APPENDIX

APPENDIX

1) Microbiological media composition

(a) Nutrient Agar Medium

Composition	**Concentration(g/l)**
Starch	10.0
Yeast extract	1.5
Beef extract	1.5
Peptone	5.0
NaCl	5.0
Agar	20.0
pH	6.0-12.0
Distilled water	1000

(b) Composition of Minimal Salt Medium(MSM)

MSM composition	**Concentration(g/l)**
Na_2HPO_4	7.8
KH_2PO_4	6.8
$NH_4(CH_3COO)_3Fe$	0.05
$NaNO_3$	0.085
$MgSO_4$	0.2
$Ca(N0_3).4H_20$	0.05
Carbon source	1.0

(c) Composition of nutrient broth

Nutrient Broth	**Concentration(g/l)**
Yeast extract	1.5
Beef extract	1.5
Peptone	5.0

(d) Composition of Luria broth

Luria Broth	**Concentration(g/l)**
Bacto-tryptone	10.0
Bacto-yeast extract	5.0
NaCl	5.0

2) Reagents, Buffers and Solutions

(a) Buffers and Reagents

(l) 50X TAE Electrophoresis Buffer	**Concentration(g/l)**
Tris base	242

Na_2EDTA	37.2
Glacial Acetic Acid	7.1 ml

Note: Final volume was adjusted to 1 litre with deionised water.

(ll) Ethidium Bromide stock solution	**Concentration(mg/ml)**
Ethidium Bromide	10

Note: Mixed well in deionised water and store at room temperature in dark

(lll) 2x Gel loading dye (DNA)	**10ml stock (ml)**
2% Bromophenol Blue	0.25
2% Xylene cyanol	0.25
Glycerol (100%)	7.0
Water	2.5

(IV) Antibiotics (Stock)	**Concentration(mg/ml)**
Ampicillin (sodium salt)	25.0
Kanamycin (sulfate)	30.0

Note: Working solution-Ampicillin(50µg/ml) and Kanamycin (50µg/ml)

(V) X-gal (Stock) **Concentration(20mg/ml)**

Dissolve 200mg of X-gal in 10ml DMSO and store at -20°C

(VI) IPTG (Stock) **Concentration(100µM)**

Dissolve 1.2g of IPTG in 50ml of deionised water abd store in aliquots at -20°C

(VII) Proteinase K (Stock) **Concentration(10mg/ml)**

Dissolve 10mg of Proteinase K in 1ml of 10mM Tris, 1mM Sodium-EDTA buffer and store at -20°C

(VIII) RNase A **Concentration(10mg/ml)**

Dissolve 100mg of RNase A in 10ml of 10mM Tris, 15mM NaCl buffer and store at-20°C

(IX) Alkaline sodium carbonate	**Concentration(g/l)**
Sodium carbonate	20.0
Sodium hydroxide	4.0

(X) Copper sulphate solution	**Concentration(g/l)**
Copper sulphate	5.0
Sodium potassium tartrate	10.0

(b) SDS-PAGE Gel Electrophoresis Composition

(I) Resolving buffer (8X)	**Concentration(g/l)**
1.5M Tris-Cl (pH 8.8)	18.17

(II) Stacking buffer (4X)	**Concentration(g/l)**
0.5M Tris-Cl(pH 6.8)	6.06

Note: Adjust the pH 8.8 with 6N HCl. Make up the final volume to 100ml with distilled water

(lll) Acrylamide mixture	**Concentration(g/l)**
30% acrylamide	30.0
0.8% bis-acrylamide	0.8

Note: Dissolve in 100ml of warm deionised water to facilitate dissolution of bis-acrylamide. Store in amber colour bottle at 4°C

(IV) Reservoir buffer (pH 8.3, 1X)	**Concentration(g/l)**
Tris-Cl	3.0
Glycine	14.4
10% SDS	10.0

Note: Dissolve in 1000ml of distilled water adjusted to pH to 8.3

(V)Sodium dodecyl sulphate(SDS)	**Concentration(g/l)**
10%SDS	10.0

(VI) Ammonium per sulphate	**Concentration(g/l)**
10%APS	0.2

(VII) Staining solution	**Volume(ml/concentration)**
Methanol	40.0
Glacial acetic acid	10.0
Distilled water	50.0
Commassie Brilliant Blue	0.4(g/ml)

(VIII) Destaining solution	**Volume(ml)**
Methanol	40
Glacial acetic acid	10
Distilled water	50

(IX) Loading buffer (50ml, 3X)	**Amount/volume**
Tris-Cl (pH 6.8)	1.296
SDS	3.0
Glycerol	1.5ml
Bromophenol blue	3.0

Note: 1ml of loading buffer mixed with 30µl of 2-mercaptoethanol (3%)

(X) Lysis Buffer (5ml)	**Amount/Volume**
Urea	2.4
CHAPS	0.2

Note: 100µl of pharmalyte mixed with 50µl of protease inhibitor mix

(XI) Rehydration buffer(5ml)	**Amount/Volume**
Urea	2.40
CHAPS	0.1
1% Bromophenol blue solution	10 µl
DTT	0.0308
Pharmalyte	25 µl

(XII) Equilibration Buffer (20ml)	**Amount/Volume**
Urea	7.207
Glycerol	6ml
10% SDS	4ml
1% Bromophenol blue	400 µl
1.5M Tris	666 µl
water	8.934ml

Note: 10ml of equilibration buffer mixed with 0.154g of DTT and 0.250g of IAA

(XIII) Resolving gel(12%) for 80ml preparation	**Volume(ml)**
Distilled water	26.4
1.5MTris-Cl (pH 8.8)	20.0
Acrylamide mixture	32.0
10% SDS	0.8
1% APS	0.8
Glycerol	0.8
TEMED	0.032

(XIV) Resolving gel(10%) for 20ml preparation	**Volume(ml)**
Distilled water	5.7
1.5MTris-Cl (pH 8.8)	5.0
Acrylamide mixture	6.7
10% SDS	0.2
1% APS	1.5
Glycerol	0.9
TEMED	0.02

(XV) Stacking gel (4X)	**Volume(ml)**
Distilled water	5.4
0.5MTris-Cl (pH 6.8)	2.5
Acrylamide mixture	1.3
10% SDS	0.1
1% APS	0.7
Glycerol	0.4
TEMED	0.015

(c) Chemicals composition for chromosomal DNA isolation

(I) Solution I	**Concentration (g%)**
50mM glucose	0.9
25mM Tris-Cl	0.30
10mM EDTA	0.37

(II) Solution II	**Concentration (g%)**
0.2M NaOH	0.0079
SDS	1.0

(III) Solution III	**Volume(ml)**
5M Potssium acetate	60.0
Glacial acetic acid	11.5
Distilled water	28.5