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

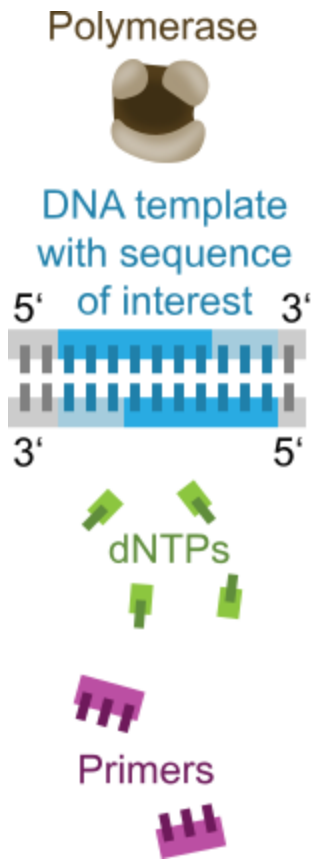
In our lab manual, we place our PCR solution into a PCR machine with the following settings:

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 polymerase, DNA, nucleic acid, and primers with some additional molecules that aid in better results.

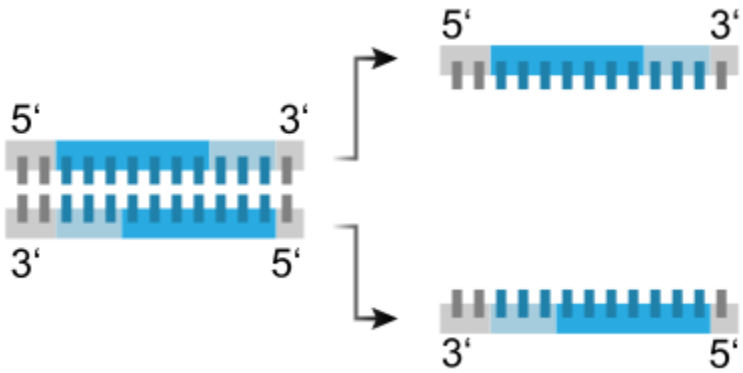


What do these steps mean for the solution?

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.

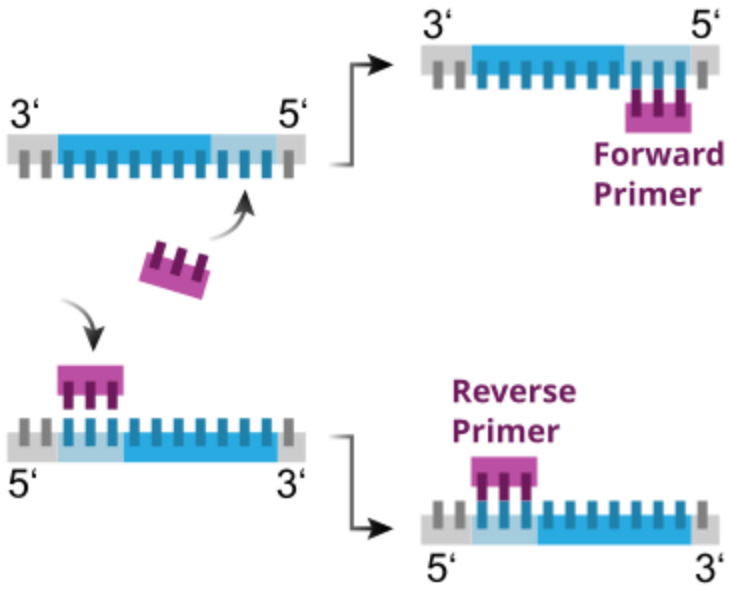


Step 2: Annealing (Primer Annealing)

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

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



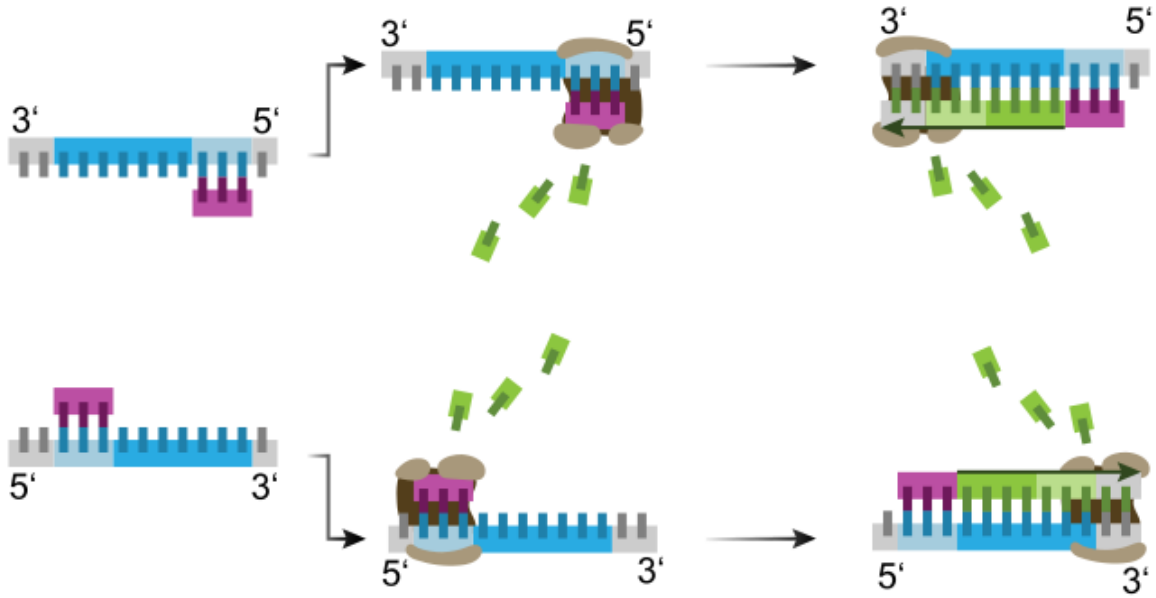
Step 3: Extension (Elongation)

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 \(72^o\)C 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

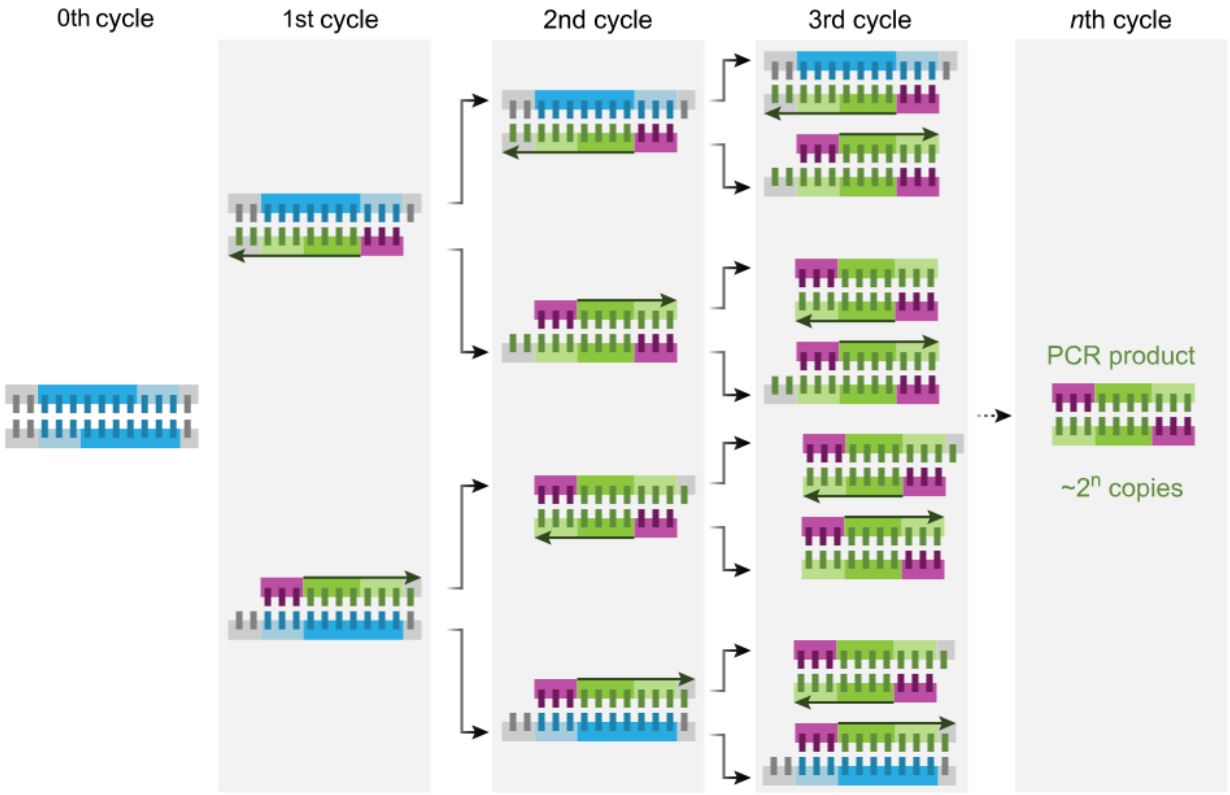
Now the steps are repeated for a given number of times to return a vast quantity of PCR targets. Namely,

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**

This process is what allows for the final PCR target to be created as the PCR target is exponetial while the variable-length DNA strands will be linear.

$$ Total\ count\ of\ \text{target DNA} = 2^n-2n $$

$$ Total\ count\ of\ \mbox{variable-length DNA} = 2n $$



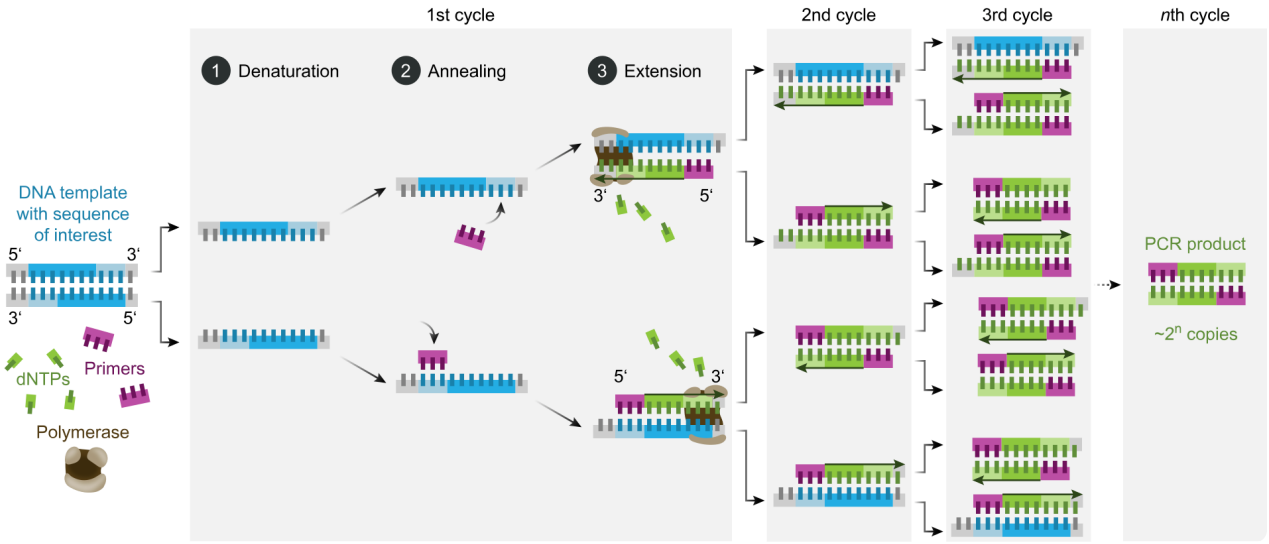
Step 5: Final extension

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 one that yields \(~2^n\) of the target

start here tomorrow with add an extended primer and restriction sites and sticky and blunt ends

The two questions I referenced are:

4. C. What's the significance of these sites' presence in the Nostoc GAF-4 sequence?

5. A. What is the expected size (in bp) of the PCR product in Experiment 4? Remember that the sequences given above does not include the sequences added by the primers

References

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