

DNA FINGERPRINTING OR: THE POLYMERASE CHAIN REACTION!

The polymerase chain reaction, or PCR, is a powerful genetic technique that allows researchers to amplify DNA sequences of interest. This amplification allows better and easier genetic characterization and can be used for a variety of tasks including those in the context of genetic disease diagnosis, DNA forensics, or simply in the production of large amounts of DNA fragments for further study.

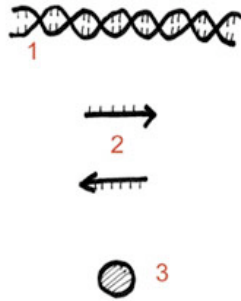
In nature, cells make more DNA by undergoing a process known as replication. This is where the amount of DNA is essentially doubled so that a cell dividing into two new cells can give each of these two cells a complete copy of the DNA. However, replication is a relatively complex process, requiring the regulated actions of over half a dozen different enzymes - technically challenging to pull off in the laboratory. PCR is therefore an experiment that simplifies the process of replication for greater efficiency in test tube settings.

THE GIST AND HOW IT MORE OR LESS WORKS:

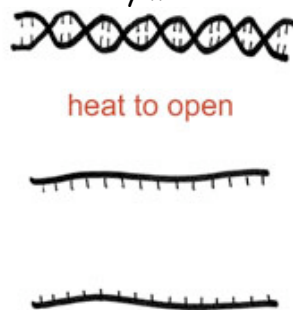
Essentially, this entails the collection of a few components needed for a successful DNA amplification reaction. Although, we'll later highlight some specific places to get them, for now, let's quickly summarize what they are. They include:

1. A sample of DNA to start with: there are many ways to get this, but you can use the cheek cell procedure outlined in the print article.
2. Two primers: These are short pieces of DNA that have been specifically designed to complement the region of your DNA that you wish to make more of. They can be ordered from places that do something called oligonucleotide synthesis (fancy talk for making small pieces of DNA synthetically).
3. A solution containing the 4 different nucleotides (the A's, T's, C's, and G's): necessary for use as the building blocks for DNA replication.
4. A heat stable DNA polymerase: This is an enzyme that is actually responsible for making a complementary DNA copy (i.e. replicating). The cheapest ones are usually those derived from the *Thermophilus Aquaticus* bacteria, and are called Taq polymerases. These polymerases will often come with a buffer solution which is a specific recipe that allows the polymerase to function at its best.
5. Equipment setup to allow a PCR reaction to cycle through three different temperatures for certain lengths of time, over and over again. This can take the form of the MAKE thermal cycler, or in a bind can be as low tech as a person moving a test tube from three different pots of water set at three different temperatures for about 3 to 4 hours!

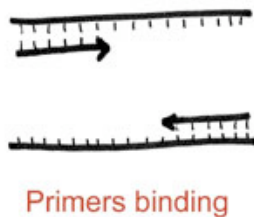
To start the PCR reaction, you'll need to mix in a plastic tube, your DNA sample, the primers, the nucleotides, and the heat stable DNA polymerase.



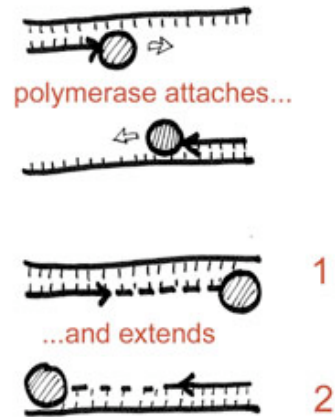
First up, is a high temperature step usually at about 95°C for about 1 minute, and often called the denaturation step. This is all about allowing your DNA to open up, since it naturally exists as a tight helical coil. The high temperature step will effectively melt the double stranded DNA strands into single strands. This step whilst elegant in its simplicity is the reason for the need of heat stable DNA polymerases, since the enzyme needs to be able to withstand the heat.



With the DNA open, we move to the second temperature step, referred to as the annealing step. Here, the primers, which have been designed to interact with the desired region of amplification, can now "get in." This binding is mediated by choosing a second temperature which ensures a specific interaction, usually around 55°C or so, and usually for about a minute.



With the primers bound, we can now move on to the third and final temperature step - the elongation step. By switching to a temperature where our enzyme works best, the heat stable DNA polymerase can extend the production of DNA from the two primers. This is the actual "replication" part, and consequently also involves uptake of the nucleotides. Note that at the end of this step you have two copies of the original piece of DNA of interest.



And there you have it! You've essentially made more of your DNA of interest (doubled it actually). However, what is cool is that you can now repeat the process over and over in the same experiment. In other words, take the two copies you've produced and subject them to the same three temperature steps. You'll hopefully see that this allow your reaction to go from two copies to four. In essence, going through these three temperatures (which is called a single cycle) will result in the doubling of your DNA of interest. The thermal cycler machine simply allows you to do this automatically and in a programmable fashion. Most PCR experiments are done with a total of 30 or more cycles (or 30 rounds of doubling), so that you can effectively increase your DNA interest by billion times or more. Factor in the fact that a single cycle is often less than 5 minutes, and what you have is an incredibly powerful, yet simple, procedure to amplify out your DNA in a relatively short period of time.

<http://scq.ubc.ca/MAKE/>

