Polymerase chain reaction (PCR)

Polymerase chain reaction (**PCR**) is a common laboratory technique used to make many copies (millions or billions!) of a particular region of DNA

Typically, the goal of PCR is to make enough of the target DNA region that it can be analyzed or used in some other way.

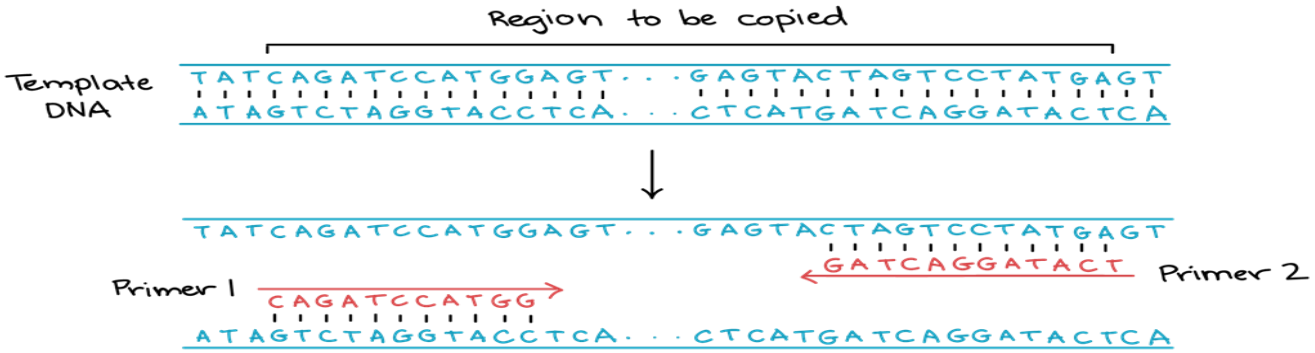
Taq polymerase

The DNA polymerase typically used in PCR is called ***Taq* polymerase**, after the heat-tolerant bacterium from which it was isolated (***Thermus aq****uaticus*).

T. aquaticus lives in hot springs and hydrothermal vents. Its DNA polymerase is very heat-stable and is most active around 70°C (a temperature at which a human or *E. coli* DNA polymerase would be nonfunctional). This heat-stability makes Taq polymerase ideal for PCR. As we'll see, high temperature is used repeatedly in PCR to **denature** the template DNA, or separate its strands.

PCR primers

PCR primers are short pieces of single-stranded DNA, usually around 2020nucleotides in length. Two primers are used in each PCR reaction, and they are designed so that they flank the target region (region that should be copied). That is, they are given sequences that will make them bind to opposite strands of the template DNA, just at the edges of the region to be copied.

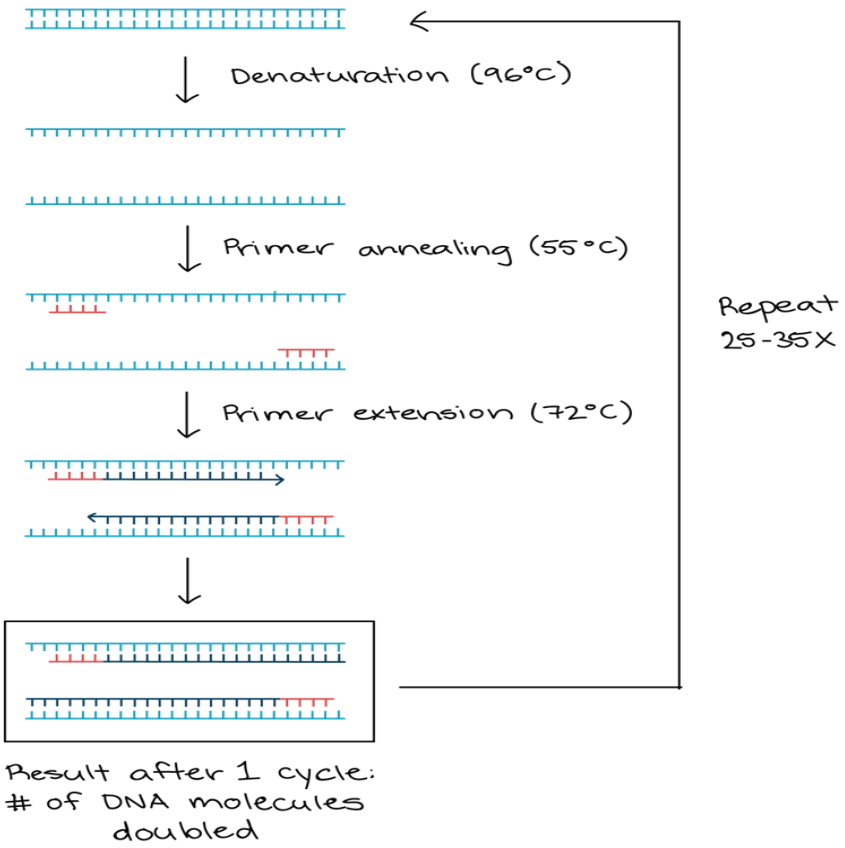


The steps of PCR

The basic steps are:

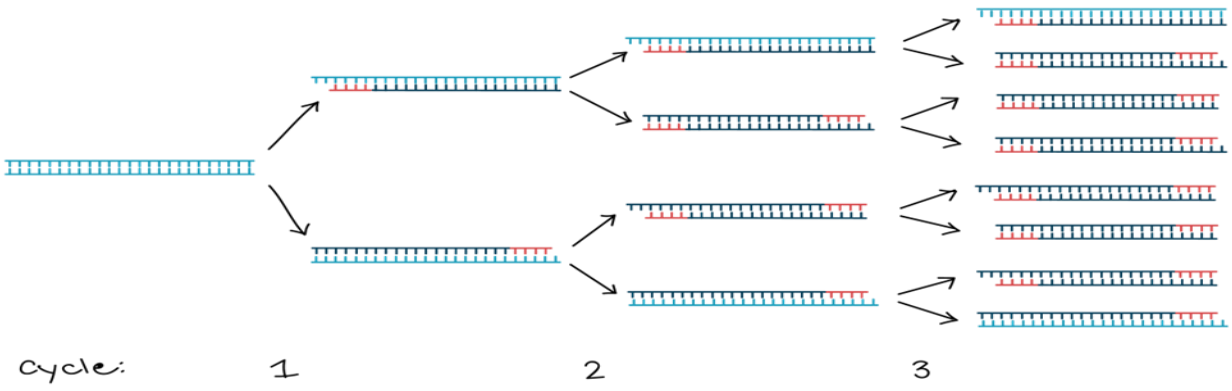
1. **Denaturation** (96°C): Heat the reaction strongly to separate, or denature, the DNA strands. This provides single-stranded template for the next step.
2. **Annealing** (55 - 65°C): Cool the reaction so the primers can bind to their complementary sequences on the single-stranded template DNA.

3. **Extension** (72°C): Raise the reaction temperatures so *Taq* polymerase extends the primers, synthesizing new strands of DNA.



This cycle repeats 25 - 35 times in a typical PCR reaction, which generally takes 22 - 44 hours, depending on the length of the DNA region being copied. If the reaction is efficient (works well), the target region can go from just one or a few copies to billions.

That's because it's not just the original DNA that's used as a template each time. Instead, the new DNA that's made in one round can serve as a template in the next round of DNA synthesis. There are many copies of the primers and many molecules of *Taq* polymerase floating around in the reaction, so the number of DNA molecules can roughly double in each round of cycling

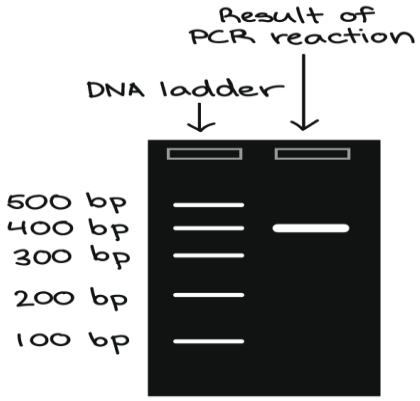


Using gel electrophoresis to visualize the results of PCR

The results of a PCR reaction are usually visualized (made visible) using gel electrophoresis.

Gel electrophoresis is a technique in which fragments of DNA are pulled through a gel matrix by an electric current, and it separates DNA fragments according to size

DNA fragments of the same length form a "band" on the gel, which can be seen by eye if the gel is stained with a DNA-binding dye. For example, a PCR reaction producing a 400 base pair (bp) fragment would look like this on a gel:



Left lane: DNA ladder with 100, 200, 300, 400, 500 bp bands.

Right lane: result of PCR reaction, a band at 400 bp.

A DNA band contains many, many copies of the target DNA region, not just one or a few copies. Because DNA is microscopic, lots of copies of it must be present before we can see it by eye. This is a big part of why PCR is an important tool: it produces enough copies of a DNA sequence that we can see or manipulate that region of DNA.

hybridization

DNA–DNA hybridization generally refers to a molecular biology technique that measures the degree of genetic similarity between pools of DNA sequences. It is usually used to determine the genetic distance between two organisms. This has been used extensively in phylogeny and taxonomy.

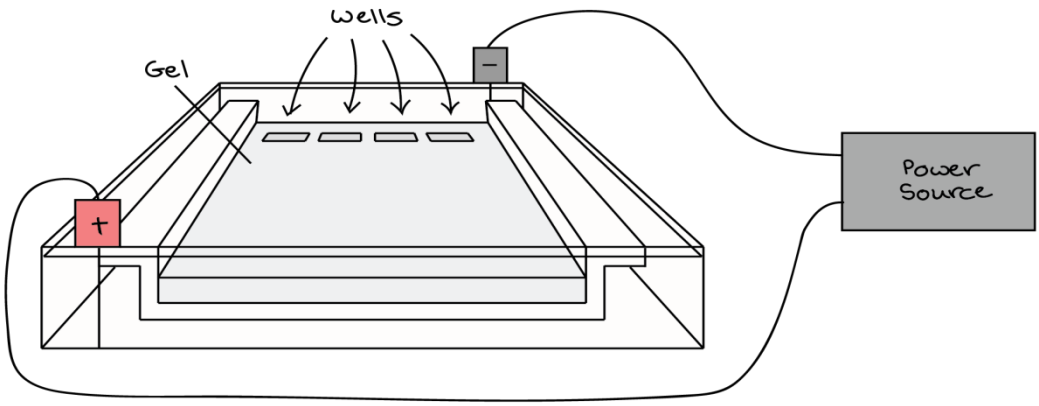
1-The DNA of one organism is labeled, 2-then mixed with the unlabeled DNA to be compared against. 3-The mixture is incubated to allow DNA strands to di-associate and then 4-cooled to form renewed hybrid double-stranded DNA. Hybridized sequences with a high degree of similarity will bind more firmly, and require more energy to separate them: i.e. they separate when heated at a higher temperature than dissimilar sequences, a process known as "DNA melting".

To assess the melting profile of the hybridized DNA, the double-stranded DNA is bound to a column and the mixture is heated in small steps. At each step, the column is washed; sequences that melt become single-stranded and wash off the column. The temperatures at

which labeled DNA comes off the column reflects the amount of similarity between sequences (and the self-hybridization sample serves as a control). These results are combined to determine the degree of genetic similarity between organisms.

Gel electrophoresis

Gel electrophoresis is a method for separation and analysis of macromolecules (DNA, RNA and proteins) and their fragments, based on their size and charge. It is used in clinical chemistry to separate proteins by charge or size (IEF agarose, essentially size independent) and in biochemistry and molecular biology to separate a mixed population of DNA and RNA fragments by length, to estimate the size of DNA and RNA fragments or to separate proteins by charge

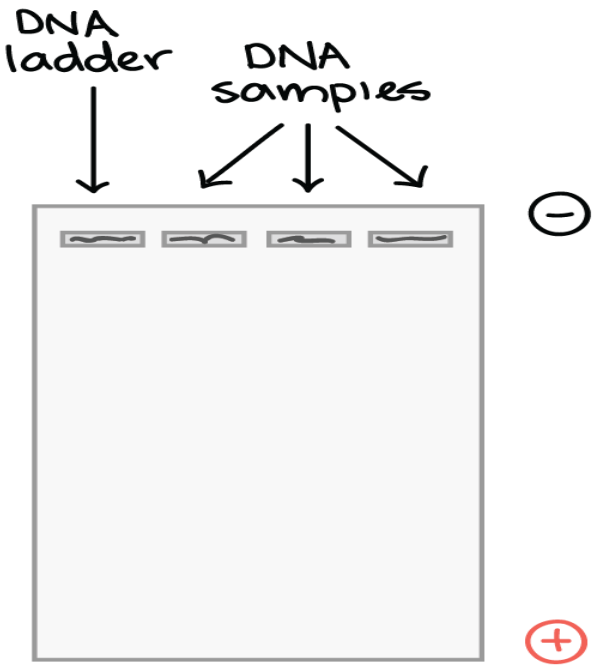


Before the DNA samples are added, the gel must be placed in a **gel box**. One end of the box is hooked to a positive electrode, while the other end is hooked to a negative electrode. The main body of the box, where the gel is placed, is filled with a salt-containing buffer solution that can conduct current

How do DNA fragments move through the gel?

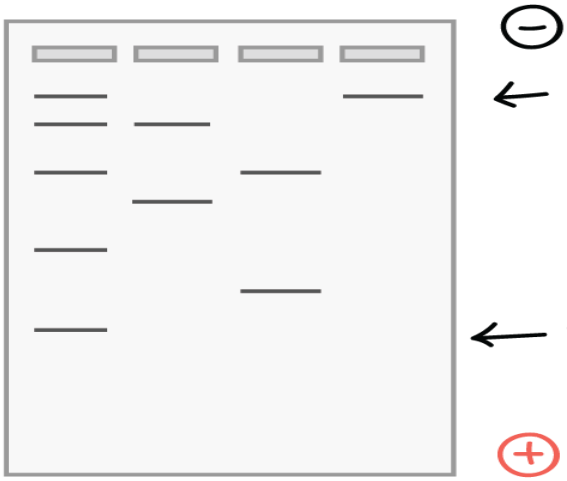
Once the gel is in the box, each of the DNA samples we want to examine (for instance, each PCR reaction or each restriction-digested plasmid) is carefully transferred into one of the wells. One well is reserved for a **DNA ladder**, a standard reference that contains DNA fragments of known lengths. Commercial DNA ladders come in different size ranges, so we would want to pick one with good "coverage" of the size range of our expected fragments.

Next, the power to the gel box is turned on, and current begins to flow through the gel. The DNA molecules have a negative charge because of the phosphate groups in their sugar-phosphate backbone, so they start moving through the matrix of the gel towards the positive pole. When the power is turned on and current is passing through the gel, the gel is said to be **running**.



DNA samples are loaded into wells

Power is turned on and DNA fragments migrate through gel



The fragments are now separated by size.

Visualizing the DNA fragments

Once the fragments have been separated, we can examine the gel and see what sizes of bands are found on it. When a gel is stained with a DNA-binding dye and placed under UV light, the DNA fragments will glow, allowing us to see the DNA present at different locations along the length of the gel.