Recombineering protocol

Preparation of competent cells

Plasmids pKD46, pKD78, and pKD119 carry the λ red genes behind the *araBAD* promoter. Expression of the λ red genes is sufficiently induced by adding 0.1% of L-arabinose to your growing culture. The plasmids themselves are temperature-sensitive to be easily cured from your strain. By transforming any *E.coli* K-12 strain with one of those three plasmids, it turns them into highly recombination efficient strains when adding arabinose. But you have to grow them at max. 30 °C to maintain the plasmid! pKD46 carries the *bla* gene (ampicillin resistance), pKD78 carries the *cat* gene (chloramphenicol resistance), pKD119 carries the *tet* gene (tetracycline resistance).

1. Grow overnight culture at 30 °C in LB containing 50 µg/mL chloramphenicol
2. Inoculate 50 mL LB containing 100 µg/mL ampicillin and 20 mM arabinose with 500 µL overnight culture (i.e.1:100 dilution; use flask with baffles). Prior to inoculation, take out 1 mL of LB as a control for the spectrophotometer.
3. Incubate in or incubator at 30 °C until the OD_{600} is approximately 0.6 (0.5 – 0.8).
4. Immediately submerse the flask in ice-water slurry while swirling. Keep swirling for a few minutes. Leave on ice for 10 min.
5. Transfer culture to a prechilled 50-mL Falcon tube.
6. Centrifuge 10 min at 4200 *g* and resuspend pellet in 35 mL ice cold ddH_2O.
7. Centrifuge 10 min at 4200 *g* and resuspend pellet in 35 mL ice cold ddH_2O.
8. Centrifuge 10 min at 4200 *g* and resuspend pellet in 2 ml ice cold 10 % glycerol.
9. Centrifuge 10 min at 4200 *g* and resuspend pellet in 500 µL ice cold 10% glycerol.
10. Use cells directly for electroporation (keep on ice!), or freeze aliquots of 90 µL at -80 °C. Best to quick chill prior to freezing with liquid nitrogen (or have 1.5-mL tubes ready that are chilled to -80 °C).

Transformation and selection

1. If competent cells are frozen, thaw them on ice. Unpack the electroporation cuvettes and chill them on ice.
2. Add up to 100ng of the purified PCR product cassette (1-3 µL) to 40 µL of competent cells.
3. Transfer the competent cells into an electroporation cuvette.
4. Electroporate with Ec2 setting.
5. Recover cells immediately in 1 mL warmed SOC, and incubate shaking for 1 h at 37 °C (now you don't need the pKD78 plasmid anymore).
6. Plate 100 µL of culture on LB plates containing the appropriate antibiotic and/or selection agent.
7. Centrifuge the remaining 900 µL (5 min, 8000 *g*), remove 800 µL supernatant, resuspend pellet in remaining 100 µL, and plate on selective plates.
8. Incubate over night at 37 °C (or longer).
9. Pick colonies, streak on new selective plates/starters, and confirm genotype by PCR (one primer in the insert, one outside of it).
10. Incubate over night at 37°C
11. Make starter for freezing the bacteria at -80°C
12. Confirm the loss of pKD78 by plating 100 µL of the over night culture on Chloramphenicol plates, incubate over night at 37 °C. Not a single colony should grow.