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ELECTROCOMPETENT C. GLUTAMICUM AND RHODOCOCCUS SP. B264-1

(Competent Cells for Electroporation)

Day 1

- 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

Day 2

- 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 OD₆₀₀ is ~ 0.2 0.25 (approx. 3 hrs)
- 6. Add 1 μl 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 µl 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. Immediately 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, p.		rol, 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	