Molecular Biology

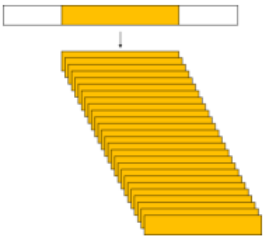
2025-2024

DR.Ahmad Al Qawasmi

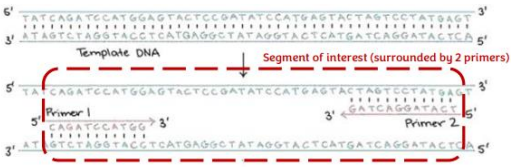
ARKAN ACADEMY

Polymerase Chain Reaction (PCR)

- It is a technique that allows the DNA from a *selected region* of a genome (even if it's tiny) to be *amplified* a billionfold and effectively *purifying* this DNA away from the remainder of the genome
  - It is ***extremely sensitive***; it can detect a single DNA molecule in a sample



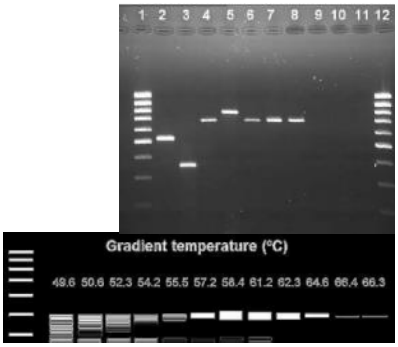
- Components of PCR:
  - *DNA template* (sample to be amplified)
  - *Pairs of primers*
    - ✓ 15-25 nucleotides-long *RNA* primers should ***surround the target sequence***
  - *All four deoxyribonucleoside triphosphates*
  - *A heat-stable DNA polymerase*
    - ✓ Suitably heat-stable DNA polymerases that have been obtained from microorganisms whose natural habitat is hot springs such as ***Taq DNA polymerase*** is obtained from a ***thermophilic*** bacterium, Thermus aquaticus, and is thermostable up to 95°C



- Steps of PCR:
  - ***Denaturation (at 95°C):*** DNA is denatured into single-stranded molecules
  - ***Annealing (50°C to 70°C):*** The primers anneal (bind, hybridize) to the DNA
    - ✓ It is done in *high temperature to ensure the specific* (perfect) hybridization
  - ***Polymerization or DNA synthesis (at 72°C):*** optimal temperature for the polymerase

- *20-30 cycles* of reaction are required for DNA amplification
  - The products of each cycle serve as the DNA templates for the next products so it is a chain reaction
  - Every cycle doubles the amount of DNA (number of fragments = 2^n)
  - After 30 cycles, there will be over 250 million short products derived from each starting molecule

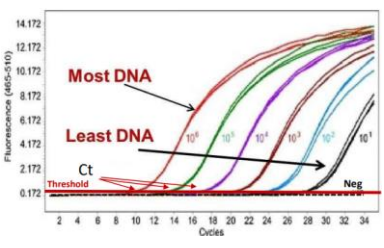
- The intended segment is amplified, and when visualized using agarose gel electrophoresis, it appears as a *discrete band*



- What is responsible for the specificity of PCR?
  - *Specific primer*
  - *High temperature* of annealing

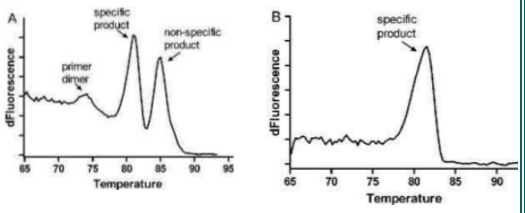
- PCR is used to study molecular fingerprinting, genotyping, genetic matching, mutations, mutagenesis, prenatal diagnosis, cloning, detection and classification of organisms, molecular archaeology

- ***Real-time quantitative PCR (qPCR):*** A way of relative ***quantitation*** of amount of DNA in a sample is by amplifying it in the presence of ***SYBR*** green which can bind dsDNA and fluoresces when bound
  - The higher the amount of DNA, the sooner it is detected

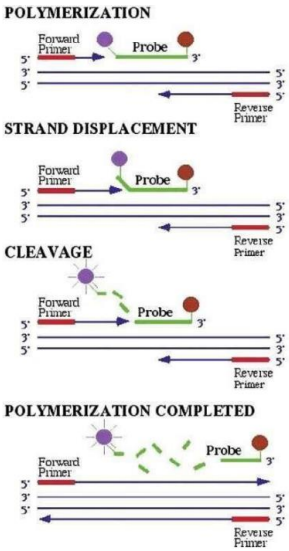


- ***Threshold cycle (Ct):*** The cycle in which the signal is firstly detected
  - It is measure and indicator of the starting amount of DNA
  - As the ***Ct is lower***, that indicates ***larger initial amount*** of DNA
  - Signal is not detected in the cycles before the Ct because the amount of DNA is very small, giving a very small amount of signals, which can't be detected due to the limitation of the instruments

- *Melting curve analysis* of qPCR is an indication of its ***specificity***
  - If there is one peak, it is a specific PCR
  - If there is more than 1 peak, the PCR is not specific



- ***Taqman qPCR:*** It is a *more specific*, *sensitive*, *reproducible* and *multiplexing* than SYBR qPCR
  - It uses ***DNA probe*** instead of SYBR green
  - The probe is complementary to a specific sequence in the intended segment
  - It is bound to a *reporter* (give signal) and a *quencher* (inhibit reporter), where the signal is only emitted when they are away from each other
  - While DNA is replicated during PCR, the polymerase will break down the probe by its 5' to 3' exonuclease activity causing the emission of signal



DNA sequencing

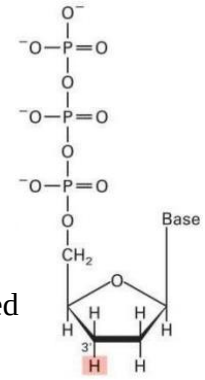
- DNA sequencing is the process of determining the exact *order of nucleotides* in a genome, important to:
  - Identification of genes and their localization.
  - Identification of protein structure and function.
  - Identification of DNA mutations.
  - Genetic variations among individuals in health and disease
  - Prediction of disease-susceptibility and treatment efficiency
  - Evolutionary conservation among organisms

- First genomes to be sequenced were *Viruses and prokaryotes* genomes, then Human *mitochondrial DNA*
- The first eukaryotic genome sequenced was that of yeast, *Saccharomyces cerevisiae*
- The genome of a multicellular organism, the nematode *Caenorhabditis elegans*
- Determination of the base sequence in the *human* genome was initiated in 1990
  - The number of protein-coding genes is less than 20,000
  - The number of regulatory elements is significant (more than 30% of the genome)
  - The non-coding genes (transcribed but not translated) such as microRNA and long noncoding RNA appear to be relevant (not mere noise)

- More complex organisms ***do not have*** to have a <u>larger genome</u> or <u>more protein and mRNA-coding genes</u>
  - More complex organisms ***usually*** have more miRNA and lncRNA

- Methodes of DNA sequencing:

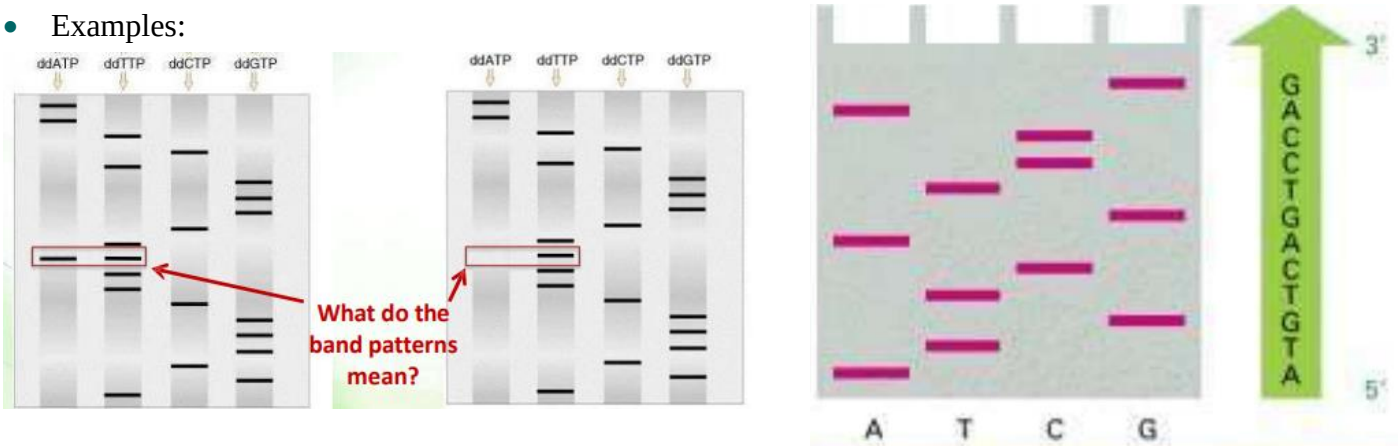
1. The basic method of DNA sequencing (Sanger Method)



- The most popular method is based on ***premature termination*** of DNA synthesis by *dideoxynucleotides (ddNTP)*
  - Dideoxynucleotides *lack OH group on the 3'* carbon impeding it from forming phosphodiester bond with the next nucleotide
- The process involves DNA synthesis which is initiated from a primer that has been labeled with a radioisotope and using ***4 separate reactions*** are run, each including *all the deoxynucleotides* plus, *one dideoxynucleotide* (either A, C, G, or T)
  - Incorporation of a dideoxynucleotide ***stops*** further DNA synthesis
  - A series of labeled DNA molecules are generated, each terminated by dideoxynucleotide
  - Fragments are then separated according to size by gel electrophoresis and detected by exposure of gel to X-ray film, where the size of each fragment is determined by its terminal dideoxynucleotide, so the DNA sequence corresponds to the order of fragments read from the gel

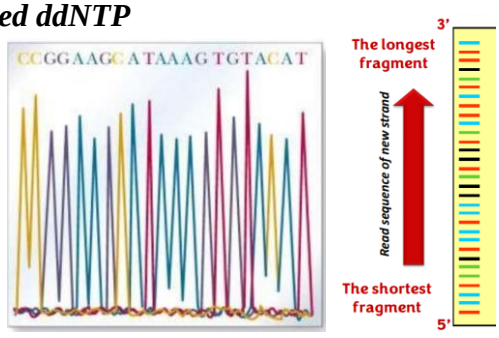
- If 2 bands in different reactions had the ***same position***, that means that the individual is ***heterozygous*** meaning that he has different alleles on the homologous chromosomes

- Examples:

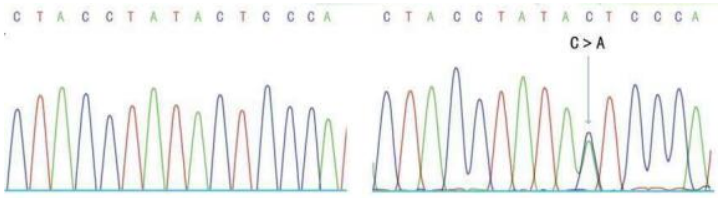


2. Fluorescence-based DNA sequencing

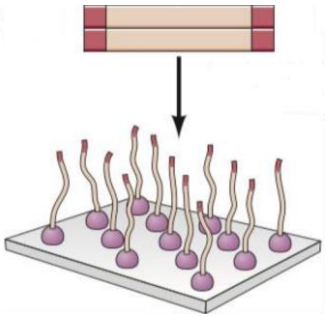
- Reactions include the four deoxynucleotides (dNTP) plus the four dideoxynucleotides (ddNTP) in the ***same reaction*** with each ddNTP labeled with a ***unique fluorescent tag***
  - It does not use radioactivity, instead it uses ***fluorescence-labeled ddNTP***
  - All fragments are then separated by gel electrophoresis and the sequence can be known using the colors
  - A computer program was done to detect the colors and analyze them into waves



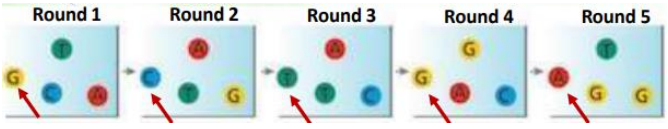
- If 2 waves or bands had the same position, the individual is ***heterozygous***



3. Next-generation sequencing



- Cellular DNA is fragmented
- ***DNA adapters*** are added to the ends of each DNA fragment
- Each DNA fragment is attached to a solid surface and amplified like PCR using primers that anneal to the adapter sequences
- Four-color ***special nucleotides (chemically modified)*** are added and a single nucleotide is incorporated
  - The color is detected by a special camera and it is the nucleotides is activated to allow for the addition of the subsequent nucleotide
  - The nucleotides are modified by *fluorophore* where laser must remove it
- Cellular DNA is fragmented, adapters are ligated to the ends of each fragment, and the double-stranded fragments are denatured to single strands which then annealed to complementary adaptors that are immobilized on the surface of a flow cell and amplified by PCR, forming fragment clusters each originating from a distinct DNA fragment
- The sequences of millions of fragments are generated and then they can be assembled into a contiguous sequence by identifying fragments with overlapping sequences



ARKAN

ACADEMY

علم في كل مكان

Arkan academy

www.arkan-academy.com

Arkanacademy

+962 790408805