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 C. GLUTAMICUM AND RHODOCOCCUS SP. B264-1

(Competent Cells for Electroporation)

<u>Day 1</u>

- 1. Inoculate a single colony of *Corynebacterium* or *Rhodococcus* (or similar strain) into 2-5 ml of rich medium (e.g. LB, 2xYT, MB)
- 2. Incubate at 30°C overnight
- 3. Autoclave two 500 ml centrifuge bottles for spinning down cells tomorrow

<u>Day 2</u>

- 4. Inoculate 2 ml of the overnight culture into 200 ml MB 3.5% Glycine in a baffled flask
- 5. Incubate the culture on a shaker at 30°C till the OD_{600} is ~ 0.2 0.25 (approx. 3 hrs)
- 6. Add 1 μ l 100 mg/ml ampicillin
- 7. Incubate 1.5 hrs at 30°C shaking
- 8. Centrifuge cells in sterile centrifuge bottles at 5 000 rpm in SS34 rotor for 10 min, 4°C
- 9. Resuspend cells in 30 ml ice cold EPB1
- 10. Repeat centrifugation
- 11. Resuspend in 30 ml EPB1 two more times and centrifuge as before
- 12. Resuspend final cell pellet in 1.5 ml ice cold EPB2
- 13. Transfer 150µl aliquots of resuspended cells into microfuge tubes
- 14. Store cells at -80°C

Electrotransformation of competent cells

- 1. Thaw electrocompetent cells on ice
- 2. Mix 1-3 μ l DNA with cells
- 3. Incubate DNA and cells on ice for 5 min.
- 4. Set Gene Pulser aparatus (electroporator) to the following:
 - 2.50 kV, 200 Ohms, 25 µFd
- 5. Transfer DNA/cell mixture to chilled 2mm electroporation cuvette (no bubbles!)
- 6. Load a P1000 with 300µl of sterile LB and carefully set aside
- 7. Place cuvette into chamber and electroporate by holding down red buttons until the beep
- 8. <u>Immediately</u> add the LB to the electroporation mixture (directly into cuvette)
- 9. Incubate the cells 1-5 hrs at 30°C
- 10. Plate aliquots of cells onto appropriate selective medium

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		EPB2 (5mM Hepes, 15% glycerol, pH7.2)	
		0.5 M Hepes stock, pH7.2	2ml
		100% glycerol	30ml
		distilled water to 200ml	

Hepes Stock Solution

Hepes	23.8g
distilled water	180ml
adjust pH to 7.2; raise volume to	200 ml