**Competent Cells**

**RF1**

for 100mls

 **ingredient: amt: [FINAL]**

Rb Cl: 1.2g: 100mM

MnCl2: 0.99g: 50mM

K0Acetate: 3ml of 1M: 30mM

CaCl2: 0.15g: 10mM

glycerol: 15ml: 15%

pH final 5.8 use 0.2 M acetic acid

Sterilize via 0.22 micron filter, pre-rinse with sterile water.

**RF2**

**ingredient: amt: [FINAL]**

MOPS: 2ml of 0.5 M pH 6.8 : 100mM

Rb Cl: 0.12g: 10mM

CaCl2 : 1.1g: 75mM

glycerol: 15ml: 15%

pH final 6.8 use NaOH

filter sterilize.

**When making competent cells, ALWAYS KEEP EVERYTHING COLD: cells and all solutions.**

1) Streak out appropriate cells on plate with correct antibiotic (e.g. XL-1tetracycline, DH-5a-no antibiotics). Pick a single colony-grow overnight with correct antibiotic.

2) Dilute O/N 1/150 into two 30mls LB in a 250 ml flask (200ul into 30ml of broth). Shake vigorously (200 rpm) at 37oC until cell densities equal 4-7 x107 (1OD550 = 8 x 108, thus grow to 0.4 OD measured at 550nm).

3) Transfer to cold 33ml tube. Chill on ice, for approximately 10 minutes.

4) Pellet cells at 3000g at 4oC (4500rpm) for 15 min. Keep pellet, remove all fluid.

5) Resuspend cells in 1/3 vol RF1-(i.e. 10 ml) by gentle vortexing. Keep cold.

6) Incubate on ice for 45 min.

7) Pellet cells at 3000g at 4oC (4500rpm) for 15 min. Keep pellet, remove all fluid.

8) GENTLY, resuspend cells in RF2, 1/12.5 of original volume (2.4 ml). Incubate on ice for 15 min.

9) Aliquot 200ul into COLD cyrotubes with O-rings.  Date and label tubes.  10) Flash freeze in liquid N2. Store at -80oC.