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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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H. S. Luker,A. J. Luker



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Daniel Johnson, 2009 Drawing from the author's own work as a lab developer coordinator and instructor this one of a kind text for college biology teachers uses the inquiry method in presenting 40 different lab exercises that make complicated biology subjects accessible to major and nonmajors alike The volume offers a review of various aspects of inquiry including teaching techniques and covers 16 biology topics including DNA isolation and analysis properties of enzymes and metabolism and oxygen consumption Student and teacher pages are provided for each of the 16 topics

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Exercises Eldon John Gardner, 1956

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