

**During Amaconing keep membrane glossy surface facing  
towards water**

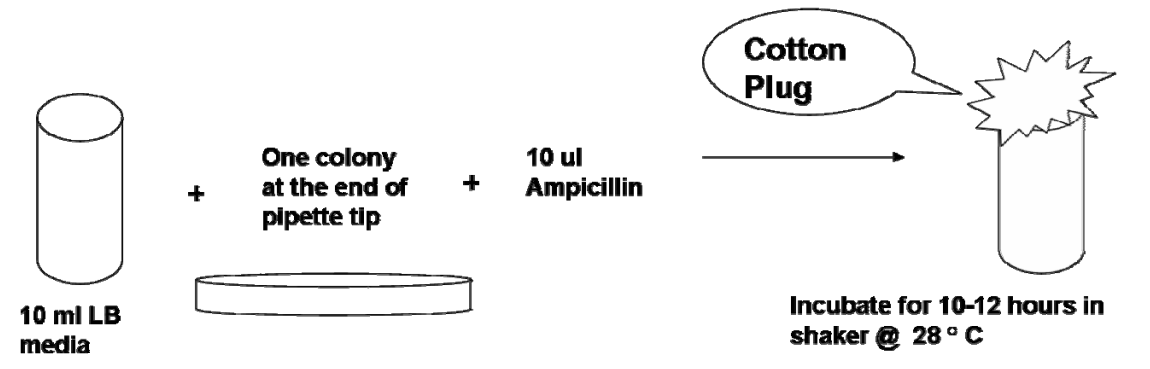
**LB Media Recipe for 1 litre**

1. Add the following to 800ml H<sub>2</sub>O
  - 10 g Bacto-tryptone- (1% w/v)
  - 5 g yeast extract-(0.5% w/v)
  - 10 g NaCl-(1% w/v)
2. Adjust pH to 7.5 with NaOH.
3. Adjust volume to 1L with dH<sub>2</sub>O
4. Sterilize by autoclaving

**M9 Media Recipe for 1 litre**

1. To 900ml H<sub>2</sub>O add
  - 12.8 g Na<sub>2</sub>HPO<sub>4</sub>-7H<sub>2</sub>O or 6.8 g Na<sub>2</sub>HPO<sub>4</sub> anhydrous
  - 3 g KH<sub>2</sub>PO<sub>4</sub>
  - 0.625 g NaCl
  - 1.0 g NH<sub>4</sub>Cl (filter sterilization with 0.22 micron membrane)
  - Stir until dissolved
  - Adjust to 1000ml with distilled H<sub>2</sub>O
  - Sterilize by autoclaving
2. **Add 2 ml of 1M MgSO<sub>4</sub> (sterile)**  
  
(for 1M → 4.93 g MgSO<sub>4</sub> .7H<sub>2</sub>O →volume make up to 20 ml)
3. **Add 10 ml of 40% glucose (or other carbon source)**  
  
(for 40 % → 4 g D-glucose →volume make up to 10 ml)
4. **Add 1 ml of 100 mM CaCl<sub>2</sub> (sterilize)**  
  
(for 100 mM → 0.294 g CaCl<sub>2</sub> .2H<sub>2</sub>O→volume make up to 20 ml)
5. **20 % CAS 1.8 ml (sterilize)**  
  
(for 20 % w/v → 4 g CAS →volume make up to 20 ml)
6. **Add the corresponding antibiotic before starting culture.**

### Small 10 ml culture:



### Small 10 ml culture in **M9 Media:**

10 ml M9 media with unlabelled  $\text{NH}_4\text{Cl}$ ,  $\text{Na}_2\text{HPO}_4(\text{anh.})$ ,  $\text{KH}_2\text{PO}_4$  and  $\text{NaCl}$  → add to it

200 ul 20 % autoclaved Glucose

10 ul of 100 mM  $\text{CaCl}_2$

20 ul of 1M  $\text{MgSO}_4$

10 ul of 100 mg/ml Ampicillin

10 ul of CAS

### 2L of TEND Buffer, pH 7.4 (for GST tagged protein prep)

1. To 1500 ml  $\text{H}_2\text{O}$  add

- 10 ml of 2 M Tris Base solution
- 4 ml of 0.5 M EDTA solution
- 30 ml of 5 N NaCl or solution 8.775 g of NaCl solid
- 1 ml of 1M DTT

2. Adjust the pH with HCl/NaOH to 7.4 and make up the volume to 2000 ml.

**Cell Lysis Buffer:** TEND with 1 % Triton-X-100

**Wash Buffer:** TEND

**Elution Buffer:** TEND with 20 mM Glutathione reduced (pH 8-8.5)

**1.5 litre TEND buffer pH 7.4**

**45 ml 5 N NaCl +15 ml 2 M Tris.base (pH 8.0) +2 ml of 0.5 M EDTA  
+rest water → adjust pH 7.4 → make up volume after that**

**Acetate Buffer pH 5.0 (for NMR of GED protein)-2Litre**

Sodium Acetate(CH <sub>3</sub> COONa)	3.48g
Glacial Acetic Acid (CH <sub>3</sub> COOH)	0.88 ml
0.5M EDTA	4.0 ml
5N NaCl	60 ml
1M DTT	2 ml

**50ml Acetate Buffer (of strength 50 m M ) pH 4.0 with 20% alcohol for storage of Thrombin removing beads [Bezimidine Saphrose Beads]**

Glacial Acetic Acid (CH<sub>3</sub>COOH) 144 ul in 40 ml of ddH<sub>2</sub>O

Adjust pH = 4 by acetic acid

Then add 10 ml of 100 % Ethanol

**Stock Solutions (Store at -20 °C)**

**Lysozyme for lysis buffer:** 50mg/ml in water

**PMSE:** 50 mM in isopropanol

**Pepstatin**: 0.5 mg/ml in DMSO

**Leupeptin**: 1.25 mg/ml in H<sub>2</sub>O

**Thrombin**: 5 units/ $\mu$ l (169 mg solid means 10KU)—bovine serum

**Ampicillin** : 100 mg/ml (dissolve 5 g in 50 ml )

**IPTG** : 100 mM ( Dissolve 2.383 g in 100ml or Dissolve **1.2 g in 50 ml**)

**DTT** : 1 M (Dessolve 7.765 g in 50 ml)

**5N NaCl** : 292.5 g NaCl in 1000 ml

**2M Tris Base** : 1 M (Dessolve 7.765 g in 50 ml)

### **Bradford's Reagent**

- 1 20 mg of CBB-G250 (Coomassie Brilliant Blue-G250) dissolved in 10 ml of Ethanol (>95%).
- 2 Add 20 ml (85%) H<sub>3</sub>PO<sub>4</sub>
- 3 Dilute to 100 ml with ddH<sub>2</sub>O and filter through whatman #1.
- 4 Use 50  $\mu$ l of this to test the protein solution in 1:1 ratio. A brilliant blue color shows the presence of protein. Always do a buffer test as negative control...you don't know if the buffer itself is contaminated.

### **5X Running Buffer-2 Litre**

1. 30.2 g **Tris Base**
2. 10 g **SDS** (Lauryl Sulphate )
3. 188 g **Glycine**
4. Adjust pH to 8.8 then make up the volume

### **Destaining Solution 2 Litre**

- 1.900 ml CH<sub>3</sub>OH +200 ml Acetic Acid
2. Filter directly into 900 ml H<sub>2</sub>O

### **Gel Loading Dye (6X) for 10 ml (DNA dye)**

0.025 g Bromophenol blue + 4g Sucrose + Rest Water

(0.25 % Bromophenol blue w/v and 40 % Sucrose w/v of water)

### **Staining Solution - 1Litre**

1. 2.5 g Coomassie Brilliant Blue R250 + 450 ml H<sub>2</sub>O + 450 ml CH<sub>3</sub>OH + 100 ml Acetic Acid.

2. Filter through Whatmann filter paper No. 2.

3. Store in a dark bottle.

### **15% SDS page (for two pages)**

<b>Components</b>	<b>Resolver (≈10 ml total)</b>	<b>Stacker (≈5 ml total)</b>
dH <sub>2</sub> O	2.3 ml	3.4 ml
Bis-acrylamide (30 %)	5.0 ml	850 ul
1.5 M Tris pH 8.0	2.5 ml	1.0 M Tris (pH 6.8) -625 ul
10 % SDS	100 ul	50 ul
10 % APS	100 ul	50 ul
TEMED	5 ul	5 ul

### **Bis-acrylamide (30 %):**

N,N'-Methylene bis-acrylamide 1g + 29 g Acrylamide → volume make upto 100ml

### **1X SDS gel loading buffer:**

50 mM Tris-Cl (pH 6.8 )

100 mM DTT

2 % SDS (w/v)

0.1 % Bromo phenol blue (w/v)  
10 % glycerol (v/v)

For 50 ml  
Add

- (1) 2.5 ml 1M tris of pH 6.8
- (2) 5 ml of 1M DTT
- (3) 1g SDS
- (4) 50 mg Bromo Phenol Blue
- (5) 5 ml glycerol
- (6) Plus rest water

[http://www.cshprotocols.org/cgi/content/full/2007/2/pdb.rec9191?text\\_only=true](http://www.cshprotocols.org/cgi/content/full/2007/2/pdb.rec9191?text_only=true)

### 6x SDS gel loading buffer

125mM Tris-HCl pH 6.8  
2% SDS  
20% glycerol  
0.2% bromophenol blue)



#### **Recipe**

### **2X SDS-PAGE gel-loading buffer**

2.5% (w/v) SDS

25% glycerol

125 mM Tris-Cl (pH 6.8)

0.01% (w/v) bromophenol blue

Immediately before use add:

100 mM fresh dithiothreitol (DTT)

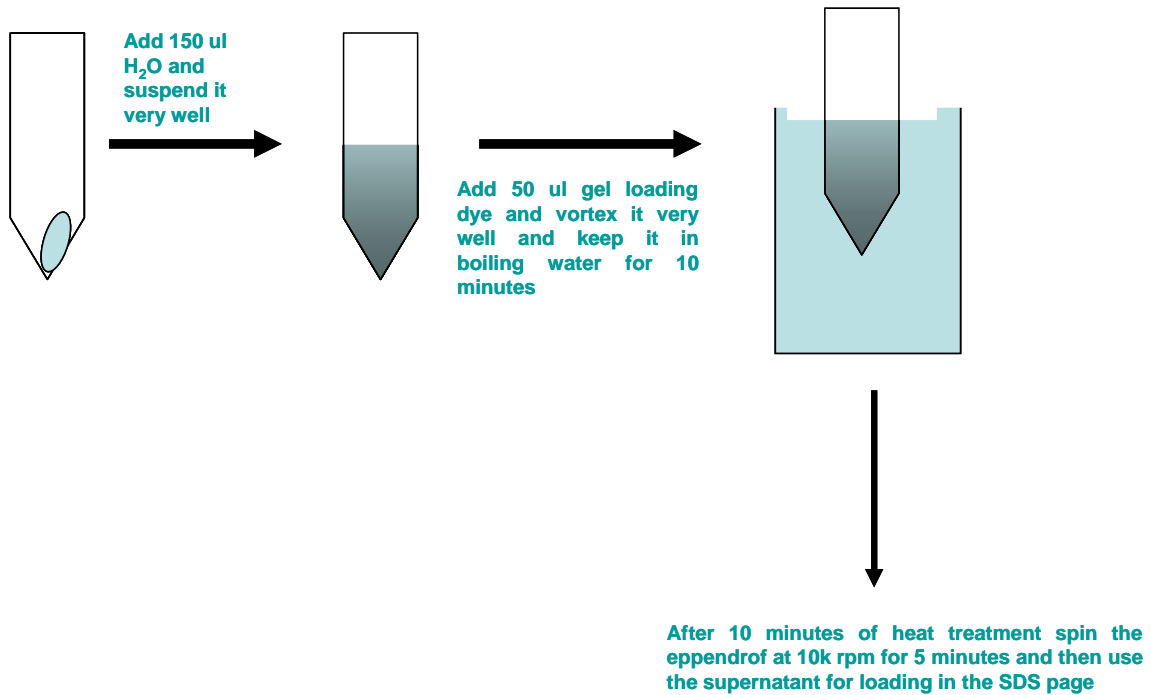
**Staining Solution:**

900 ml Methanol + 900 ml ddH<sub>2</sub>O + 200 ml glacial acetic acid + 5 g Coomassie Brilliant Blue R-250.(0.25% w/v)

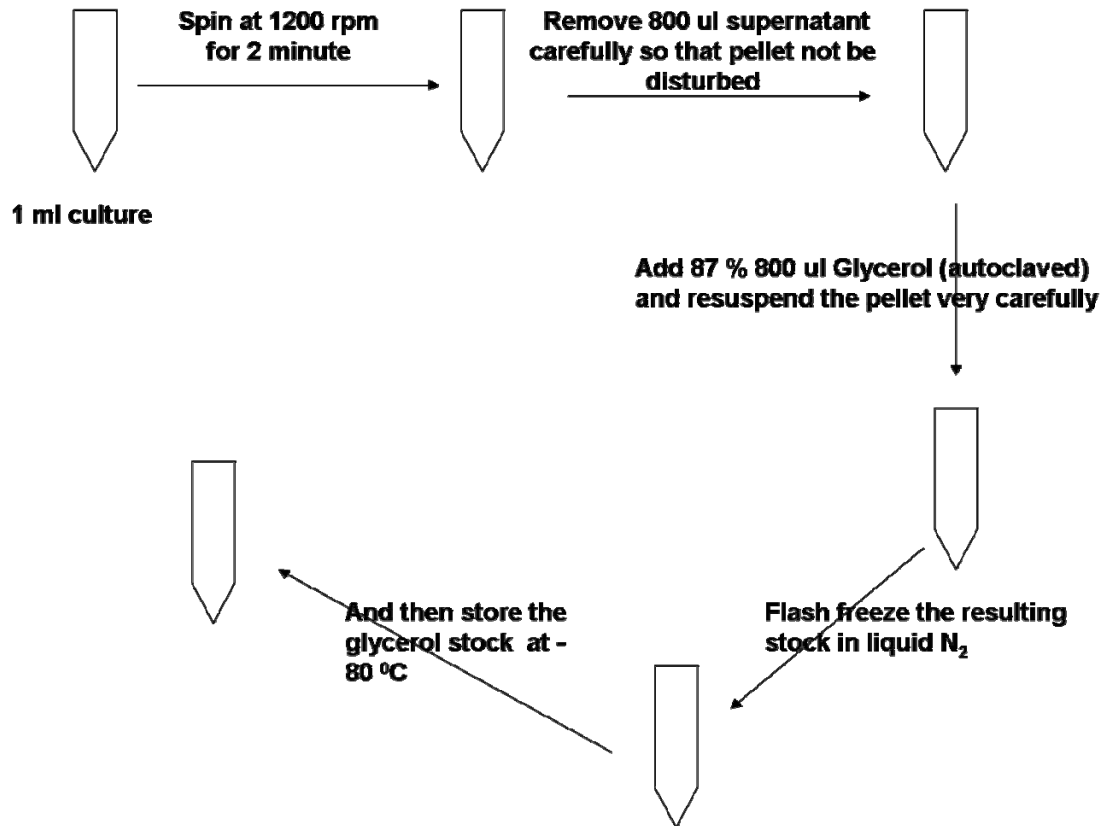
**1.5 M Tris.HCl:** 36.33 g in 200 ml ddH<sub>2</sub>O

**1.0 M Tris.HCl:** 24.22 g in 200 ml ddH<sub>2</sub>O

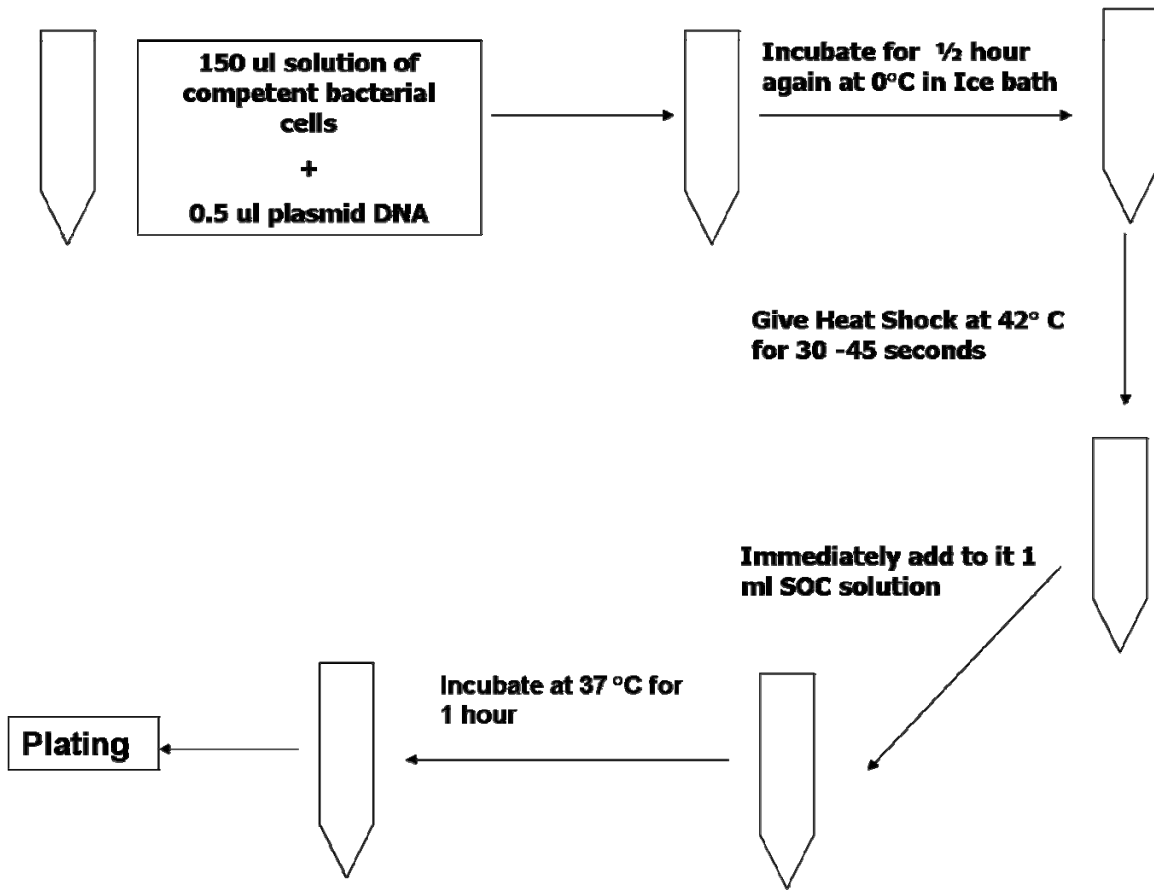
### Induction check:



### Glycerol Stock Preparation:



**Transformation Procudure:**



## Agar Plate formation:

7.5 g agar + 5 g tryptone + 2.5 g Yeast extract + 5 g NaCl → Total 500 ml with distilled water → then autoclave it → and then spread resulting soup on sterile petridishes → don't cover the petridishes → allow agar to acquire some hardness → after that close each petridish with parafilm strip → keep the plates at room temperature.

# Plasmid Preparation:

## Harvesting and lysis of Bacteria:

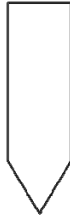
1. Transfer a single bacterial colony into 10 ml of LB medium containing the appropriate antibiotic in a loosely capped 25 ml tube. Add 10 ul of 100 mg/ml Ampicillin (final concentration of Ampicillin should be 100 ug/ml).
2. Incubate the culture overnight at 37 ° C within shaker with vigorous shaking for 10 to 12 hours
3. **Pellet formation :**
  - a. Take six eppendorf tubes (microfuge tube)
  - b. Put 1.5 ml culture solution in each eppendorf tube.
  - c. Place tubes in a balanced configuration in a microfuge rotor and centrifuge at 13200 rpm (should be >12000) for 10 minute. Align the tubes in the rotor so that the cap hinges point outward
  - d. Through out supernatant fluid and we will end up with pellets in each eppendorf. Make it as dry as possible.

## Plasmid DNA isolation

- a. Resuspend the bacterial pellet present in each eppendorf ( obtained in step 2d) with 100 ul GTE solution by vigorous vortexing.
- b. Now add 200 ul of freshly prepared solution (A). Close the tube tightly and mix the contents by inverting the tube rapidly five times. Make sure that the entire surface of the tube comes in contact with solution A. Do not vortex. Store the tube on ice.
- c. Now add 150 ul solution B. in each eppendorf tube. Close the tube and vortex it gently in an inverted position for 10 seconds to disperse solution B. through the viscous bacterial lysate. Store the tube on ice for 3-4 minutes.
- d. At this point, most of the cell membrane material, proteins and the genomic DNA precipitate to form a plasma like mass. The cell contents [including plasmid] can be separated from this material by centrifugation. The resultant supernatant is then extracted to purify the plasmid.
- e. Centrifuge at 13200 rpm for 10 minutes at 4°C and supernatant fluid is collected in six another eppendorf tubes. This supernatant is having plasmid DNA.

### **GTE Solution:**

- Glucose 50 mM
- Tris HCl (pH=8.0)-25 mM
- EDTA (pH=8.0)-10 mM



**10 ml H<sub>2</sub>O + 90 mg Glucose +125 ul 2 M Tris HCl (pH=7.4) + 200 ul 0.5 M EDTA (pH 8.0)**

**Glucose/Tris/EDTA** :Glucose functions to maintain osmotic pressure,while te tris buffers the cells at pH=8.0.

**EDTA:** Binds divalentcationsin the lipid bilayer, thus weakening the cell envelope. Following cell Lysis, EDTA limits DNA degradation by binding Mg<sup>2+</sup> ions that are necessary cofactor for bacterial nucleases.

Ensure that the bacterial pellet is completely dispersed in solution GTE. This can be achieved rapidly by vortexing the microfuge tubes.

### **Solution A : 1.5 ml solution**

**300 ul 1N NaOH + 75 ul 20% SDS + 1.125 ml ddH<sub>2</sub>O = 1.5 ml solution A**

**(4.0 g in 100 ml →1N NaOH ; 2 g SDS + H<sub>2</sub>O so that volume reaches to 10 ml)**

**SDS/NaOH:** The Alkaline mixture lyses the bacterial cells. The detergent SDS dissolves the lipid components of the cell envelope and the cellular proteins. NaOH denatures the chromosomal and Plasmid DNA into single strands; the intact circles of plasmid DNA remain intertwined→ shake the eppendrof tubes properly so that when you open the lid of eppendrof, you will see swings or threads of gels.

**Solution B : CH<sub>3</sub>COOK / CH<sub>3</sub>COOH buffer (10 ml)**

<b>5M CH<sub>3</sub>COOK (FW=98.15)</b>	<b>6.0 ml</b>
4.9075g in 10 ml H <sub>2</sub> O → 5 M CH <sub>3</sub> COOK	
<b>Glacial Acetic Acid</b>	<b>1.5 ml</b>
<b>ddH<sub>2</sub>O</b>	<b>2.85 ml</b>

**Potassium acetate/Acetic acid** : The acetic acid brings the pH to neutral, allowing the DNA strands to renature. The large, disrupted chromosomal strands cannot rehybridize perfectly but instead collapse into a partially hybridized tangle. At the same time, the CH<sub>3</sub>COOK precipitates the SDS from the cell suspension along with the associated proteins and lipids. The renaturing chromosomal DNA is trapped in the SDS/lipid/protein precipitate. Only smaller plasmid DNA fragments of chromosomal DNA and RNA molecules escape the precipitate and remain in solution

## 4. Extraction of DNA

**a.** The soup having plasmid is extracted with 500 ul PCI (25 ml Phenol +24 ml CHCl<sub>3</sub> + 1 ml Isopropyl alcohol). In each eppendorf tube, 500 ul PCI is added and vortexed and they are subjected to centrifugation at 12000 rpm for 5 minute. After centrifugation, upper aqueous layer having plasmid DNA is collected in another new six eppendorf tubes. This step should be performed with a very care.

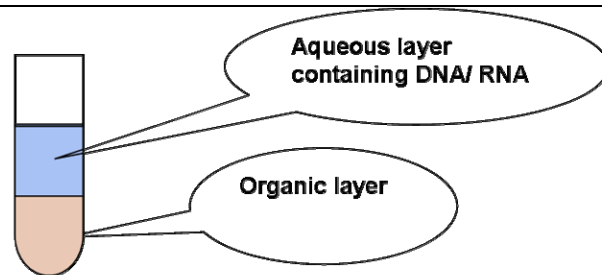
**b.** Now in each eppendorf tube we add 1 ml of 100 % ethanol (to ppt DNA). Shake and keep them at - 20°C for 1 hour.

**c.** Take them out, thaw and again centrifuge at 12000 rpm for 10 minutes.

**d.** Throw out the supernatant and again add 500 ul 70% ethanol in each eppendorf and centrifuge at 8000 rpm for 5 minutes ( to remove salts). Repeat once again.

**e.** Throw out the whole ethanol from each eppendorf and make the plasmid DNA( which is left at the bottom of each eppendorf) as dry as possible and then keep the eppendorf tubes with their open lids at 37°C for complete evaporation of alcohol (for about ½ hour).

**After centrifugation**



**The organic solvents (Phenol/CH<sub>3</sub>Cl) have two effects:**

1. They dissolve hydrophobic molecules and
2. They denature proteins[which makes them insoluble in water ]

As a result, the cell membranes and cellular proteins are either dissolved in the Phenol/CH<sub>3</sub>Cl [ which is then discarded ] or trapped in the interface between the two phases. DNA and RNA remain in the aqueous phase and are easily separated.

**Note:** The purpose of this procedure is to separate the plasmid DNA from its associated proteins so that further manipulations can be done to it. Enzymes added to purified DNA in vitro can have unhindered access to it. These enzymes might be used for restriction mapping, ligation, sequencing or other procedures to modify the DNA. A poorly purified plasmid preparation will only be partially accessible to the enzymes and this will cause many headaches in these later steps.

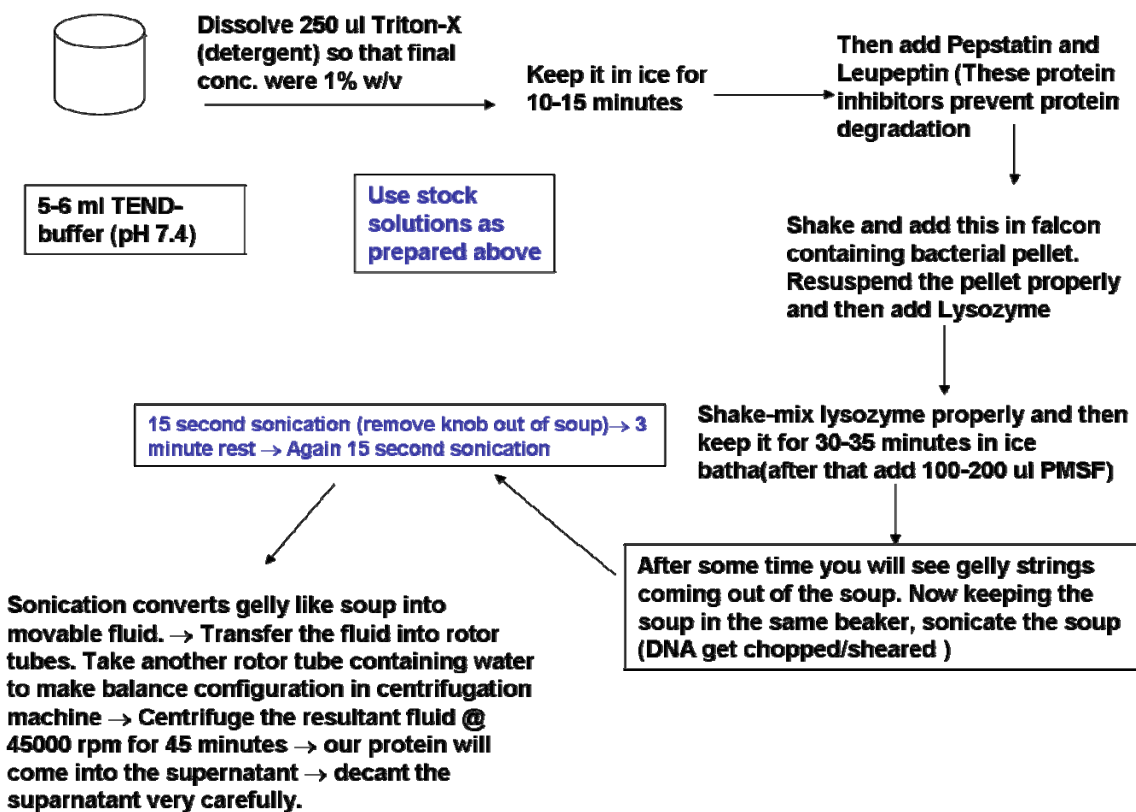
- f. Redissolve the nucleic acids in 25 ul of TE [Tris EDTA] (pH 8.0) containing DNA ase free pancreatic RNA ase [final concentration should be 20 ug/ml]. Keep it at 37°C for 10-12 hours.
- g. Vortex briefly. Store the DNA at -20°C until you are ready to continue. Thaw before using.

**100 % ethanol :** The alcohol rapidly precipitates nucleic acids but precipitates proteins slowly. Thus, a quick precipitation preferentially brings down nucleic acids.

**Ethanol:** A wash of ethanol removes some remaining salts and SDS from the precipitation.

**Tris/EDTA:** Tris buffers the DNA, EDTA protects the DNA from degradation by DNA ase by binding the divalent cations [especially Mg<sup>2+</sup>] that are necessary cofactors for the DNA ase activity

## Cell pellet processing:



## Phosphate Buffer pH 5.0 (buffer strength 20mM)

12 ml phosphate solution stock + 22.768 ml ddH<sub>2</sub>O + 72 ul 0.5M EDTA + 36 ul DTT + 1.08 ml 5M NaCl → 36 ml phosphate buffer.

**Phosphate Buffer of pH6.5** : For Phosphate buffer of pH 6.5 and buffer strength 40 mM:

Monosodium phosphate, monohydrate    **3.856**    (**3.856\*0.87 = 3.35** g of anhydrous)  
grams/liter

Disodium phosphate, heptahydrate    **3.231**    grams/liter

**Phosphate solution stock:** For Phosphate buffer of pH 5.6 and buffer strength 100 mM:

Monosodium phosphate, monohydrate **13.0892** (11.4 g of anhydrous) grams/liter

Disodium phosphate, heptahydrate **1.3804** grams/liter

**Phosphate solution stock:** For Phosphate buffer of pH 6.0 and buffer strength 20 mM:

Monosodium phosphate, monohydrate 2.43 g (2.43\*0.87= 2.11g of anhydrous)  
grams/liter

Disodium phosphate, heptahydrate 0.644 g grams/liter

**For 1 Litre:**

**2.97 g** Monosodium phosphate, anhydrous + **350 mg** Disodium phosphate, heptahydrate + **2 ml** 0.5 M EDTA + **1 ml** 1 M DTT + **30 ml** 5M NaCl + Rest DD Water → adjust pH with ortho phosphoric acid / NaOH to 5.7 → adjust volume to 1 Liter.

**Phosphate Buffer of pH6.5 :** For Phosphate buffer of pH 6.5 and buffer strength 100 mM:

Monosodium phosphate, monohydrate **9.6402** (**9.6402\*0.87 = 9.04** g of anhydrous)  
grams/liter

Disodium phosphate, heptahydrate **8.0784** grams/liter

**Phosphate Buffer of pH6.0 :** For Phosphate buffer of pH 6.0 and buffer strength 100 mM:

Monosodium phosphate, monohydrate **12.14 g** (12.14\*0.87=10.6 g of anhydrous)  
grams/liter

Disodium phosphate, heptahydrate

3.22

grams/liter

## Preparation of triply labeled sample:

**1. Acclimatization of cells:** Step by step we go towards 100% D<sub>2</sub>O. Prepare LB Media with increasing amount of D<sub>2</sub>O as shown below:

5x LB media ( 20 ml) + 100 ul Ampicillin	D2O	H2O	Total
1 ml	0.25 ml→ 5%	3.75 ml	5 ml
1 ml	0.50 ml→ 10%	3.50 ml	5 ml
1 ml	0.75 ml→ 15%	3.25 ml	5 ml
1 ml	1.00 ml→ 20%	3.00 ml	5 ml
1 ml	1.25 ml→ 25%	2.75 ml	5 ml
1 ml	1.50ml→ 30%	2.50 ml	5 ml
1 ml	1.75ml→ 35%	2.25 ml	5 ml
1 ml	2.00ml→ 40%	2.00 ml	5 ml
1 ml	2.25 ml→ 45%	1.75 ml	5 ml
1 ml	2.5ml→ 50%	1.50 ml	5 ml
1 ml	2.75 ml→ 55%	1.25 ml	5 ml
1 ml	3.00ml→ 60%	1.00 ml	5 ml
1 ml	3.25ml→ 65%	0.75 ml	5 ml
1 ml	3.50ml→ 70%	0.50 ml	5 ml
1 ml	3.75ml→ 75%	0.25 ml	5 ml
1 ml	4.00ml→ 80%	0.00 ml	5 ml
Now prepare 10 ml of	LB Stock in D <sub>2</sub> O + 20 ul Ampicillin	Tryptone:yeast extract:NaCl	=1% : 0.5%: 1%
Stock ( %age)	D2O	H2O	total
2.5 ml (85 %)	1.75 ml	0.75 ml	5 ml
2.5 ml (90 %)	2.00 ml	0.50 ml	5 ml
2.5 ml (95 %)	2.25 ml	0.25 ml	5 ml
2.5 ml (100 %)	3.00 ml	0.0 ml	5 ml

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For safety prepare glycerol stock in each and every step.

Then transfer 20 ml of 100 % inoculum in 10 ml of 100% D<sub>2</sub>O LB media with appropriate antibiotic. Grow overnight and then transfer in 1 litre M9 media with labeled glucose and NH<sub>4</sub>Cl.

## **SOB Preparation (Competent cells):**

**(SOB-Mg)→  
100ml**

**1 litre.**

**500ml**

Bactotryptone	20 g	10g	2g
Yeast Extract	5g	2.5g	0.5g
NaCl	0.5g	0.25g	0.05g
250 mM KCl (1.86%)	10 ml	5ml	1 ml

Adjust pH to 7.0 with NaOH → after that make up the volume



Autoclave and after that add.

<b>SOC →</b>	<b>1 litre</b>	<b>500 ml</b>	<b>100 ml</b>
2M MgCl <sub>2</sub> (A/C)	5 ml	2.5 ml	0.5 ml
1M Glucose (F/S)	20 ml	10 ml	2 ml

<b>CCMB-80 (100 ml) →</b>	<b>100ml</b>
100 % Glycerol	10 ml
1M KOAc (pH=7.0)	1 ml
CaCl <sub>2</sub> ·2H <sub>2</sub> O	1.56 g (Anh. CaCl <sub>2</sub> → 1.18 g)
MnCl <sub>2</sub>	0.4 g
MgCl <sub>2</sub>	0.2 g

Adjust pH to 6.4 with KOH/HCl → volume make up to 100 ml → 0.2 µ filter

## **Making Competent Cells :**

- 1.** Pick one colony of BL21 cells from freshly streaked plate into 5 ml SOB-Mg media and grow at 37° C overnight in test tube.
- 2.** Inoculate half of the media into 250 ml SOB-Mg in a 2L flask and grow at 275 rpm at 37° C for 1½ hrs until OD @600>0.3 ( $5 \times 10^7$  cells/ ml).
- 3.** Pellet the cells at 750 -1000 g(2000-3000 rpm in a clinical centrifuge/~ 3500 rpm in Sorvall) for 10 mins, at <4C
- 4.** Decant supernatant and remove x's culture inverting the container carefully.
- 5.** Disperse the cells into 1/3 vol (~80 ml) CCMB-80(<4°C) by gentle swirling
- 6.** Incubate in ice for 20 minutes.
- 7.** Centrifuge 10 mins, 3500 rpm, <4°C and remove supernatant as before.
- 8.** Resuspend the cells in ~5ml (or 1/12) CCMB-80
- 9.** Now competent cells can be used immediately (keep in ice) or stored as ~200ul aliquots in 2 ml eppendroffs---flash freeze in Liq.N<sub>2</sub>----store at (- 80° C)

## **Preparation and Transformation of chemically competent cells:**

SOB (1 litre)

20 g bactotryptone + 5 g yeast extract + 0.5 g NaCl + 10 ml 250 mM KCl (1.86 % stock) → adjust pH = 7.0 with NaOH → make up 1 litre → autoclave → after that add 5 ml sterile (autoclaved) 2M MgCl<sub>2</sub>

## SOC

Sterile autoclaved SOB with a 1/100th volume 2M glucose added

## TB buffer

10mM Pipes  
15mM CaCl<sub>2</sub>  
250mM KCl  
pH to 6.7 with 1M KOH  
55mM MnCl<sub>2</sub>

TB is really nasty to make because if you don't pH before you add the Manganese chloride, it falls straight out of solution as a brown precipitate. I always dissolve the manganese chloride in milliQ water, then add it gradually with the solution stirring and yet still, some precipitates anyway in my hands. I always prepare a fresh stock before use, and filter through a 0.2um filter using an elephant sized 50mL syringe into Falcons which I dunk straight into ice. If you do get precipitation, try making up the PIPES buffer below first, adding pre-dissolved PIPES to the solution.

## 0.25 M PIPES buffer pH 6.4

7.55 g Pipes are dissolved, and pH adjusted with 1M NaOH to pH 6.4  
Add Millipore Water to 100 ml  
Sterilfilter  
Store at 4°C  
Thanks to Isabelle Chassignet of Univ. Freiberg for the PIPES workaround

## 2M glucose

Sterile filter (10mL stock is sufficient)

## 1M Magnesium salts

Sterile filter a solution containing both 1M MgCl<sub>2</sub> and 1M MgSO<sub>4</sub> (10mL stock is sufficient)

## NOTES:

This protocol works well with commonly used DH5alpha and XL1-Blue strains, the competent cells stay good for at least 3 months when stored at -80°C (1 x 10<sup>7</sup> to 1 x 10<sup>8</sup> transformants per microgram). I have also used this method to make competent cells using strains of a different genetic background with lower but still useful efficiency (5 x 10<sup>5</sup> transformants per microgram). An important consideration for transformations with ligations is the size and efficiency of the ligation, its optimal to use a lot of material in a ligation (200-500 ng) but to transform with only a fraction of that ligation (5-50 ng).

Once you have your cells, the prep part takes only about 1-2 hours. The cells will grow up dead slow compared to what you might be used to at 37°C, so if you are impatient, turn up the heat. I have done this protocol at 18, 19, 24, and 27°C and the resulting cells are usefully competent at all temperatures.

Notes – if you are not bothered about whether you get 100 or 300 colonies, just use LB, which is far less painful and time-consuming than using SOC and SOB. If its critical that it works well use SOC.

I do the heat shock in 200ul (v.small) thin walled plastic tubes on a pre-heated PCR block, and transfer straight back to ice after 30 secs. This works well compared to a dry block or water bath since the PCR block conducts the heat faster and its temperature control is far more accurate and repeatable than a water bath.