### Composition of Minimal salt agar medium

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Amount (gm/litre)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dipotassium hydrogen phosphate</td>
<td>0.5</td>
</tr>
<tr>
<td>Potassium dihydrogen phosphate</td>
<td>0.04</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>0.5</td>
</tr>
<tr>
<td>Magnesium sulphate</td>
<td>0.05</td>
</tr>
<tr>
<td>Ammonium dihydrogen phosphate</td>
<td>0.1</td>
</tr>
<tr>
<td>Ferrous sulphate</td>
<td>0.001</td>
</tr>
<tr>
<td>Agar</td>
<td>20.0</td>
</tr>
</tbody>
</table>

### Composition of Nutrient agar medium

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Amount (gm/litre)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone</td>
<td>10.0</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.0</td>
</tr>
<tr>
<td>Beef extract</td>
<td>10.0</td>
</tr>
<tr>
<td>Agar</td>
<td>20.0</td>
</tr>
</tbody>
</table>

### Composition of Potato dextrose agar medium

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Amount (gm/litre)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potato infusion (200 g of sliced potato)</td>
<td>4.0</td>
</tr>
<tr>
<td>Dextrose</td>
<td>20.0</td>
</tr>
<tr>
<td>Agar</td>
<td>20.0</td>
</tr>
</tbody>
</table>

### Plasmid Isolation

**Alkaline lysis solution I**

- 50mM glucose
- 25mM Tris Cl (pH 8)
- 10mM EDTA (pH 8)
Alkaline lysis solution II
0.2 N NaOH
1% SDS

Alkaline lysis solution III
5M potassium acetate (60ml)
Glacial acetic acid (11.5ml)
Water (28.5ml)

Components used for setting up SDS-PAGE

Separating gel:
Distilled water 1ml
30% acrylamide 6ml
1.5 M Tris 5.5 µl
Ammonium per sulphate 400 µl
20% SDS 20 µl
TEMED 400 µl

Stacking gel:
Distilled water 1ml
30% acrylamide 3ml
1.0 M Tris 400 µl
Ammonium per sulphate (10%) 600 µl
20% SDS 20 µl
TEMED 400 µl

Anode buffer:
12.11g Tris base in 500 ml of distilled water.

Cathode buffer:
6.055g Tris base, 8.96g tricine and 0.5g of SDS in 500ml of distilled water.

Following solutions were used for the extraction
Solution 1: 25 mM tris HCl (0.394%), glucose, Tris HCl and EDTA
Solution 2: 1% SDS and 0.2 N NaOH
Solution 3: 5M potassium acetate, glacial acetic acid.
1. Agarose
   - Agarose: 1.00g
   - TAE buffer (1X): 100ml
   - Ethidium bromide: 5 µl

2. TAE buffer (50X)
   - Tris base: 24g
   - Glacial acetic acid: 5.71ml
   - EDTA: 3.72g in 100ml of distilled water

   1X TAE buffer can be prepared by adding 10ml of 50X TAE buffer to 490ml of distilled water.

**Agarose gel electrophoresis**

The following reagents were used for agarose gel electrophoresis:

i) Agarose:
   - Agarose: 1.00g
   - TAE buffer (1X): 100ml
   - Ethidium bromide: 5 µl

ii) TAE buffer: (50X)
   - Tris base: 24g
   - Glacial acetic acid: 5.71ml
   - EDTA: 3.72g in 100ml of distilled water

**LB Medium:**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein enzymic hydrolysate</td>
<td>10.0</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>5.0</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>10.0</td>
</tr>
<tr>
<td>Agar</td>
<td>15.0</td>
</tr>
<tr>
<td>Final pH (at 25°C)</td>
<td>7.5±0.2</td>
</tr>
</tbody>
</table>
Potassium Phosphate Buffer (0.1 M) Ph 7:

\[
\begin{align*}
1 \text{ M Potassium dihydrogen phosphate} & \quad - 61.5 \text{ ml} \\
1 \text{ M Dipotassium hydrogen phosphate} & \quad - 38.5 \text{ ml}
\end{align*}
\]

Made upto 1000ml

Potassium Phosphate Buffer (20 Mm) Ph 7:

\[
\begin{align*}
1 \text{ M Potassium dihydrogen phosphate} & \quad - 12.3 \text{ ml} \\
1 \text{ M Dipotassium hydrogen phosphate} & \quad - 7.5 \text{ ml}
\end{align*}
\]

Made upto 1000ml

Staining solution- 100ml

- Coomassie brilliant blue - 200mg
- Ethanol - 50ml
- Acetic acid - 7 ml
- Distilled water - 43 ml

Destaining solution – 500 ml

- Ethanol - 150ml
- Acetic acid - 35 ml
- Distilled water - 315 ml