Preparation of electrocompetent activated EL350/E250 cells

Day One: This step can be done at the end of a day.

1. Inoculate a single colony of *E. coli* from a fresh agar plate into a 50-ml flask containing 15 ml of LB medium. Incubate the culture overnight at 32ºC in a rotary shaker (250 rpm).

Day two: It starts in the morning and takes about ~ hours. Have these solutions ready: prewarmed 1 liter LB medium, pre-cooled 500 ml of Ultra-pure water, 10% glycerol, and GYT medium.

2. Inoculate 500 ml of prewarmed LB medium in a 2-liter flask with 10 ml of the overnight bacterial culture. Incubate the flasks at 32ºC with agitation (300 rpm in a rotary shaker). Measure the OD_{600} of the growing bacterial cultures every 20 min.

   This density is usually achieved after ~2.5 hours of incubation for DH5a (or DH10B?). It is essential that the density does not exceed OD_{600}, 0.4.
3. When the OD_{600} of the cultures reaches 0.5, transfer the flask to a 42C shaker for 15 min.
4. Immediately transfer the flasks to an ice-water bath for 15-30 minutes. Swirl the culture occasionally to ensure that the temperature drops as fast as possible. Incubate on ice for an additional 10 min. In preparation of the next step, place the centrifuge bottles in an ice-water bath, and pre-cool the rotor and chamber of centrifuge to 4ºC.

   For maximum efficiency of transformation, it is crucial that the temperature of the bacteria not rise above 4ºC at any stage in the protocol.
5. Transfer the cultures to ice-cold centrifuge bottles (2X 250 ml). Harvest the cells by centrifugation at 1,000g (2,600 rpm in a Sorvall SLA 1500 rotor) for 8 min at 0ºC. Decant the supernatant, resuspend the cell pellet in 250 ml of ice-cold pure H_2O (1X 250 ml bottle).
6. Harvest the cells by centrifugation at 1,000g for 10 min at 0ºC. Decant the supernatant and resuspend the cell pellet in 50 ml of ice-cold 10% glycerol in a 50-ml Falcon tube.

   This should be done immediate after the centrifugation finishes. Take care when decanting because the bacterial pellets lose adherence in 10% glycerol.
7. Harvest the cells by centrifugation at 1,000g for 20 min at 0ºC in a bench top centrifuge. Decant the supernatant and resuspend the cell pellet in 10 ml of ice-cold 10% glycerol.
8. Harvest the cells by centrifugation at 1,000g for 20 min at 0ºC. Carefully decant the supernatant and use a Pasteur pipette attached to a vacuum line to remove any remaining drops of buffer. Resuspend the pellet in 4 ml of ice-cold GYT medium.

   This is best done by gentle swirling rather than pipetting or vortexing.

9. Transfer 40 μl of the suspension to an ice-chilled electroporation cuvette (0.2-cm gap) and test whether arching occurs when an electrical discharge is applied. If so, wash the remainder of the cell suspension once more with ice-cold GYT medium to ensure that the conductivity of the bacterial suspension is sufficiently low.
10. Dispense 50-μl aliquots of the cell suspension into sterile, ice-cold 0.5-ml microfuge tubes, drop into a bath of liquid nitrogen, and transfer to a –70ºC freezer.

Solutions:

Glycerol (10% v/v)

GYT Medium

10% (v/v) glycerol
0.12% (w/v) yeast extract
0.25% (w/v) tryptone
Sterilize the medium by passing it through a prerinsed 0.22-μm filter. Store in 2.5 ml aliquots at 4ºC.