What is DNA Sequencing?

Worksheet

DNA sequencing reads the precise order of A, T, G and C bases in a DNA molecule, most commonly using Sanger chain-termination or next-generation sequencing (NGS) technologies.

Questions

1. Which four bases does DNA sequencing determine the order of?

A) A, T, G, C
B) A, U, G, C
C) A, T, G, U
D) C, G, U, T

2. What stops DNA synthesis in Sanger sequencing?

A) Restriction enzymes
B) Dideoxynucleotides (ddNTPs)
C) DNA ligase
D) Helicase

3. What technology separates DNA fragments by size in Sanger sequencing?

A) PCR
B) Capillary electrophoresis
C) Mass spectrometry
D) Centrifugation

4. Compared to Sanger sequencing, next-generation sequencing (NGS) is generally

A) Slower and more expensive
B) Faster and can process many fragments in parallel
C) Only useful for RNA
D) Unable to detect mutations

5. A Sanger sequencing reaction produces fragments ranging from 1 to 800 bases long. How many distinct fragment lengths are theoretically possible?

6. A human genome has about 3.2 billion base pairs. If a sequencer reads 150 bases per run and covers the genome 30x, how many total bases must be sequenced?

7. A researcher sequences a 1,200 base-pair gene using Sanger sequencing with a 700-base read length in both directions (forward and reverse). Is single-pass coverage enough?

8. Define: What is DNA sequencing?

9. Define: What is Sanger sequencing?

10. Define: What is Next-Generation Sequencing (NGS)?

Answer Key

1. A) A, T, G, C - DNA uses adenine, thymine, guanine and cytosine; RNA replaces thymine with uracil (U).

2. B) Dideoxynucleotides (ddNTPs) - ddNTPs lack the 3'-OH group needed to add the next nucleotide, terminating the chain.

3. B) Capillary electrophoresis - Fragments migrate through a capillary at speeds based on their length.

4. B) Faster and can process many fragments in parallel - NGS parallelizes millions of reactions, massively increasing throughput and lowering cost.

5. Fragments can end at any base position from 1 to 800 Number of distinct lengths = 800 1 + 1 = 800 Each length corresponds to one base call in the final read

6. Total bases needed = genome size coverage Total bases = 3,200,000,000 30 = 96,000,000,000 bases At 150 bases per read: 96,000,000,000 150 640,000,000 reads needed

7. Forward read covers bases 1-700 Reverse read covers bases 501-1,200 (reading backward from the end) Overlap region (501-700) confirms accuracy of 200 bases Combined, both reads span the full 1,200 bases with overlap for error-checking

8. Determining the exact order of A, T, G, and C nucleotide bases in a DNA molecule.

9. A chain-termination method using fluorescently labeled ddNTPs to stop DNA synthesis at each base, then reading fragment lengths.

10. High-throughput methods that sequence millions of DNA fragments in parallel, much faster and cheaper than Sanger sequencing.