APPENDIX

I. Reagents required

1. **0.5 M Tris-HCl (pH 8.0)**
   - Tris base
   - Distilled water
   - Adjust the pH to 8.0 using 1N HCl
   - Makeup final volume to 50ml
   - Store at 4°C.

   - 3.028 g
   - 40.0 ml

2. **0.5 M Tris-HCl (pH 8.3)**
   - Tris base
   - Distilled water
   - Adjust the pH to 8.3 using 1N HCl
   - Makeup final volume to 50ml
   - Store at 4°C.

   - 3.028 g
   - 40 ml

3. **0.5 M EDTA (pH 8.0)**
   - Na$_2$ EDTA·2H$_2$O
   - Distilled water
   - Adjust the pH to 8.0 using 0.5M NaOH.
   - Makeup final volume to 50ml
   - Store at room temperature.

   - 9.34 g
   - 40 ml

4. **0.5 M Tris-HCl (pH 7.5):**
   - Tris base
   - Distilled water
   - Adjust the pH to 7.5 using 1N HCl
   - Makeup final volume to 50ml
   - Store at 4°C.

   - 3.028 g
   - 40 ml
5. 5X TAE:

Tris base - 12.10 g
0.5 M Na₂ EDTA. 2H₂O (pH 8.0) - 5.0 ml
Glacial Acetic acid - 2.85 ml
Make up the solution to 500 ml with distilled water
Store at room temperature.

6. 0.5X TAE (Gel running buffer):

5 X TAE (stock) - 25 ml
Distilled water - 225 ml
Make fresh every time it is required.

7. Bromophenol Blue dye:

Bromophenol blue - 2.5 mg
Sucrose - 40.0 mg
Dissolve in 1 ml distilled water
Autoclave.
Store at 4°C.

II. Reagents required for genomic DNA isolation

1. High TE:

Stock 0.5 M Tris-HCl (pH 8.0) - 20 ml
Stock 0.5 M Na₂ EDTA. 2H₂O (pH 8.0) - 8 ml
Makeup the solution to 100 ml with distilled water
Autoclave it
Cool it down to room temperature
Store at 4°C.
Appendix

2. Lysis Buffer

Stock 0.5 M Tris-HCl (pH 8.3) - 2 ml
Stock 0.5 M Na₂ EDTA. 2H₂O (pH 8.0) - 0.2 ml
NaCl - 2.337 g

Make up the solution to 100 ml with distilled water
Autoclave it
Cool it down to room temperature
Store at 4°C.

3. Proteinase K

Proteinase K - 10 mg
Autoclaved distilled water - 500 μl

Dissolve Proteinase K in autoclaved distilled water.
Store at -20°C.

4. RNAase Buffer

0.5M Tris-HCl (pH 7.5) - 0.2 ml
NaCl (0.292 g in 10 ml) - 0.3 ml
Distilled water 9.5 ml

Autoclave it
Cool it down to room temperature
Store at 4°C.

5. RNAase

RNAase - 10 mg
RNAase buffer (autoclaved) - 1 ml

Dissolve RNAase in RNAase buffer.
Keep the tube in boiling water for 15 minutes.
Allow to cool at room temperature
Store at -20°C.
Appendix

6. Saturation of Phenol with Tris-HCl (pH 8.0)

6.1 Reagents required

Water saturated Phenol - 500 ml
0.5 M Tris-HCl (pH 8.0) - 1000 ml
(60.56 g of Tris base in 1000 ml)
0.1 M Tris-HCl (pH 8.0) - 1500 ml
(For 300 ml of 0.5 M Tris-HCl (pH 8.0) add 1300 ml of Distilled water).

6.2 Procedure

- Add 0.1% 8-hydroxyquinoline to 500 ml of water saturated phenol.
- Cover flask containing phenol with aluminium foil to avoid light reaction.
- Add 500 ml 0.5 M Tris-HCl.
- Stir solution using magnetic stirrer for 15 minutes.
- Keep the solution for 30 minutes to allow phenol to settle.
- Decant the supernatant.
- Add 500 ml of 0.1 M Tris-HCl.
- Repeat the steps of stirring, settling and decanting twice with 0.1 M Tris-HCl.
- Check pH of decanted supernatant using pH paper.
- The final pH should be 8.0.
- Add 500 ml of 0.1 M Tris-HCl to phenol.
- Store at 4°C in dark bottles covered with aluminium foil.

7. Chloroform: Isoamyl alcohol (24:1 V/V)

Chloroform - 96 ml
Isoamyl alcohol - 4 ml
8. **3M Sodium acetate (pH 5.2)**

- Sodium acetate: 12.4 g
- Distilled water: 20 ml

Adjust the pH to 5.2 using glacial acetic acid

Makeup final volume to 50 ml

Autoclave it

Cool it down to room temperature

Store at 4°C.

9. **TE buffer**

- Stock 0.5 M Tris-HCl (pH 8.0): 2.0 ml
- Stock 0.5 M Na₂ EDTA.2H₂O (pH 8.0): 0.02 ml

Make up the solution to 100 ml with distilled water.

Autoclave it

Cool it down to room temperature

Store at 4°C.

**III. Reagent for colony lysis on membrane**

1. **10%SDS**

- SDS: 1.0 g
- DDW: 10 ml

2. **Denaturing solution**

- Sodium hydroxide: 2.0 g
- Sodium chloride: 8.76 g

Dissolve in 100 ml DDW and store at 4°C.

3. **Neutralizing solution**

- Sodium Chloride: 8.76 g
- 1M Tris-HCl (pH 7.4): 50.0 ml
4. 2X SSC

10X SSC  -  20 ml
DDW  -  80 ml

IV. Reagent for hybridization and DIG detection

IV.I. Hybridization buffers

1. Standards hybridization buffer

N-Lauryl sarcosine  -  0.1 g
SDS  -  0.02 g
Blocking reagent (Roche kit)  -  1.0 g
10X SSC  -  50 ml

Make up final volume 100 ml in DDW.

2. 5X SSC

SSC (10X)  -  50 ml
DDW  -  50 ml

3. 0.1X SSC

SSC (10X)  -  1 ml
DDW  -  99 ml

4. 1X SSC

SSC (10X)  -  10 ml
DDW  -  90 ml

IV.II. Reagent for DIG detection

IV.II.1 Stock solution

1. 0.1M Maleic acid buffer

Maleic acid  -  11.06 g
Sodium chloride  -  8.76 g

Dissolve in 700 ml of DDW and set pH 7.5 with solid sodium hydroxide.
2. Blocking solution (10X)

Blocking reagent - 10 g
Maleic acid buffer - 100 ml
Dissolve by constant stirring on a heating stirrer with magnet at 65°C. Autoclave and store at 4°C.

3. Detection buffer

1M Tris-HCl (pH 9.5) - 10 ml
NaCl - 0.584 g
MgCl₂·6H₂O - 1.01g
DDW - 90 ml

4. Sodium Citrate (10X SSC)

Sodium citrate - 44.1 g
NaCl - 87.65 g
Dissolve in 600 ml DDW set pH 7.0 and make up to 1000 ml.

IV.II.2 Working solution for DIG detection

1. Washing buffer

Maleic acid buffer - 485 ml
Twin-20 - 15 ml
Make up to 500 ml in DDW.

2. Blocking solution (1X)

Maleic acid buffer - 180 ml
Blocking solution (10X) - 20 ml

3. Color Substrate solution

NBT/BCIP (kit stock) - 0.2 ml
Detection buffer - 10 ml
Appendix

4. Antibody conjugate solution

Antibody conjugate (kit stock)  -  1.0 μl
Blocking solution (1X)  -  10 ml

V. Reagent for bacterial growth

1. 2X YT media

Tryptone  -  1.6 g
Yeast extract  -  1.0 g
NaCl  -  0.5 g
Agar Agar  -  1.5 g

Dissolve in 100 ml DDW, set pH 7.0 and autoclave at 15 psi for 15 minutes. Store at 4°C.

2. X-gal solution

20 mg X-gal powder dissolve in 1 ml dimethyl formamide. Sterilize by filter and store at -20°C. Add 40 μl per plate.

3. IPTG solution

200 mg IPTG powder dissolve in 1 ml DDW, sterilize by filter and store in -20°C. Add 4 μl per plate.

4. Ampicillin solution

100 mg ampicillin powder dissolve in 1 ml DDW, sterilize by filter and store in -20°C. Add 1 μl per ml of medium.

VI. Reagent for plasmid isolation

1. Solution-I

Glucose  -  0.9 g
0.5M Tris-HCl (pH 8.0)  -  5.0 ml
0.5M EDTA (pH 8.0)  -  2.0 ml

Make up final volume in 100 ml DDW and store at 4°C.
Appendix

2. STE buffer

Sodium chloride - 1.16 g
0.5M Tris-HCl (pH 8.0) - 4.0 ml
0.5M EDTA (pH 8.0) - 0.4 ml
Make up final volume in 100 ml DDW and store at 4°C.

3. Solution-II

2N sodium hydroxide - 1.0 ml
SDS - 0.1 g
Make up final volume in 10 ml DDW and use fresh condition.

4. Solution-III

Potassium acetate - 56.4 g
Glacial acetic acid - 11.5 ml
Make up final volume in 100 ml DDW and store at 4°C.

VII. Reagent required for PAGE

1. 20% Acrylamide

Acrylamide - 1.9 g
Bis-acrylamide - 0.1 g
Make up the final volume to 10 ml with distilled water.

2. 10% Ammonium persulphate

APS - 100 mg
Make up the final volume to 1 ml with distilled water.

3. 5X Tris Boric EDTA

Trisbase - 27 g
Boric acid - 13.7 g
0.5 M EDTA - 12.5 ml
Make up the final volume to 500 ml with distilled water.
4. 1X Tris Boric EDTA

- 5X TBE: 60 ml
- DDW: 240 ml

5. Agarose (For sealing of 2 gels)

50 mg / 5 ml (for one set of 2 gel).

6. Different concentrations of polyacrylamide gels for microsatellites study.

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<th>Component</th>
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