Improving Student Outcomes with an Adaptable Molecular Cloning Course-Based Undergraduate Research Experience

Experiment A Planning Worksheet (Individual)

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Solutions for the individual worksheet corresponding to sequences provided in **Supplementary File 8** are noted in red.

Please complete this worksheet using information on Benchling (or another sequence analysis tool) and what you know about primer design and PCR. Your primer solutions will become available once you have turned in this worksheet.

1.Write the name/number of the plasmid you have been assigned to clone: _____9_____

2. What gene(s) will you be targeting? Name the gene(s) according to the DNA sequence file provided by your instructor.

_____polyketide hydroxylase_____

3. Draw a diagram of the DNA template to which your primers bind. Note coordinates within the template for the start and stop of the gene(s), and the coordinates of your primers within the template.

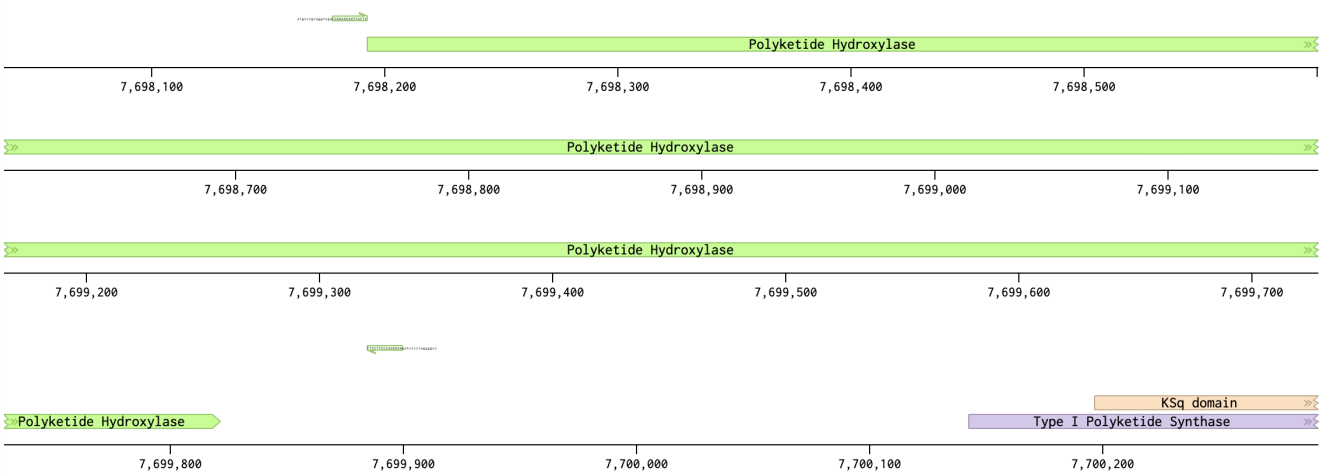

Image generated in Benchling. Numbers below thin blank lines represent the nucleotide position in the genomic DNA template (LT607413.1). Bases of primer that are complementary to template are highlighted in green. Those that are non-complementary are lower case and not highlighted. Open reading frame corresponding to the gene sequence is also highlighted in green. Primers were designed to bind upstream and downstream of the start and stop codons, respectively, to ensure specific amplification of the polyketide hydroxylase gene from a complex genomic DNA template.

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4. What size do you expect for your amplicon? ___1752 bp: includes gene (1722 bp and overhangs with vector (15 bp on either end)_

5. What annealing temperature would you choose for your PCR program to amplify this gene? Briefly justify your answer.

62 °C. Using the NEB Tm calculator, enter only the bases that are identical to the template DNA (ie CGGGAGGAGTCACTC for the forward primer, and AGGGGACCCTTCCTC for the reverse primer). Select the appropriate polymerase (Q5 in this case), and final primer concentration of 500 nM.

6. What extension time would you choose for your PCR program to amplify this gene? Briefly justify your answer.

For complex genomic DNA templates, as would be used in this reaction, an extension time of 20-30 seconds per kilobase should be used for the Q5 polymerase. For an expected amplicon of 1752 bp, use an extension time of ~50 seconds.

7. If your instructor has also assigned you primers and a DNA template sequence for a vector backbone, what size do you expect for your vector amplicon?

____with overhangs for gene: 2714 bp_____without overlaps for gene: 2684 bp_____

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Experiment A Planning Worksheet (Team-based)

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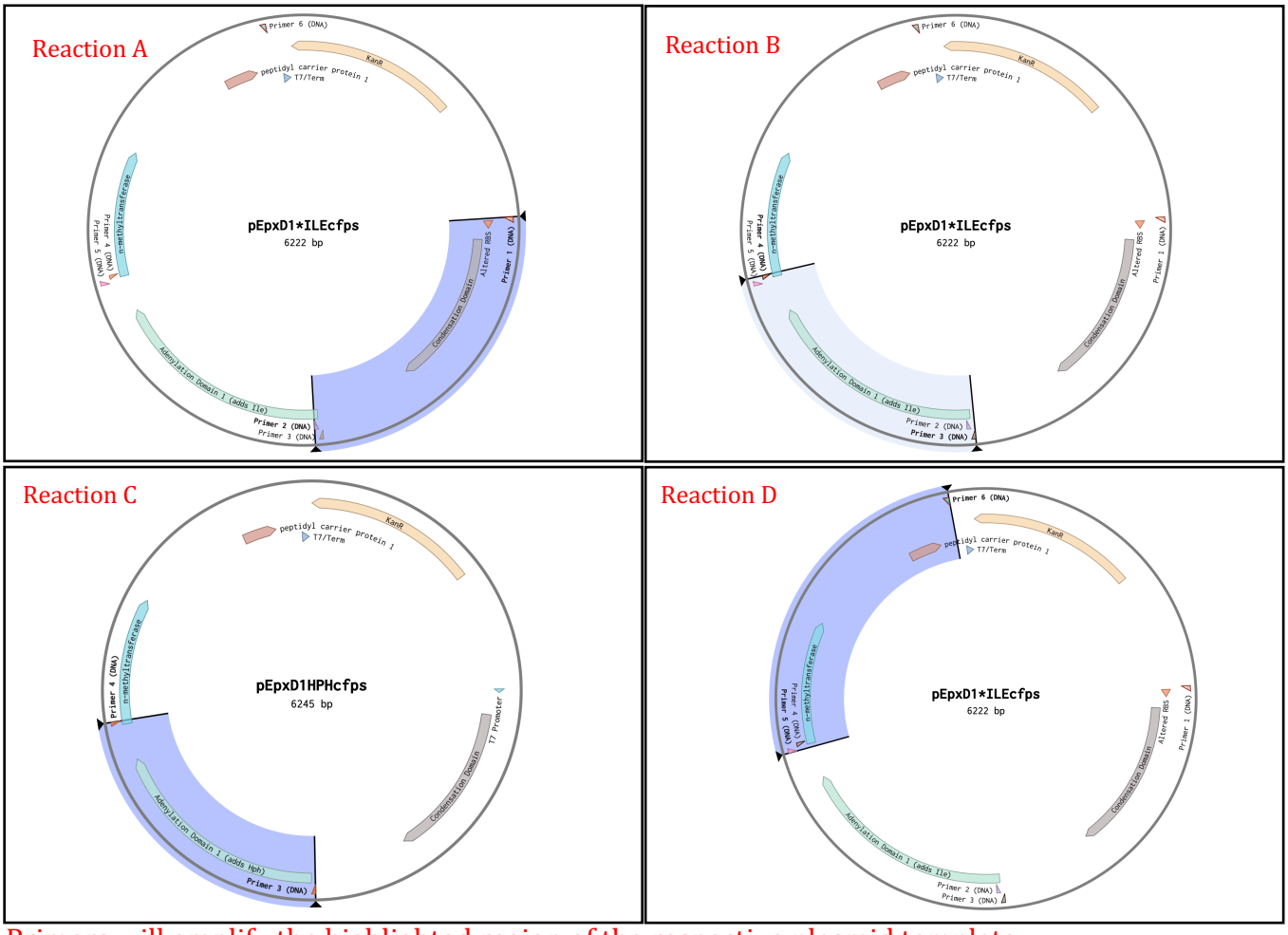
Examples of simulated student responses from the team-based Gibson Assembly module in Spring 2023 are shown in red.
Please complete this worksheet with your group using what you know about primer design, PCR, and your specific primer set.

1.Write the name(s) of the plasmid(s) your group has been assigned:

pEpxD1ILElac and pEpxD1HPHlac

2. For each of the primer pairs your table has been assigned, draw a diagram of where your primers will bind to the designated DNA template. Note coordinates of your primer binding sites within the template sequence.



Primers will amplify the highlighted region of the respective plasmid template.

3. For each primer pair you have been assigned, summarize the following data.

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Experiment A Planning Worksheet (Team-based)

Reaction Label	Amplicon Size (in base pairs)	Gene(s) Contained Within Amplicon	Proposed Annealing Temperature*	Proposed Extension Time**
A	1564	*epxD1,* encoding ribosomal binding site and condensation domain	66 °C	30 seconds
B	1423	*epxD1,* encoding adenylation domain selecting for isoleucine	71 °C	28 seconds
C	1423	*epxD1,* encoding adenylation domain that selects for homophenylalanine	71 °C	28 seconds
D	1642	*epxD1*, encoding peptidyl carrier and *N*-methyltransferse domains	65 °C	33 seconds

*According to NEB Tm calculator with Q5 polymerase and 500 nM primer concentration.
**Used 20 seconds per kilobase, based on Q5 polymerase recommendations. Templates are plasmid DNA, but relatively large.

4. Draw a diagram of how you will combine the group's amplicons in a Gibson Assembly reaction to create your assigned plasmid(s). (If your instructor did not assign you a primer pair and DNA template for a vector backbone, it will be provided to you separately. Be sure to include the vector backbone in your drawing.)