We talked previously that we use a way to cut the genome which give us **cohesive or sticky ends i.e.:** there are overhands with complementary ends, so the hydrogen bond could be restored easily and then the only thing remain is for the ligase to do its job forming the phosphodiester bond.

And this is the main way to cut & paste a gene or cloning it from one place to another

When we want to cut the plasmid –where we want to insert our new inserts- we have to cut it using the same enzyme so they give compatible cohesive ends.

Ex:if I use EcoR1 to cut in first place, I should use it in the second in order to get two TAA so they could attached to each other.

And we talked also about another way to obtain DNA molecule is to reverse transcript it from RNA. Here we take the cRNA (copied) not the gRNA (genomic) which is shorter as it's free from introns (only exons).

Or we can simply synthesize our DNA using a machine called DNA synthesizer, but in this way we can only make small DNA fragments so it's called sometimes oligo- synthesizer.

Techniques to identify DNA sequencing

Now we are going to talk about techniques to identify DNA sequencing. And here we have two ways:

1- **Direct techniques**
   Here we are reading the DNA sequencing directly.
2- **Indirect techniques**
   Here we use a probe in order to identify the sequence.
   A Probe: is single stranded piece of DNA or RNA, synthetic DNA, cDNA. Its short (about 30-40 nucleotides). And the most imp its complementary for the piece I want to detect on the genome.

   Although putting the probe with a radioactive or a florescence material and see either it binds or not is look a simple thing, we have an imp step after putting the probe to be sure either it binds or not which is: WASHING to separate bound from unbound.

   Now washing is not as simple as its seems, here it should happen in a dry environment and the genomic material must be fixed on a solid material in order to wash the unbinding probe only without washing the genomic material we are studying.
   So there is a very imp step which is **FIXATION**: to fix my DNA on a solid material.

Now, in order to this we have two steps:

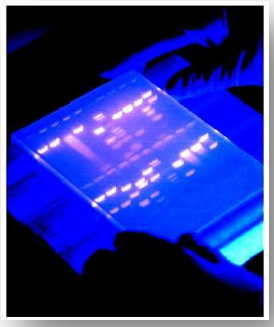
1- **Electrophoresis**
2- **Blotting**

Electrophoresis here we use a gel material like **agarose or polyacrylamide,** these materials have pores within them allow the DNA to move through when we put it under electrical current. Electrophoresis could be vertical or horizontal

Here we put the DNA in wells in the gel then the DNA is start migrating under the influence of the electrical current from the cathode (-ve charge) to the anode (+ve charge) as the DNA is negatively charged thanks to the phosphate groups.

Here the smaller bands are going to migrate faster while longer bands won't migrate as far as the others according to the pore size of the gel.

***NOTE:** I have already put **Ethidium bromide** -an intercalating agent commonly used as a fluorescent tag- with the DNA before starting the process.



So after finishing the electrophoresis I take it and put it on a box with UV light the DNA will Shines because of the ethidium bromide.

Now the strands are **inside** the gel so I can't do the probing step yet as the DNA is still inside the gel and not on the surface (they are unexposed to hybridization).

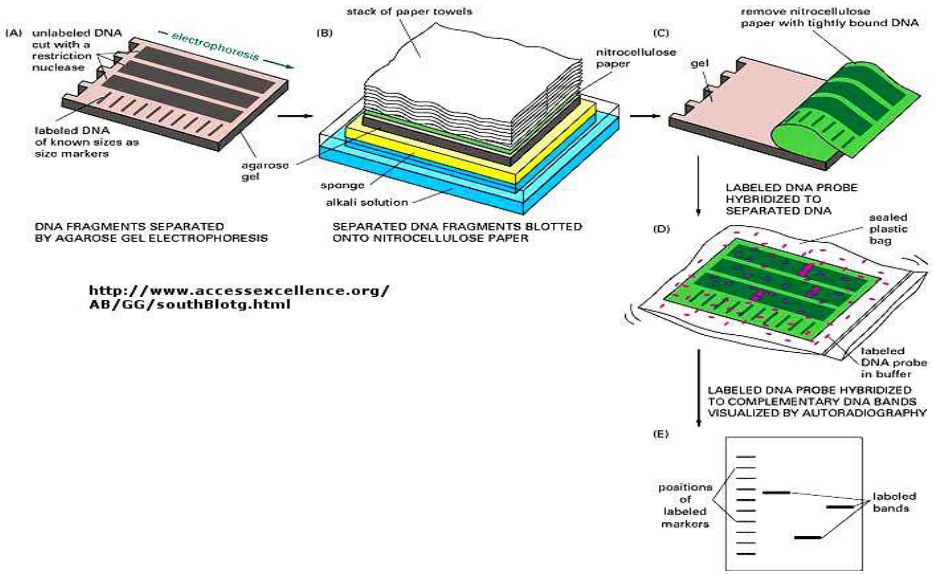
So we should move to the next step: **BLOTTING**:

Here we bring the gel and put it in a container with a buffers … etc.

But on the gel immediately we put a nylon membrane made up of **nitrocellulose** which is known to cross link with DNA.

Now, we want to move the DNA bands from inside the gel to the membrane. How**?**

Edwin Southern found a simple solution for this: by bringing a Tupperware (تبروير /طشت:P) and filling it with some water. He put the gel in the water with the buffer and put the cellulose membrane then packed tissues (cleenex) above it, a weight could be used to hold the tissues in place. Tissues will absorb the buffer and water in the solution, the buffer will pass though the gel, while it does, it will dissolve the DNA (carries the DNA with it), the gel then reaches the nitrocellulose membrane. The water will keep passing but the DNA will bind to the membrane. And that's it ☺ this technique was named **Southern Blotting** according to him.



After the DNA is fixed on the membrane, now I can start the other steps:

- **Denaturation**

By putting it –simply- in a nylon bag with a buffer with high pH, this will cause the two strands of DNA it separate from each other

- **Probe hybridization**

I put the probe then if its target is there it will bind it.

- **Washing**

After that if the probe is there we can detect it by X-ray film, we put it on the membrane then the labeled probe will make a mark on the paper indicating that our target gene or sequence is there.

***NOTE:** there are some animations you could find their links in the slides they could be helpful to understand this process perfectly.

Now, this same technique was used **for RNA and proteins.**

For RNA they called it **northern blotting** -_- And for protein also and they called it **western blotting**. nahfeh el3adeh -_-

- **<u>Northern Blotting: for RNA</u>**
  - Same as in DNA:
  Electrophoresis → Blotting → Denaturation → Hybridization → Washing → Detection
- **<u>Western Blotting: for proteins</u>**
  Electrophoresis → Blotting → Hybridization → Washing → Detection. Notice: no denaturation, and in hybridization the probes are antibodies.

- **USES of blotting:**

1- In Southern Blotting:
- For detection of mutations, for example:
A deletion caused Cystic Fibrosis in a family: we design the probe of the mutation then do the blotting and if we could detect the probe then there's a mutation and vice versa.

2- Northern Blotting:

a) Detection of gene expression:

> Genes are always there, but what we need to know is that wither they are expressed or not, therefore southern blotting does not help us see if the gene is active and expressing proteins properly. For example, MIC, always positive in Southern Blotting but if expressed (and that is what we need to know) it will be Northern Blotting positive.

b) Diagnosis of Viruses:

RNA viruses such as HIV (retrovirus). We obtain a blood sample, then apply a probe against the viral RNA and do the blotting.

3- Western Blotting:
A- Detection of Protein Expression (allows us to detect certain protein presence), or in the case of an oncogene we could check for a cancer.
B- Detection of Virus Presence → By looking for the protein products of that virus, as in HIV detection where we synthesize antibodies (probes) against an HIV protein. One of the most reliable test for HIV.

*** Colony Hybridization:**
- We insert a gene in a bacteria, for commercial purposes. Example: like hybridizing a bacteria with Insulin Gene allowing the production of commercial amounts of insulin.

How to do that?
1- We obtain a plasmid with insulin gene and apply it on a culture of thousands of different bacterial cells.
2- Some of the cells will take the insulin gene in, others won't.
3- Wait for the cells to for colonise, then we apply a special membrane allowing it (the membrane) to touch the culture and take few cells from each colony.
4- Apply a chemical material to the cells at the membrane that would cause the formation of pores

in the cells (pores for the upcoming uptake of applied probes).
5- Throw probes (complimentary to the insulin gene) which enter the cell. Then we perform washing and detection of bacterial cells with gene via X-Ray. Spots indicate cells with insulin.
6- Culture the identified cells to start the insulin production in large amounts

*The previous methods were **indirect** methods of identifying the DNA sequence. Because the signal you're receiving is coming from the probe. The problem with probes is that they are short and can only give me information on part of the gene. Therefore, we can also use other methods such as:*

- **DNA Sequencing**

- The whole sequence can be read and we can search for mutations instead of only detecting one.
- Also called: Chain Termination method, or Sangar Method. It is **direct.**
- The previously mentioned methods were all indirect ones with the problem of "specifity", in other words:

In order to use blotting with a probe, you need to know the exact mutation; its type and site, in order to detect it. But that is not always what we need. In many cases (e.g in BRCA1 and BRCA2 of familial breast cancer or thalassemia) there are too many possibilities, so we will need to sequence the entire DNA to identify the exact problem.

- The Mechanism:

We will need 4 small tubes, in each tube we will be replicating the gene we wish to sequence (imitating cell division but only replicating genes we need), they will have the following contents:
- The DNA template we want to sequence (of a suspected patient, e.g. with relatives with breast cancer)
- DNA polymerase
- Primers of the suspected gene (e.g. BRCA1 primes)
- 4 normal dNTPs (Deoxy Nucleotides Tri-Phosphate) + 1 ddNTP (A, T, C, G)
- Buffers (MgCl2..etc)

ddNTP: 2',3' dideoxynucleotides

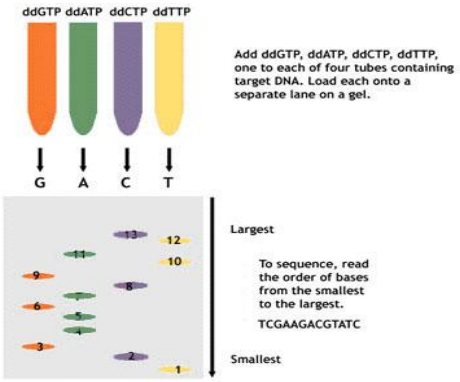
We only replicate the gene we need, not the entire genome. This is done by targeting the DNA polymerase toward that gene by our primers; specific primers for that suspected gene.

In each tube, a 5th different ddNTP is added.
That ddNTP (Di-Deoxy Nucleoside Tri-Phosohate) got it's "di" since carbon number 3 has H instead of OH as well as carbon no. 2 and hence called "Di-Deoxy".

The absence of the 3'-OH group on the ddNTP will terminate the replication as no phosphodiester bond can be created by the DNA polymerase

The ratio of ddNTP to dNTP is 1:1 so there is a 50% chance of incorporating the normal dNTP and continuing the replication and a 50% chance for termination.

So, in that tube, we'll have different copies of DNA strands with different lengths (this is since they were terminated at different points, depending on whether the polymerase added the normal nucleotide or the ddNTP). If we take one test tube as an example, such as the one with guanine (G), the DNA strands will all be of different lengths but they will all end with ddC. The resulting DNA fragments are heat denatured and separated by size using capillary electrophoresis. Each of the four reactions run in one of four individual lanes (lanes A, T, G, C) and the first one to precipitate is the shortest one which means that the ddNTP was added at that point and terminated the replication. This means that the shortest one will be in the tube which has the first nucleotide in the sequence and will be the primer +1, example: If the shortest copy (primer +1) was in the T lane ⇨ the first nucleotide was (A) –since it's a complimentary strand-. And then we continue to the next one, the second shortest will be the second nucleotide (primer +2) and so on.



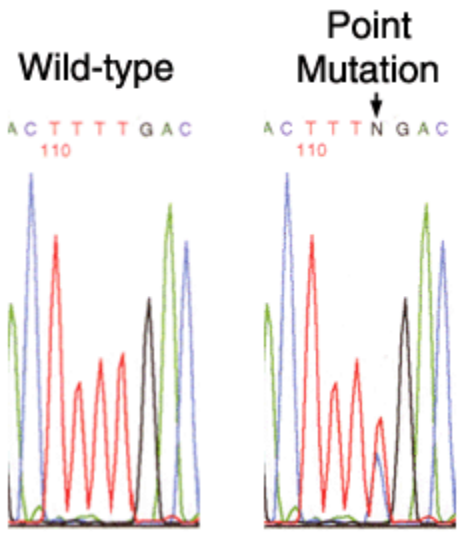
- **Automated sequencing:**

It sequences 400 bases at a time, so if you want to sequence a whole gene it will take 10 reactions. It's just the same as the Sanger method but the 4 reactions are mixed and electrophoresed on the **same lane**

- The primer in each tube is labeled with a **different fluorescent tag** to identify on which nucleotide the replication was terminated

-Then Fluorescence is detected electronically and signal are converted into base sequence.

Diagnosis:



The point mutation is shown by the arrowhead and is indicated as an N in the printed sequence. In the wild-type sequence the base is a T, but in the mutated sequence we see two overlapping peaks of flourescence, representing both a T and a C. This sample is heterozygous at this locus (one mutant and one wild-type allele).

○ If a certain disorder always had the same problem/mutation in the same location in the gene, a simple sequence for this location is made to identify the mutation.

○ If the location of the mutation is unknown, we sequence for everything.

○ Sometimes there's an increasingly diverse range of problems, these problems are found in different locations in the gene every time, and they could be in one gene out of 30 genes, sometimes even in 4 or 5 genes out of 30 genes. And so, *Next-Generation Sequencing* technology is used to identify these mutations.

- Next-Gene Sequencing is a number of different modern sequencing technologies.
- For example, lymphomas are very diverse and of multiple types, and each type is a different mutation in a different location in the gene. So if a person has lymphoma of an unknown type, we will use Next-Generation Sequencing to identify what type it is by buying one of the chips that has 400 main oncogenes, and we put this person's DNA in it, and then put it in the machine, the machine will sequence these 400 oncogenes in one go, and will tell us what oncogenes the mutations occurred in.

This is the future of medicine in which babies will be born and their own maps are ready to be used and read by certain machines and softwares, and it'll be easy to know what this baby is predisposed to and take precautions.

- **Techniques for obtaining DNA:**

□ Restriction enzymes □ Reverse transcriptase □ Chemical synthesis

- **Techniques for identifying DNA sequences:**

□ Probes (indirect) □ Sequencing (direct)

Identification DNA sequences is done either for research (to find out the sequence of the gene) or for diagnostic purposes (to find the mutation in this gene)

- **Techniques for amplifying DNA sequence:**

Anything we want to do in the molecular level has to be amplified first for us to be able to work on it.

Amplification is used for *diagnostic purposes* in the following cases:

1. **To diagnose someone with a certain virus:**

►the traditional/serological way: through detection of the antibody that is against hepatitis C (for example). However, it would take a long time for these antibodies to reach a titer high enough to be detected be ELISA. It will be too late by then because it takes months for the virus to enter the cells and replicate and be noticed by the immune system that has to give rise to antibodies that take a long time to reach a proper noticeable titer. For this reason and multiple others, PCR is better.

► PCR and molecular biology techniques: we can detect the virus at much earlier stages, instead of months it would be a couple of weeks, it's needless to say that early diagnosis in viruses -not just cancer- is very crucial, and in most cases, life saving. If HIV, for example, was discovered in the first few weeks the patient would be given polyclonal antibodies and the virus would be eliminated, and the healing percentage is 100%. But how can we diagnose a virus while it's in very very low titers in the blood? By *amplification*. If we take a blood sample from that person we won't notice the virus unless we amplify it (example for diagnostic purposes for viral interacions)

2. **To diagnose cancer**:
   By detecting cancer while it's still a few cells, we take their DNA and amplify it to see it.

3. **forensic medicine** Used to collect evidence in a crime scene.

We can use a few skin cells/ a few hair cells/ a blood spot that we can hardly see/remnant cells from the scratching the victim did while trying to escape -> amplify any of them and employ DNA finger printing.

There are two ways to amplify DNA sequences:

1. DNA cloning

DNA cloning is the old method.

Mechanism:

- we isolate the gene we want to amplify and cleave it using restriction enzymes/endonucleases
- this gene is incorporated into the plasmid
- recombinant plasmid is incorporated into bacterial cells by transformation
- the bacteria will copy its plasmid including the gene.

(you put one copy of the gene and 2-3 days later you get billions of copies).

How can we get the gene back from the plasmid?

We cleave it with the same restriction enzymes that we cloned with.

The whole process actually takes 3-4 days, because we have to cut our DNA and then cut the plasmid , then ligate the gene into the plasmid, then incorporate the plasmid and transform the bacteria, then select the bacteria that took in the plasmid, and then grow that bacteria and break down its cell membrane, extract the plasmid and cut again with the same restriction enzyme to get our gene back, not to mention the likeliness of the cloning to fail the first time, so because it takes a long time it's not used anymore to amplify the DNA. However, it's still used to amplify the protein product of the DNA;

◊ By doing this process, we're actually after translating our DNA into proteins and not after copying the DNA: All the steps aforementioned are done and we grow the bacteria just because we're after the protein product such as insulin, growth hormone, and other therapeutic proteins that are being made in bacterial cells. So yes, we're still using the bacteria because we need a cell to make the protein.

(We're still using this technique but for a different reason which is amplifying protein products and not amplifying DNA).

● Cloning components:

A host cell (e.g. bacteria)

A gene (e.g. insulin)

A vector (e.g. plasmid)

A vector: a vehicle that carries the gene into the host cell.

If the insulin gene was thrown into the bacterial cell without the vector (plasmid), the bacteria will break it down, and the best vector to express a protein in a bacterial cell is the plasmid.

● If the protein is too large for the bacterial cell to handle, then mammalian cells, or even human cells are used instead because they're the only ones able to produce such large and complex proteins.

● Since bacterial cells are useless in this case, the plasmid would be useless as a vector as well, and viral vectors will be used instead.

- From slide 314 the doctor is interested in the following only:

Plasmids are the vectors used in bacterial cells

Viral vectors are used in mammalian and human cells.

So, DNA cloning isn't used anymore UNLESS we need the DNA protein and not the DNA itself, if we want the DNA we use the PCR.

2. PCR

▪ PCR is also known as **Thermal Cycler.**

▪ PCR is the most important molecular biology technique. It's being run in each and every molecular lab around the world for it's the most efficient, easiest, least expensive method to amplify DNA into billions of copies in a couple of hours.

▪ PCR was first thought of in mid 90s. In 2000s, everyone started making PCRs. And nowadays, every lab must have at least one.

▪ PCR attempts to mimic the DNA replication that occurs inside the cell.

▪ PCR is a machine where we insert tubes, and in these tubes we put the wanted-amplified gene, and the machine copies the gene into billions of copies. (The whole reaction happens inside a tiny tube that holds about 25-50 microlitre)

▪ Reaction components:

-The template we want copied

-A primer that marks the region we want copied

-Nucleotides

-Polymerase (No other enzymes are wanted, we certainly don't want helicase, because the DNA will be subjected to heating. We don't want helicase because if we put helicase we will need single strand binding proteins and topoisomerases and many other enzymes, so we just need a polymerase and we replace the helicase by heating.)

And here is the problem; to denature the DNA we need to reach a temperature around 80-90 degrees and this temperature is capable to denature the polymerase (which works at 37 degree).

As a solution for this problem they use the DNA polymerase of certain bacteria "**Thermus Aquaticus** " which lives in boiling hot springs, and their polymerase is said to be "**Thermo-stable**" and can withstand high temperatures, and then they called it **TAQ Polymerase**. (Kary Mullis is the scientist who thought of this)

After they check that this polymerase could be used for our DNA, they bring the tube with its contents (DNA, Primer, Nucleotides) and add the TAQ polymerase to them. After that they raise the temperature to 94 degree → the DNA is denatured and the polymerase still functions.

- Because it is a bacterial polymerase its accuracy will be low; every 10000 nucleotides we will have an error, but what really happens that we only need to replicate about 300-400 nucleotides, so it will be difficult to have an error in this short strand.

P.S. This polymerase possesses 3' to 5' exonuclease, but they remove it to increase the speed of replication.

- They start the process and after the first round they have 2 copies → by repeating the process (heating and adding the polymerase) → they have 4 copies → → → they reach their goal.
- PCR is a chain rxn; 1 copy → 2 → 4 → 8 → → → it is an exponential amplification.
- Each round (where DNA doubles) of this process needs 3 steps:

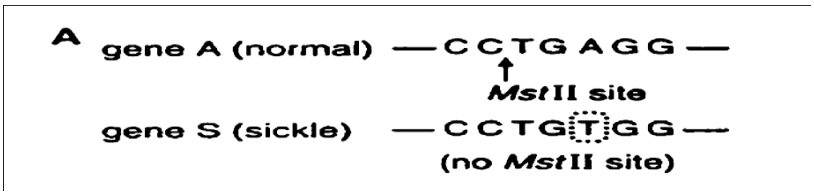
1) Heating to have the DNA **denatured** (raise the temperature to 94 degree)
2) At this temperature (94) the primers will not attach to the strands so we decrease the temperature to 55 degrees to have this attachment (**annealing**)
- But why not to 30-40? Because the DNA molecules will re-nature if we do that.
3) Now we will raise the temperature to 72, why?
Because we need a high temperature for the polymerase to start its job (**extension**), and we can't raise it to 90 or more because the primers will detach, 72 will allow the polymerase to work.

As a summary for these 3 steps:
1) Denaturing DNA (94 degree)
2) Annealing of the primer (55 degree)
3) Extension by polymerase (72 degree)

- Each step takes about 15-60 seconds.

- In the past it had been a difficult procedure (they used 3 water baths with those 3 temperatures) but now they invented the "**Thermal Cycler**", which repeats multiple cycles of PCR by itself, we program the time, temperature, and the number of cycles we need. This machine was made of metals that absorb and release heat in a very quick manner.

Detection of DNA polymorphism



1- RFLP (Restriction fragment length polymorphism) is used if the point mutation on a sequence in a gene happens to delete or create a restriction site.

Please use the picture on the top right depicting sickle cell anemia as an example

- A point mutation that substitutes an "A" base with a "T" base is a single nucleotide polymorphism (SNP) causing sickle cell anemia.
- The normal sequence is affected by the restriction enzyme MST II (It cuts normal β globin DNA at a particular site), but the mutated form isn't affected.
- So in this case it's used in simple diagnosis:
  a. First, we amplify the region " PCR it " (consider the space between primers is 400 in length)
  b. Position of the mutation is on the 250th nucleotide for example in the 400 length gene.
  c. We add the restriction enzyme, and then electrophoresis
     - if there was no mutation then the enzyme will cut the gene and gives two bands a 150-nucleotide and 250-nucleotide length
     - but if there was a mutation, there will be no cutting, and we will end up with a band of 400 nucleotide length "single band."

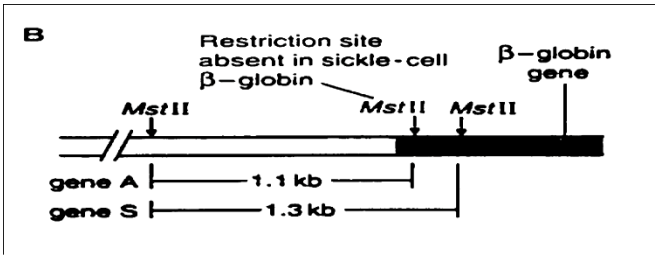
- Sometimes it's the opposite thing, if the gene was normal no cutting, but if it was mutated it will be cut.
- This is the method of choice to diagnose so many SNPs in the lab (Blood clots and abortion, thalassemia, FMF –familial Mediterranean fever-)
- We have many restriction enzymes that it's very frequent that any SNP has a restriction site, so we manage to know all that through computer software, we only have to enter the gene sequence and the software will give us the location of the restriction site.
- RFLP: PCR → restriction and digestion → electrophoresis. By the size of the band we can decide, if there is a mutation of not.
- We can do it for the whole family and observe the product bands

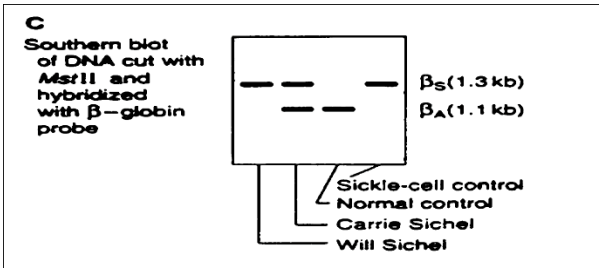
  For the previous example, if we find the following:

  (400N. 150N. 250N.) → Heterozygote (Carrier)

  (400N.) → Homozygote mutated for the wild type "long band"

  (150N. 250N.) → Homozygote normal. "Short Band"





The gel isn't a good example in the book, because there is no PCR and there are too many MST sites, and we should amplify the region we want to work with so it becomes dominant not just add the genome

2- Detection of mutation by specific probes
- Here we design the probe so it binds to the region where there is a mutation (We use 2 probes- normal and mutated- to confirm our results)
  → if the normal probe binds then Homozygote normal

  → if the mutated probe binds then homozygote mutated

  → if both probe bind then heterozygote.

- We can do it by southern plotting, but there is an easier way by isolating DNA, then we do PCR and then we add the product of the PCR to a special nitrocellulose membrane that will attach the DNA to it directly without need for plotting. The membrane has wells and for each person in the family we add two DNA copies, then we add a normal probe and a mutated one and wash to remove unbound probes, then observe the signals.
- The mutation most likely to cause cystic fibrosis is a deletion in ΔF_{508}

The father is heterozygote, Child #1 normal , Child #2 is heterozygote

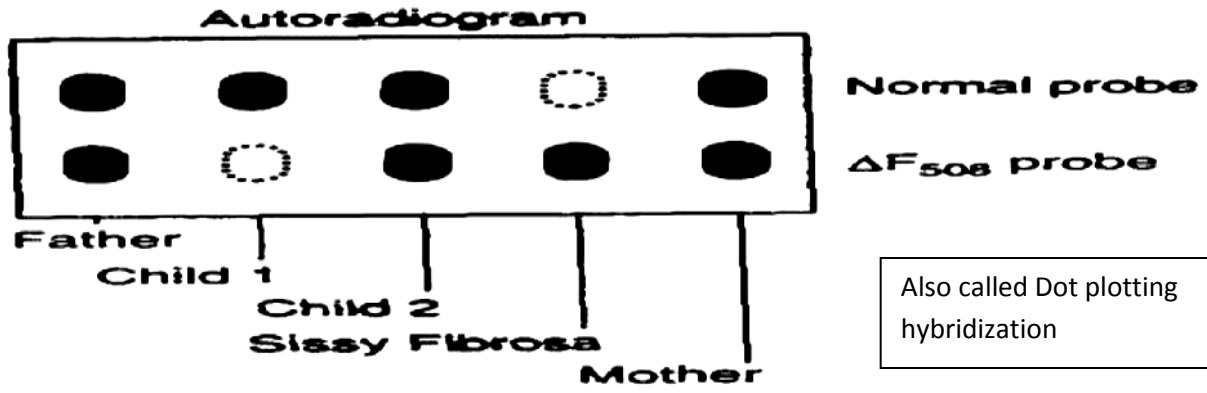
Child#3 is affected. And the mother is heterozygote.

The disease is cystic fibrosis, and it's AR so at least 2 carrier parents are needed for an affected child

3- detection of mutation by PCR (Just skip it)

4- Detection of mutations that contain highly variable regions: there are mutations that are caused by an increase (expansion) in the no. of tandem repeats like Huntington and fragile X syndrome.

-These sites have many names, sometimes they are called **VNTRs (variable number tandem repeats),** if the repeating unit was short, we call it **STR (short tandem repeats),** the repeating unit can be of 2 nucleotides, 20 or even 300. That's why sometimes we call them **micro-satellite**



Also called Dot plotting hybridization

regions and sometimes **mini-satellite regions**, but forget about all of these names, the name that the world is using is **STRs**, not VNTRs because in VNTRs the length of the repeating unit is 10-20 nucleotides, our book is still using it which is wrong, no one uses them anymore. While in the STRs the repeating unit is about 4-5, so that it would be shorter and the PCR would be faster so the time we need is much less.

*Note: In the exam, the questions will include **only** STRs **not** VNTRs even though the book uses it.

-To detect the mutation In this case, we measure the length of the area containing the repeats, if it is short then it is normal, if it is long then it is mutated, we determine the length using electrophoresis. But of course we should do PCR step first for the region we need (the region containing repeats); so that we won't do electrophoresis for the whole DNA.

-The book doesn't mention that we should take the specific region and apply PCR on it to amplify it before putting it on the gel to see its length directly. Instead, the book says that the whole genome undergoes restriction digestion then we put it on the gel then we do blotting, then we use a probe, to see the band, no one does this anymore (working on the genome), it will take about 3 days.

-Another importance for tandem repeats:

-Tandem repeats are most important in DNA fingerprinting. Most of them (99%) are found on the non-coding regions, as we said before, most of our non-coding sequence is made of repetitive sequences; tandem repeats, we don't know their function but we can use them in fingerprinting.

-Someone called Sir Alex Jeffreys didn't want to know their function or why they are present, but he wanted to make use of them; when he studied them, he took one of 10,000s of sites where tandem repeats are present, and he found out that all of us have the same sequence at that spot; for example the short arm of chromosome 17, we all have ATC repeats, but the difference was in the number of repeats, so, we have the same repetitive sequence at that single spot, but we differ in the no. of repeats, which can be used to distinguish between us.

-If we did this and found the same no. of repeats at that certain site between two people (which could happen by coincidence), we move to another site that contains repetitive sequences and if also this time -by coincidence- they had the same no. of repeats, we move to a 3rd site and so on until we find a difference. So the more sites of tandem repeats we study we are more likely to distinguish between individuals.

-And since we have two copies of each position, for each STR we have two different number of repeats (one maternal and one paternal) in each individual, so we should include this while comparing the STR between 2 individuals.

-Example (check slide #328): individuals A to C: at a certain STR (#1925)

A has on his maternal copy 6 repeats while on the paternal one 9.

B: maternal 3 paternal 15

C: maternal 12 paternal 18

Run them on a gel and you will find the small ones (6, 9) below, while 12 and 18 are higher, 3 will be below 6 and 9 while 15 will be above 12 and below 18. From one look you can know that these DNAs belong to different people.

-By coincidence 2 individuals can have the same numbers of repeats on both copies (A: 6, 9 – B: 6, 9) because we are 7 billion people. So, it's better if we take 2 STRs, each one has two numbers of course (maternal and paternal) and still two random individual can have similar no. of repeats in the 2 STRs. So now we will try to amplify (using PCR) 3 STRs (a total of 6 different numbers for each person), the chances are very low to find two individuals with similar 3 STRs/ 6 matching numbers (maybe only 2 or 3 people in the whole world), so to be more sure they didn't take 4, they took 13 STR positions (26 different numbers), in this case you can never find two similar individuals even if we included all humans that have died, the ones living now and the ones yet to live.

*Who chose the number 13?

Americans; Federal Bureau of Investigation (FBI)

They were the first people to use it widely in forensic medicine. They agreed to a specific 13 STRs out of 10s of thousands and the whole world followed them using the same 13 STRs.

To find a suspect they should have a database, and that's why they take the DNA fingerprints for people with previous criminal records in case they committed another crime.

Some countries have already started making a database for all their citizens (England, USA, Jordan). Jordan is about to build a large forensic medicine lab from a USA grant which is suspected to be aimed towards creating a DNA base for the Jordanian population.

They have agreed that out of tens of thousands of different STRs we will take those 13 regions. And we need this because every time we do finger printing, we should not use different STRs for different people, it is just that those STRs are the best choice and the whole world followed them including Jordan.
Of course, to find a suspect we need to have a data base and what have happened so far is that they have data bases for people with a criminal record who have done criminal acts so they can catch you next time. But some countries have started to take finger prints for the whole population and the leading countries that started this are USA , UK and JORDAN!! Yes Jordan have started this project and got a big grant and the goal is to have a data base for the whole population.

So again as we have said this is the old method , we get the DNA , cut it with restriction enzymes ,you run it on a gel and then you plot it from the gel to the membrane and from the membrane and within sudden plotting you highlight the band you want …. Nowadays no one does this anymore, we use one PCR for the 13 STRs or what is called multiplex PCR … they throw the primer of each position all together in one tube by one PCR and when you use the gel the band will be seen isolated in one shot so we don't need the sudden plotting any more ,instead we use gel electrophoresis .

So when you go to the crime scene and take a sample from a drop of blood or from nails of the victim or from hair that you have found there. you now obtain the DNA and do the finger printing and then we take the victim's DNA to know that this sample is not from him so when we know that this sample is not for him and it must come from another source , and it is not from the people that live in that crime scene after obtaining and testing their DNA so it is from unknown source ,then you see the suspects and you do fingerprinting and the one whose DNA is matched with the sample can't deny that he was in the crime scene, and this is the most definite evidence in any investigation and the only case that have acquitted this is the actor and athletic O.J Simpson . This man killed his wife and the man that she was cheating him with, and a helicopter filmed his car in the nearby street at the time of the crime, and they found his finger print in the scene, and for about a year people watched his trial .He denied the accusation and said that he was passing nearby accidently . They brought the Chinese scientist who confirmed that he did the test, but unfortunately Simpson escaped from the punishment he have a dream team of 11 lawyers and the defense was based on questioning of the reliability of the testing that even the scientist became very doubtful for his work ,and they asked him how he did this finger printing and the answer was PCR ,and they claimed that PCR do amplification for a small sample of DNA and if a very small piece of DNA was flying around in the lab and contaminate the sample from the crime scene it would become billions of copies after PCR so they destroyed the reliability of finger printing for the juries ,and became innocent in a ridiculous way . And this man after couple of years he went to jail for doing another crime!!
So finally the doctor said that your DNA finger print is like your barcode.
And to avoid any similar cases, labs have become very alert for any contamination in such cases using special kind of vacuums over the tubes in case molecules escaped.
finger printing has other applications , **paternal testing** and paternity fraud ,and they were doing this in JUH but they have stopped doing this, and police is doing it now for security reasons , so any child would have a mixture of band lengths that have either come from his father or from his mother ,so any band length that is found in the baby and is not found in neither the father nor the mother ,then they are not his biological parents .In the example in slide #330: daughter number 1: her band have similar copies either from the father or from the mother ,daughter number 2 have foreign bands and have bands that only the mother has so she is her mother but he is not her father.

And a famous story was that for Charlie Chaplin, a woman claimed that her son was his after many years, and there was no finger printing and even no blood group testing at that time, and unfortunately he paid a lot of money for this child and after years and years they found that he wasn't his son (after the discovery of blood grouping).

The professor asked to concentrate on these points about the techniques:

1- *Know the steps for each technique and which step is found in this technique not that.*
2- *The level at which the technique is used for (DNA, RNA, expression,…).*
3- *The uses of the technique.*

5- DNA chips (Microarrays):

This technique was invented by **Sir Edwin Mellor Southern** which after 20 years gained him 400 million.

What makes this technique different from other previous ones, which were working on a gene or a mutation at a time, is that this one can study diseases that are caused by many genes (20-40 genes) or cancers (200-400 oncogenes).

→ So simply the 400 genes can be fixed on this chip as **single strands** then the probe of the patient will be thrown on it to know which gene is mutated.

The chip is about (1.2 cm X 1.2 cm), all the 30,000 genes can be fixed on it with all the mutated forms (20-30) forms → there will be about (0.5×10^6)spots in the chip containing all the possible mutations for all genes, each spot contains a certain mutation in a certain gene.

This technique is used for diagnostic & research purposes:

Diagnostic: I- SNPs detection II- Expression profiling

I- SNPs detection/profiling

Steps:

1- Put the genes and all their mutated forms on the chip
2- Label the patient`s DNA (probe)
3- Throw it on the chip

→ DNA starts swimming on chip, catches the complementary for it and fluoresces.

→ So in one trial we can identify all our SNPs in all our genes.

II- expression profiling

Used to know which genes are working and which are not.

Steps:

1- Put the genes on the chip
2- Label the patient`s RNA (Why RNA? Because we are dealing with expression)
3- Throw RNA on the chip

→ Then Detect:

a- Which gene will be expressed/ON/ will fluoresce
b- Which gene won't be expressed /OFF / wont fluoresce
c- The level of expression by the intensity of fluorescing (higher level of expression →more RNA that binds → more fluorescence)

At the research level: I- to understand a new disease II- pharmacogenomics

I-understanding a new disease

→ Assume that we are studying a cancer, there will be some oncogenes that are up regulated and some tumor suppressor genes that are down regulated, so which is which?

Steps:

1- Bring 2 cells, one is cancerous and the other is normal
2- Extract the RNA of both cells
3- label each cell's RNA with different color (cancer RNA: Red, normal RNA: Yellow)
4- Mix both RNAs together and throw them on the chip

→ Then detect (remember: each spot on the chip represents a gene):

a- Dark spots: these genes aren't expressed (no RNA did bind to them)
b- Red spots: more RNA from cancer cells than from the normal cell (these genes are up regulated in cancer cells) (most likely to be oncogenes)
c- Yellow spots: more RNA from normal cells (these genes are down regulated in cancer cells) (most likely to be tumor suppressor genes)
d- Intermediate color: equal amounts of RNA from both cells (this gene`s expression didn't change by transforming from normal to cancer cell) (has nothing to do with cancer)

II_ Pharmacogenomics:

→Used to know how the drug works at the cellular level. Many drugs have been used by humans for 10s of years without knowing its mechanism at the cellular level, such as aspirin.

Steps:

1- Bring 2 cells, one is drug treated cell and other isn't
2- label each RNA with different color.
3- Mix both RNAs together and throw them on the chip

→ Then detect the genes that are up regulated and the genes that are down regulated and by that we know how the drug works on the cellular level.

Recombinant DNA Technology

And Treatment

<u>* Prevention of disease:</u>

E.g.: production of Hepatitis B vaccine

Before: the vaccine was a live attenuated microorganism (RNA/DNA lacking virus due to putting it in an organic solvent to degrade the RNA/DNA and the protein shell will be left and we will use it as a vaccine) but what if the solvent wasn't enough for degrading the DNA, then when injecting the vaccine it will contain the active virus so it will cause a disease instead of protecting from it.

Now:

1- Bring the virus
2- Determine the most antigenic protein on its surface and take the gene which encodes it
3- Clone this gene in bacteria so the bacteria will produce that protein
4- Put the protein in ampules and give it to people as a vaccine

→ So HB vaccine is the surface antigen of the virus that's gene is cloned in a bacteria and the surface protein is produced by it (this is genetic engineering)

→When infected with the virus after vaccination the body recognizes it as it saw it before and so on …

***Production of therapeutic proteins**

* As we took before Insulin & GH as simple proteins that can be produced in bacteria, but the complications appear with large proteins that require many post translational modifications, those proteins cannot be produced in bacteria and they have to be in a mammalian or a human cell.

To be in a mammalian or a human cell has many disadvantages:

a- The cell is dividing slowly
b- It need a viral vector
c- The quantity of protein produced is low
d- So, It will cost more

→ But because it is very useful we use it.

→Such as hemophilia A (**Hemophilia A** is a genetic deficiency in clotting **factor VIII**, which causes increased bleeding and usually affects males "X-linked recessive". Most patients affected produce factor VIII in insufficient amounts). 3ammo wiki :p

→ Before, the patients were treated by platelets transfusion, which will cause rejection, immune reactions, and most importantly after repeated transfusions this may transmit many diseases such as HIV, hepatitis, etc.

→ Now instead of doing that, **factor VIII** is produced in mammalian cells (because it can't be don't in bacterial cells) and given to the patient, the patient doesn't need the platelets, he only needs the protein which is factor VIII.

Si RNA ,, the dr. talked about it before, but it is not required in the exam.

Genetic counseling: just read the slide

The rest of the topics just skip them

Sorry for any mistakes