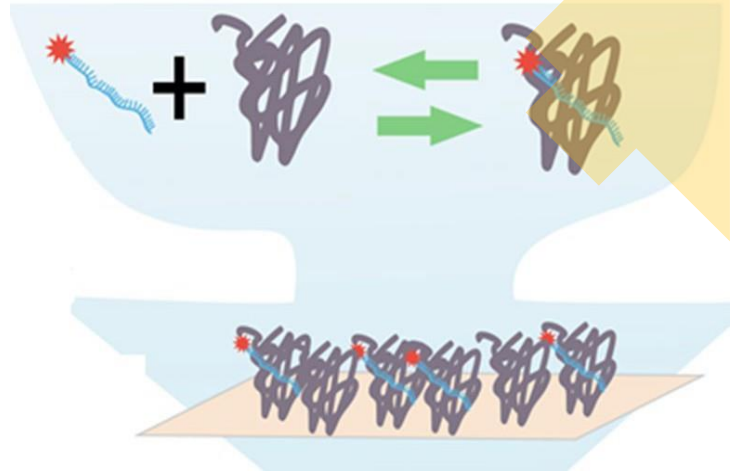
Pedigree(family tree)



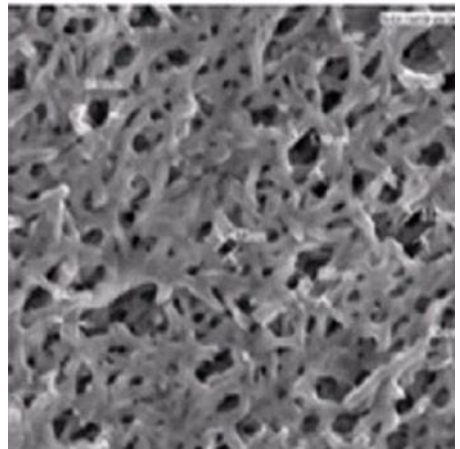


-the doctor said" that there are more than 15 genes that affect the eye color however we will assume that it is affected only by one and let us assume that it is located at chromosome number 5".
-there is always a specific location of the allele at the chromosome and this gene is located at the same chromosome at the same location for everyone of us our cells have diploid chromosomes meaning that they have homologous chromosomes
-Even though child (B) has blue and brown alleles, he has blue eyes; it means that the blue allele is dominant and brown allele is recessive.(it is a hypothetical example!)
-An individual should have two recessive alleles for it to appear as a phenotype. Notice child (C).

Dot blotting blot:لطخة



- 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.
- We add the DNA to the membrane(piece of paper, can see under EM) and it will be imbedded and attached we add many DNA molecules not just a single DNA molecule.
- The probe is labeled so where the hybridization occurs there will an emitted signal.
- We spot DNA on the membrane, a lot of the same type.
- Spotted DNA could be complex (the whole genome) or simple.
- When we add the probe:
- **1-If the DNA sequence is complementary with the probe, hybridization occurs & signal is produced.**
- **2-If the sequence is not complementary, hybridization doesn't occur and signal isn't produced.**

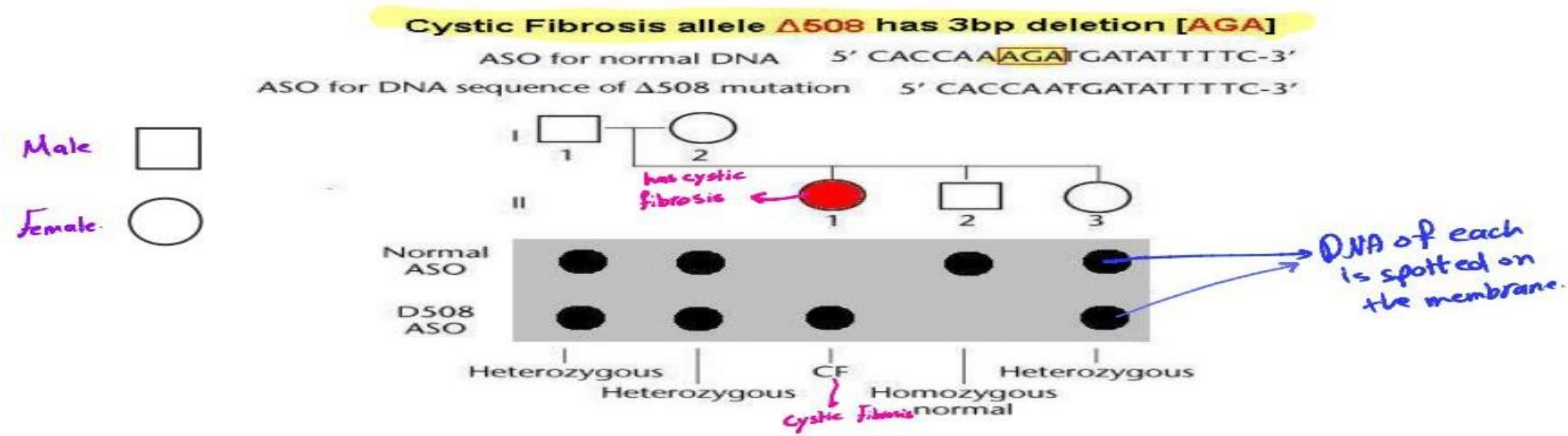


Disease detection by ASO(cystic fibrosis)

- Cystic fibrosis(from google): A genetic disorder, in which the lungs and the digestive system get clogged with mucus.it is fatal in early ages
- ASO: Allele-specific oligonucleotide which is a short piece of synthetic DNA complementary to the sequence of a variable target DNA and it acts as a probe.(for eg it will bind to blue eye allele not to brown cause it is specific)

Two ASO probes :

- 1. ASO for normal DNA.
- 2. ASO for DNA sequence of Δ508 mutation. ** Deletion of nucleotide delta 508. (The main reason for the disease).
- ASO probe can distinguish between normal and mutated allele.
- We hybridize on strict conditions so ASO probe of normal DNA only binds to normal DNA & vice versa.(by preventing imperfect hybridization each probe will bind only to the DNA fragment 100% complementary to it.)
- The whole genomic DNA is spotted on a solid support (a membrane) and hybridized with two ASO's, one at a time.
- The delta 508: means that deletion occurs at position 508 within the gene(AGA).
- Notice that there are some parts of the probe that are complementary to the normal gene but because we are using strict conditions hybridization between them which is imperfect won't occur.
- The ASO for non normal will not bind to the normal.



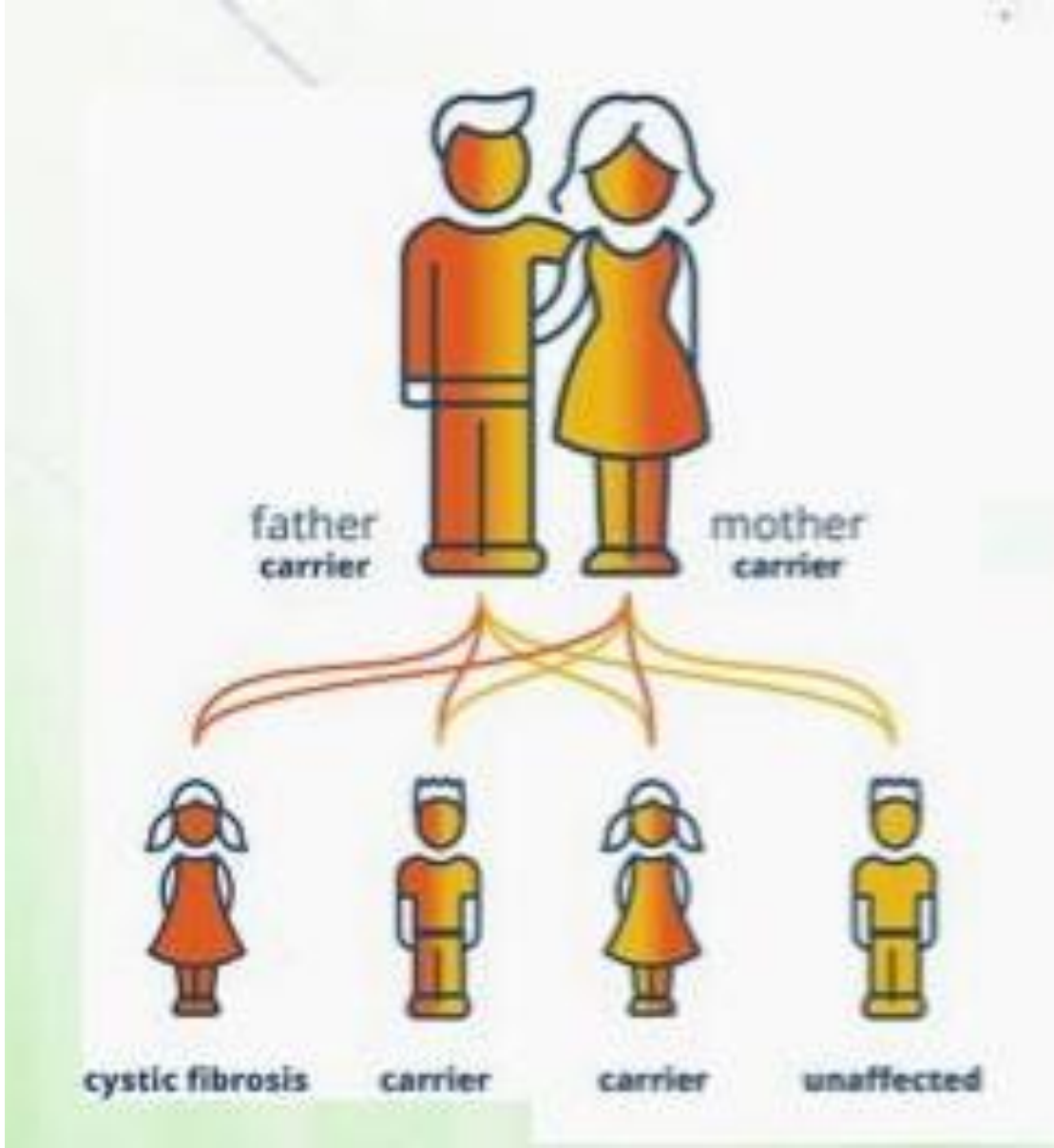
-when ever you see black there is a signal which means that hybridization happened.

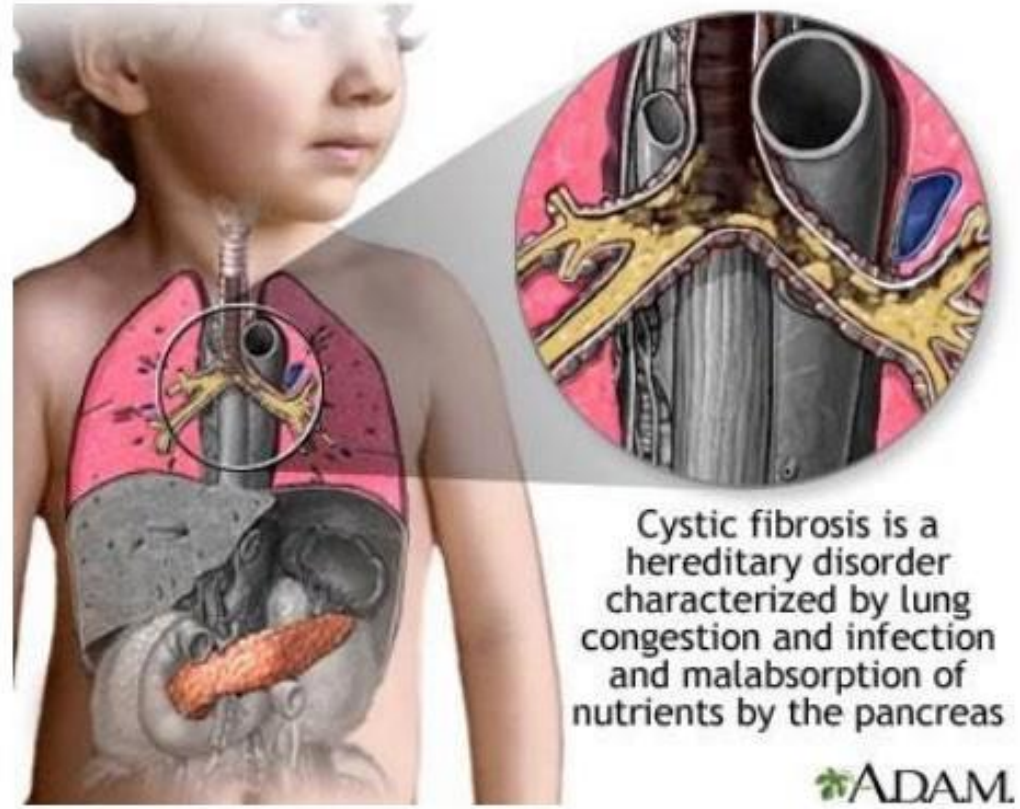
-the father DNA bind to the ASO normal and to the ASO D508(D: disease) this means the father has normal allele and the disease allele but he isn't affected by the abnormal allele because it is recessive and the normal one is dominant so he is carrying the disease we say he is heterozygous and carrier same thing goes for the mother.

-Since the father,mother and child (3) have both normal and D508 mutated DNA sequence , they are considered as carriers but not affected by the disease.

-Since child (1) is homozygous for the mutated allele, she has the disease.

- The sick girl or child (1) showed no signal when the normal ASO was added so she doesn't have the normal alleles look at the picture.


father
carrier
mother
carrier
cystic fibrosis
carrier
carrier
unaffected


Cystic fibrosis is a hereditary disorder characterized by lung congestion and infection and malabsorption of nutrients by the pancreas
A.D.A.M.

Southern blotting

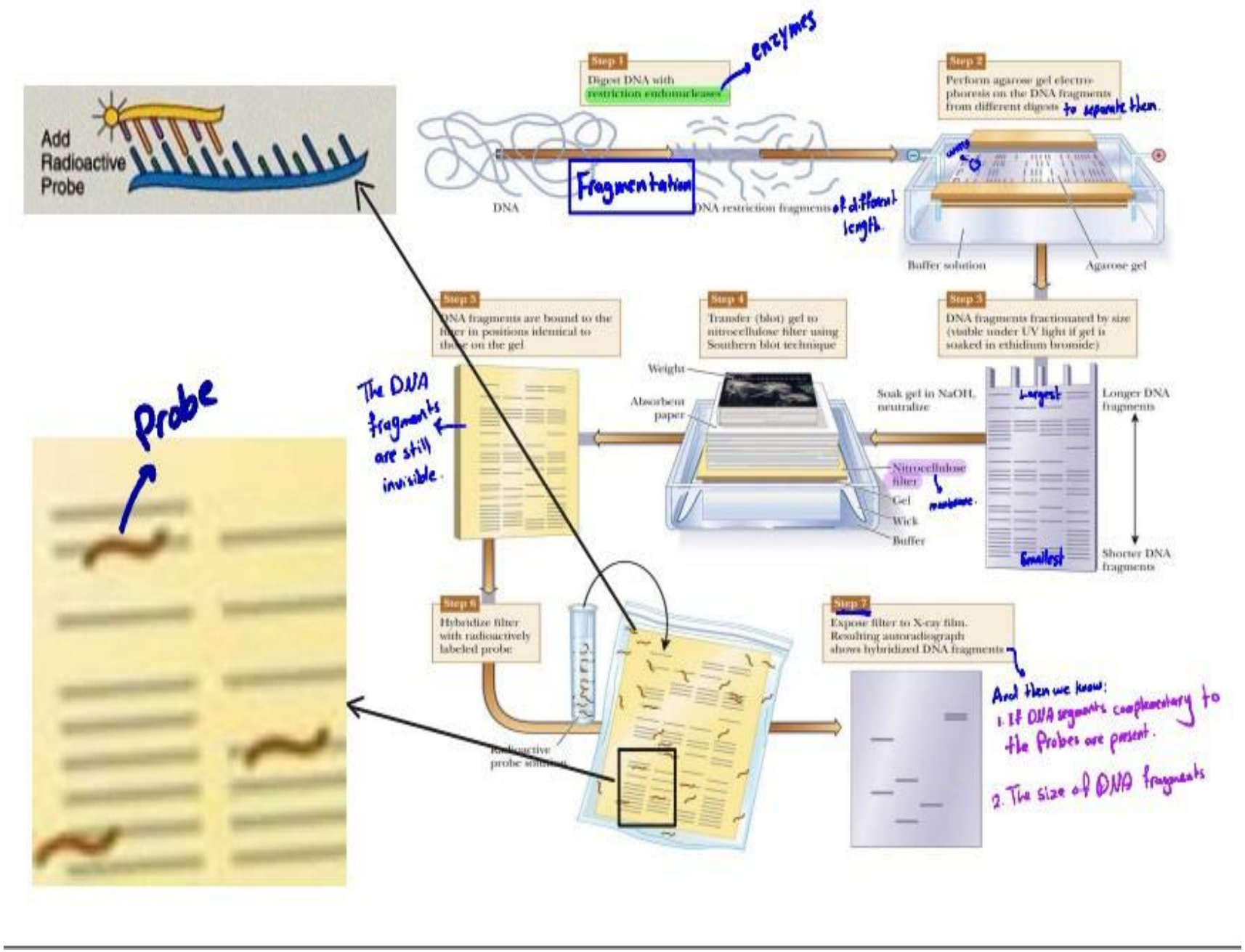
southern :a scientist

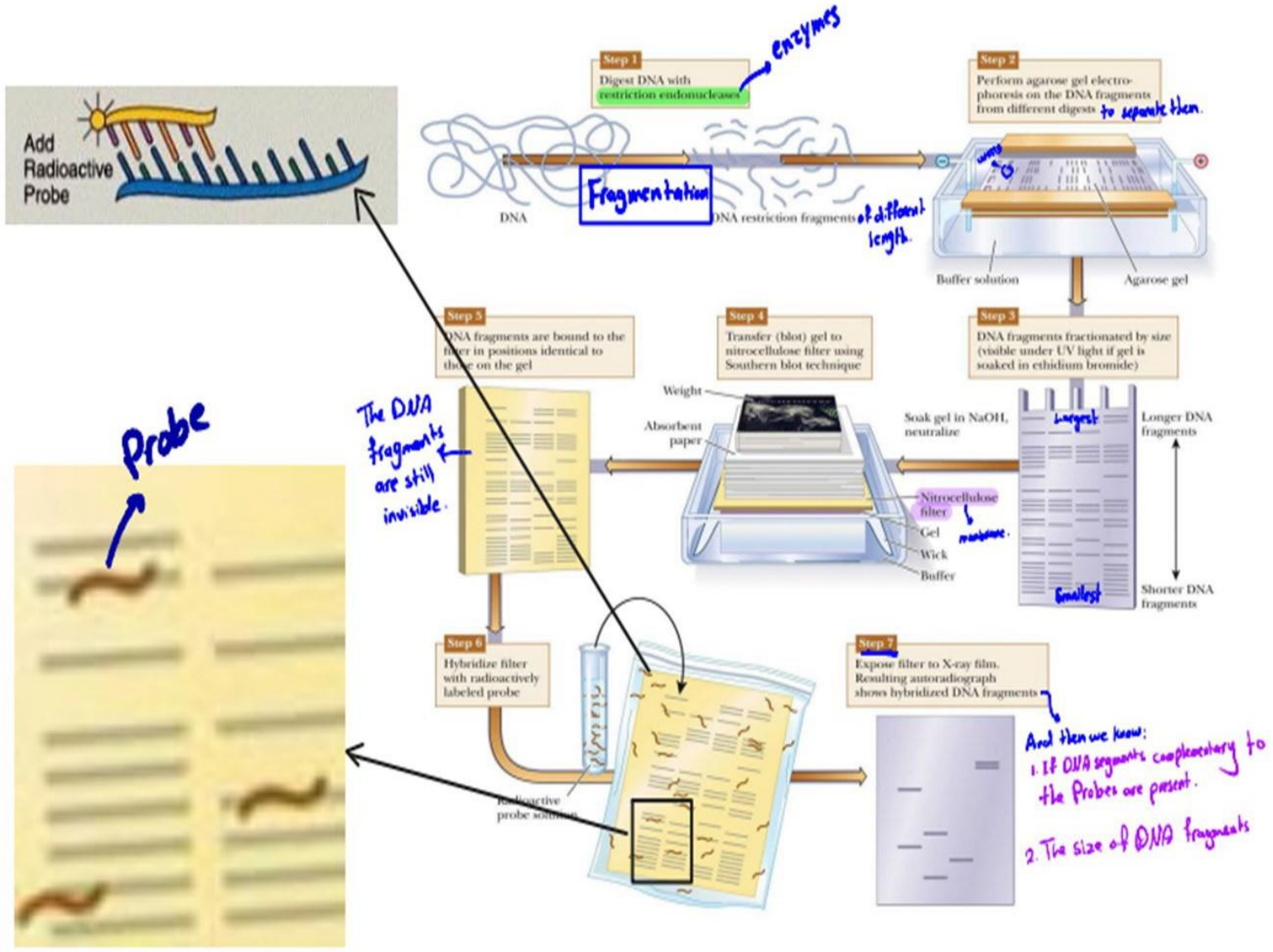
- This technique is a combination of DNA gel electrophoresis and dot blotting.
- Used to detect:
- the presence of a DNA segment complementary to the probe.(signal)
- the size of the DNA fragment. (The advantage of this technique).

Southern blotting done by 2 processes:

1)Gel electrophoresis

2)Dot blotting

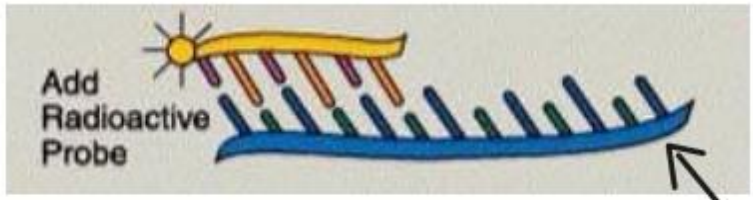




1)Fragmentanization
2)Put fragments in gel so it will separate according to size
- In this image there is 4 samples

3) Put fragments on membrane
4) Put probe so it will bind if it is complementary
6) Possess the membrane to X-ray so you will see signal

Dot blotting shows signal only
Southern blotting : shows DNA size and signal



Step 1
Digest DNA with restriction endonucleases → enzymes

Step 2
Perform agarose gel electrophoresis on the DNA fragments from different digests to separate them.

DNA

Fragmentation

DNA restriction fragments of different length.

wells

Buffer solution

Agarose gel

Step 3
DNA fragments fractionated by size (visible under UV light if gel is soaked in ethidium bromide)

Largest

Longer DNA fragments

Shorter DNA fragments

Smallest

Soak gel in NaOH, neutralize

Step 4
Transfer (blot) gel to nitrocellulose filter using Southern blot technique

Weight

Absorbent paper

Nitrocellulose filter

membrane.

Gel

Wick

Buffer

Step 5
DNA fragments are bound to the filter in positions identical to those on the gel

The DNA fragments are still invisible.

Step 6
Hybridize filter with radioactively labeled probe

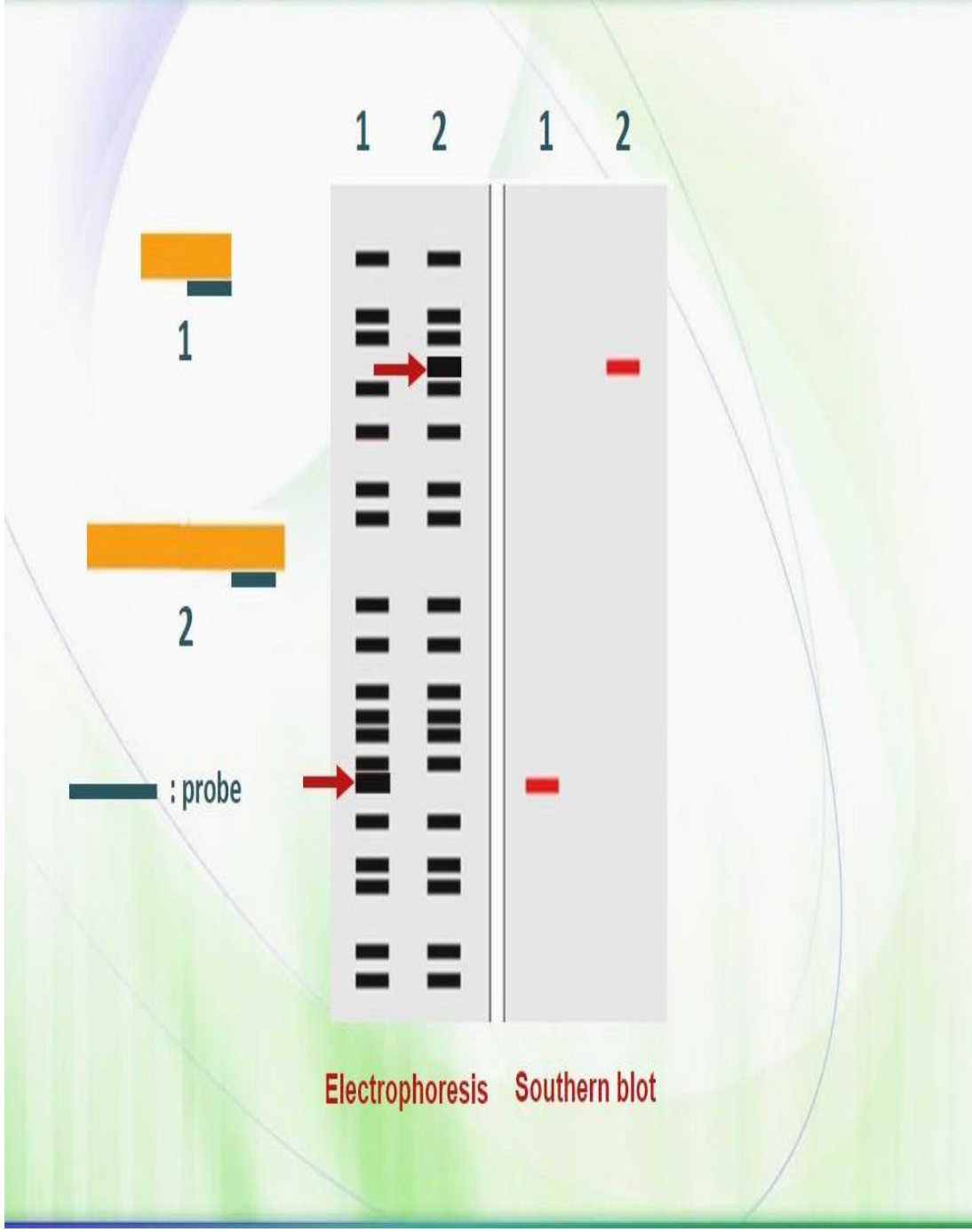
Radioactive probe solution

Step 7
Expose filter to X-ray film. Resulting autoradiograph shows hybridized DNA fragments

And then we know:
1. If DNA segments complementary to the Probes are present.
2. The size of DNA fragments

Probe

-we first separate the DNA fragments based on the size(fragmentanization) and then we do dot blotting by adding a probe.
-if there is a band that appears on the membrane we will know that there is a complementary region.
-and we will know the size because the fragments were already separated according to the size.
-the position of the DNA in the membrane is exactly the same as in the gel.
-so basically the membrane looks like a replica of the gel and so in the membrane they(DNA fragments) are organized according to the size
-the fragments that have the probe bound to them will emit the signal.
********important notes:
1-the size of the fragment don't reflect the size of the probe
2- when we present a gel we show it in away that the large fragments are shown at the top and the small ones are at the bottom.
3-We use labeling in southern blotting while we use staining in Electrophoresis since labeling is more sensitive.
4-Sizes that we see on the membrane do NOT represent the size of the probes, but the size of the DNA fragments detected.



- 14.Which of the following about ASO is incorrect?
- A) two types of probes are used.
- B) it’s used in the detection of cystic fibrosis.
- C) when a signal is produced on both membranes after DNA hybridization
- this indicates heterozygous person where only the dominant allele is
- expressed
- D) the defection in cystic fibrosis is the deletion of 2 nucleotides in a
- specific gene.
- E) all of the above
- Answer: D

Dear all don’t focus on the length of the road just enjoy the journey ☺

- Best wishes my fellow doctors ☺