Protocol for high efficiency E. coli chemically competent cells

1. SOB medium (250ml)

Tryptone	5g	2%
Yeast extract	1.25g	0.5%
NaCl	0.146g	10mM
KCl	0.0466g	2.5mM
$MgCl_2•6H_2O$	0.508g	10mM
$MgSO_4$	0.301g	10mM
Total volume		250ml

2. TB buffer (200ml)

10mM	PIPES	0.604g
15mM	$CaCl_2•2H_2O$	0.441g
250mM	KCl	3.7275g

- Add H_2O to 150ml
- Adjust pH to 6.7 by 5M KOH

55mM	$MnCl_2•4H_2O$	2.18g
H_2O	to 200ml	

Method

1. Pick a single colony from a LB/Tet (or other requisite antibiotic) plate and inoculate to 3ml SOB medium + 12.5μg/ml tetracycline (or other antibiotic) and culture at 37°C for 6-7 hours
2. Transfer the 3ml SOB/culture to 250ml SOB and shake at 18°C for 20-24 hours
3. When OD_{600} reach 0.4-0.6*, put the culture vessel to ice-water bath for 10 minutes
4. Chill down centrifuge for 15min, then harvest cells by 2500g centrifugation for 15min at 4°C
5. Pour off the medium and resuspend pellet with 40mL cold TB buffer on ice
6. Harvest cells by 2500g centrifugation for 15min at 4°C
7. Pour off the medium and resuspend cells with 10mL TB buffer on ice and add 0.75mL DMSO
8. dispense aliquot of the cells into pre-chilled 1.5ml tube and freeze the cells with nitrogen liquid, store them in -80°C