

## Electroporation

### Materials:

1. Ice
2. Electro-competent E. coli (~10uL of cells per DNA sample)
3. Electroporation cuvettes (0.1cm)
4. SOC media at 37°C (250uL per sample)
5. DNA/ plasmids
6. 15mL Falcon tubes
7. LB+antibiotic resistance plates
8. shaking and non-shaking 37°C incubators

### Methods:

1. Slowly thaw electrocompetent cells from the -80°C freezer on ice. It is important for the cells to stay ON ICE until use.
2. Cool all electroporation cuvettes on ice.
3. Thaw frozen SOC media (-20°C freezer) in the 37°C water bath.
4. Warm LB plates in 37°C incubator.
5. Add 10uL of competent cells to an electroporation cuvette. The cells need to rest on the bottom of the cuvette and touch the metal plates on either side of the cuvette. NO BUBBLES!
6. Add 3uL of ligated DNA to the cells. **MAKE SURE THAT THE DNA IS IN AN EXTREMELY LOW SALT SOLUTION!** For ordinary ligations the amount of salt is **far too much**. Typically a 1:5 dilution of a ligation reaction in deionized water is required to prevent electrochemical arcing during the electroporation process. If you see a \*spark\* or if you hear a loud click, or if you see the work “ARC” show up on the screen, you did not dilute your salt enough.
7. For electroporating non-ligated (stock) DNA you need SUBSTANTIALLY LESS DNA. Typically 1µl of a 1:10 dilution of a DNA stock (stock is usually 1mg/ml) is sufficient for thousands of colonies.
8. Electroporate at these settings:
  - a. High Voltage; 2.75KV; 99usec; 5 pulses; 1 sec interval
9. Add electroporated cells and 250 uL of 37°C SOC media to a sterile 15mL tube.
10. Incubate @ 37°C in a shaking incubator (200-250rpm). Incubation should be for 30 minutes if using Ampicillin selection. Incubation should be for 60 minutes if using Kanamycin selection.
11. Make serial dilutions onto LB (*selective!*) plates and incubate at 37°C overnight.
12. Select several single colonies, grow liquid cultures in 5ml LB, isolate plasmids with minipreps, and check by restriction digest.