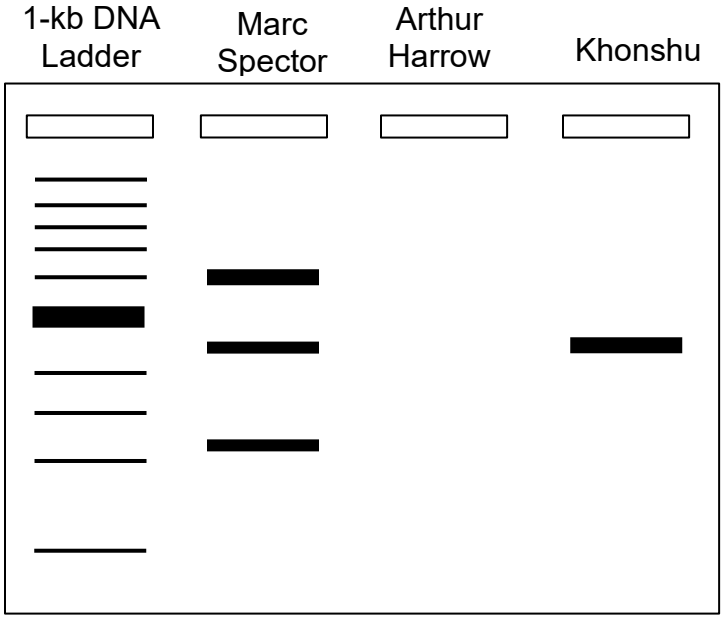
7.003 Spring 2022
Day 12 In-Lab Questions

1) What size DNA band(s) do you expect to see for your pRSQ2 PCR control sample when you run it on a gel today? (Note: You may find it helpful to use SnapGene and the "PCR…" function under the "Actions" menu toolbar.)

2) Three of your classmates have run their pRSQ2 PCR control sample on a gel, as shown below.



A) Which student got the expected result for their pRSQ2 PCR control sample?

All three students used the proper reagents and set up their PCR sample correctly, but it turns out they each altered the conditions of the PCR program they used by changing either the temperature or time of one of the steps.

B) For each of the two students who did not get the expected gel result, what condition might they have potentially altered in their PCR program? Explain.

3) For each of your αF-resistant mutant PCR samples, how many DNA bands do you expect to see when you run them on a gel today? What size(s) do you expect these bands to be? If you do not know what exact size(s) to expect, what would be the minimum size you would expect for each PCR product?

4) Open your mTn3 + pRSQ2 .dna file in SnapGene and use the "Add primers…" function in the "Primers" menu to add the M13(-40) sequencing primer.

A) What ideally will be the first 10 bases of the Sanger sequence readout using this primer?

B) In reality, the first 50 or so bases of a Sanger sequence readout are usually unclear and are simply read as "NNNs" (i.e. could be any nucleotide) in the final readout result. Explain why.

C) How many different binding sites for the M13(-40) primer are there in the mTn3 + pRSQ2 construct?

D) Your instructor is concerned that the multiple M13(-40) primer binding sites in the mTn3 + pRSQ2 construct may be a problem for your plasmid recovery procedure. Do you agree? Explain why or why not.

5) When setting up a sequencing reaction, the amount of DNA template used is important – having too little or too much DNA template can impact the success of the reaction. Explain why it might be unfavorable to have too much DNA template in a sequencing reaction.

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