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

Polymerase Chain Reaction (PCR)

Field Trip Background

Background Information

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.

PCR is based on the natural cellular event of DNA replication. Key events in DNA replication include:

1. The enzyme helicase separates the complimentary strands of DNA.
2. The enzyme primase builds a complimentary short fragment of RNA called a primer.
3. DNA polymerase binds to the double-stranded region made of the primer and complimentary DNA and travels along the DNA strand, adding in complimentary DNA nucleotides (Adenine, Thymine, Guanine, Cytosine).
4. The RNA primer is removed and replaced with DNA nucleotides.

The final product of DNA replication is two identical copies of an entire genome (all the DNA in the cell).

PCR recreates these key replication events, combining in a tube:

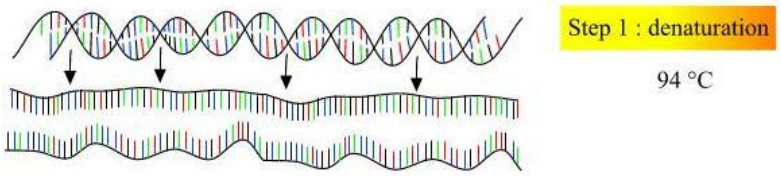
- **template DNA** - the starting DNA of interest
- **two primers (oligonucleotides)** - short, single-stranded, synthesized pieces of DNA that complement sequences on each side of the region of the template DNA that is being amplified.
- **thermostable DNA polymerase** - typically *Taq* (from *Thermus 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.

The role of other cellular enzymes is taken on by different temperatures, which are controlled by a thermal cycler. There are three key events and temperatures: denaturation, annealing, and elongation or extension. See diagrams and descriptions of what happens at each step in a thermal cycler on the following page.

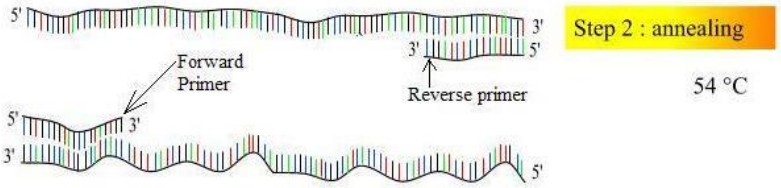
Unlike in the cell, where the replication process stops once one copy of DNA is made, the purpose of PCR is to make *many* copies of a section of DNA, so the thermal cycler continues through many cycles of these three temperatures. This process will generate exponential copies of the DNA segment of interest. 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.

Since the anticipated product length is known, PCR products can be evaluated using agarose gel electrophoresis when run alongside a DNA size standard, or marker, with DNA bands of known sizes.

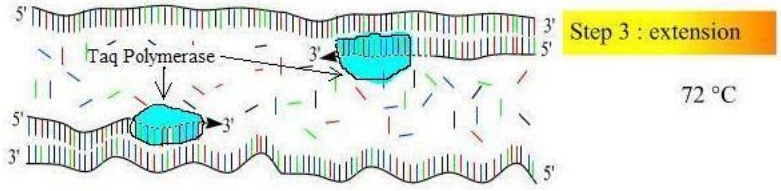
Denaturation separates the double-strands of the DNA molecule at a relatively high temperature of 90-96°C.



Annealing allows the primer sequences to find their complimentary region on the template DNA at a moderate temperature between 40-70°C.



Elongation or extension occurs as the polymerase adds nucleotides to the growing strand at 68-72°C.



Polymerase Chain Reaction Graphics were derived from http://commons.wikimedia.org/wiki/File:PCR_Steps.JPG where it has been released by its copyright holder for unrestricted use.

Supplementary Resources

DNA Replication
Your Genome video animation: https://www.youtube.com/watch?v=TNKWgcFPHqw
Amoeba Sisters video: https://www.youtube.com/watch?v=Qqe4thU-os8
CK-12 Flexbook reading: https://flexbooks.ck12.org/cbook/ck-12-biology-flexbook-2.0/section/4.3/primary/lesson/dna-structure-and-replication-bio/

Polymerase Chain Reaction
1993 Nobel Prize in Chemistry: https://www.nobelprize.org/prizes/chemistry/1993/summary/
Amoeba Sisters video: https://www.youtube.com/watch?v=a5jmdh9AnS4
DNA Learning Center Interactive: https://dnalc.cshl.edu/resources/animations/pcr.html
DNA Learning Center video: https://www.youtube.com/watch?v=2KoLnIwoZKU

Gel Electrophoresis:
Amoeba Sisters video: https://www.youtube.com/watch?v=ZDZUAleWX78

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