ELECTROCOMPETENT *RHODOCOCCUS ERYTHROPOLIS* AN12

Competent cells for electroporation

1. (Day or two before) Inoculate small (5-10 ml) LB or NBYE cultures of *Rhodococcus erythropolis* AN12; grow at 30°C
2. Transfer 0.1-5 ml of overnight culture of *Rhodococcus* to 200 ml NBYE, 0.05% Tween-80 in a 1L baffled flask
3. Incubate shaking at 30°C overnight or until $O.D._{600}$ is between 0.8 and 1.0
4. Pellet the cells by centrifuging for 5 min at 6 000 rpm in a GSA rotor using sterile centrifuge bottles or 50 ml conical tubes and proper adapters (may have to spin twice to pool)
5. Resuspend the cell pellet in 30 ml ice-cold distilled, sterile water; transfer cells to sterile 50 ml conical tube (if you haven't already done so); Recentrifuge as in step 4 and discard the supernatant
6. Resuspend cell pellet one more time in distilled, sterile water; centrifuge as before; discard supernatant
7. Wash pellet once in 10 ml ice-cold, sterile 10% glycerol; centrifuge as before except at 8000 rpm; discard supernatant
8. Resuspend final cell pellet in 1 ml or less of ice-cold, sterile 10% glycerol
9. Aliquot 150 µl into sterile microfuge tubes and store at -80°C

Electroporation of *Rhodococcus*

1. Thaw aliquots of electrocompetent *Rhodococcus* cells on ice
2. Mix DNA with 70µl cells in a sterile microfuge tube and incubate on ice for 5 min.
3. Electroporate DNA at 2.5 kV, 25 µF and 400 Ω
4. Immediately add 300 µl NBYE
5. Incubate cells for recovery at 30°C for 1-6 hours
6. Spread cells onto plates with appropriate antibiotics

NBYE

0.8% nutrient broth (Difco)

0.5% yeast extract (Difco)

Tween-80 stock

5ml Tween-80

95 ml distilled water

Filter sterilize