**Name:**

## PCR assignment

1. (6 pts) Attach a printout of your gel. Label each lane to indicate what sample was run in each lane.

2. (12 pts) Complete the table below.

|  |  |  |  |
| --- | --- | --- | --- |
| **PCR product** | **Are you expecting a PCR product?** | **If so, what is the expected size (bp)?** | **Actual size (bp; estimate from the gel picture)** |
| TPCR PC |  |  |  |
| TPCR NC |  |  |  |
| FPCR PC |  |  |  |
| FPCR NC |  |  |  |
| BB |  |  |  |

3. (3 pts) The positive control (PC) consists of a plasmid and two primers that have been successfully amplified in the past. Why do you include this control when running a templateless or finish PCR reaction?

4. (3 pts) The negative control (NC) contains only DNA primers and water. Why do you include this control when running a templateless or finish PCR reaction?

5. (6 pts) The only PCR reaction that we did not run on a gel is the BB PCR reaction after templateless PCR but before finish PCR. What would you expect to see if you ran this PCR reaction on a gel? Therefore, why is there little point in running the templateless PCR BB product on a gel?

6. (6 pts) After DNA fragments have been separated by gel electrophoresis, how does the dye enable us to visualize the DNA (which is normally clear and colorless)? Why do we need to use both loading dye and also ethidium bromide?

7. (6 pts) When we set up the templateless PCR, we add all of the oligonucleotides to the reaction (the TPM), but when we set up the finish PCR we only add two of the primers. Why? Is finish PCR a template-dependent or template-independent PCR and what is the difference between the two in terms of what we are trying to accomplish with each reaction?

8. (6 pts) Look at the reaction conditions for the templateless PCR. Notice that the reaction begins at an annealing temperature of 69 degrees and then uses annealing temperatures of 65 and 61 degrees. In contrast, the finish PCR uses only one annealing temperature that is much lower (55 degrees). Why do the annealing temperatures differ and how does this enable the goals of each PCR reaction?

9. (6 pts) If you run your building block DNA on a gel after finish PCR and you get no PCR product, what are two things that you could change (in terms of the reaction conditions) to improve your results to get a PCR product?

10. (6 pts) When performing PCR, you want to maximize the yield of specific product and minimize the amount of nonspecific product that can result from primers binding to non-identical regions of DNA. If your PCR is not specific enough and you get more than one PCR product, what are two things that you could change (in terms of reaction conditions) to improve your results to get only one PCR product?