Transformation of chemically competent cells Entered by Cheryl Borgman
Janes Lab Protocols 3/15/17

Before starting:

- Thaw chemically competent cell suspension on ice
- Prepare LB/antibiotic plates and allow to dry if necessary
- Turn on the shaker temperature to equilibrate

1. Aliquot 20 μl of competent cell suspension (located in pre-aliquoted microcentrifuge tubes in the –80°C freezer) into a pre-chilled Falcon tube (BD #352059) and incubate for 2 minutes on ice.
   - *The size and brand of the tube used can change effectiveness due to plastic thickness.*
   - *Competent cells are frozen as 50 μl aliquots sufficient for two transformations.*
2. Add 1 μl plasmid DNA to the Falcon tube and incubate for 30 minutes on ice.
3. Heat shock the bacteria by placing the Falcon tube in a 42°C water bath for exactly 45 seconds; then, immediately place on ice for 2 minutes.
   - *There are many different variants of chemically competent cells. Check the protocol for the cells being used for recommended heat shock conditions.*
4. Add 1 ml SOC medium to each tube and incubate at 37°C with agitation for 60 minutes.
   - *Volume, temperature, and/or time may change based on the cells and/or the plasmid used.*
5. Plate 0.5 ml onto LB/antibiotic plates, and allow them to sit for approximately 10 minutes.
   - *Be sure to use the antibiotic appropriate for the plasmid.*
   - *Less transformation mix can be added if high transformation efficiency is expected.*
6. Incubate overnight at 37°C. Make sure to store the plates upside down in the dry heat incubator.
   - *Temperature and/or time may be different based on the cells and/or the plasmid used.*
7. Remove plates from the incubator, wrap in parafilm, and store at 4°C.
   - *Plates can only be stored at 4°C for a few weeks before they will begin to grow mold.*

Buffer recipes

- SOC medium (for 1 L total volume):
  - To 950 ml Milli-Q water, add:
    - 20g Tryptone
    - 5g Yeast Extract
    - 0.5g NaCl
  - Mix with a magnetic stir bar unti dissolved.
  - Add:
    - 10 ml of dissolved 250 mM KCl
  - Adjust the pH to 7.0 with 5 N NaOH.
  - Adjust the volume to 1 L.
  - Autoclave
  - Once cooled, add 20 ml of 1 M glucose.

- LB/antibiotic Plates:
  - LB Media (Luria-Bertani media):
    - To 950 ml Milli-Q water, add:
      - 10 g Tryptone
      - 5 g Yeast Extract
      - 10 g NaCl (Janes Lab concentration)
      - pH to 7.0
      - *(CSH protocol recommends 5 g NaCl)*
  - To LB Media, add:
    - 15 g/L Agar
    - Autoclave
    - Add appropriate antibiotic when agar has cooled to 50–60^{o}C.
    - Pour 20–30 ml onto each plate and allow to harden at room temp.
    - Invert and seal (bagged or with parafilm) plates, then store at 4^{o}C.