Rubidium Competent Cells

Solutions:

TFB I (30mM KOAc, 50mM $MnCl_2$, 100mM RbCl, 10mM $CaCl_2$, 15% (Glycerol)

		250mL
KoAc	1.472g	0.736
$MnC1_2$	4.95 g	2.475
RbC1	6.05 g	3.03
$CaC1_2$ (Dihydrate)	0.735 g	0.368
Glycerol	75 ml	37.5

Adjust pH to 5.8 with acetic acid. Open in hood!! Very Careful!! (CAREFUL, not buffered well!!!) Did not need much dilute 1:10, few drops. Up to 500 ml with dH2O. Sterile filter and store at 4°C.

TFB II (10mM NaMOPS, pH 7.0, 75mM $CaC1_2$, 10mM RbC1, 15% Glycerol)

		250mL
MOPS	1.046 g	0.523
$CaC1_2 \cdot 2H_2O$	5.513 g	2.757
RbC1	0.605 g	0.303
Glycerol	75 ml	37.5

Adjust pH to 7.0. Up to 500 ml with dH_2O. Sterile filter and store at 4°C.

Protocol:

1. Grow a 5ml O/N culture of the desired strain.

2. Add 1 ml of the O/N culture to 2 separate flasks with 100 ml LB each and grow with shaking at 37°C to $OD_{600} \sim 0.6$. (Takes about 2 hours.)
   *Add antibiotic if needed!!

3. Transfer the culture to two 50 ml conicals and chill on ice for 20 min. (Be very careful not to contaminate the caps. WEAR GLOVES.)

4. Spin down the cells at 4°C @ max (5100 RPM) in the Allegra 25R centrifuge for 5 min.

5. Pour off the supernatant and resuspend each pellet in 15 ml of TFB I (gently by hand) and shake on ice for 20 min. (taped to spinner in 4°C coldroom).

6. Spin as in step 4 and discard the supernatant.

7. Gently resuspend each pellet in 2 mls of TFB II and incubate on ice for 30 minutes.

8. Aliquot the cells in 200 ul aliquots and freeze at -70°C in autoclaved labeled eppendorfs.

***Try to do steps 3-8 in the cold room as much as possible.**