Standard transformation using heat-shock method

Frozen competent cell preparation

→Overnight culture *E. coli* in SOB medium (eg. DH5α) (5ml)

→Subculture to 50 ml SOB medium (1:100), shake at 37°C, 30 mins (O.D.=0.3)

→Transfer culture into centrifuge tube, put on ice for 10-15 mins

→Centrifuge 2000-3000rpm for 12-15 mins at 4°C

→Collect pellet, and resuspend in 16.6ml FSB, put on ice for 10-15 mins

→Centrifuge 2000-3000rpm, 12-15 mins at 4°C

→Resuspend in 4ml FSB

→+ 140 μl DMSO (i.e. to 3.5%), put on ice for 10 mins

→+ 140 μl DMSO (i.e. to 7%), put on ice for 10-20 mins

→Quick freezing by placing on dry-ice or liquid nitrogen, then store at -70°C

Transformation

-Take 100 μl (or 200) competent cell, + 5-10 μl ligased DNA

-Put on ice for 30 mins

-Heat shock at 42°C, 2 mins

-Put back on ice for 2 more mins

-Add 400 μl SOC medium (or LB) per tube

-Incubate and shake at 37°C, 30-60 mins

-Spread the cells to plates (50μl, 200μl)

SOB medium (for 500 ml):

tryptone	2%	10g
yeast	0.5%	2.5g
NaCl	10mM	0.3g (5M, 1ml)
KCl	2.5mM	0.093g (1M, 1.25ml)
$MgCl_2$	10mM	1M, 5ml
$MgSO_4$	10mM	1M, 5ml

SOC medium: SOB + 20mM glucose