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 OD_{600} 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)

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

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	

Hepes Stock Solution

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