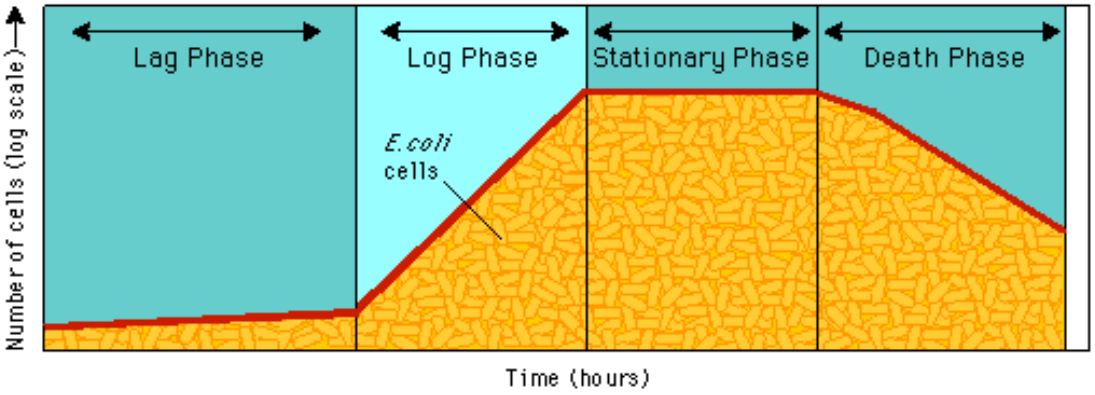
Preparation of Competent Cells

Competent cells:

E.coli are more likely to incorporate foreign DNA if their cell walls are altered so that DNA can pass through more easily. Such cells are said to be "**competent**". Cells are mode competent by a process that uses **calcium chloride ($CaCl_2$) and heat shock**. Cells that are undergoing very rapid growth (@ Log phase) are made competent more easily than cells in other stages of growth.

http://www.phschool.com/science/biology_place/labbench/lab6/competen.html



Procedure:

1. Streak from DB 3.0 stock onto LB plate. Incubate overnight at 37°C, 200 rpm
2. Pick a single colony and inoculate a 2 ml culture of SOB. Incubate overnight at 37°C
3. Add 20 ul overnight culture to **50 ml** SOB. Grow until OD_{600}=0.6-0.8 (37°C, 200 rpm)
   Protocol from Yang Lab: add 2 ml overnight culture to 500 ml SOB, and shake at 23°C; 80 ml TfbI resuspension; 30-50 ml TfbII resuspension
4. Put the flasks on ice for **10 min** to arrest the *E.coli* growth. (*From now on, keep everything as cold as possible and work on ice*)
5. Divide the bacterial solution into 16 sterile 1.5 ml Eppendorf tube; add **1 ml** bacterial solution into each tube; centrifuge at **6,000 rpm for 5 min at 4°C**; repeat 3 times to pellet all 50 ml SOB
   Protocol from Yang Lab: centrifuge at 4,000 rpm for 10 min at 4°C
6. Discard supernatant carefully, and for each tube, *gently* resuspend cell pellet in **500 ul TfbI** while keep on ice
7. Incubate on ice for **15 min** (Protocol from Yang Lab: 30 min)
8. Pellet cells at **4°C for 10 min at 4000 rpm**
9. For each tube, resuspend cell pellet in 312 ul TfbII while keep on ice
10. Incubate on ice for **15 min** (Protocol from Yang Lab: 30 min)
11. Aliquot **100 ul** into new sterile 1.5 ml Eppendorf tube
12. Flash freeze in liquid nitrogen and store at -80°C

SOB solution

	For 1 L	For 500 ml	Final [c]
Tryptone	20.0 g	10.0 g	
Yeast extract	5.0 g	2.5 g	
NaCl (Sodium chloride)	0.6 g	0.3 g	10 mM
KCl (Potassium chloride)	0.5 g	0.25 g	6.7 mM
$MgSO_4$ (Magnesium sulfate)	1.2 g	0.6 g	10 mM
$MgCl_2•6H_2O$ (Magnesium chloride hexahydrate)	2.0 g	1.0 g	10 mM
pH 7.0, NaOH			

* sterilize the solution through autoclaving
* Tryptone (胰蛋白胨) is the assortment of peptides formed by the digestion of casein by the protease trypsin. It provides a source of amino acids for the growing bacteria.
* Yeast extract (酵母粉) is a complex and widely used hydrolysate of yeasts. It provides nitrogenous compounds, carbon, sulfur, trace nutrients, vitamin B complex and other important growth factors, which are essential for the growth of diverse microorganisms.

TfbI (transformation buffer I)

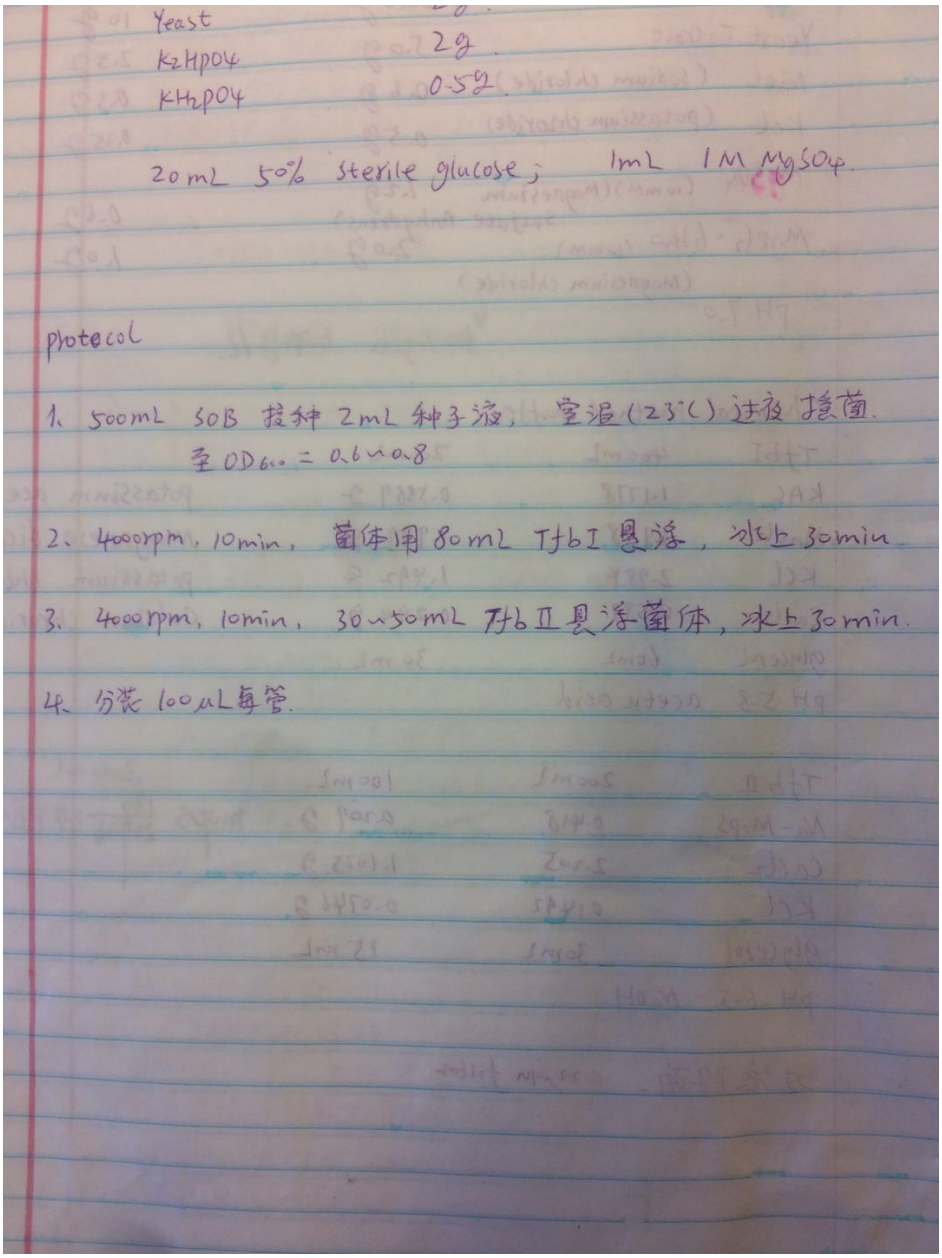
	For 400 ml	For 200 ml	Final [c]
KAc (Potassium acetate)	1.1778 g	0.5889 g	30 mM
$MnCl_2$ (Manganese(II) chloride)	3.958 g	1.979 g	79 mM
KCl (Potassium chloride)	2.984 g	1.492 g	100 mM
$CaCl_2$ (Calcium chloride)	0.588 g	0.294 g	13 mM
Glycerol	60 ml	30 ml	15% (v/v)
pH 5.8, Acetic acid			

* sterilize the solution using syringe filter with a 0.22 μm pore size

TfbII (transformation buffer II)

	For 200 ml	For 100 ml	Final [c]
MOPS	0.418 g	0.209 g	10 mM
$CaCl_2$ (Calcium chloride)	2.205 g	1.1025 g	100 mM
KCl (Potassium chloride)	0.1492 g	0.0746 g	10 mM
Glycerol	30 ml	15 ml	15% (v/v)
pH 6.5, NaOH			

* sterilize the solution using syringe filter with a 0.22 μm pore size
* MOPS is frequently used as a buffering agent in biology and biochemistry.



Yeast
K_2HPO_4 2g
KH_2PO_4 0.5g

20mL 50% sterile glucose; 1mL 1M $MgSO_4$

protocol

1. 500mL SOB 接种 2mL 种子液，室温(23°C)过夜摇菌。至 OD_{600} = 0.6~0.8
2. 4000rpm, 10min, 菌体用 80mL TfbI 悬浮，冰上 30min。
3. 4000rpm, 10min, 30~50mL TfbII 悬浮菌体，冰上 30min。
4. 分装 100μL每管。

SOB	1 liter	500 mL	250mL
Tryptone	20.0 g	10 g	5
Yeast Extract	5.0 g	2.5 g	1.25
NaCl (Sodium chloride)	0.6 g	0.3 g	0.15
KCl (potassium chloride)	0.5 g	0.25 g	0.125
$MgSO_4$ (10mM) (Magnesium Sulfate Anhydrous)	1.2 g	0.6 g	0.3
$MgCl_2 \cdot 6H_2O$ (10mM) (Magnesium chloride)	2.0 g	1.0 g	0.5

pH 7.0

↓ for $MgCl_2$ 0.95 g/L

Chemical method buffer

Tfb I	400mL	200mL	
KAc	1.1778	0.5889 g	potassium acetate
$MnCl_2$	3.958	1.979 g	Manganese dichloride
KCl	2.984	1.492 g	patassium chloride
$CaCl_2$	0.588	0.294 g	Calcium chroride
glycerol	60mL	30 mL	

pH 5-8 acetic acid

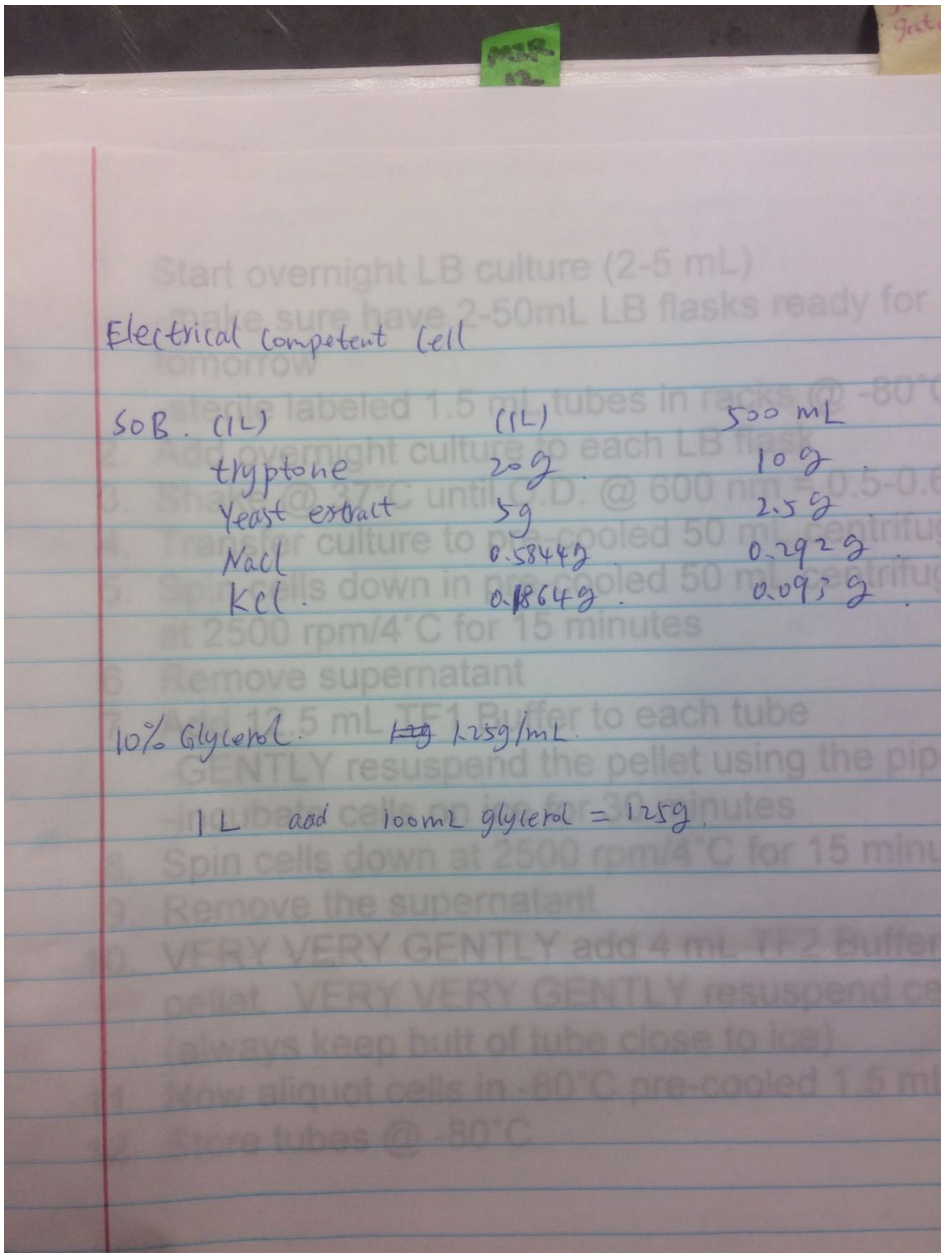
Tfb II	200mL	100mL	
Na-Mops	0.418	0.209 g	
$CaCl_2$	2.205	1.1025 g	
KCl	0.1492	0.0746 g	
glycerol	30mL	15 mL	

pH 6-5 NaOH

200mL

mops $\frac{186}{209.26} \times 0.418 = 0.3715$

过滤除菌. 0.22μm filter



Electrical competent cell

SOB.	(1L)	(1L)	500 mL
tryptone		20g	10g
Yeast extract		5g	2.5g
NaCl		0.5844g	0.292g
KCl		0.1864g	0.093g

10% Glycerol: ~~1.5g~~ 1.25g/mL

1L add 100mL glycerol = 125g