HEAT SHOCK TRANSFORMATION OF *E. COLI*

TREAT COMPETENT CELLS GENTLY - THEY ARE VERY FRAGILE

1. Inoculate 5 ml broth (plus antibiotic) with a single colony. Incubate shaking at 37°C overnight. Prewarm 2 x 50 ml LB at 37°C overnight.
2. Add antibiotic to prewarmed broth and inoculate each 50 ml broth with 500 µl overnight culture.
3. Grow at 37°C with shaking until OD_{550} = 0.5.
4. Transfer cells to 4 x 30 ml centrifuge tubes. Chill on ice for 30'.
5. Pellet cells in a benchtop centrifuge at 4000 rpm for 5' at 4°C.
6. Discard supernate and resuspend each pellet very gently in 10 ml ice cold, sterile 100 mM $CaCl_2$, 20mM $MgCl_2$
7. Combine cells in 2 tubes, place on ice for 1 hr and then pellet cells as in 5.
8. Discard supernate and resuspend each pellet in 1.25 ml ice cold, sterile 100 mM $CaCl_2$, 20mM $MgCl_2$. Combine cells so that they are in one tube.
9. Place on ice at 4°C overnight.
10. Add 0.375ml of cold, sterile 100% glycerol. Leave on ice for 15-20'
11. Make aliquots of 250µl in 0.5ml tubes. Use directly or store at –80°C.
12. Add 20–100 ng DNA to 250 µl cells. Mix gently & store on ice for 30'.
13. Heat shock at 42°C for 2'.
14. Add 1 ml broth to the tube. Incubate 1 hr at 37°C without shaking.
15. Spread 500µl on a plate and incubate overnight at 37°C.

$CaCl_2$ +$MgCl_2$ solution

100mM $CaCl_2$	10 ml 1M
20mM $MgCl_2$	2 ml 1M

dH_2O to 100ml.
Autoclave to sterilise