Preparing Electrocompetent BJAdEasy1 Bacterial Cells
by TCH 1/24/02

Preparation: LB/Streptomycine/Amp: 1000ml, kept at room temp for next morning use;
10% Glycerol (v/v): 2000-3000ml, autoclaved, kept at 4°C;
6-8 of -liter flasks, autoclaved;
4 of 200ml-centrifuge bottles for ***Eppendorf 5810***, autoclaved.

Note: The BJAdEasy1 cells are BJ5183 cells transformed with pAdEasy-1 plasmid. It is very important to characterize the integrity of the pAdEasy-1 in the cell stock. It is advised to perform restriction digestions with enzymes, such as *Hind*III or *Pst*I, on the DNA purified from the stock culture, and compare the digestion patterns with that of the pAdEasy-1 stock plasmid.

1. Use a fresh colony or frozen stock of BJAdEasy1 cells to inoculate 10ml of LB/Strep/Amp medium in a 50ml conical tube. Grow cells in a shaker overnight at 37°C. ().

2. Dilute 1 ml of cells into 1000 ml of LB/Strep/Amp medium in eight 1-liter flasks (or 125 ml each). Grow for 4 to 5 hours with vigorous aeration at 37°C until the OD_{550} reaches 0.8. (**Monitor the OD_{550} closely once the reading reaches 0.3, i.e., every 20 min**).

3. Combine cells to one flask and incubate on ice in the cold room for at least 1.0hr to overnight (Note: the longer the cells are incubated, the higher the competency).

4. Divide cells into four 200ml-centrifuge bottles. Pellet bacterial cells by centrifuging at 3,900 rpm (***Eppendorf 5810***) at 4°C for 30 min. (If the culture is more than 800ml, it is possible to pellet the cells twice using the same bottle).

5. Decant the supernatant (<u>It should be clear! If it's cloudy, longer centrifugation may be needed</u>). Resuspend the cell pellet in 1,000ml of sterilized, ice-cold 10% glycerol (or 200ml per centrifuge bottle). Make sure mix well (by vortexing or pipetting up-and-down) and always keep cells on ice.

6. Centrifuge the cell suspension at 3,900 rpm (***Eppendorf 5810***) for 30 min at 4°C.

7. Carefully decant most of the supernatant. Use 10ml pipettes to remove the remaining 20-50ml liquid.

8. Add about 20ml of ice-cold 10% glycerol to each bottle, mix well and combine the cell suspension to one bottle. Rinse other three bottles with 10ml ice-cold 10% glycerol and combine the rinsing solution to the remaining one. Fill the bottle with 10% glycerol.

9. Centrifuge the cell suspension at 3,900 rpm (***Eppendorf 5810***) for 30 min at 4°C.

10. Carefully decant most of the supernatant. Use 10ml pipettes to gently remove the remaining approx. 50ml liquid.

11. Repeat the washing step once by resuspending the pellet in 200ml of ice-cold 10% glycerol. Centrifuge the cell suspension at 3,900 rpm (***Eppendorf 5810***) for 30 min at 4°C.

12. Carefully pour off the supernatant, gently pipette most of supernatant off leaving about 20 ml. Transfer the cell suspension to a 50ml sterile conical tube and fill the tube with ice-cold 10% glycerol. Spin at 3,900 rpm x 20 min, and pipette all but 2ml of the supernatant out. (Note: it is important to keep the competent BJ5183 cells concentrated because of their lower competency).

13. Resuspend cell pellet in the remaining liquid in the tube. Aliquot 20ul per 1.5ml tube and store the aliquots at -80°C. (**Optional:** you can achieve better competency by pre-chilling the 1.7ml microfuge tubes at -80°C, and by snap-freezing the aliquots in dry ice-methanol bath before storing them at -80°C).