**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<sub>600</sub> 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 5000 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 Pulse apparatus (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 15g
- 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