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:
   - 50 ml culture
   - → 2.5 ml
   - store as 500 μl aliquots

4) Ice 10 minutes.

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. Mix gently. Ice 10-20 minutes.

7) Heat shock at room temperature 10 minutes.

8) Express as Usual. Or, 50°C at 37°C shak 1 hr.
   - 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: Make 10% 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.