ELECTROCOMPETENT *RHODOCOCCUS* I24 AND KY1

1. (Day or two before) Inoculate small (5-10 ml) cultures of *Rhodococcus* I24; grow at 37°C
2. Transfer 0.1-5 ml of overnight culture of *Rhodococcus* to 200 ml MB 1.5% Glycine (for I24 strain) or TSB Glycine (for KY1 strain) in a 1L baffled flask
3. Incubate shaking at 37°C overnight or until $O.D._{600}$ is approximately 0.25
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 EPB1; Recentrifuge as in step 4
6. Wash cell pellet one more time in EPB1; centrifuge as before; discard supernatant
7. Wash pellet once in 10 ml ice-cold EPB2; centrifuge as before except at 8000 rpm; discard supernatant
8. Resuspend final cell pellet in 1 ml or less of EPB2
9. Aliquot 150 µl into sterile microfuge tubes and store at -80°C

Electroporation of *Rhodococcus*

7. Thaw aliquots of electrocompetent *Rhodococcus* cells on ice
8. Mix DNA with 70µl cells in a sterile microfuge tube and incubate on ice for 5 min.
9. Electroporate DNA at 2.5 kV, 25 µF and 400 Ω
10. Immediately add 300 µl LB
11. Incubate cells for recovery at 30°C for 1-20 hours
12. Spread cells onto plates with appropriate antibiotics

MB 1.5% Glycine medium (per liter)

Yeast extract	5g
Bacto tryptone	15 g
Bacto soytone	5g
NaCl	5g
Glycine	15g

TSB Glycine(per liter)

Per liter	
Bacto Tryptone	17 g
Bacto Soytone	3 g
Sodium Chloride	5 g
Dipotassium Phosphate	2.5 g
Yeast Extract	5 g
Glycine	15 g

Hepes Stock Solution

Hepes	23.8g
distilled water	180ml

adjust pH to 7.2; raise volume to 200 ml

EPB1 (20 mM Hepes, 5% glycerol, pH7.2)

0.5 M Hepes stock, pH7.2	20ml
100% glycerol	25ml

distilled water to 500 ml

EPB2 (5mM Hepes, 15% glycerol, pH7.2)

0.5 M Hepes stock, pH7.2	2ml
100% glycerol	30ml

distilled water to 200ml