Bacterial Transformation of DH5a

Materials Needed

- 42° C water bath
- Ice
- LB plates with appropriate antibiotic
- Competent Cells (DH5a, XL1 Blue, or other as appropriate)
- Sterile SOC or LB (no antibiotics)

Protocol

1. Thaw competent DH5a cells on ice
2. Gently mix cells with the pipet tip (do NOT pipet up and down) and aliquot 50µl of cells for each transformation into 1.5ml tubes that have been pre-chilled on ice
    - Bacteria may be re-frozen and returned to the -80° freezer by freezing in the Papa Cooler.
3. Add 1-5µl DNA (1-100ng) to the cells and mix gently (do NOT pipet up and down)
    - If doing a ligation, it's OK to add 10µl)
4. Incubate the tubes on ice for 30 minutes
5. Heat shock at 42°C for exactly 30 seconds without shaking
6. Place tubes on ice for 2 minutes
7. Add 250µl of pre-warmed (37°C) SOC or LB (make sure this does not contain antibiotics)
8. Shake at 37°C for 1 hour
9. Spread 20µl and 200µl of each transformation onto LB plates with appropriate antibiotic
10. Allow plates to dry and incubate inverted at 37°C overnight

Notes

- Thaw cells in ice. DO NOT thaw by warming them with your hands
- To maintain competency, bacteria should be kept on ice at all times. Once they have warmed up, they are no longer competent and will not take up DNA.
- Add LB or SOC using sterile procedures (ie in the hood)