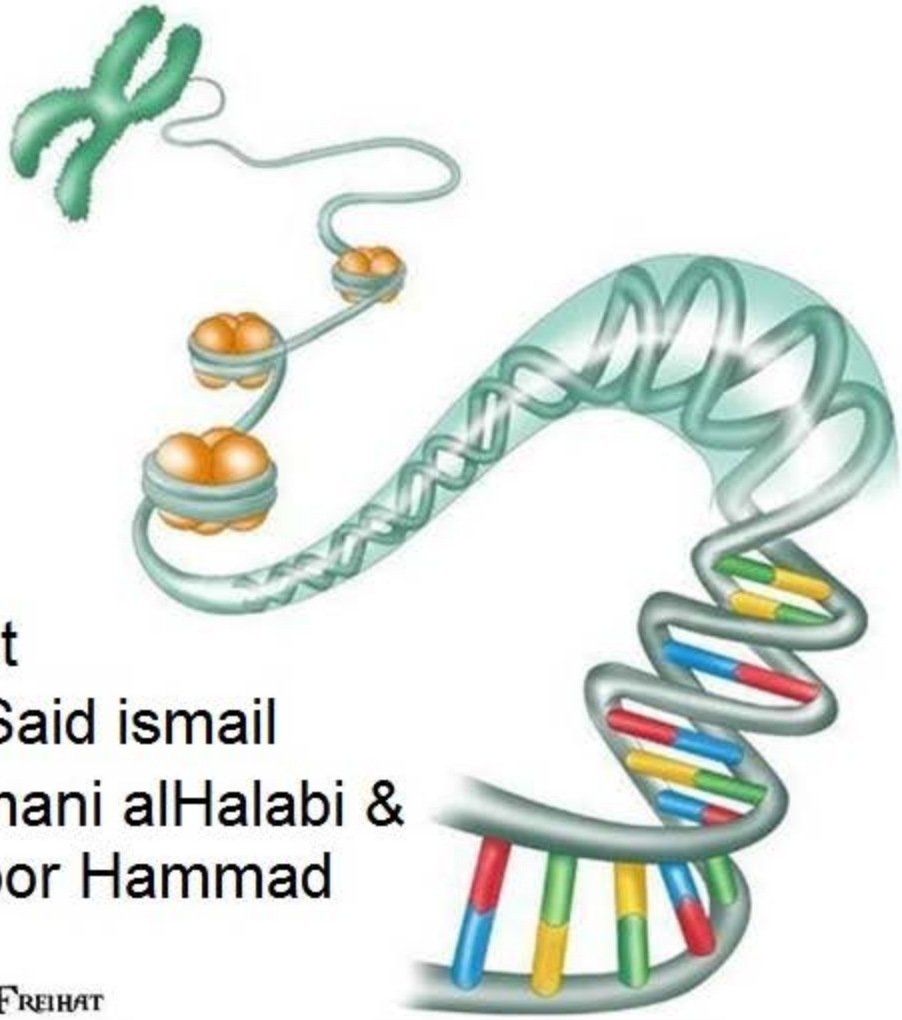
University of Jordan
Faculty of Medicine
Batch 2013-2019

Medical Committee
The University of Jordan

Genetics & Molecular Biology

○ Slides ● Sheet ○ Handout ○ other.....



Sheet#: Last

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Applied molecular biology - Chapter 17

This sheet is covering only some topics in chapter 17, you're concerned only with these topics not the whole chapter, so kindly pay attention. Also notice that the doctor skipped some slides and you don't need to refer to them. The number of the needed/discussed slide will be written beside the corresponding subtitle.

- Applications and Techniques

- The Molecular biology can be categorized into two sections;

1. Basic Molecular Biology

2. **Applied Molecular Biology**, aka **Recombinant DNA Technology** or **Genetic Engineering**.

→ These are techniques that enable the experts to manipulate the DNA sequences, for diagnostic, therapeutic, or preventive purposes.

- These techniques start to be developed through 1970s & 1980s, but the majority was developed in the 1990s and 2000s.
- This field of molecular biology is in a continuous change in an alarming speed, that the workers in the field themselves are finding it hard to keep pace with the changes. New techniques are emerging and will be established in the coming decades, while other old ones won't be used anymore.

- Let's start discussing some techniques:

1) DNA Sequencing – (Slide 314)

- A very first technique of sequencing: "**Sanger's sequencing**":
sequencing the DNA in a method related to a scientist called Sanger, Cambridge, UK.

- Sangers sequencing has been there for 30-40 years, it is very complicated, and luckily we won't discuss it.
- It reads 400-500 base pairs in each reaction, so if you want to sequence the whole genome "6 billion nucleotides", you need a huge number of reactions to be repeated and that consumes timeeeeee.
- The final draft of the Genome project for example was published in 2007/2008, it took them a decade to finish it, it took 6-7 billion dollars, and 4 main countries (US, UK, France, Japan) have been working on it to finally sequence one genome.
- It was followed by the emergence of the "Next Generation Sequencing".

- **Next (2^{nd}) Generation Sequencing**

- In around 2010.
- New techniques enable us to sequence a whole genome within 10 hours, in 1000 dollars, with only one small device.

- ***3rd* Generation Sequencing**

❖ By Oxford Nano-pore company, these days.
❖ The new sequencer is similar to a flash memory in size, it contains small chips and a very small hole in which you put your blood sample, and you insert it into a laptop and wait for hours. Or you can directly, at the real time, see your genome' sequencing on a screen.
❖ These chips will start to isolate your blood cells, amplify its DNA and sequence it for a million times or more. Highly Precise.
❖ The sequences of nucleotides are coming out through tiny pores (nanometers in diameter, and hence the name), and there simultaneously will be some guys (proteins) to read the nucleotides

that appear one by one. Imagine that you have millions of pores that work in the same manner.

➔ Sequencing your Genome will be available in labs as a routine test within the coming few years.

Implications and results?

- Clinically, you can read your medical future. Then you can look for SNP profiling (SNP = Single nucleotide polymorphism) to determine if a certain localized point mutation will increase the susceptibility to a certain diseases or not.
- Economic/social aspects: Health-insurance companies may start here to ask you to pay more, for you are in an increased risk for certain disease in the future. Pharmaceutical companies will also look for more earnings out of this.
- The good thing is that you'll be able to do something to prevent an expected disease.

2) PCR techniques, Polymerase Chain Reaction - (Slides 325-329)

- One of the important, highly used techniques that won't be replaced.

- "Polymerase": DNA polymerase.

- "Chain reaction": continuous reactions.

- In PCR, there are 25-40 cycles, each cycle amplifies the DNA many times. After 30 cycles, you will end up with 1 billion copies, assuming that you start the reaction with only one copy. Most times we originally start with thousands of copies..

- In all molecular techniques for different purposes, we need to amplify the DNA and work with a large amount of it. Small amounts won't be enough/useful.

- It mimics the DNA replication in a normal cell, but it's much faster.

- In a tube, you put: the selected **DNA** to be amplified, the **Polymerase**, **Primers** flanking the gene of interest to direct the polymerase to copy it only -or even some time the primers may define only one point mutation-, **dNTPs** (nucleotides in excess), and some **Buffers** (to afford a suitable pH for the enzyme.)

→ You can watch a lot of animations that show the full process smartly.

http://www.youtube.com/watch?v=2KoLnIwoZKU

- The polymerase will start to copy the selected area between the two primers and will finish the task in around two hours.

- Each cycle of the reaction has the following steps:

1- Denaturation of the two strands of DNA at **94** degrees. *(Remember that in a biological cell, the separating of the strands is done by the helicase. Since we don't have a helicase in the mixture, we heat the strands to separate.)*

2- Annealing step: Cooling the mixture to **50-60** degrees to allow the primers to anneal to the DNA strand. (*The degree is determined in respect to the length of the primer and its G-C content.)*

- ➔ Pay attention that there won't be a chance for the two DNA strands to anneal. If you cool it to 40 degrees, then the two DNA strands may re-nature!

3- Polymerization (Extension): Raising the temperature to **72** degrees for the polymerase to start working. (*The polymerase that we're dealing with is a **thermo-stable** one, it can tolerate high temperatures in the first step (not like our polymerases, or those of original viruses). It's obtained from a thermophillic bacteria named "**Thermus Aquaticus**" found around the boiling hot springs. The polymerase is called "**Taq Polymerase**".*

- ➔ We need to raise the temperature so that the Taq polymerase will be activated and will start its work. It won't work at low temperatures.

➔ Although this enzyme would prefer a temperature more than 85 degrees, but we can't afford it because the primers will break away.

- Each step of these takes from 15-60 seconds, thus each cycle takes around 2 minutes, and the whole 30 cycles will need 2 hours and end up having millions of copies; ***Exponential Amplification***.
- The whole reaction volume is about 25-50 microliters.
  (50 microliters = half a small droplet of a fluid)
- The whole mixture is placed in a machine, it's a cycler that will establish all the steps and repeat the cycles by itself.

- The PCR is important in almost all aspects of molecular biology.
  - ➔ **Cloning**; The insulin that's used by diabetics is a product of a human gene. This gene was isolated from human cells and cloned in bacterial cells, then these bacteria started to synthesize it. We take it, purify it, and give it again to patients.
  - Before we insert the gene to bacterial cells, we need to amplify it and insert millions of copies in millions of cells, then select some bacterial cells that acquired the gene and enrich them. We can't use only 1 or 2 copies of the gene.

  - ➔ **Diagnosis;** when a medical student in his clinical years, a doctor, or a nurse accidently get exposed to a blood from a +ve HCV patient, you need to know directly if he becomes infected or not. You can't wait for serology for it could take months to detect a viral antigen or an antibody.
  - **What to do?** Take a sample from his blood, although he's infected recently and the viral antigens are really low in amount in the blood, BUT you can amplify them.
  - Isolate the patients' DNA and the viral RNA from the sample, then introduce a polymerase and primers that will only attach to the viral RNA, the viral RNA will be amplified and noticed in tests.

ملاحظة :فقرات السواليف تم اقتطاعها ,فش وقت للسواليف بدنا نخلص

Note: we exclude the SAWALEEF part

By using PCR technique what I can do is the following, I will take a blood sample from a leukemic patient and DNA extraction procedures. Then depending on our previous knowledge about mutations and each type of leukemia has a specific mutation on a particular gene, so we amplify this particular gene. After two hours, we get the final result.

PCR in diagnosis of diseases

The PCR technique increases the sensitivity for early detection of leukemias and lymphomas. PCR allows for rapid and highly specific diagnosis of infectious disease, such as HIV and HCV.

A Student asked: what stops the DNA polymerase from continuing its working?

The doctor answered: great question, but there is no time to explain this topic .The primers differ from one segment to another and they direct the DNA polymerase to start working but do not control the time it stops working.

PCR can be used for genetic fingerprinting; it is used in, for example, parentage testing and criminal investigation.

. *Detection of mutations that contain highly variable regions*

- ✓ **It is used in DNA fingerprinting**
- ✓ **By detection of STRs** (Short tandem repeats)

- ➢ Most of our genome is non–coding (junk) and the vast majority of this non–coding is repetitive sequences.
- ➢ What do we mean by saying **repetitive sequences?** Tandem repeats (تكرارات متتابعة) that are exact in identity and repetition. **For example, when (ATTCATTCATTC) sequence is repeated several times in different regions on different chromosomes.**

- We all have the same **Repetitive DNA sequence in the same locations but we vary in the number of these repetitions** (These repeat sequences tend to vary in length among different individuals and are called Short tandem repeats).

- ➢ The cause for this difference is that DNA polymerase may forget copying a certain number of the repetition. For example, if you write your full name 500 times, you may make a mistake in the total number.

- ➢ **Scientists benefitted from this phenomenon to discriminate between individuals** (Enabling scientists to distinguish one DNA sample from another.)

☒ **Someone has 12 repeats of a specific sequence (band A) and someone else has 18 repeats from the same sequence on the same chromosome (band B)** These STR loci (locations on a chromosome) are targeted with

sequence-specific primers and amplified using PCR. The DNA fragments that result are then separated and detected using electrophoresis . The DNA fragments that result are then separated and detected using electrophoresis.

☒ **We find that band B is larger than band (A) (the length of bands depends on the number of repeats they contain). Even if we have the same number of repetitions in this region, we should not be similar in many other repetitions** (people almost certainly have different numbers of repeat units)

☒ **Each individual has two homologues of every somatic chromosome and thus two genes each containing repetition region with a variable number of tandem repeats, so when I compare 4 chromosomes between two individuals, actually I take 8 regions and the possibility of similarity in the number of these repetitions is decreased.**

☒ **The true power of STR analysis is in its statistical power of discrimination. Because of the 13 chromosomes (26 loci) that are currently used for discrimination in APF.**

☒ **Concentrate here please**
The book says that
1-We use VNTR (variable number of tandem repeats), but we use STR (short tandem repeat) and the difference between them is:
The repeat unit in VNTR is larger **than the STR** (the most common is 4 bases repeated)

- **How do we know the number of repetitive units in each person?**
  By using electrophoresis that is used to move the molecules through the gel matrix

✓ **The shorter length is the faster movement of the band (it will be at lower position).**

✓ **The longest bands are located in higher places than shorter bands**

✓ **If there is two bands at the same line that means they have the same length**

✓ **2-The second difference is; the book says we cut the whole genome into small segments by specific enzymes then they will be added to the GEL in order to get thousands of bands and this technique became a part of the past.**

✓ The system of DNA profiling used today is based on PCR and uses short tandem repeats (STR). This method uses highly polymorphic regions that have short repeated sequences of DNA (the most common is 4 bases repeated, because unrelated people almost certainly have different numbers of repeat units, STRs can be used to discriminate between unrelated individuals. These STR loci (locations on a chromosome) are targeted with sequence-specific primers and **amplified using PCR**. The DNA fragments that result are then separated and detected using electrophoresis. There are two common methods of separation and **gel electrophoresis.**

Forensic DNA Fingerprinting

✓ STR analysis is a tool in **forensic analysis** that evaluates specific STR regions found on nuclear DNA. The variable nature of the STR regions that are analyzed for forensic testing intensifies the discrimination between one DNA profile and another. Fingerprints are

used to identify an unknown victim and most importantly, as links and matches between a suspect and a crime.

Paternity DNA Fingerprinting

✓ **Fingerprints** are widely applied to determine genetic family relationships such as paternity and maternity

- Each person's DNA contains two copies of these markers—one copy inherited from the father and one from the mother

Are one's fingerprints similar to those of his or her parents in any discernable way?

- Since a child receives half of his or her DNA from the mother and the other half from the father, paternity can be established. A DNA fingerprint is obtained from the mother, the child, and father. These fingerprints are compared with each other. Every band that appears in the child's fingerprint must come from either the mother or the father. If there is a band in the child's fingerprint that does not appear in the mother's or the father's fingerprint, then. Then the child is not their son.

- Note

- **You should study:**
  - **DNA chips (Microarrays):**
    - **Slide number 339**

Dedication to: Salsabeela Ban i Hammad ,Sireen Al –khatib Majeda Al -Foqara

امنة حماد و منار المحسييري

جميل أن تكون من الذين يسعون للتغيير ,ولكن الأجمل أن تبدا بنفسك

Written by

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Noor Hammd