Electrocompetent cell preparation for Shewanella (4/04 revision)

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(Ref: No clear ref for this one- modified from several standard electrocomp cell prep methods).

Required Reagents:
LB (500 mls) (or Psi Media* may help a bit- still checking that out)
15% Glycerol solution (500 mls)
10% Glycerol solution (4 mls)
Liquid Nitrogen or a dry ice/ethanol bath

Cell Growth:
Grow cells overnight in 3mL LB.
Dilute the 3mL culture into 500 mls of LB in a 4L flask. (Keep media to flask vol ratio ~ 1:10)
Incubate on shaker at 37°C until OD_{600} ~ 0.3-0.5 (about 2-4 hours in LB).

Electrocompetent cell preparation:
Chill flasks on ice for 30 min.
Pour cells into 2- 250 mL centrifuge bottles.
Centrifuge at 4000 x g for 20 min at 4°C.
Pour off supernatant. Resuspend each pellet in 200 mls of cold 15% glycerol.
(Resuspend **VERY** gently with a wide bore pipette)
Centrifuge cells at 5000 x g for 10 min at 4°C.
Pour off supernatant.
Gently resuspend each pellet in 30 mL 4°C 15% glycerol and transfer to 4°C 50 mL tubes.
Spin cells at 5000 x g for 10 min at 4°C.
Pour off supernatant.
Resuspend cells in 4 mL chilled 10% glycerol (add 2 mls to each tube and then pool).
(Measure OD of a 1:100 dilution or do a plate titer determination (should be around 10^9 or 10^{10} cfu/ml)).

Freezing:
Chill 40 0.5 mL microfuge tubes on ice.
Aliquot 100µl of cells into each tube. (Pipette gently using a P-1000 or something with a wide opening to avoid damaging the cells).
Immediately freeze by dropping tube into liquid nitrogen.
Store at -80°C. (Cells perform well for at least 3 months)

Expected efficiency ~ 10^2 to 10^3 with plasmid isolated from *E. coli* and 10^7 to 10^8 with plasmid isolated from *Shewanella.* (*Shewanella* has a very active Restriction-Modification system)

Tips:
Growth stage is critical- OD_{600} ~ 0.4 is optimal. Start over if the cell density goes above 0.5.
Good aeration during growth is important- use a media:flask vol ratio of ~ 1:10
Treat the cells **VERY** gently throughout and work quickly.
Before starting this protocol be sure to chill the glycerol solutions in the fridge or cold room. I usually put the glycerol solution in the cold room at the same time I start the overnight culture. Also, make sure the centrifuge and rotor are kept cold. Keeping the cells cold at all times during the washes is **ESSENTIAL**.
The initial low osmotic strength washes commonly used with E. coli should NOT be used with *Shewanella* as cell viability drops dramatically- (use 10-15% glyerol).
The pellets are kind of loose- avoid jostling the centrifuge tubes following the spins to prevent excessive cell loss. I find that it's best to do everything right at the centrifuge itself.
Snap freezing (liquid nitrogen or dry ice/methanol) is also very important for viability and efficiency. Don't cheat on this.

***Psi Media:** (For 500 mls) Psi media improved transformation efficiency by about 2 fold in one comparison (still need to check that out again).
If you want to try it, here's the recipe (From Hanahan, Ch 6 pp109-135 DNA Cloning Vol 1 Glover)

2% bacto-tryptone (Difco)	10g
0.5% yeast extract (Difco)	2.5g
10mM NaCl	0.3g
2.5mM KCl	0.093g
10mM MgCl2	1.17g
10mM MgSO4	1.23g

pH to 7.6 with KOH and filter-sterilize