U.S. Dept Commerce/NOAA/NMFS/NWFSC/Molecular Biology Protocols

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 min.

3. Pellet cells in appropriate centrifuge tube 3-5000 x g 5 min (~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, resupend and ice 15 min.

5. Pellet cells as in #3.

6. Discard supernatant and resuspend in 0.04 volume TfbII, ice 15 min and either use immediately or quick freeze at -70C for storage. I usually save these in 0.25 to 0.5 ml aliquots. Quick freeze in ethanol-dry ice or liquid nitrogen prior to storage in a -70 to -80 C freezer. Thaw on ice just before using in a transformation experiment.

I typically transform 50 ul cells with 2-10 ul of a ligation reaction, and you should get between $1x10^8$ to $1x10^9$ cfu's/ug DNA.

Medium and Buffers

Psi broth (per liter)

compound	amount
Bacto yeast extract	5 g
Bacto Tryptone	20 g
magnesium sulfate	5 g

pH 7.6 with potassium hydroxide

TfbI (per 200 ml)

compound	amount	final molarity/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

TfbII (per 100 ml)

compound	amount	final molarity/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

I don't remember the original source for this. I'm sure there are published protocols that are the same or similar, but this has always worked well for us.

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