

#### Transformation:

1. Thaw competent cells on ice
2. Add DNA to competent cell PCR tubes (3uL from BioBrick suspensions or 20 uL from ligations)
3. Vortex to homogenize
4. Place in ice for 30 minutes
5. Heat shock at 42 C for 90 seconds
6. Place back in ice for 2 minutes to recover
7. Add 100 uL of recovery broth and resuspend
8. Incubate at 37 C while shaking at 300 rpm
9. Take 2 agar plates, pipet 100 uL onto each and streak with inoculation loops
10. Incubate overnight at 37 C

#### Mini-Prep:

1. Pour 6 mL cell broth into 15 mL tubes
2. Centrifuge at 3500 rpm for 5 minutes
3. Decant completely and discard supernatant
4. Add 250 uL Buffer 1 to pellet
5. Vortex to resuspend
6. Place 250 uL Buffer 2 into a new 1.5 mL tube
7. Take 250 uL of resuspended cells+Buffer 1 and pipet into the tube with Buffer 2
8. Mix by inverting ten times
9. Quickly open and add 350 uL of Buffer 3
10. Mix by inverting twelve times
11. Place in freezer for 10 minutes
12. Centrifuge at max speed for 10 minutes
13. Transfer supernatant to silica DNA bind column
14. Incubate at room temperature for 1 minute
15. Spin at max speed for 1 minute
16. Remove 'flow through' and reassemble column
17. Pipet 700 uL onto the silica disc at top of column
18. Quickly spin at max speed for 1 minute
19. Discard 'flow through' and spin at max speed for 1 minute again
20. Transfer top of silica column to a new 1.5 mL tube
21. Dispense 200 uL of 60 C diH<sub>2</sub>O onto center of silica disc
22. Incubate at room temperature for 1 minute
23. Spin at max speed for 1 minute
24. Discard silica top, label tube and place in freezer