PCR and DNA Sequencing

Polymerase Chain Reaction (PCR) is a process that can be used to amplify (copy) the number of copies of a target fragment of DNA.

- First, the DNA is heated, which causes the two strands to denature (come apart).
- Primers are then added, and they form a type of bracket around the region of DNA that is to be amplified.
- A polymerase, which synthesizes DNA, is added so that a new DNA strand can be created. This process (shown in Figure 1) is repeated as many times as needed to get the amount of DNA that is necessary for further experimentation.

One method of sequencing a strand of DNA is called “Dye-Terminator Sequencing” (Figure 2). Dye-Terminator Sequencing allows scientists to sequence DNA much quicker. This benefit of Dye-Terminator Sequencing is one reason why it is a preferred method by today’s scientists.

- First, modified versions of nucleotides are used to separate the DNA that was amplified during PCR into four distinct groups based on their nucleotide base.
- Then, each chain terminator nucleotide is labeled with a different fluorescent dye.
- Each of these dyes will fluoresce at a different wavelength, allowing the reactions to be distinguished from one another.
- Scientists are then able to sequence the DNA by recognizing whether an A, T, G, or C is present based on the color of the fluorescent reaction (Figure 3).
Figure 1: Steps in PCR: http://www.flmnh.ufl.edu/cowries/amplify.html

Figure 2: Steps of Sequencing
http://www.dnasequencing.com/tag/dna-sequencing-image/

Figure 3: Fluorescent peaks detected by sequencing machine
http://en.wikipedia.org/wiki/DNA_sequencing#Dye-terminator_sequencing

Information from:
http://www.bio.davidson.edu/Courses/Molbio/MolStudents/spring2003/Obenrader/sanger_method_page.htm

http://en.wikipedia.org/wiki/DNA_sequencing