E. coli Transformation

1) Grow cells in LB or 2YT to OD_{550} 0.3-0.6.

2) Spin 5 minutes at 4,000 RPM and 4° C.

3) Resuspend cells on ice in 1/20 volume:

50ml culture
→ 2.5mls
store as 500 λ aliquots

LB broth, pH 6.1
10 % PEG 3350
5 % DMSO
10 mM $MgCl_2$
10 mM $MgSO_4$

} keeps indefinitely in the frige.

4) Ice 10 minutes.

1µL

5) Mix DNA in 100 µl KCM (100 mM KCl, 30 mM $CaCl_2$, 50 mM $MgCl_2$).
Keep on ice.

6) Add 100 µl competent cells to DNA/KCM. (divide in 1/2)
Mix gently.
Ice 10-20 minutes.

7) Heat shock at room temperature 10 minutes.

8) Express as Usual. or. 50c at 37°c shake 1hr.
For amp^r plasmids I leave at room temp 15 minutes, then plate.
For other drugs, add 1 ml 2YT and roll at 37 ° C for 1 hour.

To Freeze Competent Cells: Make10% glycerol at step 4, freeze in dry ice/ethanol bath, and store at -80° C. Thaw cells on ice, then continue with step 5.

Derived from Chung and Miller, 1988, NAR, 16#8, p.3580.

Procedure used at Genentech
I freeze cells without adding glycerol and they work well