



APPENDIX

A) Reagents for genomic DNA extraction

1. Sodium dodecyl sulphate (10%)

| | |
|--|----------|
| SDS | 10.0 gm |
| Deionized water | 100.0 ml |
| Warmed to dissolve completely and adjusted pH to 7.0 | |

2. Proteinase K 20 mg/ml

| | |
|-----------------------------------|---------|
| Proteinase K | 20.0 mg |
| Distilled Water | 1.0 ml |
| Stored at -20°C in small aliquots | |

3. Tris HCl (1M: pH 8.0)

| | |
|--|----------|
| Tris base | 121.1 gm |
| Dissolved in 800 ml water, adjusted the pH to 8.0 by adding concentrated HCl. Made the volume to 1000 ml. Autoclaved and stored at 4°C | |

4. Tris EDTA (TE buffer pH 8.0)

| | |
|---------|-------|
| TrisHCl | 10 mM |
| EDTA | 1 mM |

5. 0.5 M EDTA (pH 8.0)

| | |
|-----------------|-----------|
| EDTA | 18.612 gm |
| Distilled water | 100.0 ml |

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B) Reagents for agarose gel electrophoresis

6. Tris-borate (EDTA BE) buffer (5x)

| | |
|----------------------|---------|
| Tris base | 54.0 gm |
| Boric acid | 27.5 gm |
| EDTA (0.5 M; pH 8.0) | 20.0 ml |
| Distilled water | 1000 ml |

7. Ethidium bromide

| | |
|------------------|----------|
| Ethidium bromide | 100.0 mg |
| Distilled Water | 1.0 ml |

8. Loading dye (6x)

| | |
|------------------|-------|
| Orange G | 0.2% |
| Bromophenol blue | 0.05% |
| Glycerol | 60% |
| EDTA | 6 mM |
| Stored at | 4 °C |

C) General purpose solutions

9. *Phosphate buffered saline (pH 7.2)

| | |
|-----------------------|---------|
| Sodium chloride | 8.0 gm |
| Potassium chloride | 0.02 gm |
| Disodium hydrogen | 1.15 gm |
| Phosphate (anhydrous) | |
| Potassium dihydrogen | 0.20 gm |
| phosphate | |
| Distilled water | 1000 ml |

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10. Normal saline solution

| | |
|-----------------|-----------|
| Sodium chloride | 8.5 gm/ l |
| Distilled water | 1000 ml |

- Sterilized by autoclaving at 15 psi, 121°C for 15 min and stored at 4°C until used.
- The above materials autoclave at 121°C for 15 min and allowed to cool to 50°C -60°C and poured into petridishes.

Media and Reagents

11. Baird Parker Agar (PH 7.0)

| | |
|------------------|---------|
| Peptone | 10.0 gm |
| Beef Extract | 5.0 gm |
| Yeast extract | 1.0 gm |
| Glycine | 12.0 gm |
| Sodium pyruvate | 10.0 gm |
| Lithium Chloride | 5.0 gm |
| Agar | 20.0 gm |
| Distilled Water | 950 ml |

The above base autoclaved and allowed to cool to 40-50°C and then about 50ml concentrated egg yolk emulsion and 3ml sterile 3.5% potassium tellurite solution added properly mixed poured in petridishes.

12. Oxidation – Fermentation Medium (g m/ml)

| | |
|--|--------|
| Peptone | 2.0 gm |
| Sodium Chloride | 5.0 gm |
| Dipotassium Hydrogen Phosphate | 0.3 gm |
| Agar | 3.0 gm |
| Bromothymol Blue (1% aqueous solution) | 3.0 ml |
| Distilled Water | 1000ml |

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The pH was adjusted to 7.1 before adding of the bromothymol blue and then medium was autoclaved in a flask at 121°C for 30 minutes. Then 0.5 ml, 1% carbohydrate solution (pre-sterilized) was added to medium aseptically. The medium was then tubed to a depth of about 4 cm.

13. Brain heart infusion Broth (pH 7.4±0.2)

| | |
|--------------------------------|----------|
| Peptic digest of animal tissue | 10.0 gm |
| Calf Brain Infusion (solids) | 12.5 gm |
| Beef Heart Infusion (solids) | 5.0gm |
| Dextrose | 2.0 gm |
| Sodium chloride | 5.0 gm |
| Di-Sodium phosphate | 2.5 gm |
| Distilled Water | 1000 ml. |

14. 5 % Sheep blood agar

| | |
|-----------------------------|--------|
| Base: blood agar (Hi Media) | 5.0 gm |
| Distilled water | 100ml |

Dissolved the ingredients by boiling and autoclave at 121°C for 15 min
For complete medium: the autoclaved base was allowed to cool to 46- 48°C and then add 10ml of aseptically collected defibrinated sheep blood was added properly mixed and pour into petriplates.

15. Nutrient broth

| | |
|-----------------------------|-------------|
| Lean meat, ox heart or beef | 500.0 gm |
| Peptone | 10- 20.0 gm |
| Sodium chloride | 5.0 gm |
| Distilled water | 1 liter |

The above materials autoclave at 121°C for 15 min and allowed to cool to 50 – 60 °C and poured into petridishes.

16. Nutrient agar:

| | |
|----------------|--------|
| Nutrient broth | 1000ml |
| Agar | 20.0gm |

The above materials autoclave at 121°C for 15 min and allowed to cool to 50 – 60 °C and poured into petridishes.

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17. DNase agar (pH 7.3)

| | |
|-----------------------|--------|
| Tryptose | 2.0gm |
| Deoxyribonucleic acid | 2.0gm |
| Sodium chloride | 5.0gm |
| Agar powder | 12.0gm |
| Toludine blue | 0.15 % |

The above materials autoclave at 121°C for 15 min and allowed to cool to 50 – 60 °C and poured into petridishes.

18. Mueller – Hinton agar (pH 7.4)

| | |
|--------------------|---------|
| Beef infusion | 300.0ml |
| Casein hydrolysate | 17.5gm |
| Starch | 1.5gm |
| Agar | 10.0gm |
| Distilled water | 1 liter |

The above materials autoclave at 121°C for 15 min and allowed to cool to 50 – 60 °C and poured into petridishes.

19. Mueller – Hinton agar with 5% Nacl (pH 7.4)

| | |
|--------------------------------|----------|
| Mueller-Hinton Agar (Hi Media) | 3.8 gm |
| Sodium chloride | 5.0 gm |
| Distilled water | 100.0 ml |

The above materials autoclave at 121°C for 15 min and allowed to cool to 50 – 60 °C and poured into petridishes.

20. Phenolphthalein phosphate agar (pH 7.4)

| | |
|----------------------------------|---------|
| Peptic digest of animal tissue | 5.0gm |
| Beef extract | 3.0gm |
| Sodium chloride | 5.0gm |
| Sodium phenolphthalein phosphate | 0.012gm |
| Agar | 15.0gm |
| Distilled water | 1000ml |

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The above materials autoclave at 121°C for 15 min and allowed to cool to 50 – 60 °C and poured into petridishes.

21. Voges-Proskauer

| | |
|---|--------|
| Peptone | 5.0 gm |
| Dipotassium hydrogen phosphate (K ₂ HPO ₄) | 5.0gm |
| Distilled water | 1000ml |
| Glucose 10% solution (sterilized separately) | 50 ml |

Peptone and phosphate were dissolved; pH was adjusted to 7.6; filtered and then dispensed in 5.0 ml amounts in test tubes and sterilized at 121 C for 15 minutes. Glucose solution was sterilized by filtrations and 0.25 ml was added to each tube (final concentration 0.5%).

22. Triptone soya broth (pH 7.3 + 0.2)

| | |
|----------------------------------|--------|
| Casein enzyme hydrolysate | 17.0gm |
| Peptic digest of Soya bean meal | 3.0gm |
| Sodium chloride | 5.0gm |
| Di- potassium hydrogen phosphate | 2.5gm |
| Dextrose | 2.5gm |
| Distilled water | 1000ml |

The above materials autoclave at 121°C for 15 min ; nd allowed to cool to 50 – 60 °C and poured into petridishes.
