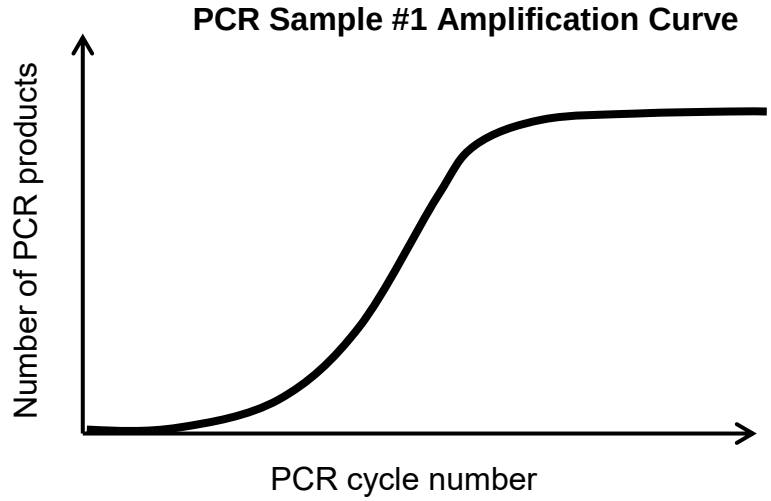
7.003 Spring 2022
Day 18 In-Lab Questions

1) The graph below shows an amplification curve for PCR Sample #1, with the number of PCR products plotted vs. the PCR cycle number.



A) Label which portions of the curve correspond to the exponential, linear, and plateau phases of PCR and explain why the curve is shaped the way it is for each phase.

B) In the graph above, draw a second potential PCR amplification curve for a hypothetical PCR Sample #2 which would have a greater amount of starting template than Sample #1. Explain your reasoning for how/where you drew this second amplification curve.

2) Explain what the C_t (cycle threshold) value represents for a qPCR sample. In the PCR amplification curve graph above in Question 1, draw an example of how you would determine the C_t values for Sample #1 and Sample #2.

3) A common goal of a qPCR experiment is to compare how much more Gene X starting template one sample has than another sample. One normally would not directly compare the C_t values of the two samples though. Instead, one can compare 2^{-Ct} values between the two samples.

A) Why do you think comparing 2^{-Ct} values is more informative than comparing plain C_t values?

It is also common when comparing qPCR samples to normalize them with a reference gene (e.g. to account for any variations in starting sample amount used, pipetting errors, etc.). This is referred to as the "$-\Delta C_t$" method, where the $2^{-\Delta Ct}$ values are used for comparison, with $\Delta C_t = C_t(\text{Target gene}) - C_t(\text{Reference gene})$.

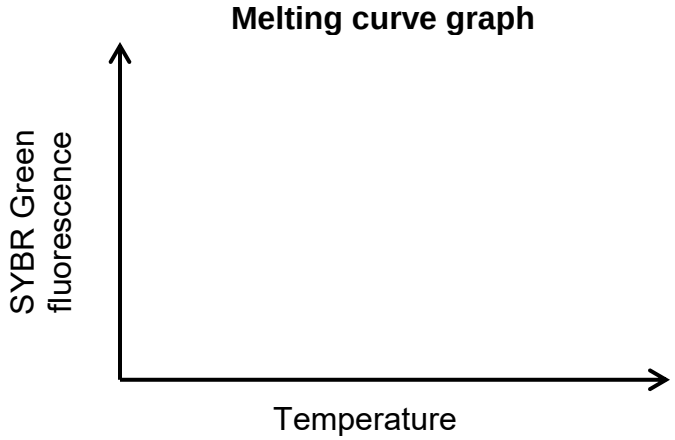
B) What key characteristic(s) would you want a good reference gene to have? How might you determine or test if a gene would make a good reference gene for your experiments?

4) For qPCR analysis to be accurate, it is important that the qPCR primers used have a high efficiency during the exponential phase of PCR.

A) What does it mean for a pair of PCR primers to have maximum (100%) efficiency? Why is this important for qPCR analysis?

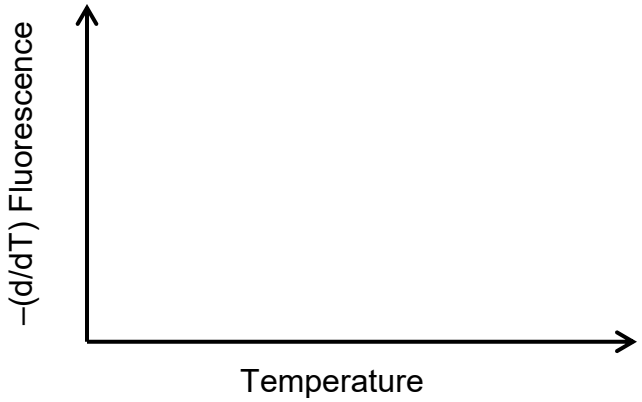
B) How might you determine or test the percent efficiency of a pair of qPCR primers?

5) You have a DNA sample consisting of SYBR Green dye mixed with two different dsDNA products that are 300 bp and 500 bp in size. You perform a melting curve analysis of this sample by gradually increasing the temperature while measuring the amount of SYBR Green fluorescence (see Steps 7 – 8 of the qPCR program on page 103 of the lab manual). A melting curve graph can be made by plotting SYBR Green fluorescence on the y-axis vs. increasing temperature on the x-axis.



A) In the graph above, draw an example of what you might expect the melting curve to look like for your DNA sample. Explain the shape of your melting curve.

It is often easier to visually interpret the melting curve by taking the derivative of the SYBR Green fluorescence over the change in temperature (i.e. the change in slope of the melting curve vs. temperature, as shown in the graph template below).



B) Fill in the graph above, based on the melting curve you drew in Part A for your DNA sample. Explain the shape of the graph you drew and what each part represents.

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