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

Experiment C Planning Worksheet

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Representative answers are shown in red for a screen designed and conducted by a student on plasmids assembled from sequences available in **Supplemental File 8**.
Use this worksheet to design a protocol for Experiment C: Screening plasmids for an insert.

In your protocol, you will need to specify the details of reaction assemblies for either a restriction digest experiment or PCR experiment, the incubation time(s), as well as how to analyze products of your reaction.

In addition to standard sterile water, pipet tips, tubes, thermocycler, etc., the following reagents are available:

- Selected Restriction Enzymes and appropriate buffers
- DNA Polymerase Master Mix (Q5 2X Master Mix)
- All PCR primers used in Experiment A

It is important to figure out what your desired plasmids look like before designing your screen! Refer back to sequences of your linear fragment(s) you would have generated for Experiment A. Using these sequences, you can perform your Gibson Assembly *in silico* using Benchling or another sequence analysis tool.

1. I will be performing a (circle your choice):

   Restriction Digest Screen *PCR Screen*

2. Summarize your recipe for your reaction assembly(ies) in the following table. If you plan to combine common reagents for multiple reactions (a master mix), summarize the master mix recipe in the table, and then draw a diagram of how you will assemble individual reactions on the back of the page. If you are pipetting multiple reactions, summarize the common reagents in the table, but describe how they are different below the table.

Reagent/Solution Name	Stock Concentration	Volume	Final Concentration
EcoRI enzyme	20 units/µL	4.50 µL	2 units/µL
HindIII enzyme	20 units/µL	4.50 µL	2 units/µL
CutSmart buffer	10X	4.50 µL	1X
Sterile Water	----------	31.5 µL	----------

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Table summarizes the recipe for a MasterMix that will be used to digest 4 samples – 3 isolated plasmids, and 1 'empty' pUC19 vector. Plasmid samples will be diluted to a concentration of 200 ng/µL. 1 µL of the diluted plasmid sample will be combined with 9 µL of the MasterMix.

3. Summarize any incubation times and temperatures required for your screen.

Samples will be incubated at 37 °C for 2 hours.

4. Use this table to summarize your expectations for your agarose gel.

Template	**Treatment** (PCR with primers X and Y, or Digest with enzymes X and Y)	**Expectation on Gel**
Plasmid with Insert	Digest with EcoRI and HindIII	Two bands, 1771 bp and 2635 bp
Plasmid without Insert or with incorrect Insert	Digest with EcoRI and HindIII	Two bands, 51 bp and 2635 bp