**Created by Yongtao Zhu (Spring 2021)**

**Transformation**

1. Label one tube of *E. coli* competent cells (kept on ice) with plasmid + primer names.
2. Add 10 µl of ligation product (centrifuged briefly before use) to the cells. Do not pipette but can stir gently to mix the cells with DNA. Keep the tubes on ice for 30 mins.
3. Set the heating block at 42oC (add water to the wells for better heat transduction and use a thermometer to monitor the temperature if needed).
4. Heat shock: Transfer the tube to the 42oC heating block and incubate exactly 90 seconds.
5. Keep the tube on ice for 3-5 mins.
6. Add 1 ml of LB broth to the tube and incubate at 37 C for 1 hour.
7. Centrifugation at 7000 rpm for 3 mins and remove the supernatant.
8. Add 400 µl of fresh LB broth and resuspend the cells.
9. Sterilize a glass hockey stick by using flame and let it cool.
10. Labe 2 LB agar (with ampicillin) plates with the plasmid + primer names, and the date.
11. Transfer 100 µl of the cells onto each plate and store the leftover cultures at 4oC.
12. Spread the cells evenly on the agar using the sterile hockey stick.
13. Incubate the plates (inverted) at 37oC for no more than 24 hours and transfer them to 4oC.