WARNING NOTICE: The experiments described in these materials are potentially hazardous and require a high level of safety training, special facilities and equipment, and supervision by appropriate individuals. You bear the sole responsibility, liability, and risk for the implementation of such safety procedures and measures. MIT shall have no responsibility, liability, or risk for the content or implementation of any of the material presented. Legal Notices

## 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.<sub>600</sub> 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  $\mu$ F and 400  $\Omega$
- 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)		EPB1 (20 mM Hepes, 5% glycerol, pH7.2)	
Yeast extract	5g	0.5 M Hepes stock, pH7.2	20ml
Bacto tryptone	15 g	100% glycerol	25ml
Bacto soytone	5g	distilled water to 500 ml	
NaCl	5g		
Glycine 35g	- 0		
Hepes Stock Solution		EPB2 (5mM Hepes, 15% glycerol, pH7.2)	
Hepes	23.8g	0.5 M Hepes stock, pH7.2	2ml
distilled water	180ml	100% glycerol	30ml
adjust pH to 7.2; raise volume to 200 ml		distilled water to 200ml	