pDONRp4p1R Preparation

The attP4 and attP1R sites can sometimes undergo self-recombination. Since this intramolecular reaction is greatly favored over recombination with an attB4-attB1 PCR product, batches of plasmid that self-recombine will give you very poor cloning efficiency. Fortunately, it is possible to select against clones that exhibit self-recombination.

Electroporation

- *you can also introduce plasmid into chemi-competent ccdB tolerant cells. They actually don't have to be that competent for introducing supercoiled plasmid.*

1. Add 1.0μL (10pg-1.0ng) of pDONRp4p1R plasmid to ccdB tolerant cells on ice.

2. Transfer mixture to chilled electroporation cuvettes and electroporate cells.

    We use 1 mm cuvettes with a BioRad Gene Pulser. Settings are: 1.8kV, 25μF, 200 ohms

3. Transfer cells to 500μL of LB and incubate at 37°C for 1h

4. Spread cells on kanamycin/chlormphenicol plates (kanamycin 50μg/mL and chloramphenicol 30μg/mL) and incubate at 37°C for 12-16h

Self Recombinant Test

1. Prepare kanamycin/chloramphenical LB broth that has a final concentration of kanamycin 50μg/mL and chloramphenicol 30μg/mL

2. Add 100mL of broth to six different 500mL flasks

3. Pick six different colonies and add to warm broth, and incubate at 37°C for 12h with constant mixing (230rpm)

4. Transfer 2.0 mL of each culture into 1.5 mL tube (for mini prep), and transfer the rest into a large centrifuge tube then spin at 6000xg for 15min at 4°C. Decant liquid and store pellets at -20°C for later.

5. Isolate plasmid DNA from each of the 2.0 ml cultures.

    We use the Qiagen miniprep kit for prepping plasmid at this step.

6. Quantify DNA using a UV spectrophotometer.

7. Set up BP reaction for each miniprep:

miniprep DNA	150 ng
10 mM Tris, pH 8.0	to 4 μl
BP clonase II	1 μl

- incubate at room temperature overnight

8. Dilute reaction by adding 1.0μL of reaction to 9.0μL of RO water

9. Add 1.0μL of each diluted BP reaction to *ccdB* tolerant cells (positive control; we recommend plating dilutions since there will be many positive clones) and 1.0μL to Top10 cells, and electroporate the cells as above.

10. Add the cells to 500μL of LB broth and then incubate at 37°C for 1h.

11. Plate cells on kanamycin resistant plates (50μg/mL) and incubate at 37°C for 12-16h.

    You should see a high number of colonies with the ccdB competent cells (will depend on the competence of your cells). In the ccdB sensitive cells (e.g. TOP10), you would like to see no colonies indicating that that particular prep does not undergo self-recombination. We usually see that about half of the clones have some degree of recombination indicated by the presence of colonies on the TOP10 plates.

12. Maxi prep pellets that didn't have self-recombination (i.e. no colonies in TOP10 cells on kanamycin plates) using QIAGEN maxi prep kit.