

Start day 15

## Making Competent Cells

### DAY 1

Grow an overnight culture in LB.

Important: Make sure autoclaved centrifuge bottles are at ~~20°C~~

Important: Make sure buffers are at 4°C

just put on  
ice before hand

### DAY 2

Important: Make sure that centrifuge is at 4°C; get dry ice; get big bucket of ice for aliquoting (in making competent cells, keep them as cold as possible)

Dilute the overnight 1:250 and re-grow until  $OD_{600} \sim 0.4-0.6$  (this should take about 4 to 5 hours if overnight has grown to saturation)

(less if in baffled flasks  $\approx 3.5$  hr)

Pour into sterile centrifuge bottles and spin down at 5K for 10 minutes (4°C).

$\wedge$   
cold

(used 5 x 500 mL bottles + 1 balance)

Resuspend in 1/5 vol. TFBII (e.g. if originally 250 ml, add 50 ml of TFBII). Let sit on ice 20-30 minutes.

Spin down and resuspend in 1/50 vol. TFBII (e.g. if originally 250 ml, add 5 ml of TFBII). Transfer to tubes in 215  $\mu$ l aliquots.

Snap freeze aliquots in dry ice and ethanol

use 100  $\mu$ l aliquots

150  $\mu$ l aliquots

### BUFFERS

or  $N_2$

TFBI (filter-sterilize and store at 4°C)

30 mM KOAc ✓  
50 mM  $MnCl_2 \cdot 4H_2O$  ✓  
100 mM KCl ✓  
10 mM  $CaCl_2 \cdot 2H_2O$  ✓  
15% Glycerol

TFBII (filter sterilize and store at 4°C)

10 mM Na-MOPS pH 7  
75 mM  $CaCl_2$  ✓  
10 mM KCl ✓  
15% Glycerol