E. coli **Transformation**

Before you begin, check for:

- 42°C Water Bath
- Room Temperature S.O.C. Medium

- Spread X-gal or antibiotics onto LB agar, if necessary.
- Warm plates in a 37°C incubator for 30 minutes.

1. Thaw competent cells on ice. (25-50µl per tube, split into several reactions if tube has more)
2. Add 1-5µl of DNA; mix gently. DO NOT PIPETTE UP AND DOWN.
3. Incubate tube for 15-30 minutes on ice.
4. Heat shock the cells for 30-45 sec in the 42°C degree water bath.
5. Place on ice for 2 minutes (no more than 2 minutes).
6. Add 250 µl of S.O.C. medium.
7. Place in 37°C room and tape tubes to shaker for 30-60 minutes.
8. Spread 125-250µl of the transformation on a pre-warmed plate with spreading beads.
9. Invert plates and incubate at 37 degrees C.

Amp/Carb Selection – should have visible growth within 8 hours
Other Antibiotics – should have visible growth after incubating overnight
TG1 Cells – should have visible growth after 8 hours

What to do next

Colony PCR or grow cultures and sequence plasmid to find colonies that have your gene of interest.