Preparation of culture media

**LB medium (Luria Bertani medium)**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tryptone</td>
<td>10 g</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>5 g</td>
</tr>
<tr>
<td>NaCl</td>
<td>5 g</td>
</tr>
</tbody>
</table>

Dissolve 20 g of LB powder (Sigma) in double distilled water. Add ddH₂O to make up the total volume to 1 litre. Sterilise the media by autoclaving for 15 minutes at 121 °C/15 lb/sq in.

**LB Agar plate**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tryptone</td>
<td>10 g</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>5 g</td>
</tr>
<tr>
<td>NaCl</td>
<td>10 g</td>
</tr>
<tr>
<td>Agar</td>
<td>15 g</td>
</tr>
</tbody>
</table>

Dissolve 35 g of LB agar powder (DIFCO) in double distilled water. Add ddH₂O to make up the total volume to 1 litre. Sterilise the media by autoclaving for 15 minutes at 121 °C/15 lb/sq in. Allow LB agar to cool and just before pouring the plates, add appropriate antibiotic to the autoclaved media in the required final concentration to be used as a selection marker.

**Ampicillin solution**

Dissolve the dry sodium salt of ampicillin in sterile water to make the stock solution of 100 mg/ml. The stock solution should be sterilised by filtration through a 0.22-micron disposable filter. Store the solutions at -20 °C. The working concentration of the antibiotic is 100 μg/ml for broth and plates.

**Solutions for plasmid isolation and purification**

**T₁₀E₁ buffer, pH 8.0**

<table>
<thead>
<tr>
<th>Buffer</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tris-Cl</td>
<td>10 mM</td>
</tr>
<tr>
<td>EDTA</td>
<td>1 mM</td>
</tr>
</tbody>
</table>

Finally, sterilise the buffer by autoclaving.
**TENS buffer**

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaOH</td>
<td>0.2 N</td>
</tr>
<tr>
<td>SDS</td>
<td>0.5%</td>
</tr>
</tbody>
</table>

Add T₁₀E₁, pH 8.0, buffer to make up the total volume. The TENS buffer should be prepared fresh just before adding into the bacterial culture.

**2 N NaOH**

Dissolve 8 g of Sodium hydroxide in 45 ml of ddH₂O. Finally, make up the volume to 100 ml with water. Sterilise the solution by autoclaving for 15 minutes at 121 °C/15 lb/sq in.

**10% Sodium dodecyl sulphate (SDS)**

Dissolve 100 g of SDS (electrophoresis grade) in 700 ml of sterile ddH₂O. Heat at 55 °C for 2 minutes. Make up the final volume to 1 litre with sterile ddH₂O.

**3 M Sodium acetate (pH 5.2)**

Dissolve 408 g of Sodium acetate in water. Adjust the pH to the desired value with Acetic acid. Add water to make 1 litre total volume. Sterilise the solution by autoclaving for 15 minutes at 121 °C/15 lb/sq in.

**DNase free RNAase A**

Dissolve pancreatic RNAase A at a concentration of 10 mg/ml in 0.01 M Sodium acetate, pH 5.2, Heat to 100 °C for 15 minutes. Allow cooling slowly at room temperature. Adjust the pH by adding 0.1 volumes of 1 M TrisCl (pH 7.4). Dispense into aliquots and store at -20 °C.

**Stock solutions of commonly used reagents**

**0.5 M EDTA**

Dissolve 186.1 g of Na₂EDTA.2H₂O powder in 700 ml of water. Keep on vigorous shaking. EDTA will not dissolve completely until the pH of the solution reaches 8.0. Adjust pH to 8.0 with 10 M NaOH. Finally add water to 1 litre and autoclave the solution.
1 M CaCl₂
Dissolve 147 g of Calcium chloride (CaCl₂·2H₂O) in 1 litre water and sterilise the solution by filtration with a 0.22- micron filter membrane.

1 M Tris (pH 6.8, 7.0, 7.2, 7.4, 7.5, 8.0, 8.8, 9.1, 9.5)
Dissolve 121.1 g Tris base in 800 ml of ddH₂O. Adjust pH to desired value by adding Concentrated HCl. Make up the final volume to 1 litre with water and sterilise by autoclaving.

50 % Glycerol
Add 50 ml of pure glycerol to 50 ml of sterile water and mix thoroughly. Finally autoclave the solution for 15 minutes at 121 °C/15 lb/sq in.

70 % Ethanol
Mix 70 ml of pure ethanol and add 30 ml of sterile water to make up the total volume to 100 ml. Store it at 4 °C.

1 M DTT
Dissolve 3.09 g of Dithiothreitol in 20 ml of 0.01 M sodium acetate, pH 5.2 and store at -20 °C after filter sterilization with 0.22-micron filter.

30% Acrylamide (29:1) and 40% (19:1)

<table>
<thead>
<tr>
<th></th>
<th>29:1</th>
<th>19:1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acrylamide</td>
<td>29 g</td>
<td>19 g</td>
</tr>
<tr>
<td>N,N'-methylenebisacrylamide</td>
<td>1 g</td>
<td>1 g</td>
</tr>
</tbody>
</table>
Add ddH₂O to make up the total volume of 100 ml for (29:1) and 50 ml for (19:1), filter the solution and keep at 4 °C.

10% Ammonium persulphate
Add 10 g ammonium persulphate powder in 100 ml of sterile ddH₂O and mix. Keep the solution at 4 °C. The solution is stable for 2-4 weeks.
**Ethidium bromide**
Dissolve 100 mg ethidium bromide tablet in 10 ml of water. Leave overnight on 37 °C shaker to dissolve the tablet completely and store in dark at room temperature.

**10 M Ammonium acetate**
Dissolve 385.4 g of ammonium acetate in 150 ml of water. Make up the volume to 500 ml. Sterilise the solution by autoclaving for 15 minutes at 15 lb/sq in.

**dNTP’s mix (dATP, dCTP, dGTP, dTTP)**
Prepare 25 mM each dNTP in T10E1 buffer, pH 7.5. Combine all the four dNTP’s at a final concentration of 2.5 mM each and store in small aliquots at -20 °C.

**X-gal solution (5-bromo-4-choloro-3-indolyl-β-D-galactoside)**
Make a stock solution by dissolving X-gal in Dimethylformamide at a concentration of 20 mg/ml. The tube containing the solution should be wrapped in aluminium foil to prevent damage by light and should be stored at -20 °C.

**100 mM IPTG solution**
Dissolve 2 g of IPTG powder in 8 ml of sterile distilled water. Make up the to 10 ml with water to a final concentration to 200 mg/ml and sterilise the solution by filtration through a 0.22-micron disposable filter. Dispense the solution into small aliquots and store them at -20 °C.

**Phenol: Chloroform: Isoamyl alcohol**
Mix 25 parts (v/v) Phenol (previously equilibrated in 150 mM NaCl/50 mM Tris.Cl, pH 7.5 and 1 mM EDTA) with 24 parts (v/v) Chloroform and 1 part (v/v) of Isoamyl alcohol. Store in a dark coloured glass bottle at 4 °C.

**1 M HEPES Buffer (pH 7.9, 8.0)**
Dissolve 23.83 g HEPES in 80 ml sterile water. Adjust the pH to the desired value with the help of 1 M NaOH. Make up the final volume to 100 ml with water. Filter the solution through a 0.22-micron disposable filter. The buffer should be stored at 4 °C.
5 M NaCl
Dissolve 292.2 g of sodium chloride in 800 ml water and make up the total volume to 1 litre with water. Finally, sterilise the solution by autoclaving.

1 M KCl
Dissolve 74.6 g of potassium chloride in 1 litre of water and autoclave. Store at room temperature.

1 M MgCl₂
Dissolve 20.3 g of magnesium chloride (hexahydrated) dry powder and make up the total volume to 1 litre with water. Sterilise the solution by autoclaving.

2.5 M Sucrose
85.5 g of Sucrose powder was added and dissolved in 100 ml of sterile water. Finally sterilise the solution by filtration through 0.22-micron filter. Store at 4 °C.

Triton X -100 (v/v)
Add 1 ml of Triton X-100 detergent solution into 99 ml of water to make up the final volume to 100 ml to get a final concentration of 1%.

3M Potassium acetate
Dissolve 29.442 g of Potassium acetate in 80 ml of water. Adjust the pH to 5.5 with 3 M Acetic acid. Add water to make up the final volume to 100 ml and store at room temperature after autoclaving.

NP-40 (Nonidet P-40)
Make 1% solution of NP-40 detergent in sterile water as a stock solution. This solution can be diluted as per requirement.

Protease inhibitors
All the protease inhibitor solutions were made as 100x stock. They should be added to the pre-cooled solutions just before the use. All the protease inhibitor solutions are
active for 3-4 weeks at a storage temperature of -20 °C. The required concentration of the solutions is 1×.

Leupeptin 100 μg/ml in water
Aprotinin 100 μg/ml in water
Trypsin inhibitor 100 μg/ml in water

100 mM Phenyl methyl sulphonyl fluoride (PMSF)
Dissolve 174 mg of PMSF powder in 10 ml of isopropanol. Divide the solution in aliquots and store at -20 °C. Wear gloves while handling the chemical. The half-life of PMSF in aqueous solution is 20 minutes. So it should be added to the solution just before use.

Sephadex G-50
Add 10 g of Sephadex G-50 to 160 ml of sterile water. Wash the swollen resin with sterile water several times to remove soluble dextran, which can create problems by precipitating during ethanol precipitation. Finally autoclave (121 °C/15 lb/sq in for 15 minutes) and store at room temperature.

5× binding buffer (Gel mobility shift assay)
Glycerol 20%
MgCl₂ 5 mM
EDTA 2.5 mM
DTT 2.5 mM
NaCl 250 mM
Tris-HCl, pH 7.5 50 mM

Ca²⁺/Mg²⁺ solution
CaCl₂ 5 mM
MgCl₂ 10 mM

2× binding buffer (DNase I Footprinting)
Tris-HCl, pH 8.0 50 mM
KCl 100 mM
<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>MgCl₂</td>
<td>12.5 mM</td>
</tr>
<tr>
<td>EDTA</td>
<td>1 mM</td>
</tr>
<tr>
<td>Glycerol</td>
<td>20%</td>
</tr>
<tr>
<td>DTT</td>
<td>1 mM</td>
</tr>
</tbody>
</table>

**Stop Solution**

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>200 mM</td>
</tr>
<tr>
<td>EDTA</td>
<td>30 mM</td>
</tr>
<tr>
<td>SDS</td>
<td>1%</td>
</tr>
<tr>
<td>Yeast RNA</td>
<td>100 μg/ml</td>
</tr>
</tbody>
</table>

**10x Binding buffer (South-Western blot assay)**

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>HEPES, pH 7.9</td>
<td>250 mM</td>
</tr>
<tr>
<td>MgCl₂</td>
<td>30 mM</td>
</tr>
<tr>
<td>KCl</td>
<td>500 mM</td>
</tr>
<tr>
<td>DTT</td>
<td>1 mM</td>
</tr>
</tbody>
</table>

**1x Denaturing buffer (South-Western blot assay)**

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>10x Binding buffer</td>
<td>1 ml</td>
</tr>
<tr>
<td>Guanidine hydrochloride</td>
<td>5.73 g</td>
</tr>
<tr>
<td>Make up the volume to 10 ml with sterile milliQ water</td>
<td></td>
</tr>
</tbody>
</table>

**1x Blocking buffer (South-Western blot assay)**

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>10x Binding buffer</td>
<td>1 ml</td>
</tr>
<tr>
<td>Non-fat milk</td>
<td>0.5 g</td>
</tr>
<tr>
<td>Make up the volume to 10 ml with sterile milliQ water</td>
<td></td>
</tr>
</tbody>
</table>

**Probe solution (South-Western blot assay)**

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-fat milk</td>
<td>0.25% (w/v)</td>
</tr>
<tr>
<td>Poly (dl-dC) · poly (dl-dC)</td>
<td>10 μg/ml</td>
</tr>
<tr>
<td>In 1x Binding buffer</td>
<td></td>
</tr>
</tbody>
</table>

83
Solutions for silver staining

Fixative A
Methanol \( 50\% \)
Acetic acid \( 10\% \)
Add ddH\(_2\)O to make up the total volume.

Fixative B
Ethanol \( 10\% \)
Acetic acid \( 5\% \)
Add ddH\(_2\)O to make up the total volume.

Oxidizer solution
Potassium dichromate \( 3.4 \) mM
Nitric acid \( 3.2 \) mM

Developer
Sodium carbonate \( 0.28 \) M
Formaldehyde (Formalin) \( 75 \mu l \) (0.05%)

Staining solution
Silver nitrate \( 12 \) mM

Fixative solution (SDS-PAGE)
Ethanol \( 40\% \)
Acetic acid \( 12\% \)
Formaldehyde \( 0.05\% \) (v/v)

Developer (SDS-PAGE)
Sodium carbonate \( 6\% \) (w/v)
Sodium thiosulphate \( 0.004\% \) (w/v)
Formaldehyde \( 0.05\% \) (v/v)
Staining solution (SDS-PAGE)
Silver nitrate 0.2% (w/v)
Formaldehyde 0.075% (v/v)

Buffers

10x Restriction digestion buffers
The restriction enzymes, which were used for digestion of DNA for different experiments of cloning and other experiments, were bought from New England Biolabs (NEB). The company provides four different buffers for various enzymes. The concentrations of various components in the 10x restriction buffers are:

<table>
<thead>
<tr>
<th>Component</th>
<th>NEB 1</th>
<th>NEB 2</th>
<th>NEB 3</th>
<th>NEB 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tris Propane-HCl</td>
<td>100 mM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tris HCl</td>
<td>100 mM</td>
<td>500 mM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tris-acetate</td>
<td></td>
<td>200 mM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Magnesium Chloride</td>
<td>100 mM</td>
<td>100 mM</td>
<td>100 mM</td>
<td></td>
</tr>
<tr>
<td>Magnesium acetate</td>
<td></td>
<td></td>
<td>100 mM</td>
<td></td>
</tr>
<tr>
<td>Sodium Chloride</td>
<td>500 mM</td>
<td>1000 mM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potassium acetate</td>
<td></td>
<td></td>
<td>500 mM</td>
<td></td>
</tr>
<tr>
<td>Dithiothreitol</td>
<td>10 mM</td>
<td>10 mM</td>
<td>10 mM</td>
<td>10 mM</td>
</tr>
<tr>
<td>pH at 25 °C</td>
<td>7.0</td>
<td>7.9</td>
<td>7.9</td>
<td>7.9</td>
</tr>
</tbody>
</table>

50x TAE buffer
Tris base 242 g
Glacial acetic acid 57.1 ml
Na₂EDTA.2H₂O 37.2 g
Add sterile water to make up the total volume to 1 litre.

10x TBE buffer
Tris base 107.8 g
Boric acid 55 g
Na₂EDTA.2H₂O 7.44 g
Add sterile water to make up the total volume to 1 litre.

5x Tris glycine buffer
Tris base 15.1 g
Glycine 94.0 g
SDS 5.0 g
Add ddH₂O to make up the total volume to 1 litre.

1x Western transfer buffer
Tris base 4.03 g
Glycine 19.2 g
Methanol 267 ml
Make up the volume to 1.333 litre with water.

6x Gel loading buffer
Bromophenol blue 0.25% (w/v)
Xylene cyanol FF 0.25% (w/v)
Ficoll (Type 400) 15% (w/v)
Add ddH₂O to make up the total volume. Store at room temperature.
Alternatively, the DNA loading dye can be made with following components.
Bromophenol blue 0.25% (w/v)
Xylene cyanol FF 0.25% (w/v)
Glycerol 30% (v/v)
Add ddH₂O to make up the total volume. Store at 4 °C.

2x Sample buffer (SDS gel loading buffer)
Tris-HCl, pH 6.8 100 mM
β- mercaptoethanol 2%
or
Dithiothreitol 200 mM
SDS 4% (w/v)
Bromophenol blue 0.2% (w/v)
Glycerol 20% (v/v)
### 10× Calf Intestinal Alkaline Phosphatase (CIAP) buffer

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tris.Cl, pH 8.0</td>
<td>200 mM</td>
</tr>
<tr>
<td>MgCl₂</td>
<td>10 mM</td>
</tr>
<tr>
<td>ZnCl₂</td>
<td>10 mM</td>
</tr>
<tr>
<td>BSA/Gelatin</td>
<td>0.5 mg/ml</td>
</tr>
</tbody>
</table>

### 10× Klenow buffer

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tris.Cl, pH 7.5</td>
<td>500 mM</td>
</tr>
<tr>
<td>MgCl₂</td>
<td>100 mM</td>
</tr>
<tr>
<td>DTT</td>
<td>10 mM</td>
</tr>
<tr>
<td>BSA/Gelatin</td>
<td>0.5 mg/ml</td>
</tr>
</tbody>
</table>

### 10× T4 DNA ligase buffer

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tris.Cl, pH 7.5</td>
<td>500 mM</td>
</tr>
<tr>
<td>MgCl₂</td>
<td>100 mM</td>
</tr>
<tr>
<td>DTT</td>
<td>100 mM</td>
</tr>
<tr>
<td>BSA/Gelatin</td>
<td>0.5 mg/ml</td>
</tr>
</tbody>
</table>

### 10× T4 PNK buffer

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tris.Cl, pH 7.5</td>
<td>500 mM</td>
</tr>
<tr>
<td>MgCl₂</td>
<td>100 mM</td>
</tr>
<tr>
<td>DTT</td>
<td>50 mM</td>
</tr>
<tr>
<td>BSA/Gelatin</td>
<td>0.5 mg/ml</td>
</tr>
</tbody>
</table>

### Buffer A

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>HEPES, pH 7.9</td>
<td>10 mM</td>
</tr>
<tr>
<td>MgCl₂</td>
<td>1.5 mM</td>
</tr>
<tr>
<td>KCl</td>
<td>10 mM</td>
</tr>
<tr>
<td>DTT</td>
<td>0.5 mM</td>
</tr>
<tr>
<td>PMSF</td>
<td>0.5 mM</td>
</tr>
</tbody>
</table>
Buffer C

- HEPES, pH 7.9: 5 mM
- Glycerol: 26% (v/v)
- MgCl₂: 1.5 mM
- EDTA: 0.2 mM
- DTT: 0.5 mM
- PMSF: 0.5 mM

MOPS [3-(N-morpholino)-propanesulphonic acid] buffer

- MOPS: 0.2 M
- Sodium acetate: 0.5 M
- EDTA: 10 mM

Adjust the pH to 7.0 with 1 N NaOH. Sterilise the solution by filtration.

Denaturation buffer

- NaCl: 1.5 M
- NaOH: 0.5 M

Add ddH₂O to make up the total volume. Finally autoclave the buffer and store at room temperature.

Neutralisation buffer

- NaCl: 1.5 M
- Tris-Cl, pH 8.0: 0.5 M

Add ddH₂O to make up the total volume. Finally autoclave the buffer and store at room temperature.

Prehybridization solution

- SSC: 5x
- Denhardt's solution: 5x
- SDS (w/v): 1%
- Sonicated Salmon sperm DNA: 100 μg/ml just before use
Wash Buffer

SSC  
2x

SDS  
1%

Made up the total volume with sterile water.

Hybridization solution

Add labelled DNA probe to the prehybridization solution having $10^7$ cpm/ml.

50× Denhardt’s solution

Ficoll 400 [2% (w/v)]  
10 g

Polyvinylpyrrolidone [2% (w/v)]  
10 g

Bovine serum albumin  
10 g

ddH₂O to make up the total volume to 1 litre. Filter and store at -20 °C.

Salmon sperm DNA (denatured)

Dissolve 100 mg salmon sperm DNA in 1 ml of water. Pass vigorously through an 18-gauge needle 20 times to shear the DNA. Place in boiling water bath for 10 minutes, then chill. Store at -20 °C in small aliquots.

20× SSC

Sodium chloride  
3 M (175 g/l)

Trisodium citrate  
0.3 M (88 g/l)

Adjust pH to 7.0 with 1 N HCl. And then autoclave the solution before storing at room temperature.

Buffers for detection of biotinylated DNA probe

Buffer 1

NaCl  
0.2 mM

Tris-HCl, pH 7.5  
0.1 M

Triton X-100  
0.05%

Bovine serum albumin  
3% (w/v)
Buffer 2
NaCl 0.2 mM
Tris-HCl, pH 7.5 0.1 M
Triton X-100 0.05% (v/v)
Added 0.125 µg/ml streptavidin-alkaline phosphatase conjugate just before using.

Buffer 3
NaCl 0.1 M
Tris-HCl, pH 9.5 0.1 M
MgCl₂ 50 mM

Buffer 4
NaCl 0.1 M
Tris-HCl, pH 9.5 0.1 M
MgCl₂ 50 mM
NBT 0.32 mg/ml
BCIP 0.16 mg/ml

Cell culture reagents

PBS
NaCl 100 mM
KCl 4.5 mM
Na₂HPO₄ 7 mM
KH₂PO₄ 3 mM
Added water to make up the total volume and sterilise the buffer by autoclaving.
Store the buffer at 4 °C.

DMEM (Dulbecco's modified Eagle's media)
DMEM dry powder 13.4 g
Sodium bicarbonate 33.3 mM
HEPES 20 mM
L-Glutamine 2 mM
Sodium pyruvate  1 mM  
Penicillin  50 U/ml  
Streptomycin  50 μg/ml  

After adding all the components of the media, adjust the pH of the media to 7.4 with the help of 1 N HCl solution. The media was sterilised by vacuum filtration using 0.22-micron filter assembly. Store the media at 4 °C. For preparation of complete media, added heat inactivated (The serum was incubated at 55 °C for 1 hour for heat inactivation) FCS to a final concentration of 10% in the media and the filter sterilized using the same 0.22-micron filter assembly.

1× Trypsin-EDTA  
The commercial solution was bought from Gibco-BRL, which is a 10× concentrated stock solution. For harvesting and splitting the cells, a 1× solution of Trypsin-EDTA in PBS was added to the cells.