Electrocompetent cell prep:

1. Fresh colony of US0 (-)F’1321-TF inoculate 300 ml 2 xYT +Tc (10 ug/ml)+ Chloramphenicol (30 ug/ml), grow at 21°C O/N
2. Next morning check O.D.600 to make sure about 0.40; if below, grow the cells at 37 °C till O.D. about 0.40
3. Collect cells in 500 ml tubes (fill the tube with ultra pure water to full) and keep on ice for 20’
4. Spin down at 4 °C for 10’, 5000 rpm
5. Discard supernatant and resuspend the pellet with 350 ml cold ultra pure water and spin down at 4 °C for 10’, 6000 rpm
6. Repeat step 5)
7. Resuspend the pellet with 50 ml cold 10% glycerol and spin down at 4 °C for 15’, 6500 rpm
8. Repeat 7)
9. Discard supernatant and resuspend the pellet with 10% glycerol in less than 300 ul total volume
10. Make 5-6 aliquot of cells, each with about 60 ul
11. Put on dry ice
12. Store at -80°C