Dye Terminator Reaction

Narrator: *Each reaction requires multiple copies of a double-stranded DNA template, which is a fragment from the genome being sequenced; many copies of a specific single-stranded oligonucleotide primer, which acts as a starting point for DNA synthesis; Taq DNA polymerase, an enzyme that can add nucleotides to a DNA strand during DNA synthesis; free floating single nucleotides; and some fluorescently labeled terminator nucleotides that are designed to stop DNA synthesis.*

Changes in temperature are the key to making the dye terminator reaction work. First, the reaction mixture is heated up to 96 degrees Celsius so that the double-stranded DNA template denatures and becomes single-stranded. Second, the reaction mixture is cooled down to 50 degrees Celsius so that the oligonucleotide primer can base pair with, or anneal to, the DNA template. Third, the reaction is warmed up to 60 degrees Celsius, so Taq DNA polymerase can perform DNA synthesis. Taq is able to put free-floating nucleotides into the correct places along the DNA template so that a new complementary strand of DNA is extended from the primer. However, when a terminator nucleotide is put in place, Taq is no longer able to add more nucleotides and DNA synthesis is stopped.

Multiple rounds of temperature cycling are necessary for the reaction mixture to generate an array of DNA fragments of differing lengths. The PCR machine is able to make rapid transitions between the different temperatures. Each time the reaction mixture is heated for denaturing, cooled for annealing, and warmed for extension, more DNA fragments are created.

The fluorescently labeled terminator nucleotides each have their own specific color. A is green, T is red, G is yellow, and C is blue. After the multiple rounds of temperature cycling, any DNA fragments that can fluoresce green must have an A as the terminating nucleotide. Those that can fluoresce red must have a T at the end. Those that can fluoresce yellow must have a G at the end and those that can fluoresce blue must have a C as the terminating nucleotide. The random assortment of free-floating nucleotides and fluorescently labeled terminator nucleotides ensures that the newly synthesized DNA fragments are each terminated at a different place along the DNA template sequence. The goal is for the reaction to result in an array of fluorescent DNA fragments that have only a single nucleotide difference in length. The sequencing reaction is considered finished when multiple copies of every possible DNA fragment have been generated.

All of the fluorescently labeled DNA fragments created in the dye terminator reaction now need to be separated by size. This is possible with gel electrophoresis. When the fragments are put in order from smallest to largest, and the terminating fluorescent base color is analyzed, then the corresponding terminating nucleotide can be identified.