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Exercise 9

DNA Sequence Analysis and the Genetic Code

Introduction:

DNA sequence analysis involves the determination of the **primary structure** of the DNA molecule in a cell or individual. The structure is the **nucleotide sequence** or the **relative order of nucleotide pairs** in a stretch of DNA, a gene, a chromosome, or the genome. The nucleotide sequence is said to be the most basic property of the gene.

All DNA sequencing protocols involve a common procedure. A protocol will generate a series of single stranded DNA molecules, each molecule one base longer than the previous one. DNA molecules of the *same sequence* but differing in length by one base at the end can be separated by gel electrophoresis on acrylamide gels. Bands corresponding to molecules of increasing length form a "ladder" that can be followed for up to 300 molecules.

The Sanger Method of DNA Sequencing

In 1975, Frederick Sanger and his colleagues developed a method for determining the nucleotide sequence of a defined piece of DNA. The method came to be known as the **Sanger method** or **dideoxy chain termination procedure**. The main feature of this procedure is the use of **enzymatic reactions**.

The process is carried out in each of 4 tubes that contain the necessary materials for DNA synthesis. These materials are the following:

1. The **single-stranded DNA** (of unknown sequence) which will serve as the template;
2. The oligonucleotide **primer** that will start DNA synthesis;
3. The **DNA polymerase** that will catalyze the reactions;
4. The **dideoxyribonucleotides (ddNTP's)** – ddATP's, ddCTP's, ddTTP's, and ddGTP's that will be added to the growing nucleotide chain.

The basic idea in the Sanger method is that at this point, each tube will be added with a different **deoxyribonucleotide (ddNTP)**. For example, ddATP is placed in tube 1 so that chain termination will occur with A's, ddCTP in tube 2 so chain termination will occur with C's, ddGTP in tube 3 so that chain termination will occur with G's, AND ddTTP in tube 4 so chain termination will occur with T's.

The result is a series of fragments of different lengths in each tube. In tube 1, all the fragments end in A; in tube 2, all end in C; in tube 3, all end in T; and in tube 4, all end in G.

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