Rubidium Chloride method for Transformation Competent *E. coli*

Procedure

1. Inoculate 1 mL from overnight culture into 100 mL Psi broth (scale up or down as needed). Incubate at 37 °C with aeration to A550=0.48

2. Ice 15 minutes

3. Pellet cells in appropriate centrifuge tubes 3-5000 x g 5 minutes (~5000 rpm in a Sorvall SS-34 rotor)

4. Discard supernatant and add 0.4 volume (ie. of original volume, here it is 40 mL) TfbI, resuspend and ice 15 minutes

5. Pellet cells as in #3

6. Discard supernatant and resuspend in 0.04 volume TfbII, ice 15 minutes and either use immediately or quick freeze at -70°C for storage. I usually save these in 0.25 to 0.5 mL aliquots. Quick freeze on ethanol-dry ice or liquid nitrogen prior to storage in a -70°C to -80°C freezer. Thaw on ice just before using in a transformation experiment. I typically transform 50 µL cells with 2-10 µL of a ligation reaction, and you should get between $1x10^8$ and $1x10^9$ cfu's/µg DNA.

Medium and Buffers:

Psi Broth (per Liter)		
Bacto yeast extract	5g	
Bacto Tryptone	20g	
Magnesium sulfate	5g	
pH 7.6 with potassium hydroxide		
TfbI (per 200mL)		
Potassium acetate	0.588g	30mM
Rubidium chloride	2.42g	100mM
Calcium chloride	0.294g	10mM
Manganese chloride	2.0g	50mM
Glycerol	30mL	15% v/v
pH 5.8 with dilute acetic acid		
TfbII (per 100mL)		
MOPS	0.21g	10mM
Calcium Chloride	1.1g	75mM
Rubidium Chloride	0.121g	10mM
Glycerol	15mL	15% v/v
pH 6.5 with dilute NaOH		