Standard Assembly (Combining Column 1 and 3)

Introduction

http://2013.igem.org/wiki/images/1/1c/Uppsala2013_BioBrick_Assembly_Manual.pdf
Still need to make it specific to columns 1 and 3

Materials

- Restriction enzymes (EcoRI-HF, XbaI, SpeI, PstI), NEBuffer 2, BSA
- 10X T4 DNA Ligase Reaction Buffer, T4 DNA Ligase B
- H2 O (not shown)
- Small PCR tubes
- 2 µl, 200µl pipet tips
- Destination plasmid as purified DNA
- Upstream and downstream parts as purified DNA
- 2 µl and 20 µl pipet
- incubator/water bath/thermocycler capable of holding 37o C and 80o C
- Rack for small PCR tubes
- -20oC freezer
- freezer box

Procedure

Preparing the reaction mix

1. Remove the DNA for the upstream part, the downstream part and the destination plasmid along with NEBuffer 2 and BSA from the freezer to thaw. Thawing is fast if the tubes are immersed in room temperature water. You can also remove the enzymes from the freezer but leave them in a cold box so they remain close to -20o C.

2. You will need three PCR tubes, one for the digest of the upstream part, one for the downstream part, and one for the destination plasmid. You should label each tube (for example, U, D, P, for upstream part, downstream part, and destination plasmid respectively).

3. To each tube, add H2 O and 500 ng of the part or plasmid to be digested. Adjust the amount of water you add such that the total volume in each tube is 42.5 µl.

4. Add 5 µl of NEBuffer 2 to each tube.

5. Add 0.5 µl of BSA to each tube.
6. Add 1 µl of the first appropriate* restriction enzyme to each tube**.
7. Add 1 µl of the second appropriate* restriction enzyme to each tube**
8. The total volume in each tube should now be 50 µl. Ensure the digest is well-mixed by flicking the tube. You can spin the tube in a microcentrifuge for a few seconds to collect the liquid in the bottom of the tube again.
9. Return all reagents and purified DNA to the -20o C freezer.

Incubations

10. Incubate the three restriction digests at 37o C for 30 min. Either a water bath, an incubator, or a thermocycler are suitable for this incubation.
11. Incubate the three restriction digests at 80o C for 20 min to deactivate the restriction enzymes. This step is most conveniently performed in a thermocycler so choose tubes for the restriction digest that fit in your thermocycler. As an easy way to confirm the digests worked, consider running 20 µl of each digest on a 1% agarose gel and look for bands of the expected length.
12. Store the three restriction digests at -20o C or proceed immediately to the ligation step.

Prepare reaction Mix

13. Remove the 10X T4 DNA Ligase Reaction Buffer* from the freezer to thaw. You can also remove the T4 DNA Ligase enzyme from the freezer at this point but leave the ligase in a cold box to keep it close to -20o C. Thawing is fast if the buffer tube is immersed in room temperature water. Once thawed, agitate the 10X T4 DNA Ligase Reaction Buffer until all precipitate goes into solution.
14. Add 11 µl of H2 O to a 200 µl PCR tube.
15. Add 2 µl from each of each of the digests to the tube**.
16. Add 2 µl of 10X T4 DNA Ligase Reaction Buffer to the tube.

Ligate

17. Add 1 µl of the T4 DNA Ligase to the tube.
18. The total volume in each tube should now be 20 µl. Ensure the ligation is well-mixed by flicking the tube. You can spin the tube in a microcentrifuge for a few seconds to collect the liquid in the bottom of the tube again.

Incubate

19. Incubate the reaction mix at room temperature for 30 min.

20. Incubate the reaction mix at 80o C for 20 min. The 80o C incubation deactivates the enzyme and improves transformation efficiency.

21. Store the ligation mix at -20o C or proceed immediately to the transformation step