Making *E. coli* Competent cells: (Rubidum chloride method)

Procedure:

1. Inoculate 1 ml from overnight culture into 100 ml LB broth (scale up or down as needed). Incubate at 37^0C with aeration to A600=0.4

2. Keep on ice 15 min.

3. Pellet cells in 50 ml falcon tube (3-5000 x g 5 min)

4. Discard supernatant and add 1/3 volume of TfbI, resupend and incubate back on ice for 15 min.

5. Pellet cells at low speed. (3000 x g 5 min).

6. Discard supernatant and resupend in 1/30 volume of TfbII (1/30 of the culture volume), Keep on ice for 15 min and either use immediately or quick freeze at -70C for storage. I usually save these in 0.1 to 0.2 ml aliquots. Quick freeze in liquid nitrogen prior to storage in a -70 C freezer. Thaw on ice just before using in a transformation experiment.

I typically transform 100 ul cells with 2-10 ul of a ligation reaction, and you should get between $1x10^8$ to $1x10^9$ cfu's/ug DNA.

TFB I (per 200 ml)		
compound	**amount**	**final conc.**
potassium acetate	.588 g	30 mM
rubidium chloride	2.42 g	100 mM
calcium chloride	0.294 g	10 mM
manganese chloride	2.0 g	50 mM
glycerol	30 ml	15% v/v
pH 5.8 with dilute acetic acid		

TFB II (per 100 ml)		
compound	**amount**	**Final conc.**
MOPS	0.21 g	10 mM
calcium chloride	1.1 g	75 mM
rubidium chloride	0.121 g	10 mM
glycerol	15 ml	15% v/v
pH 6.5 with dilute NaOH		