E. coli Transformation Protocol

(Klein, October 2016)

If using S17 electrocompetant cells, thaw one vial on ice for 15 minutes prior to transformation. Proceed to either section B or C.

A) If transforming any other strain:

1. Grow recipient strain overnight in LB (2 ml culture per transformation)
2. Subculture the strain 1:100 in fresh LB and grow for 3 hours (5 ml per transformation)
3. Pre-chill 2 mm electroporation cuvette (blue cap)
4. Transfer the culture into a 15 ml tube and centrifuge for 10 min at 4000 rpm at 4°C
5. Keep tubes on ice for remainder of protocol
6. Decant the supernatant. Wash the pellet in 1 ml cold-sterile water and transfer to a microcentrifuge tube
7. Centrifuge for 2 min at 8000 rpm at 4°C
8. Repeat steps 6-7 once more
9. Resuspend pellet in a total of 50 µl water

B) For replicating plasmids:

1. Add 20 ng of miniprepped plasmid to bacteria
2. Pipette bacteria into cuvette
3. Pulse on EC2 setting
4. Recover in 1 ml LB for 1 hr at 37°C
5. Place a 10-200 µl of transformation onto selective plates and streak/spread for single colonies

C) For ligation reactions:

1. Add 4 µl of dialyzed ligation mixture to bacteria
2. Pipette bacteria into cuvette
3. Pulse on EC2 setting
4. Recover in 1 ml LB for 1 hr at 37°C
5. Place a 10-200 µl of transformation onto selective plates and streak/spread for single colonies