DNA Sequencing (Lec7)

٣ طرق لمعرفة تسلسل ال DNA:-

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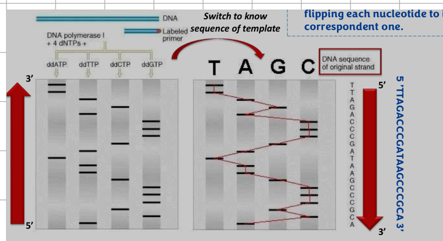
*Sanger method of DNA sequencing involves premature termination of DNA synthesis using dideoxynucleotides (ddNTPs), which lack a 3' hydroxyl group and stop further elongation. Here's a summary of the process:

this is the basic methode.

Reaction Setup: Four separate reactions are performed, each containing:
DNA template (single-stranded),
Radiolabeled primer,
DNA polymerase,
Regular deoxynucleotides (dNTPs), and
A small amount of one specific ddNTP (A, C, G, or T) for each reaction.
DNA Synthesis: DNA polymerase starts synthesizing a complementary strand from the primer. It incorporates normal dNTPs unless it encounters a ddNTP, which causes premature termination at that point because ddNTPs lack the 3' OH group needed to add the next nucleotide.
Fragment Generation: Each reaction generates a series of DNA fragments, all terminated at different points based on where the ddNTP was incorporated. These fragments are labeled with a radioactive isotope.
Gel Electrophoresis: The fragments are separated by size using gel electrophoresis. Smaller fragments migrate faster, and the gel is then exposed to X-ray film to detect the labeled fragments.
Bottom to top of the gel gives you the complementary strand sequence (5' to 3').
To find the sequence of the original template strand, you need to flip the complementary strand's sequence.



When two DNA fragments (bands) align at the same level, it indicates that the person is heterozygous at that specific gene or sequence. Since humans are diploid (having two copies of each chromosome—one from each parent), one allele is inherited from the mother and the other from the father. In this case, the difference between the two bands could be due to a single nucleotide polymorphism (SNP).
Case 2:
In contrast to the previous case, if there is only one band in the gel for a particular sequence, this indicates that the person is homozygous for that position, meaning both alleles have the same nucleotide. For example, if both alleles have a T at the same position, there will only be a single band at that level, because there is no SNP or variation between the two alleles. This suggests that the person is homozygous at this locus due to the specific polymorphism or mutation.

• هاي أول طريقة استعملناها لنعرف التسلسل لل DNA ، فيها radioactive probe (مضرة) لذلك دورنا على طريقة أخرى.

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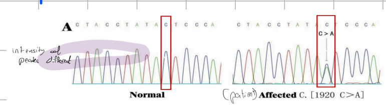
fluorescent tags on dideoxynucleotides (ddNTPs), making it safer and more efficient.
Key Steps:
Single Reaction: Unlike the older method with four separate reactions, this technique uses one reaction. It includes:
Single-stranded DNA templates,
DNA polymerase,
Primers,
A mix of dNTPs (normal nucleotides) and ddNTPs (fluorescently labeled).
DNA Synthesis: The DNA polymerase synthesizes a complementary strand from the primer. When the polymerase encounters a nucleotide (e.g., A), it adds either a normal nucleotide (e.g., dTTP) or a fluorescently labeled ddNTP (e.g., ddTTP). When ddNTP is added, the synthesis stops.

فوق ، رحنا الجيل و X-ray لنشوف القطع

Fluorescent Labels: Each ddNTP is labeled with a different fluorescent color, allowing for detection by a laser. This makes it safer than using radioactive labels.
Electrophoresis: The DNA fragments, which differ in length by one nucleotide, are separated by gel electrophoresis. As they pass through a laser beam, the fluorescent labels emit light, which is detected and recorded by a computer.



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*Next-Generation Sequencing (NGS) involves two main stages: cluster formation and sequencing by synthesis.

1. Cluster Formation:

This stage prepares the DNA for sequencing by creating clusters of identical DNA fragments.

DNA Fragmentation: The cellular DNA is first fragmented into small pieces.

Adapter Addition: Short DNA adapters are added to the ends of each DNA fragment. These adapters are essential for the next steps (e.g., attaching to the sequencing surface).

Denaturation: The double-stranded DNA fragments are then denatured (separated into single strands).

Attachment to Solid Surface: The single-stranded fragments are attached to a solid surface (e.g., a flow cell). This surface contains complementary sequences (adaptors) that bind to the DNA fragments, ensuring each fragment is anchored at a specific location.

PCR Amplification: Using PCR-like amplification, each DNA fragment is copied many times, creating a cluster of identical DNA fragments at each position on the surface. Each cluster comes from a single original DNA fragment.

Barcoding: The adapters can also contain barcode sequences, which can label samples from different individuals or conditions, allowing for multiple samples to be processed together.

بتعرف تسلسل أكتر من Sample بنفس التجربة

اي بعين هاي الطريقة

2. Sequencing by Synthesis:

This is where the actual sequencing takes place, and it involves determining the nucleotide sequence of the DNA.

Primer Binding: A primer is added that binds to the adapter sequence on the DNA fragment.

Addition of Special Nucleotides: Four types of special fluorescently labeled nucleotides (A, T, C, and G) are added, but these are different from regular nucleotides (they are not dideoxynucleotides or normal deoxynucleotides).

DNA Polymerase Action: DNA polymerase adds a nucleotide to the growing strand, based on the template DNA. Only one nucleotide is added per cycle.

Fluorescent Detection: After each nucleotide is incorporated (= joining), the fluorescent signal (color) corresponding to that nucleotide is detected by a special camera. Each color represents a specific nucleotide (A, T, C, or G).

Clearing and Repeating: After detecting the fluorescence, the chemical modifications on the nucleotides are removed (The fluorophore and chain-terminating modifications are then removed,), allowing the addition of the next nucleotide in the sequence. This process repeats in cycles.

Cycle Repetition: The cycle is repeated multiple times to build up the entire sequence of the DNA fragment. Each cycle adds one nucleotide, and the sequence is read from the emitted fluorescent signals.

Cluster Sequencing: Since each cluster represents many identical DNA fragments, the sequence of each cluster can be determined independently. These sequences range from 50 to 300 nucleotides in length.

Final Steps:

Sequence Assembly: After sequencing millions of fragments, the sequences are assembled by identifying overlapping regions. This allows the fragments to be pieced together into a complete contiguous sequence (the full genome or target region).

Massive Parallel Sequencing: The key advantage of NGS is that it can sequence millions of DNA fragments simultaneously, making it much faster