BioPharmaceutical Technology Center
I N S T I T U T E

PCR to Detect Genetically Modified Organisms (GMOs)

Field Trip Background

Background Information

A genetically modified organism (GMO) is when an organism's genetic make-up has been changed using recombinant DNA technology, to produce something non-native to the species. There are many examples of GMOs including transgenic animals, fish, plants, and microorganisms. A specific example of genetic modification would be the introduction of the human insulin gene into *E.coli* to produce human insulin. Today GMOs are being developed and used world-wide; in the United States most of our corn and soybeans have been genetically modified.

Farmers have been doing selective breeding in crops for thousands of years. In early farming, seeds from plants that produced desirable traits (taste, color, size, etc) were saved and used the following season. Also, cross-bred plants or "hybrids" have been developed for many types of crops. These plants are not considered GMOs, because they are naturally manipulated, the introduction of the new gene or trait does not require recombinant DNA techniques. To create a GMO there are many methods of introducing a new gene into a plant. The most common recombinant DNA technique utilizes *Agrobacterium*, a soil bacteria, and a plasmid (Ti) which has a promoter from the cauliflower mosaic virus (CMV). We will use the Polymerase Chain Reaction in this lab to detect whether the food samples (corn chips and corn meal) have been genetically modified by amplifying a region of the 35S promoter region of the CMV (195 bp).
[a]

In 1983 Kary Mullis, a scientist at the Cetus Corporation in California, imagined a way to replicate (copy) DNA in the lab. He worked on the idea for two years, and in 1985 published and filed a patent for his idea. In 1993 he won the Nobel Prize in Chemistry for his work developing the Polymerase Chain Reaction. The Polymerase Chain Reaction (PCR) is now a very widely used technique for copying DNA. Starting with only a small amount of a DNA sample, PCR can generate many copies of a specific DNA segment to be used for further analysis. This process is also called DNA amplification. PCR has revolutionized molecular biology, and is now routinely used in biological research, forensics (criminal investigations), medical testing, and anthropology.

During the PCR-GMO Field Trip, students will isolate DNA from food, set-up and perform a PCR reaction, learn the underlying principles of PCR, and analyze their results on an agarose gel.

PCR utilizes:

- **template DNA** - the starting DNA of interest.
- **two primers (oligonucleotides)** - short, single-stranded, synthesized pieces of DNA that complement sequences on each side of the region of the template DNA that is being amplified.
- **thermostable DNA polymerase** - typically *Taq* (*Thermus aquaticus)*, a heat stable enzyme capable of adding nucleotides to a growing DNA strand.
- **dNTPs** - a supply of the 4 nucleotides (dATP, dTTP, dCTP, dGTP) needed to make the new DNA strands.
- **magnesium** - a cofactor for the *Taq* polymerase.
- **a buffered solution** - to maintain the pH and salt concentrations appropriate for the polymerase.

Background Information (continued)

Once these components are combined they go through a series of temperature changes (cycles), repeatedly, in a machine called a thermal cycler. This process will generate exponential copies of the DNA segment of interest. In other words, if you start with 2 copies of the DNA segment of interest, after 20 cycles you will theoretically have 2(nth) = 2(20th) = 1,000,000 copies of that segment.

Each cycle consists of three parts: denaturation (D), annealing (A), and elongation or extension (E). Denaturation separates the double-strands of the DNA molecule at a relatively high temperature of 90-96°C, annealing allows the primer sequences to match and bind to the flanking regions of the target area at a moderate temperature between 40-70°C, and elongation or extension occurs as the polymerase adds nucleotides to the growing strand at 68-72°C. The initial denaturation may last from 2-5 minutes, then is typically 30 seconds during subsequent cycles. Annealing and elongation steps are typically 15-60 seconds each, with a final elongation that may last up to 10 minutes. After the cycling is complete the PCR product is held at 4°C. The number of cycles, temperatures and time lengths are programmed into the thermal cycler, which is somewhat like a computerized heat block.

Since the anticipated product length is known, PCR products can be evaluated using an agarose gel when run alongside a DNA size standard, or marker, with DNA bands of known sizes. Template DNA will be longer in length then the desired PCR product, run more slowly through the gel, and appear as a band closer to the top of the gel. Unbound primers or "primer dimers" will be shorter, run faster, and create smears of DNA closer to the bottom of the gel. The amplified DNA in the PCR field trip ranges in size from 180 to 1500 base pairs in length, the primers are 22 and 24 base pairs in length.

You can find some useful information on the web about PCR, including animated computer tutorials. One tutorial sponsored by Cold Spring Laboratory can be found at

- http://www.dnalc.org/shockwave/pcranwhole.html

Note that this requires a Shockwave Plug-in to view, you need to download this plug-in when you visit the site. For more information on GMOs check out the following internet links:

- http://en.wikipedia.org/wiki/Genetically_modified_organism
- http://www.saynotogmos.org/
- http://www.newscientist.com/channel/life/gm-food

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