*Electro*Competent Cells

Protocol:

(1) Streak E. coli strain onto LB plate.

(2) Pick O/N into 5 ml LB.

(3) Inoculate 1.0 ml of the O/N into 1000ml LB.

(4) Grow to mid log phase (OD_{590} ~ 0.600; about 3-5 hours). (To determine the cells' OD_{590} use the DU640 spec.)

(5) Chill cells by placing the flask into an ice bath.

(6) Spin cells down at 2,500 X g (~4200 RPM in SLA1500 rotor) for 5min at 4°C.

(7) Resuspend cells in 1000ml ice cold sterile dH_2O.

(8) Spin cells down 10,000 X g (~8500 RPM in SLA1500 rotor) for 15min at 4°C.

(9) Repeat with a second 1000ml volume of ice-cold sterile dH_2O.

(10) Repeat with 500ml ice-cold sterile dH_2O.

(11) Resuspend in 20ml ice-cold sterile 10% Glycerol.

(12) Transfers cells to an Oak Ridge tube.

(13) Spin cells down at 10,000 X g (~9000 RPM in SS34 rotor) for 15min at 4°C.

(14) Resuspend in 2ml ice-cold sterile 10% glycerol.

(15) Aliquot into convenient volume (50 - 125µl).

(16) *Quick-freeze tubes in liquid N_2.

(17) Store cells at -80°C.

Media and Stuff:

(1) LB (1000 ml)

Bacto tryptone	10.0g
Yeast extract	5.0g
NaCl	2.5g
NaOH	2 pellets
dH_2O	to 1000ml

(2) Sterile dH_2O ~2.5L
(3) 10% glycerol
(4) 0.5 ml Microfuge tubes
(5) 250 ml centrifuge bottles
(6) Oak Ridge Tubes
(7) Sterile pipettes (pre-chilled)
(8) Sterile pipette tips (pre-chilled)
(9) SLA1500 rotor (pre-chilled)
(10) SS34 Rotor (pre chilled)

Notes:

All media, solutions, centrifuge tubes, microfuge tubes and things that come into contact with the cells need to be sterile.

I keep the NaCl concentration low in my LB when making *Electro*competent cells.

Cells must be kept on ice the entire time cold after harvest (work in cold room or keep cells on ice).
I wash my cells 2 X with equal vol dH_2O whereas other protocols only do it once.

Cells don't pellet as well after being washed in H_2O; hence the increase in g forces; alternatively the length of the centrifuge run may be increased.

*Optional; i.e., cells can be placed directly into the -80^oC freezer without freezing them first in N_2.