APPENDIX

Composition of Culture Media:

**LB Medium (Luria Bertani Medium)**

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tryptone</td>
<td>10 gm</td>
</tr>
<tr>
<td>Yeast Extract</td>
<td>5 gm</td>
</tr>
<tr>
<td>NaCl</td>
<td>5 gm</td>
</tr>
</tbody>
</table>

25 gm of LB powder (Difco) was dissolved in double distilled water. ddH₂O was added to make up the volume to 1 litre. The media was sterilized by autoclaving for 15 minutes at 121°C/15 lb/sq. in.

**LB Agar Plate**

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
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<tbody>
<tr>
<td>Tryptone</td>
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<tr>
<td>Yeast Extract</td>
<td>5 gm</td>
</tr>
<tr>
<td>NaCl</td>
<td>10 gm</td>
</tr>
<tr>
<td>Agar</td>
<td>15 gm (1.5%)</td>
</tr>
</tbody>
</table>

35 gm of LB Agar powder was dissolved in double distilled water. dd H₂O was added to make up the total volume to 1l. The media was sterilized by autoclaving for 15 minutes at 121°C/15 lb/sq. in. The LB Agar was allowed to cool before pouring the plates; appropriate antibiotic was added to the autoclaved media in the required final concentration to be used.

**GC Agar plate**

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protease peptone No. 3</td>
<td>1.5 g</td>
</tr>
<tr>
<td>Potassium phosphate dibasic</td>
<td>0.4 g</td>
</tr>
<tr>
<td>Potassium phosphate monobasic</td>
<td>0.1 g</td>
</tr>
<tr>
<td>NaCl</td>
<td>0.5 g</td>
</tr>
<tr>
<td>Soluble starch</td>
<td>0.1 g</td>
</tr>
</tbody>
</table>
**Haemoglobin**

An autoclavable preparation of beef blood. The 2% solution is ready for use in the preparation of media for the cultivation of fastidious organisms.

**BBL™ VCNT Inhibitor**

Formula Per 1 mL Vial

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vancomycin</td>
<td>300 µg</td>
</tr>
<tr>
<td>Colistin</td>
<td>750 µg</td>
</tr>
<tr>
<td>Nystatin</td>
<td>1,250 units</td>
</tr>
<tr>
<td>Trimethoprim Lactate</td>
<td>500 µg</td>
</tr>
</tbody>
</table>

7.2 gm of GC medium agar powder was dissolved in double distilled water. dd H₂O was added to make up the total volume to 100 ml. The media was sterilized by autoclaving for 15 minutes at 121°C/15 lb/sq. in. The GC Agar was allowed to cool and mixed with 100ml of 2% autoclaved Haemoglobin solution before pouring the plates. VCNT (2ml) was added to the autoclaved media before pouring the plates.

**Ampicillin Solution**

Ampicillin salt was dissolved in one ml of sterile water to a concentration of 100mg/ml and stored at minus 20.

**Stock Solutions of Commonly Used Reagents:**

**0.5M EDTA (pH 8.0)**

9.3g of Na₂EDTA.2H₂O powder was dissolved in 40 ml of water. EDTA dissolves completely the pH 8.0. pH of solution was adjusted to 8.0 with 10M NaOH. Volume was made up to 50ml and the solution was sterilized by autoclaving for 15 minutes at 121°C/15 lb/sq. in.

**1M Tris (pH 8.0)**

121.1 gm of Tris base was dissolved in 800 ml of ddH₂O. The pH was adjusted to the desired value by adding concentrated HCl. The final volume was made up to 1l with water and the solution was sterilized by autoclaving for 15 minutes at 121°C/15 lb/sq. in. 1.5M
1M Tris (pH 6.8)
121.1 gm of Tris base was dissolved in 800 ml of ddH₂O. The pH was adjusted to the desired value by adding concentrated HCl. The final volume was made up to 1 l with water and the solution was sterilized by autoclaving for 15 minutes at 121°C/15 lb/sq. in.

1M Tris (pH 9.2)
121.1 gm of Tris base was dissolved in 800 ml of ddH₂O. The pH was adjusted to the desired value by adding concentrated HCl. The final volume was made up to 1 l with water and the solution was sterilized by autoclaving for 15 minutes at 121°C/15 lb/sq. in.

1.5 M Tris (pH 8.8)
181.5 gm of Tris base was dissolved in 800 ml of ddH₂O. The pH was adjusted to the desired value by adding concentrated HCl. The final volume was made up to 1 l with water and the solution was sterilized by autoclaving for 15 minutes at 121°C/15 lb/sq. in.

30% Acrylamide (29:1)

<table>
<thead>
<tr>
<th>Acrylamide</th>
<th>29 gm</th>
</tr>
</thead>
<tbody>
<tr>
<td>N, N’-methylenebisacrylamide</td>
<td>1 gm</td>
</tr>
</tbody>
</table>

ddH₂O was added to make up the total volume of 100 ml for (29:1) and the solution was filtered through Whatman no.1 filter paper and stored in dark bottle at 4°C.

10% Ammonium persulphate
1.0 g of ammonium persulphate powder was dissolved in 10 ml autoclaved ddH₂O and stored at –20°C.

70% Ethanol
70% of pure ethanol was taken and sterile water was added to make up the total volume to 100 ml.

1M IPTG (Isopropylthio-β-D-galactoside)
0.238 g of IPTG powder was dissolved in 1 ml of sterile distilled water. The solution was stored at -20°C.
5M NaCl

292.2 gm of Sodium Chloride was dissolved in 800 ml water and the volume was made up to 1l with water. The solution was sterilized by autoclaving for 15 minutes at 121°C/15 lb/sq. in.

Buffers Used

5X Running Buffer
Tris Base: 15.1 g
Glycine: 94 g
10% SDS: 50 ml

a/c ddH₂O was added to make the total volume to 1L.

10X Sample buffer (Lammelli’s buffer)
Tris-HCl, pH 6.8 2ml
β-mercaptoethanol 0.1ml
10% SDS 1ml
Bromophenol Blue 0.04
Glycerol 5ml

Made upto 10ml using autoclaved water.

50X TAE
Tris Base - 242 g
Glacial Acetic Acid - 57.1 ml
0.5 M EDTA (pH 8.0) - 100 ml

Distilled water was added to make final volume of solution 1000 ml. For use buffer was diluted to 1X concentration.

5X TBE
Tris Base - 54 g
Boric Acid - 27.5 g
0.5 M EDTA (pH 8.0) - 20 ml
Distilled water was added to make final volume of solution 1000 ml. For use buffer was diluted to 1X concentration.

**SDS-PAGE Reagents**

**10% Resolving gel (15 ml):**
- 30% acrylamide solution: 5.0 ml
- 1.5M Tris-Cl pH 8.8: 3.8 ml
- ddH₂O: 5.9 ml
- 10% SDS: 150 µl
- 10% APS: 150 µl
- TEMED: 6 µl

**4% Stacking gel (5 ml):**
- 30% acrylamide solution: 0.830 ml
- 1M Tris-Cl pH 6.8: 0.630 ml
- ddH₂O: 3.4 ml
- 10% SDS: 50 µl
- 10% APS: 50 µl
- TEMED: 5 µl

**Staining solution**
- Coomassie brilliant blue: 2.5 g
- Methanol: 450 ml
- Glacial acetic acid: 100 ml

ddH₂O was added to make the total volume to 1L. The solution was filtered using Whatman no.1 filter paper and stored at RT.

**Destaining solution**
- Methanol: 350 ml
- Water: 550 ml
- Acetic acid: 100 ml

Methanol: water: acetic acid: 35:55:10. The solution was stored at RT.
Western blotting

DAB western blot development: DAB development system was purchased from Bangalore Genei. The blots were developed according to the manufacturer’s protocol.

Transfer Buffer

Tris base 3g
Glycine 7g
Methanol 200 ml

added ddH₂O was added to make up the total volume to 400 ml.

Phosphate buffered saline

Na₂HPO₄: 10 mM
KH₂PO₄: 2 mM
NaCl: 137 mM
KCl: 2.7 mM

PBST: PBS with 0.04 % Tween-20.

Agarose Gel Electrophoresis

TAE buffer

Tris: 242g
0.5M EDTA (pH8.0): 100ml
Glacial Acetic acid: 57.1 ml

a/c ddH₂O was added to make up the total volume to 1l.

1% Agarose

Agarose: 1g
a/c water: 100ml
EtBr: 4µl
Plasmid preparation solutions

Solution I (pH 8.0)
Tris. HCl: 25mM
EDTA: 10mM
Glucose: 50mM
RNase: 20µg/ml

Solution II
SDS: 1 % (wt./vol.)
NaOH: 0.2N

Solution III (per 100ml)
Potassium acetate 5M: 60ml
Glacial acetic acid: 11.5ml

Phenol Chloroform mix
1:1 ratio of pH equilibrated Phenol mixed with chloroform.

Tris EDTA Solution
Tris-Cl: 10mM
EDTA (pH 8.0): 1mM

Protein Purification Buffers

Sonication Buffer
Tris-Cl pH8: 30mM
NaCl: 50 mM
Sucrose: 25%
Protease Inhibitor cocktail: 0.5%
Sodium Orthovandate: 1mM
(Protease Inhibitor cocktail, Sodium Orthovandate)

Imidazole (2M)
Imidazole: 6 gm
dissolved in autoclaved ddH2O to make the total volume to 50 ml.
Charge buffer
NiSO₄ (Nickel Sulphate) 10.51 gm
dissolved in autoclaved ddH₂O to make the total volume to 50 ml.

Binding buffer
NaCl (500mM)
Tris Cl (pH 8.0) 10mM
Imidazole (10mM)

Binding buffer
NaCl (500mM)
Tris Cl (pH 8.0) (10mM)
Imidazole (10mM)

Wash buffer 1
NaCl (500mM)
Tris Cl (pH 8.0) (10mM)
Imidazole (40mM)

Wash buffer
NaCl (500mM)
Tris Cl (pH 8.4) (10mM)
Imidazole (60mM)

Wash buffer
NaCl (500mM)
Tris Cl (pH 8.8) (10mM)
Imidazole (60mM)

Elution buffer
NaCl (500mM)
Tris Cl (pH 9.2) (10mM)
Imidazole (100mM)