***E. coli* transformation** Rahul Patharkar

1. Thaw competent cells at room temperature until they just begin to thaw (note: shake droplets of thawed competent cells on the sides of the tube to the bottom). Once the cells begin to thaw, place them on ice.
2. Add between 0.5-2 µL ligation or DNA to the cells. Keep on ice for 2-30 minutes and gently mix once by flicking.
3. Heat shock at 42°C for 30 sec in a water bath. Immediately place cells back on ice for 2 minutes.
4. If your plasmid confers ampicillin resistance, plate the cells directly on LB amp plates. Otherwise add 0.5-1mL LB or SOC to the cells and shake at 37°C for 1 hour. Then plate your cells on appropriate media after spinning them down to remove most of the media.
5. Grow plates at 37°C O/N or at room temperature over the weekend.

Notes:

- 100-200 µL of competent cells made by the Inoue method *Gene, 96, (1990) 23-28* should be used to obtain the maximum transformants when dealing with difficult ligations.
- 10 µL of competent cells (even ones that have been thawed and re-frozen) are more than sufficient for obtaining plenty of colonies from supercoiled plasmid DNA.
- SOC recovery media give a slightly better number of colonies than LB, however, this difference rarely fixed a no colonies situation for me. Therefore, I typically use LB because I always have LB.
- 20 min. recovery time is sufficient for antibiotics that require it. It is possible that longer incubations simply allow multiplication of transformants rather than producing extra transformants.

For video protocol see: https://rahulpatharkar.000webhostapp.com/2018/10/e-coli-transformation-and-plating