**Making Molecular Clones**

*Walker Orr, 03/31/2023*

**Application**

**SOPs Used**

Golden Gate

NEB 10-beta Competent cells (C3019H)

Plasmidsaurus

**Reagent Output**

Glycerol stock of bacterial clone with plasmid (-80C)

Prepared plasmid (-30C)

**Data Output**

Sequence file associated with clone

**Other Resources**

**Workflow**

Day 1:

* Use Golden Gate cloning to produce the plasmid of interest. Make sure to use the correct enzyme, i.e. BsaI or BsmbI.

Day 2:

1. Transform chemically competent cells with products from the Golden Gate reaction. Follow the protocol. You may wish the measure the concentration of DNA in your Golden Gate products beforehand. **2 µL** is a good amount of GG reaction to add to **50 µL** bacterial cells.
   * If this is an unfamiliar reaction, use the pUC19 control DNA for a positive transformation control.
   * Retain the rest of the Golden Gate products at -30C until you have successfully sequenced a clone.
   * When plating outgrowth (step 9), especially if you aren’t sure of the transformation efficiency, use multiple dilutions. **50 µL** is a good amount to start with; if plating multiple dilutions take 100 µL of outgrowth and make 10-fold serial dilutions in 10-beta/stable outgrowth medium.
   * Spread 50 µL of each dilution onto a selection plate and incubate overnight at 37C, or at room temperature for 48 hours.

Day 3:

1. If transformation was successful, isostreak 3 colonies from each plate onto fresh selective plates.

Day 4.

1. Select colonies from the isostreaked plate and grow a 5-mL culture of the colonies.

Day 5

1. Save 500 µL of culture for a glycerol stock and make the glycerol stock. Save this at -80°C in yourstorage area.
2. Do a miniprep using the remaining 4.5 mL culture.
3. Measure the concentration of miniprep eluent by Qubit.
4. Send the plasmid for sequencing (Plasmidsaurus); preserve the rest of the plasmid at -30°C in your temporary storage.

Once colonies are sequence-confirmed:

1. Choose one successful colony’s Glycerol Stock and move it to permanent lab storage at -80°C. Make sure to assign it the next sequential number in the Teams plasmids sheet.
2. Transfer the corresponding plasmid to permanent lab storage at -30°C.