What is PCR?

Two questions I got today on the upcoming Lab 4 report could be answered with a better understanding of what is happening in our PCR reaction. Therefore, this write-up:

Information presented in our manual

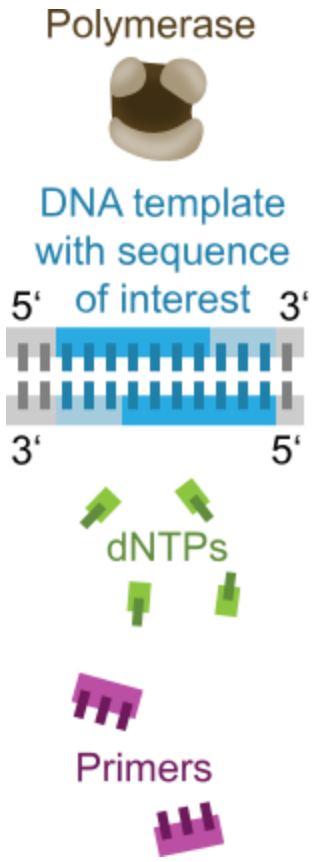
In our lab manual, we place our PCR solution into a PCR machine with the following settings:

Programmed PCR reaction cycles:

- Step 1. 95^{o}C for 30 seconds
- Step 2. 55^{o}C for 30 seconds
- Step 3. 72^{o}C 1 minute
- Step 4. Repeat steps 1 – 3, for 24 additional cycles
- Step 5. 72^{o}C for 10 minutes
- Step 6. RT

What is in the solution?

The PCR solution contains PCR buffer (not depicted), DNA polymerase, double-stranded DNA, nucleic acid, and primers. The buffer contents aid in the function of polymerase for better results.

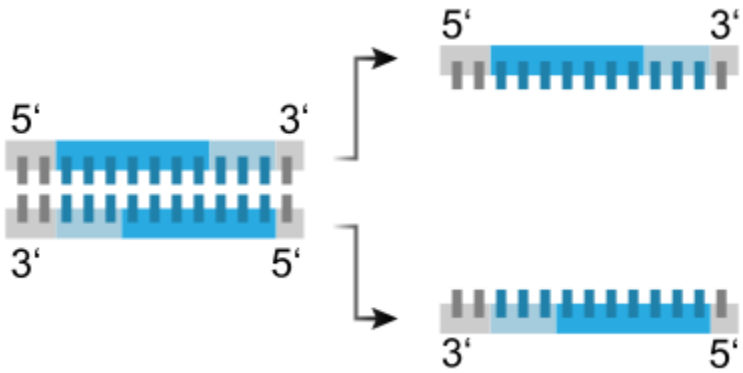


What do these steps mean for the solution?

In otherwords, how do these temperatures effect the contents of our solution?

Step 1: Denaturation (DNA melting)

The short burst (30 seconds) of intense heat (95^o C) will cause ***any*** double-stranded DNA to denature – separate into single-stranded DNA.

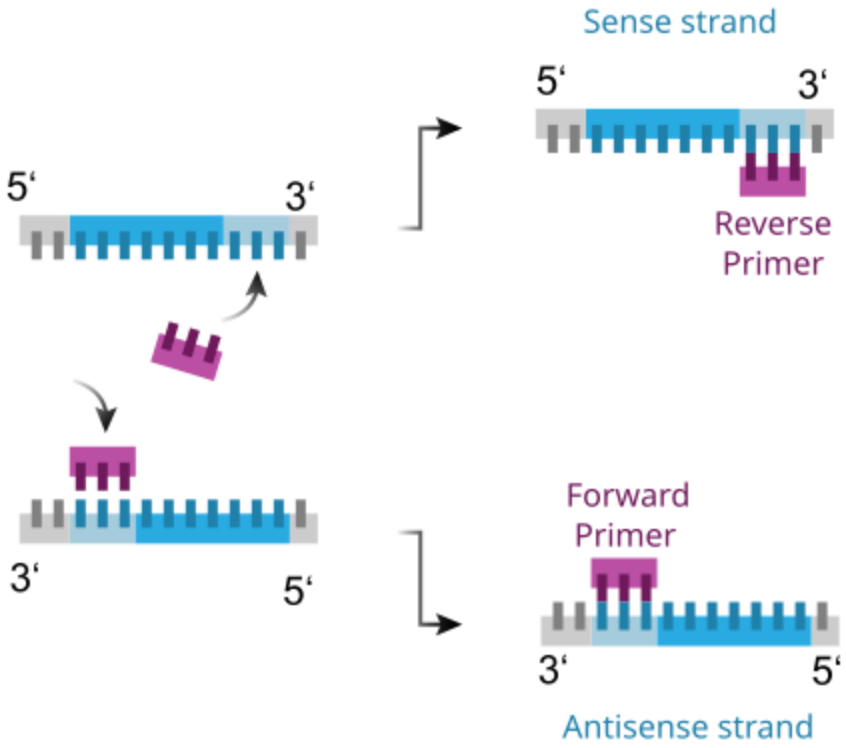


Step 2: Annealing (Primer Annealing)

Now that our solution only contains single-stranded DNA, another short burst (30 seconds) of milder heat (55^o C) allows the primers to anneal to the single-stranded DNA. The forward and reverse primers will be bound to their complimentary DNA strands.

The 5'-3' DNA strand is known as the *sense* strand and is annealed to by the *antisense* primer (AKA the reverse primer).

The 3'-5' DNA strand is known as the *antisense* stand and is annealed to by the *sense* primer (AKA the forward primer).

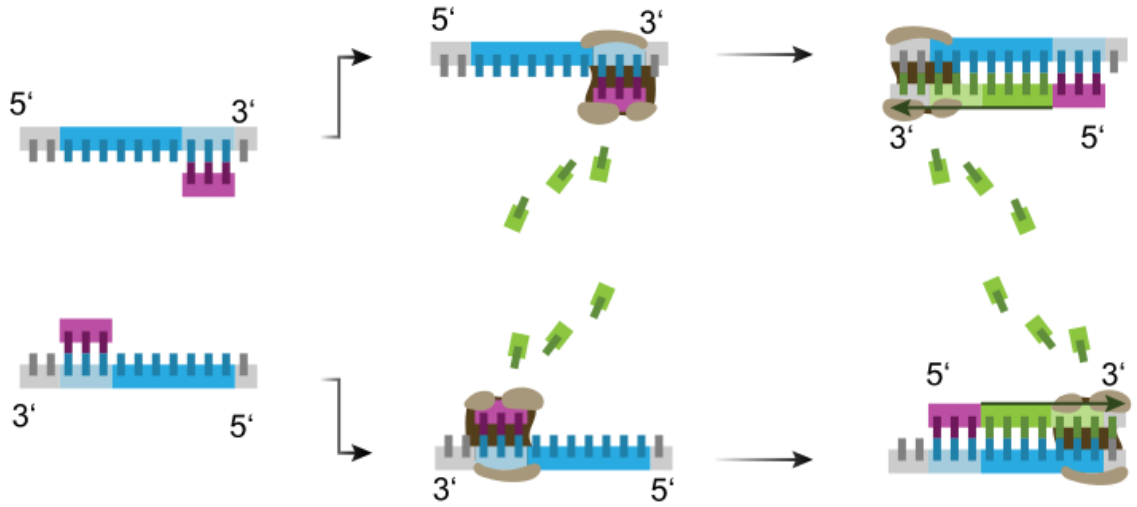


Step 3: Extension (Elongation)

The solution now contains only single-stranded DNA with primers annealed (a duplex primer-template DNA strand).

The taq polymerase requires a double-stranded segment of DNA followed by a single-stranded segment of DNA to work. The primers provide an additional requirement for taq polymerase to operate – a 3' hydroxyl group. This -OH group allows polymerase to create the phosophdiester bond between the primer 3'-OH end and the 5'-phospate for the incoming dNTP.

The temperature of 72^oC serves to set the taq polymerase at the peak of its enzymatic activity (the polymerase is most active at this temperature). Allowing for an approximate of 1000 bp/min.

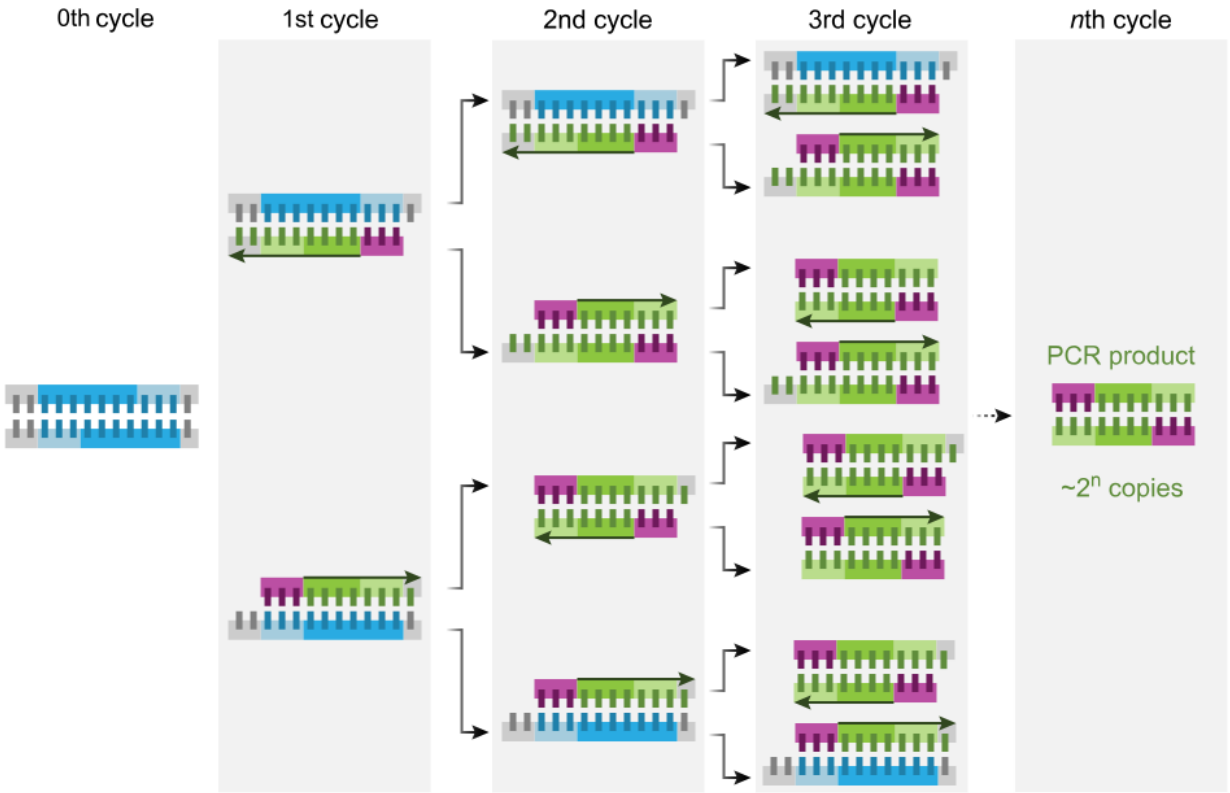


Step 4: Cycling

Now the steps are repeated for a given number of times to return a vast quantity of PCR targets. Namely,

1. Denaturing ***every*** double-stranded DNA molecule into a single-stranded DNA molecule (even the PCR targets will be denatured in subsequent steps)
2. Annealing primers to ***every*** single-stranded DNA molecule to create a duplex molecule of primer-template DNA
3. Extending the primer strand of the molecule **until the polymerase runs off the template strand or the cycle begins again**

$$Total\ count\ of\ \text{target DNA} \approx 2^n$$
$$\text{where } n \text{ is the number of cycles}$$



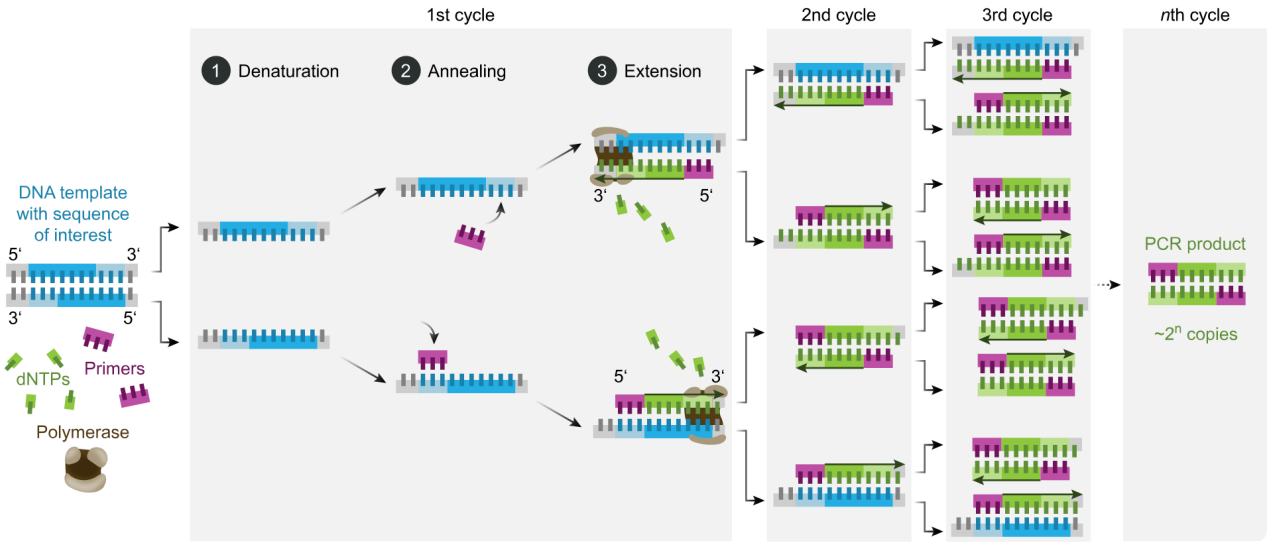
Step 5: Final extension

In this step, the settings are similar to **Step 3** except for the longer time. This will allow any single-stranded DNA in solution to be polymerized.

Step 6: Stabilization

Allowing the solution to reach room temperature is for stable storage of the PCR products until they are retrieved for further experimentation.

The full process



But why is it called PCR, or Polymerase Chain Reaction.

- Polymerase – an enzymatic reaction that creates a polymer like DNA
- Chain Reaction – An exponential reaction like this one that yields 2^n of the target PCR product

Primers with extra sequence

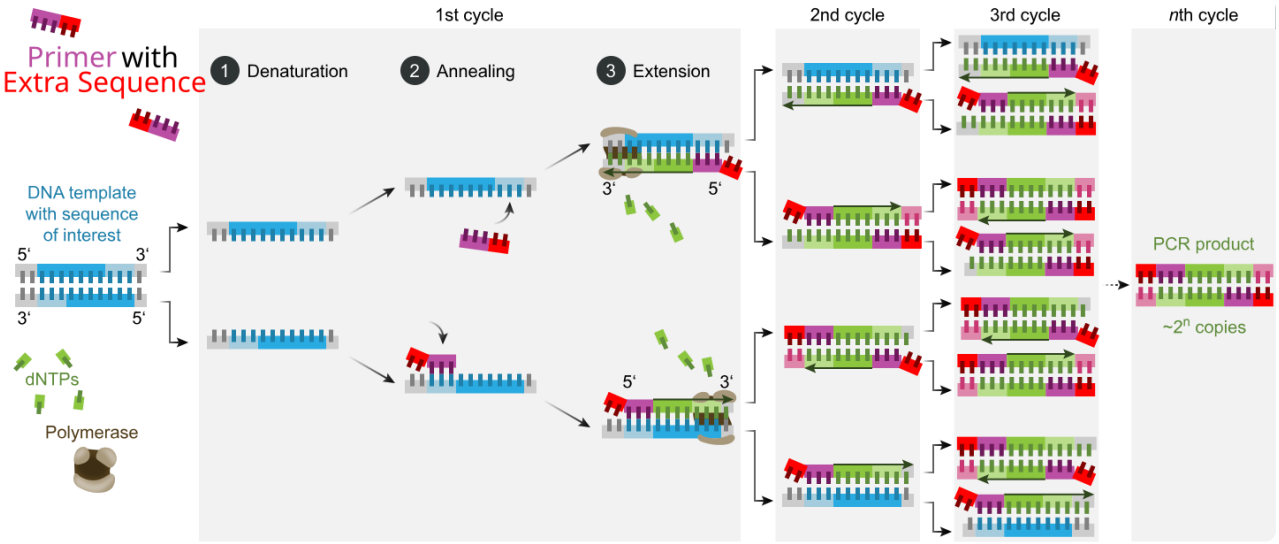
In this lab, the primers have an extra 8 base pairs that do not align with the DNA template. These extra base pairs serve as new base pairs that are added on to the end of the PCR product.

For our experiment:

- The **forward primer addition** adds a site for the `NcoI` restriction enzyme to cut. Creating cohesive ends at the beginning of our gene target.
- The **reverse primer addition** adds a site for the `SmaI` restriction enzyme to cut. Creating blunt ends at the end of our gene target.

It is important that of the hundreds of options of restriction enzymes that the ones selected have:

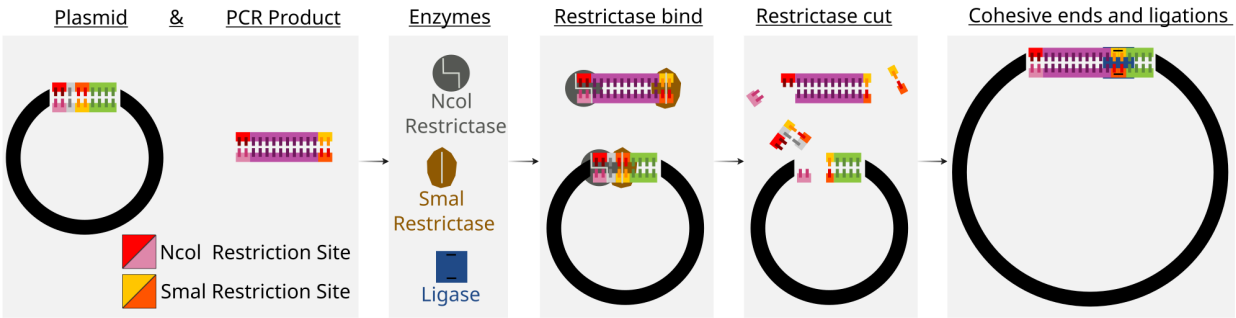
1. Different target sequences
2. Different final DNA ends (e.g. cohesive and blunt)
3. Do not target sequences within our gene target



Adding our PCR gene target to a plasmid

The use of these restriction sites was strategic. The NcoI site allows the PCR product and the plasmid to both have a single cohesive end. Our cohesive ends find and adhere to one another. While the blunt ends created at the SmaI site require a ligation enzyme to repair the plasmid to a circular state.

This combonation of NcoI and SmaI sites allow our PCR gene to be placed in the correct position and orientation on our plasmid.



References

All images were adapted from the work of Enzoklop, CC BY-SA 4.0 https://creativecommons.org/licenses/by-sa/4.0 (https://creativecommons.org/licenses/by-sa/4.0), via Wikimedia Commons