Section: PSY6B Score:

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;
- 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.

Lab Exercise 9a Genetics Answers

Léo Pomar

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