Protocol 906_Electroporation Protocol

- Electrocompetent cells (aliquots are in 80 µL, stored in 10% glycerol at -80°C)
- High quality plasmid DNA (prepared via Qiagen kit)
- 1 cm diameter electroporation cuvette(s)
- Electroporator
- 5 mL warmed LB broth in glass flask (warmed SOB media works well for sensitive cells)
- LB plates with indicator/selective agent (i.e.: antibiotics)

Protocol

1. Thaw competent cells on ice
2. Pre-chill cuvettes on ice (must be cold)
3. Add 0.5-1.0 µL plasmid DNA (approx. 100ng) to the cells, and allow to sit on ice for 1 min. (do not invert/mix)
4. Transfer aliquot to chilled cuvettes, then place in electroporator (settings: preset at "bacteria 1", 1.8kV, 200Ω, 25µF)
5. Pulse; optimal time constant 4.7-5.0 (record constant)
6. Rinse cuvette with fresh LB broth and transfer cells to flask with warmed LB media
7. Incubate at 37°C for 1 hr stationary (!!)
8. Remove 100 µL of supernatant, create dilution series and plate on appropriate selective media
9. Centrifuge for 10 min. at 4,000 rpm (4°C)
10. resuspend pellet in 100 µL of leftover supernatant or sterile PBS or sterile broth
11. Plate re-suspended pellet on Kan-LB media, incubate overnight at 37°C