APPENDIX

Luria Broth (LB) and Luria Agar (LA) Media

Formula / Liter

Standard Recipe for 1 Liter of LB

- Tryptone: 10 g
- Yeast Extract: 5 g
- NaCl: 10 g

Final pH: 7.3 ± 0.2 at 25°C

Mineral Salt Medium MSM (per L)

- KH$_2$PO$_4$: 170 mg
- Na$_2$HPO$_4$: 980 mg
- (NH$_4$)$_2$SO$_4$: 100 mg
- MgSO$_4$: 4.87 mg
- FeSO$_4$: 0.05 mg
- CaCO$_3$: 0.20 mg
- ZnSO$_4$: 0.08 mg
- CuSO$_4$5H$_2$O: 0.016 mg
- H$_3$BO$_3$: 0.006 mg

pH 7.4

Solutions for DNA Extraction

Solution I

- Glucose: 50 mM
- Tris Cl (pH 8.0): 25 mM
- EDTA (pH 8.0): 10 mM

Autoclaved and stored at 4°C

Solution II

- NaOH: 0.2 N
- SDS: 1%

Phenol

- Tris saturated phenol (pH 7.5), stored in dark at 4°C
**Chloroform: Isoamyl alcohol (24:1)**
- Chloroform 24 ml
- Isoamyl alcohol 1 ml

**Ethanol (70%)**
- 95% Ethanol (Merck) 75 ml
- Distilled water 25 ml

**Tris EDTA (TE buffer)**
- 1M Tris buffer (pH 8.0) 5 ml
- 0.5M EDTA 1 ml
- Distilled water 494 ml

**Solutions for Agarose Gel Electrophoresis**

**10X Tris Borate EDTA (TAE)**
- Tris Base 108 gm
- Acetic acid 55 gm
- 0.5M EDTA (pH 8.0) 40 ml

Volume made upto 1000 ml with H₂O. Working concentration was 0.5X

**Ethidium bromide (EtBr)**
- EtBr 10 mg
- Distilled water 1 ml

Solution stored in dark at 4ºC. Working concentration was 0.5 µg/ml

**Loading buffer**
- Bromophenol blue 0.25%
- Xylene cyanol 0.25%
- Glycerol 30%

Solution stored at 4ºC

**Solutions for DNA Extraction**

**Lysis buffer**
- Sucrose 0.32 M
Tris-HCL (pH 7.6) 10 mM
MgCl$_2$ 5 mM
Triton X-100 1%
Solution stored at 4ºC

**Proteinase buffer**

EDTA (pH 8.0) 25 mM
NaCl 75 mM

**10% SDS**

SDS 10 mg
Distilled water 100 ml

**NaCl**

NaCl 5 M

**Phenol**

Tris saturated phenol (pH 7.5), stored in dark at 4ºC

**Chloroform: Isoamyl alcohol (24:1)**

Chloroform 24 ml
Isoamyl alcohol 1 ml

**Sonication buffer**

Tris-sulfate pH 7.0
EDTA 1 mmol l$^{-1}$
DTT (dithiothreitol) 1 mmol l$^{-1}$

**Solutions for Southern Hybridization**

**Denaturation solution (for Southern transfer hybridization)**

0.5 M NaOH
1.5 M NaCl

**Neutralization solution 1 (for Southern transfer)**

0.5 M Tris-HCl, pH 7.5
1.5 M NaCl
**Maleic Acid Buffer**

Concentration Amount of Stock solution

- 0.1M Maleic acid 11.60g
- 0.15M NaCl 8.77g

pH to 7.5 using NaOH pellets.

- Add slowly until pH is reached, then add the rest of ddH20 to 1 liter.

**Washing Buffer**

Concentration Amount of Stock solution

- Maleic Acid Buffer Make the same as above
- 0.3%Tween 20 3ml for 1 Liter

**2X SSC**

- Sodium chloride 300 mmol l⁻¹
- Sodium citrate 30 mmol l⁻¹
- pH 7.0