Appendix

Appendix I

• **L.B. medium: (Liquid)**

Bactopeptone-10g/lt. (Hi-media, Cat#RM001)

NaCl-10g/lt. (Hi-media, Cat#RM1150)

Yeast Extract-5g/lt (Hi-media, Cat#RM027)

The above mentioned components were dissolved in 1 litre of double distilled water and then autoclaved.

• **L.B. medium: (Solid)**

Bactopeptone-10g/lt. (Hi-media, Cat#RM001)

NaCl-10g/lt. (Hi-media, Cat#RM1150)

Yeast Extract-5g/lt. (Hi-media, Cat#RM027)

Agar-1.2% (Hi-media Cat# RM201)

The above mentioned components were dissolved in 1 litre of double distilled water and then autoclaved.

• **MS-104 medium: Liquid** (Hi-media Cat# PT0018)

Macroelements	**Amount required**
KH_2PO_4	170.0mg/l
KNO_3	1900.00mg/l
$MgSO_4$	180.54mg/l
NH_4NO_3	1650.00mg/l
Microelements	**Amount required**
$CoCl_2.6H_2O$	0.025mg/l
$CuSO_4.5H_2O$	0.025mg/l
FeNaEDTA	36.70mg/l
H_3B0_3	6.20mg/l
KI	0.83mg/l
$MnSO_4.H_2O$	16.90mg/l
$Na_2Mo0_4.2H_2O$	0.25mg/l
$ZnSO_4.7H_2O$	8.60mg/l

Vitamins	Amount required
Glycine	2.00mg/l
Myoinositol	100.0mg/l
Nicotinic acid	0.50mg/l
Pyridoxine HCl	0.50mg/l
Thiamine HCl	0.10mg/l

To it was added 30gm/l sucrose (Hi-media Cat# RM1158), 0.332mg/l $CaCl_2$ (Hi-media Cat# MB034), 500mg/l MES buffer (Hi-media Cat# RM1128). pH was adjusted to 5.6 and the volume was made up to 1000ml with double distilled water. It was then autoclaved for 15 minutes at 15psi and cooled to room temperature and hormones were added.

- **MS-104 medium: Solid** (Hi-media Cat# PT0018)

Macroelements	Amount required
KH_2PO_4	170.0mg/l
KNO_3	1900.00mg/l
$MgSO_4$	180.54mg/l
NH_4NO_3	1650.00mg/l
Microelements	**Amount required**
$CoCl_2.6H_2O$	0.025mg/l
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Vitamins	**Amount required**
Glycine	2.00mg/l
Myoinositol	100.0mg/l
Nicotinic acid	0.50mg/l
Pyridoxine HCl	0.50mg/l
Thiamine HCl	0.10mg/l

To it was added 30gm/l sucrose, 0.332mg/l $CaCl_2$, 500mg/l MES buffer and 1% agar. pH was adjusted to 5.6 and the volume was made up to 1000ml with double distilled water. It was then autoclaved for 15 minutes at 15psi, cooled to room temperature and hormones were added.

• **MSO medium: Liquid** (Hi-media Cat# PT0018)

Macroelements	**Amount required**
KH_2PO_4	170.0mg/l
KNO_3	1900.00mg/l
$MgSO_4$	180.54mg/l
NH_4NO_3	1650.00mg/l
Microelements	**Amount required**
$CoCl_2.6H_2O$	0.025mg/l
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Vitamins	**Amount required**
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Myoinositol	100.0mg/l
Nicotinic acid	0.50mg/l
Pyridoxine HCl	0.50mg/l
Thiamine HCl	0.10mg/l

To it was added 30gm/l sucrose, 0.332mg/l $CaCl_2$, 500mg/l MES buffer. pH was adjusted to 5.6 and the volume was made up to 1000ml with double distilled water. It was then autoclaved for 15 minutes at 15psi and cooled to room temperature. No hormones were added.

• **MSO medium: Solid** (Hi-media Cat# PT0018)

Macroelements	**Amount required**
KH_2PO_4	170.0mg/l
KNO_3	1900.00mg/l
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H_3B0_3	6.20mg/l
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$MnSO_4.H_2O$	16.90mg/l
$Na_2Mo0_4.2H_2O$	0.25mg/l
$ZnSO_4.7H_2O$	8.60mg/l
Vitamins	**Amount required**
Glycine	2.00mg/l
Myoinositol	100.0mg/l
Nicotinic acid	0.50mg/l
Pyridoxine HCl	0.50mg/l
Thiamine HCl	0.10mg/l

To it was added 30gm/l sucrose, 0.332mg/l $CaCl_2$, 500mg/l MES buffer and 1% agar. pH was adjusted to 5.6 and the volume was made up to 1000ml with double distilled water. It was then autoclaved for 15 minutes at 15psi, cooled to room temperature and no hormones were added.

• **YEM medium: (Liquid)**

Yeast extract-0.4g/lt (Hi-media, Cat#RM027)

Mannitol-10.0g/lt (Hi-media, Cat#RM570)

NaCl -0.1g/lt (Hi-media, Cat#RM1150)

$MgSO_4.7H_2O$-0.2g/lt (Hi-media, Cat#RM1281)

K_2HPO_4-0.5g/lt (E Merck India, Cat#17885)

- **YEM medium: (Solid)**

Yeast extract-0.4g/lt (Hi-media, Cat#RM027)

Mannitol-10.0g/lt (Hi-media, Cat#RM570)

NaCl -0.1g/lt (Hi-media, Cat#RM1150)

$MgSO_4.7H_2O$-0.2g/lt (Hi-media, Cat#RM1281)

K_2HPO_4-0.5g/lt (E Merck India, Cat#17885)

Agar-1.2% (Hi-media Cat# RM201)

Appendix II

Composition of Buffers:

- **C-TAB Extraction buffer**

2% (W/V) C-TAB (Hi-media Cat# RM164)

1.4M NaCl (Hi-media, Cat#RM1150)

20mm EDTA (pH-8.0) (Hi-media Cat# RM1197)

100mM Trizma base (pH-8.0) (SIGMA Cat# T1503)

0.2% Mercaptoethanol (Hi-media, Cat#RM2895) just before use

54g of molecular biology grade Trizma base (Sigma, USA Cat#T-1503, Tris (hydroxymethyl) aminomethane, $C_4H_{11}NO_3$ FW-121.1) was dissolved in 800ml of sterile distilled water, pH was adjusted to 8.0. It was divided into two parts; to one part 7.44g EDTA was added. In other part 81.82g NaCl, 20g CTAB (Hexadecyl trimethyl ammonium bromide, $C_{19}H_{42}NBr$) was added. Both the parts were then mixed and to it 0.2% Mercaptoethanol was added.

- **DNA Loading buffer (6X Concentration)** (Fermentas Cat# R0611)

(TypeIII, Sambrook *et al.*, 2001)

0.25% Bromophenol blue

0.25% Xylene cyanol FF

30% Glycerol in DD H_2O

Two and a half grams of Bromophenol blue and Xylene cyanol was dissolved in 1000ml of 30% Glycerol.

- **dNTP mix** (Finnzymes Cat#F560L)

10mM dATP, 2'-Deoxyadenosine 5'-Triphosphate, minimal diphosphate, sodium salt.

$C_{10}H_{12}N_5O_{12}P_3Na_4$, F.W-579.2

10mM dGTP, 2'-Deoxyguanosine 5'-Triphosphate, minimal diphosphate, sodium salt

$C_{10}H_{12}N_5O_{13}P_3Na_4$, F.W-595.1

10mM dCTP, 2' Deoxycytidine 5'-Triphosphate, minimal diphosphate, sodium salt

$C_9H_{12}N_3O_{13}P_3Na_4$, F.W-555.1

10mM dTTP, 2' Deoxythymidine 5'-Triphosphate, minimal diphosphate, sodium salt

$C_{10}H_{13}N_2O_{14}P_3Na_4$, F.W-570.1

• **GUS staining solution** (SIGMA USA, Cat# GUS-S)

Reagent A, Product No. R6147

200mM sodium phosphate, pH 7.0 with 4mM EDTA-2.5ml

Reagent B, Product No. R6272

100mM potassium ferricyanide-10µl

Reagent C, Product No. R6397

100mM potassium ferrocyanide-10µl

Deionized water-5.5ml

Methanol-2.0ml

5-Bromo-4-Chloro-3-Indolyl-ß-D-Glucuronide (X-GlcA), Cyclohexylammonium Salt, Product No. ß 0522-20µl

The staining solution may be stored at 2-8°C in the dark for one month.

• **Taq buffer (10X) (Supplied with Taq polymerase, Finnzymes Cat#F501L)**

10mm Tris-HCl (pH-8.8)

1.5mM $MgCl_2$

50mM KCl

0.1% Triton X-100

• **TE-Tris EDTA Buffer (pH-8)**

10mM Tris (pH-8.0)

10mM EDTA (pH-8.0)

1.21g molecular biology grade Trizma base (Sigma, USA Cat#T-1503, Tris (hydroxymethyl) aminomethane, $C_4H_{11}NO_3$,FW-121.1) was dissolved in 400ml of double distilled water and the pH was adjusted with Conc. HCl (Hi-media, Cat#RM5955) to 8.0 and sterilized by autoclaving. Similarly 0.372g Di-Sodium EDTA was dissolved in 400ml of double distilled water. The solution was stirred properly and the pH was adjusted with NaOH (Hi-media, Cat#RM1183) pellets and sterilized by autoclaving. Both the solutions were then mixed and the volume was made upto 1 litre sterilized double distilled water.

- **TBE- Tris-Borate EDTA Buffer (5X Concentration)**

0.045M Tris-Borate

0.001M EDTA (Hi-media Cat# RM1197)

Preparation of 5X stock:

54g of molecular biology grade Trizma base (Sigma,USA Cat#T-1503, Tris (hydroxymethyl) aminomethane, $C_4H_{11}NO_3$, FW-121.1) and 27.5g Boric acid (Hi-media, Cat#MB007) were dissolved in 800ml of sterile double distilled water. To it 20ml of 0.5 EDTA (pH-8.0) was added.

TBE was used in a final concentration of 1X, so the 5X stock was diluted to 1X.

- **Washing solution**

70% Ethanol (E Merck Cat# 101076HBD)

10mM Ammonium acetate (Hi-media, Cat#MB033)