[iGEM 2017] Gibson Assembly

Introduction

Combine linear fragments of double stranded DNA to create a circular plasmid.
Each fragment must have overlap regions (of ~20 bp) in the way that you wish to combine them. See the Primer Design procedure for how to design primers that will amplify fragments in this way.

The typical cloning pipeline is:
PCR -> Gel -> DpnI -> PCR Purification -> **Gibson Assembly** -> Transformation -> Colony PCR -> Inoculation -> Miniprep

Materials

- 2X Hi-Fi DNA Assembly Master Mix
- PCR Purification Product
- Nuclease-Free Water (NFW)
- 0.2 mL Tube

Procedure

Compute Volumes using the Gibson Volume Calculator

1. Go to the 5µl calculator, which is a spreadsheet on Google Drive/iGEM 2017/Calculators.

2. The spreadsheet calculates requisite volumes for one gibson reaction. In the yellow cells, enter in the requisite information for your reaction.

   The calculator accomodates 1 backbone fragment and 4 insert fragments. If you are not using all four insert fragments, for the unused rows set their desired moles and length to 0 and set their concentration to 1.
   If your fragment is a gBlock, enter "G" (without quotes, capitalized) into the "Concentration" cell in that row. We are assuming that gBlocks are diluted to 0.1 pmol/uL.

3. Ensure that the constraints in the red cells are satisfied-- these are total moles, total volume, and ratio of insert fragment to backbone fragment.

   If the constraints are not all simutaneously satsified, modify your "How much fragment would you like? (pmol)" values until they are.

4. Add each fragment (as a PCR Product), and NFW, to a 0.2 mL tube according to the volumes specified by the blue cells.

   Sometimes the calculator returns volumes which are very small. If the NFW volume is small (<= 0.4 uL), feel free to ignore it. If the fragment volume is small (< 0.5 uL) and you can't resolve the issue by changing your "How much fragment would you like?" values, it's probably okay to round up to 0.5 uL.

5. Record the volumes you used for each reaction in your notebook! Make sure you are also specific about which PCR products you are using.

6. *Once you get comfortable with using the calculator, you can go to the 'Bulk' tab in the spreadsheet and compute many gibson reaction simultaneously. There is no set protocol for how to do this, so always double-check that your formulas have been copied correctly in each cell.*

7. CRITICAL You may find that one of the PCR products has too small of a volume to pipette. (<.2µl). If this is the case, your solution is over concentrated, and you can simply 1:5 dilute a small volume (ex: .5µl DNA to 2µl NFW), and use that concentration in the calculator instead. In the rare case where you are over concentrated, you may use the 10µl gibson reaction, which is larger.

Perform the Assembly

8. Your tube now contains 2.5 uL of PCR product - NFW solution. Add 2.5 uL of 2X HiFi DNA Assembly MM.

9. Place the tube in the thermal cycler and set the program to "Gibson Assembly", and run with 10 uL total volume.

   The program is 50C for 1 hour, then hold at 4C.
   Gibson products are stable for a few hours, but not overnight!

10. Proceed relatively quickly to Transformation.