7.003 Spring 2022
Day 17 In-Lab Questions

1) In which lanes of your cDNA PCR gel do you expect to see any DNA bands? How many bands? What size and intensity do you expect these bands to be? Explain your reasoning.

2) The 7.003 lab has run out of 2X KAPA SYBR Fast Master Mix, so instead, your instructor says you can just use the 2X Phusion High-Fidelity Master Mix (from Day 16) to set up your qPCR samples today. Do you agree? Explain why or why not.

3) What is the purpose of the qPCR samples you set up today using the *ACT1* primers?

4) You are designing primers to use for qPCR to measure expression of GeneX. You have the following forward primer sequence:

Forward Primer: 5' – atc gaa tcg gta cct gtc acg – 3'

Your labmate suggests two possible reverse primers:

Reverse Primer #1: 5' – cgt gac agg tac cga ttc gat – 3'

Reverse Primer #2: 5' – gct tag ctc aag tgc gat ctg – 3'

A) Which of the two reverse primer options would be best to use? Why?

Your labmate sets up two different qPCR reactions, one using Reverse Primer #1 and another using Reverse Primer #2. Unfortunately, they forgot to properly label their reaction samples.

B) Explain how you might use the melting curve analysis (Steps 7 – 8 in the qPCR program) to differentiate between the two qPCR reactions your labmate set up.

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