

APPENDIX

PBS (Phosphate buffer saline, (pH7.4)

NaCl	8 gm
KCl	0.2 gm
Na ₂ HPO ₄	1.44 gm
KH ₂ PO ₄	0.24 gm

Dissolve in 800 ml of auto DW, adjust the pH to 7.4 with HCl and make volume to 1000 ml. Sterilize by autoclave. Store at 4°C.

Lysis buffer (pH 8.0)

Tris Hcl	10mM
NaCl	100 mM
EDTA	1mM
SDS	0.5%

10% SDS (Sodium dodecyl sulphate)

Sodium dodecyl sulphate	10 gm
Auto DW	100 ml

Store at room temperature. Heat at 60°C before use.

5 M NaCl

NaCl	292.9 mg
Auto DW	1000 ml

Autoclave and store at 4°C.

1 M Tris-HCl (pH 8.0)

Tris HCl	121gm
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Dissolve in 800 ml of DW. Make volume upto 1000 ml. Adjust the pH 8.0 by HCl, sterilize by autoclave. Store at 4°C.

0.5 M EDTA (pH 8.0)

EDTA	186.1 gm
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Dissolve in 800 ml of auto DW with the help of magnetic stirrer for 1-2 hr and adjust the pH 8.0 by NaOH pellets. Make the volume upto 1000 ml, sterilize by autoclave. Store at 4°C.

1X TE (Tris-EDTA::10:1)

Tris	10mM
EDTA	1mM

Chloroform-Isoamyl Alcohol (24:1)

Chloroform	24 ml
Isoamyl alcohol	1 ml

Mix thoroughly and store at 4°C.

Phenol-Chloroform-Isoamyl Alcohol

Tris saturated phenol	25 ml
Chloroform isoamyl alcohol	25 ml

Mix thoroughly and store at 4°C

Tris saturated phenol

Melt phenol at 66°C by keeping in waterbath.

Measure the required volume. Add 8-hydroxy quinoline to a final concentration of 0.1% (It is an anti-oxidant, gives yellow colour to phenol).

Extract phenol several times with equal volume of 1 M Tris (pH 8.0)

Then with 0.1 M Tris, until the pH of the aqueous phase is more than 7.6. Add 0.2% β-mercaptoethanol. Mix thoroughly and store in amber coloured bottle at 4°C.

Proteinase K (20 mg/ml)

Proteinase K	20 mg
Autoclaved distilled water	1 ml

Store at -20°C.

5 X Tris borate EDTA (TBE)

Tris base	54 gm
Boric acid	27.5 gm
0.5 M EDTA (pH 8.0)	20 ml

Auto DW upto 1000 ml. Sterilize by autoclave. Store at room temperature.

6 X gel loading dye

Bromophenol blue	0.25%
Xylene cynol	0.25%
Sucrose	40% (w/v)

20,000 X Ethidium bromide (100 mg/ml)

Ethidium bromide 10 mg

Auto DW 1 ml

Wrap in an Aluminium foil (photo-sensitive). Store in a dark place at room temperature.

70% Alcohol

Ethanol 70 ml

distilled water 30 ml

30% Acrylamide

Acrylamide 29 gm

N, N' methylene bisacrylamide 1 gm

Distilled water upto 100 ml

Heat the solution to dissolve the chemicals.

10% APS

Ammonium persulphate 1 gm

Distilled water upto 10 ml

3 M Sodium Acetate

Sodium acetate (Anhydrous) 24.6 gm

Distilled water upto 100 ml

Adjust pH 5.5 using glacial acetic acid. Sterile by autoclaving in batches of 20 ml.

Antibiotics

Ampicillin 100mg/ml

Autoclaved D.W.

Sterile filter

LB medium

Tryptone 10 gm

Yeast extract 5gm

NaCl 5gm

Add deionized water to approximately 1 litre. Adjust the pH to 7.5 with 10 N NaOH and autoclave.

IPTG (0.1M)

IPTG (isopropyl - β -D-thiogalactopyranoside) 1.2gm

Add deionized water to make 50ml final volume. Filter to sterilize (0.2 μ m) and store at 4^oC

X-gal

X-gal (5 bromo-4chloro-3 indolyl - β -D- galactoside) 100mg.

Dissolve in 2ml of N'N'-dimethyl formamide.

LB/Antibiotic Agar plates

Add 15 gm of agar to 1 litre of LB medium and autoclave. Allow the medium to cool to 55°C before adding antibiotic to specified concentration. Let the agar harden store at 4°C till further use.

10% Ethanol

Ethanol 10 ml

D.W. 90 ml

10% Glacial acetic acid

Glacial acetic acid (CH₃COOH 99.7%) 10 ml

D.W. 90 ml

1% Nitric acie

Nitric acid 1ml

D.W. upto 100ml

Silver Nitrate (0.006M)

Silver Nitrate 1gm

D.W. upto 1lit

Sodium carbonate (0.28M)

Sodium carbonate (Na₂CO₃) 30gm

D.W. upto 1lit

Formaldehyde (0.019%)

Formaldehyde (HCHO 40%) 400ul

D.W. 1000ml

PCR Buffer

Tris-HCL, 10 mM

Polymerase 0.01%

KCl, 50mM

pH 8.3

Publications

- **Sumit Kumar**, K.A Ahmed, M. Saxena, N.R. Sundaresan, R.V.Singh, B.P. Singh, M.U. Charaya, and V.K. Saxena (2006). Estimation of genetic divergence between immuno-divergent broiler parent line using RAPD markers. *Indian J.of Poult. Sci.*, 41: (1) 16-20.
- **Kumar, S.**, Ahmed, K.A., Saxena, V.K., Charaya, M.U. and Saxena, M. Molecular characterization of the intron IV region of growth hormone gene in immunodivergent chicken lines. Nucleotides Sequence, NCBI, GenBank Accession: **AM262338**.
- **Kumar, S.**, Ahmed, K.A., Saxena, V.K., Charaya, M.U. and Saxena, M. Molecular characterization of the intron IV region of growth hormone gene in immunodivergent chicken lines. Nucleotides Sequence, NCBI, GenBank Accession: **AM262339**.

ABSTRACT

- **Sumit Kumar**, Ahmed K.A, Nandedkar P.V., Saxena M, Charaya M.U., Singh R.V., B.P. Singh, M.U. Charaya, and. Saxena V.K (2006) *MspI* PCR-RFLP reveled polymorphism in intron IV Region of Growth hormone gene in immunodivergent broiler lines. In National Symposium on Technological Interventions for Livestock Improvement and Production. Thrust: Disaster Management. New Delhi Feb 17-19.
- **Sumit Kumar**, Ahmed K.A, Nandedkar P.V., Saxena M, Charaya M.U., Singh R.V., B.P. Singh, M.U. Charaya, and. Saxena V.K (2006). Genetic divergence analysis of immuni-divergent broiler parent lines by RAPD-PCR. In National Symposium on Technological Interventions for Livestock Improvement and Production. Thrust: Disaster Management. New Delhi Feb 17-19.

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