Transformation of *E. coli*

Use aseptic techniques!

Calcium chloride method:

For pure plasmid DNA clones, transform 10 ng of plasmid into 10 μL of competent cells. For ligation reactions, use 10 μL of cells for each μL of ligation mix.

(1) Thaw the competent cells on ice.
(2) Mix the DNA and competent cells in a sterile 1.5-mL microfuge tube.
(3) Incubate in ice for 30 min.
(4) Heat shock at 42 °C for 30 sec (1 min for volumes ≥50 μL).
(5) Incubate in ice for 2 min.
(6) Add 9 volumes of SOC or LB medium containing **no antibiotics**. Media pre-warmed to 37 °C work best, but this is not required. Shake for at least 30 min (45-60 min if the plasmid encodes resistance to an antibiotic other than ampicillin). When transforming pure plasmids encoding ampicillin resistance, steps 6 and 7 are not required.
(7) If the total volume is >100 μL, spin down the cells in a microcentrifuge for 1 min at 8,000 *g*. Remove all but ~100 μL of the supernatant. Resuspend the cells in the remaining liquid.
(8) Plate on LB-agar (preferably pre-warmed) containing the appropriate antibiotics.
(9) Allow the plate to dry for around 10 mins, then incubate upside-down at 37 °C overnight.

Things to look up / think about:

How does calcium chloride make *E. coli* competent to take up DNA?
Why do we pre-grow the cells in media with no antibiotics prior to plating? Why can we skip this step if the antibiotic is ampicillin?