ELECTROCOMPETENT *RHODOCOCCUS ERYTHROPOLIS* SQ1 AND XO1

1. (Day or two before) Inoculate small (5-10 ml) cultures of *Rhodococcus*
2. Transfer 0.1-5 ml of overnight culture of *Rhodococcus* to 200 ml MB 3.5% Glycine supplemented with 1.8% sucrose and 0.01% isonicotinic acid hydrazide (isoniazid) in a 1L baffled flask
3. Incubate shaking at 30°C overnight or until $O.D._{600}$ is approximately 0.5-0.6
4. Add sterile ampicillin to a final concentration of 1 mg/L; continue inccubating for 1.5 hr at 30°C
5. 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)
6. Resuspend the cell pellet in 30 ml ice-cold EPB1; Recentrifuge as in step 4
7. Wash cell pellet one more time in EPB1; centrifuge as before; discard supernatant
8. Wash pellet once in 10 ml ice-cold EPB2; centrifuge as before except at 8000 rpm; discard supernatant
9. Resuspend final cell pellet in 1 ml EPB2
10. 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 LB
5. Incubate cells for recovery at 30°C for 1-20 hours
6. Spread cells onto plates with appropriate antibiotics

MB 3.5% Glycine medium (per liter)

Yeast extract	5g
Bacto tryptone	15 g
Bacto soytone	5g
NaCl	5g
Glycine	35g

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