7.13 Experimental Microbial Genetics

Fall 2008

PSEUDOMONAS ELECTROPORATIONS

Materials:

- o/n culture of recipient strain
- plasmid for electroporation
- 300mM sucrose (filter sterilized)
- LB
- 2 mm gap width cuvette

Protocol:

- grow overnight culture of *Pseudomonas* strain in LB at 37°C (1ml/electroporation)
- transfer 1ml o/n culture into a microcentrifuge tube and spin for 1 minute at 13,000 rpm
- carefully aspirate off supernatant and resuspend in 1ml 300 mM sucrose
- spin again at 13,000 rpm for 1 minute and repeat sucrose wash
- final spin at 13,000 rpm for 2 minutes, aspirate remaining liquid and resuspend in 100 ul sucrose
- if working with a replicative plasmid, add ~10 - 50 ng plasmid to 100 ul cell suspension. If working with a non-replicative plasmid (i.e. suicide plasmid) add ~ 300 – 500 ng plasmid.
- slowly mix cell suspension several times by pipetting and transfer into a 2 mm gap width cuvette
- gently tap and/or flick cuvette several times until cell suspension settles at the bottom between the two metal plates
- insert cuvette into electroporation apparatus and slide cuvette holder all the way back until cuvette is in contact with the electrodes
- electroporation settings should be 2.5 kV, 25 uF, 200 Ω
- press and hold two red buttons simultaneously until tone sounds
    - time constant (displayed after electroporation) should be 4-5 msec
- immediately resuspend cells in 1 ml room temperature LB and grow at 37C in rotator for 1-1.5 hours
- transfer cells to a microcentrifuge tube, spin at 13,000rpm for 2 minutes and aspirate supernatant leaving the cell pellet in ~50 ul LB
- resuspend cells in remaining 50 ul LB and plate on LB + antibiotic plate, incubate plates overnight at 37°C
    - antibiotic should correspond to resistance conferred by plasmid