Appendices
APPENDICES

PREPARATION OF BACTERIAL CULTURE MEDIA

- **LURIA-BERTANI (LB) MEDIUM**
  
  Components g/l
  
  Tryptone 10.0 gm
  Yeast Extract 5.0 gm
  Sodium chloride 10.0 gm
  
  The pH was adjusted to 7.2 using NaOH and autoclave the medium at 15lbs/ sq. in for 15 minutes at 120°C.

- **2XYT MEDIUM**
  
  Components g/l
  
  Tryptone 16.0
  Yeast Extract 10.0
  Sodium Chloride 5.0
  
  Adjust the pH to 7.2 – 7.4 using NaOH and autoclaved.
  
  Autoclave the medium at 15lbs/ sq. in for 15 minutes at 120°C. After cooling, aseptically add 20 ml of 50X ADC enrichment to the medium.

ANTIBIOTIC SOLUTIONS

- **AMPICILLIN**: 100 mg/ml stock solution was made in sterile distilled water and filter sterilized.
- **KANAMYCIN**: 10 mg/ml stock solution was made in sterile distilled and filter sterilized.

ELECTROPHORESIS BUFFERS (Per Litre)

- **TAE (50 X STOCK SOLUTION)**
  
  Tris base 242.0 gm
  EDTA 18.6 gm
  Glacial acetic acid 57.1 ml
➢ **SDS-PAGE RUNNING BUFFER**

- Tris base: 3.0 gm
- Glycine: 14.4 gm
- SDS: 1.0 gm

➢ **TRANSFER BUFFER**

- Tris base: 3.0 gm
- Glycine: 7.0 gm

Dissolved in 500 ml distilled water, added 200 ml methanol and adjusted the volume to 1 litre.

**SOLUTION FOR PLASMID DNA ISOLATION**

➢ **SOLUTION I (100ML)**

- 1 M Tris-Cl pH 8.0: 1 ml
- 0.5 M EDTA: 0.2 ml
- Sterile water: 98.2 ml

➢ **SOLUTION II (100ML)**

Prepared 1% SDS solution in 0.2 N NaOH

➢ **SOLUTION III (100ML)**

- 5M Potassium acetate: 60 ml
- Glacial acetic acid: 11.5 ml
- Triple distilled water: 28.5 ml

**COMMONLY USED BUFFERS AND REAGENTS**

➢ **5X SDS-GEL LOADING BUFFER**

- Tris-Cl [pH 8.0]: 250mM
- β-mercaptoethanol: 5%
- SDS: 5%
- Glycerol: 50%
- Bromophenol blue: 0.2%
30% ACRYLAMIDE MIX (100ML)

- Acrylamide: 29.0 g
- N, N’ dimethylene-bisacrylamide: 1.0 g

Adjust the volume to 100ml using double distilled water and store at 4°C.

STAINING SOLUTION FOR SDS-PAGE

- Commassie brilliant blue R250: 0.25%
- Methanol: 45%
- Glacial acetic acid: 45%

The solution is made up with triple distilled water filtered through whatman No.1 filter paper and stored at room temperature.

DESTAINING SOLUTION FOR SDS-PAGE

- Methanol: 30%
- Glacial acetic acid: 10%

Make up volume in triple distilled water.

6X DNA GEL LOADING DYE

- Tris-Cl, pH 8.0: 100mM
- Bromophenol blue: 0.25%
- Glycerol: 40%

PHOSPHATE BUFFER SALINE (PBS, 1X) (1 LITRE)

- NaCl: 136 mM
- KCl: 2.6 mM
- Na₂HPO₄: 10 mM
- KH₂PO₄: 1.76 mM

10 x TBE FORMULA

- Tris base: 108 g (89 mM)
- Boric acid: 55 g (89 mM)
- 0.5 M EDTA (pH 8.0): 40 ml

Use dH₂O to bring total volume to 1000 ml
BINDING BUFFER, 5X (EMSA)

1 mL 1 M Hepes pH 8.0
2.5 mL 1 M KCl
25 μl 1 M DTT
5 μl 0.5 M EDTA
50 μl 1 M MgCl₂
2.5 mL glycerol
dH₂O to 10 mL

Aliquot into small volumes and store at -20°C.

BUFFERS USED FOR 2D-TLE

» pH 1.9 Buffer

88% Formic acid 50 ml
Acetic acid 156 ml

Make up volume to 2 L in triple distilled water.

» pH 3.5 Buffer

Pyridine 10 ml
Acetic acid 100 ml
100mM EDTA 10 ml

Make up volume to 2 L in triple distilled water.