Gibson Assembly

Overview

This protocol covers the assembly of plasmids from overlapping DNA fragments via Gibson Assembly with NEB's 2x HiFi DNA Assembly Master Mix.

Materials

- Nuclease Free Water (NFW)
- Purified DNA Backbone
- Purified DNA Insert(s)
- .2mL Tube
- 2x NEB HiFi DNA Assembly Master Mix.
    - Do not remove from freezer until just before use. Keep in stratacooler. Do not contaminate. Do not waste. Each µL costs ~1.25 dollars when bought in bulk.

Procedure

1. Compute volumes for the reaction by going to the 5µl[i] calculator, which is a spreadsheet on google drive under calculators.
2. The spreadsheet calculates requisite volumes for one Gibson reaction. In the yellow cells, enter in the requisite information for your reaction.
    - There is also a bulk version which can be used for inputting a large number of 2 component Gibson reactions.
3. The calculator accommodates 1 backbone fragment and up to 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.
4. 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/µL.
5. Ensure that the constraints in the red cells are satisfied-- these are total moles, total volume, and ratio of insert fragment to backbone fragment.
6. If the constraints are not all simultaneously satisfied, modify your "How much fragment would you like? (pmol)" values until they are.
    - Volumes smaller than .2µL cannot be pipetted. If you get fragment volume that is less than .2µL, simply make a dilution of the sample (recommended 1:5). It is also recommended to dilute if volume is below .5 µL. If NFW volume is below .5µL either change dilution, scale up reaction, reduce total fragment moles, or be clever about volume mixing.
7. Add each fragment (as a PCR Product), and NFW, to a 0.2 mL tube according to the volumes specified by the blue cells.

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[i] NEB's protocol calls for you to perform a 20µL reaction. Which is of course absurd. We use 5µL because we cannot pipette smaller volumes accurately. Andy has told me he uses an acoustic liquid handler to do 2µL reactions with no problems

8. Record the volumes you used for each reaction in your notebook! Make sure you are also specific about which PCR products you are using.
9. *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.*
10. Your tube now contains 2.5 µL of PCR product - NFW solution. Add 2.5 uL of 2X HiFi DNA Assembly MM.
    - Ensure tube is not **frozen**. If frozen, thaw on ice. Resuspend any precipitate if present.
11. Place the tube in the thermal cycler and set the program to "Gibson Assembly", and run with 5 µL total volume.
    - The program is 50C for 1 hour[ii], then hold at 4C.
12. *Gibson products are stable for a few hours, but not overnight![iii]*
13. Proceed relatively quickly to transformation.

[ii] NEB says that 15 minutes is fine, but we've found that 1 hour performs vastly better.
[iii] Allegedly you can freeze them overnight (20C) and use the next day.