Competent Cell Preparation Protocol

1. Plate cells onto agar gel which contains NO antibiotic. Incubate Overnight.
2. Make 50ml of buffer (NO H2O) by adding
   a. 10% PEG 3350
   b. 20mM MgCl2
   c. 5% DMSO
   d. Fill to volume with LB
3. Filter in steriflip (Or using Milipore Vacuum Filtration.)
4. Place buffer in fridge, place on ice the next day.
5. Set up overnight from colony with NO antibiotic.
6. Use a 500ml flask to shake a mixture of 100ml LB and 2ml of the overnight.
7. Measure OD of starting solution. Starting OD should be between .05 and .1. If starting OD is >.1, dilute with LB until appropriate OD is achieved.
8. Shake @37 degrees Celsius until the Optical Density (Measured on spectrophotometer) is between 0.3 and 0.4. Test 1ml per IR test.
9. Spin down in 2 50ml falcon tubes at 3000RPM for 10 minutes
10. Pour off supernatant, re-suspend (gently) in 5ml of Ice Cold TSS Buffer
11. Aliquot into pre-frozen Micro centrifuge Tubes (Either 50ul or 100ul per aliquot) and place in -80.