**Transformation of Chemically Competent Cells**

Solutions

* 0.1M CaCl2 – should be stored at 4°C.
* 20% glycerol/0.1M CaCl2 – should be stored at 4°C.

Making Competent Cells

Day 1:

* Prepare a 5mL overnight sample (5mL LB + 1 colony desired bacteria).

Day 2:

* Grow day culture: add 3mL overnight to 300 mL LB.
  + Grow to 0.7 O.D.600, then chill on ice.
* Spin cells using F14 rotor at 5.5k and 4°C for 10 minutes to pellet.
* Re-suspend cells in 80 mL of ice cold 0.1M CaCl2.
* Incubate cells on ice for 60 minutes.
* Spin cells using F14 rotor at 5.5k and 4°C for 10 minutes to pellet.
* Re-suspend cells in 4mL of 20% glycerol/0.1M CaCl2.
* Make 100μL aliquots. Store at -80°C.

Heat Shocking Competent Cells

* Thaw cells on ice.
* Add 5μL DNA into 100μL cells. Transfer to 14 mL Polypropylene Round-Bottom Tube.
* Incubate for 15 minutes on ice.
* Heat-shock cells in water bath at 42°C for 45 seconds-2 minutes.
* Add 1mL SOC media. Incubate at 37°C for 60 minutes.