

## CaCl<sub>2</sub> Chemical Competence (for *E. coli*)

### DAY I: STREAK OUT

### DAY II: O/N CULTURE

- (1) Inoculate 2 mL O/N culture from single colony from part I

### DAY III: GROW

- (2) Subculture (back-dilute) 1:25-1:50 in 100mL fresh media  
2mL culture → 100 mL media for 102 mL total is fine
- (3) Grow cells to mid-log (OD<sub>600</sub>=0.3-0.5)  
Put in shaker at 200 rpm at 37C  
Grow for ca. 1.5 hours
- (4) Harvest cells by centrifugation, pour off supernatant  
4000 rpm for 20 min, or 5000 rpm for 10 min; more time as needed  
Use two 50 mL Falcon conicle tubes  
Fast-cool the centrifuge to 4C
- (5) Resuspend cells in COLD (4C), STERILE CaCl<sub>2</sub>  
Put 10 mL CaCl<sub>2</sub> in each conicle, for a total of 20 mL  
Use 100 mM (.1 M) CaCl<sub>2</sub> for most cases  
Use 50 mM (.05 M) CaCl<sub>2</sub> for M13 hosts, i.e. TG1 or JM103)
- (6) Incubate O/N in the cold room

### DAY IV: FINISH

- (7) Pellet cells by centrifugation, pour off supernatant  
4000 rpm for 20 min, or 5000 rpm for 10 min; more time as needed  
Fast-cool the centrifuge to 4C
- (8) Resuspend cell pellet in COLD (4C), STERILE CaCl<sub>2</sub>  
Put 2 mL CaCl<sub>2</sub> in each conicle, for a total of 4 mL  
Use 100 mM or 50 mM as needed

If using immediately: store on ice for 2-12 hours prior to transformation; after 24 hours, efficiency of transformation drops.

If storing: add 0.5 mL COLD (4C), STERILE 50% glycerol to each tube, for 1 mL total. Aliquot 310 µL into eppi. tubes and store at -80C.