

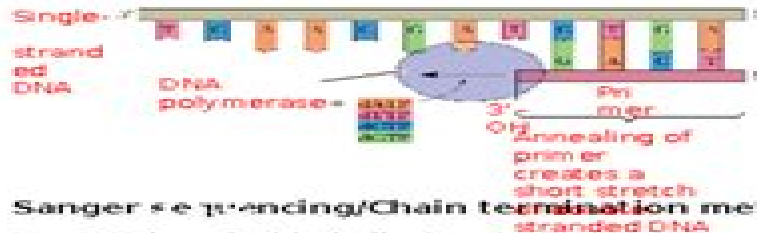
Study Section 3 - DNA

Technique used to determine structure of DNA/order of nucleotides

1. Restriction Enzymes
 - Cleave DNA at specific sites → fragments of manageable size
2. Electrophoresis
 - Method separate fragments based on size

These tools paved the way for more efficient technologies for nucleotide sequencing

1. Using DNA replication as model, the chain termination method allowed for the generation of polynucleotide fragments
- Focus on Sanger sequencing method because it is the technique used most modern
- 2 strands separate
 - Each strand now serves as a template strand
 - Template strand
 - Incubated with 4dNTPs
 - Enzyme: DNA polymerase (DNA polymerase because DNA replication is taking place)
 - Nucleotide sequence of 1 strand copied in complementary fashion to new strand → 2 daughter strands
 - Primer is the starting point
 - In vitro: reaction will take place provided: primer
 - Primer: Oligonucleotide capable of forming a short stretch of dsDNA when it bp ssDNA
 - Primer important: free 3'OH to which allows for next set nucleotide to be added.
 - Chain grows in 5' to 3' direction



Sanger sequencing/Chain termination method

- DNA is a double helical molecule.
- In the course of its replication, the sequence of nucleotides in one strand is copied in a complementary fashion to form a new second strand by the enzyme DNA polymerase.
- Each original strand of the double helix serves as a template for the biosynthesis that yields two daughter DNA duplexes from the parental double helix.
- DNA polymerase will carry out this reaction in vitro in the presence of the four deoxynucleotide monomers and copy single-stranded DNA, provided a double-stranded region of DNA is artificially generated by adding a primer.
- This primer is an oligonucleotide capable of forming a short stretch of dsDNA by base pairing with the ssDNA.
- The primer must have a free 3'-OH end from which the new polynucleotide chain can grow as the first residue is added in the initial step of the polymerization process.

Study Guide Section 3 Dna Technology Continued

Jianjun Gao



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