PREPARATION OF COMPETENT CELLS

From Maggie 6/20/08

Reagents:

Transformation/Storage Buffer (TSB)	LB with:	5.5 mL	2xTY (or 2xYT)
	43 mM NaCl	95 µL	5 M NaCl
	10% PEG	1.1 gm	PEG (MW 3350)
	5% DMSO	550 µL	DMSO
FILTER STERILIZE	10 mM $MgCl_2$	110 µL	1 M $MgCl_2$
	10 mM $MgSO_4$	110 µL	1 M $MgSO_4$
	10% glycerol	1.1 mL	100% glycerol
		3 mL	water

5x KCM buffer			
	0.5 M KCl	5.1 mL	1 M KCl
	0.15 M $CaCl_2$	1.5 mL	1 M $CaCl_2$
FILTER STERILIZE	0.25 M $MgCl_2$	2.5 mL	1 M $MgCl_2$
		0.9 mL	water

Protocol:

Making competent cells:

1. Inoculate 5mL 2xTY or LB with a single colony or 10uL competent cells from previous batch. Grow overnight, shaking at 37°C. (plus tet OK with XL-1).

2. Inoculate 200 mL 2xTY or LB (no selection) w/ 1 mL of the overnight culture.

3. Grow, shaking, at 37°C for ~4 hr until OD_{600} = 0.3 -0.6. Use 2xTY or LB as the "blank" reference for the spec.

4. Spin cells to a pellet in JLA 10.5 rotor, 2300 rpm (1000g), 15 min., 4°C.

5. Resuspend pellet in 10 mL TSB.

6. Incubate on ice, 10 min. Aliquot 100µL into sterile 0.5 mL or 0.2mL tubes. Keep cells on ice while aliquoting.

7. Freeze tubes in dry ice/ethanol bath. Store at -80°C.

Test competency of cells by transformation:

1. Thaw a vial of competent cells on ice. [NOTE: OK TO REFREEZE; EFFICENCY WILL DECREASE SOMEWHAT]

2. Mix 10 ng plasmid DNA, 5 µL 5x KCM buffer, and water to bring volume to 25 µL. Add 25 µL competent cells. The total volume is now 50 µL.

3. Incubate on ice for 20 min. then at room temperature for 20 min.

4. Add 200 μL SOC medium. Incubate 30-60 min., shaking at 37°C.
   or LB

5. Plate 10 μL, 5 μL, and 1μL onto LB-Amp50 plates. Plate with enough SOC medium to spread a total of 50 μL. Use sterile glass beads or flamed cell spreader to spread the cells evenly on the plates.

6. Incubate overnight at 37°C.

7. Count the colonies on the plates. Calculate the total number of colonies per microliter of plated cells, then calculate the total number of colonies per ug of DNA used for the transformation. This is the efficiency of the competent cells.

Example: If there are 2000 colonies on the plate where 5 μL was plated, there are 400 colonies per μL of culture.

Since the culture was 250 μL (50 μL transformation reaction plus 200 μL SOC), there are 400 x 250 colonies (= 100,000) in that culture.

Each transformation reaction used 10 ng DNA (=0.01 μg), so the number of colonies per μg DNA = 100,000 / 0.01 = 1 x 10^7 colonies / μg DNA. An efficiency of 10^7 is acceptable.

References:
1. Chung and Miller (1988) NAR 16:3580.
2. DAW protocol
3. see 4/26/96 (KSU); 11/4/98 (LH)