Amamoto Lab, 2022

Protocol: Gibson Cloning

Primer Design

1. In SnapGene, go to Actions -> Gibson Assembly -> Insert One Fragment.
2. Select the vector to use (i.e. the plasmid with the promoter) and select the restriction enzymes used to replace the insert.
3. Select the plasmid or cDNA sequence to use as the insert.
    a. Note: cDNA can be generated by extracting RNA with Trizol and performing RT with Superscript IV. Both Trizol and Superscript IV protocols can be found online.
4. Under Product, select Choose Overlapping PCR Primers. Use the default setting.
5. Go back to Fragment, and copy the primer sequences. Make sure to generate primers that are less than 60 nt, if possible.
6. Assemble the final product and save.
7. Order the primers.

PCR and Cloning

8. Use a 25 uL Q5 reaction protocol for the PCR. If possible, run 2 PCR reactions in parallel, one with annealing temperature of 55°C and another with 65°C. Alternatively, use Touchdown.
9. Concurrently, run a restriction digest of the vector, following the NEB protocol.
10. Run both the digested vector and the PCR product on a 1% agarose gel.
11. Gel extract and elute with 15 uL water.
12. Add 1 uL of the vector and 1 uL of insert (No need to Nanodrop) into 7.5 uL of homemade Gibson mix.
13. Incubate at 50°C for 15 minutes.
14. Transform 2 uL of the reaction into DH5a e.coli.
    a. Mix the DNA and e.coli. Incubate on ice for 5 minutes.
    b. Heat shock at 42°C for 30 seconds.
    c. Incubate on ice for 2 minutes.
    d. Add 200 uL of LB broth (without antibiotic).
    e. Incubate at 37°C for 30 minutes. Concurrently, warm the antibiotic plates at 37°C.
    f. For Gibson cloning, pipette 200 uL of the e.coli onto the plate with beads. Shake and flip upside down.
    g. Incubate overnight at 37°C (~16 hours).
15. Pick a colony using a pipette tip and put it in 3 mL of LB+Carb. Incubate in 37°C shaker for 8-16 hours.
16. For Maxipreps, add 2 mL of the above into 200 mL of LB+Carb. Incubate in 37°C shaker for 16 hours.
17. Maxiprep following the protocol.
    a. Note: After adding P3, there will be a lot of foamy white substance in the tube. Before pouring that into the syringe, put it in a 50 mL conical and centrifuge for 3 minutes at 500xg. Pipette the supernatant into the syringe.