CaCl2 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 (OD600=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 CaCl2
   Put 10 mL CaCl2 in each conicle, for a total of 20 mL
   Use 100 mM (.1 M) CaCl2 for most cases
   Use 50 mM (.05 M) CaCl2 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 CaCl2
   Put 2 mL CaCl2 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 mL into eppi. tubes and store at -80C.