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

Polymerase Chain Reaction (PCR)

Field Trip Background

The **Polymerase Chain Reaction (PCR)** is a widely used technique for copying DNA. Starting with only a small sample of DNA, 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 field trip, students set-up and perform a PCR reaction, learn the underlying principles of PCR, and analyze their results on an agarose gel.

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.

PCR utilizes:

- **Template DNA** - the starting DNA of interest.
- **Two Primers** – 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 needed to make the new DNA strands.
- **Cationic Magnesium** – a cofactor for the polymerase.
- **Appropriately Buffered Solution** – to maintain the pH and salt concentrations appropriate for the polymerase

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 copies of the DNA segment of interest exponentially. In other words, if you start with 2 copies of the DNA segment of interest, after 20 cycles you will theoretically have $2^n = 2^{20} = 1{,}000{,}000$ 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 1 minute 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.

Background Information (continued)

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.

PCR has become part of the popular culture. "Jurassic Park" and "CSI" are just two examples where PCR is crucial to the plot. Some fun PCR facts to share with your students*: …PCR has been used to amplify DNA from…

- poached moose meat in hamburger
- a preserved quagga (an zebra relative that became extinct 100 years ago)
- crime scenes
- eight-celled human preembryos, to detect cystic fibrosis
- the brain of a 7000 year old American mummy
- patients for disease diagnosis

*Life third edition, Ricki Lewis, WCB McGraw-Hill, 1998.

You can also find some useful information on the web about PCR, including animated computer tutorials. One tutorial sponsored by Cold Spring Laboratory can be found at: www.dnalc.org/shockwave/pcranwhole.html. Please note that this requires a Shockwave Plug-in to view, you will be prompted on how to download this plug-in when you visit the site.

The PCR Field Trip includes three major components; students set up a PCR reaction, while the reaction is in the thermal cycler they learn the theory behind PCR, after the reaction is finished, they analyze their PCR products on an agarose gel. They will also receive a photocopy of their gel to take with them. During this field trip, students learn about a variety of techniques including; pipetting, using a centrifuge, making an agarose gel, running and analyzing the gel.

If you have any questions about this field trip, please give us a call, or bring your questions along and we can discuss them during the lab. Thank you for your interest in BTCI's Biotechnology Field Trips program!

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Revised August 2018