

## APPENDIX I

### Z.N. staining

#### Solution (A)

- basic fuchsin ..... 3 g
- ethanol 95% .....100 ml

#### Solution (B)

- phenol crystals .....5 g
- distilled water .....100 ml

Solution (A) .....10 ml

Solution (B) .....90 ml

#### Decolourizing agent

- sulfuric acid .....25 ml
- distilled water .....100 ml

#### Counter stain

- methylene blue .....3 g
- distilled water ..... 100 ml

## **Appendix II**

### **PCR Reagents and Equipments**

- PCR Reagents:
- Go Taq DNA polymerase M830B 23372207 Promega, Madison WI USA
- dATP 100 mM U120A 22741205 Promega, Madison WI USA
- dCTP 100 mM U122A 22123211 Promega, Madison WI USA
- dTTP 100 mM U123A 22631905 Promega, Madison WI USA
- dGTP 100 mM U121A 21944512 Promega, Madison WI USA
- MgCl<sub>2</sub> 25 mM A351H 22535622 Promega, Madison WI USA
- 5 X Green Go Taq Flexi Buffer M891A 22967815 Promega, Madison WI USA
- PCR equipments:
- power supply BluePower 500 SERVA
- SIGMA centrifuge 1- 15 GERMANY
- Water bath, SCOTT SCIENCE UK
- PCR machine TECHNE TC-312, TECHNE, DUXFORD, CAMBRIDGE, UK
- SYNGENE gel documentation system, Synoptics Ltd, UK
- GENWAY 1000 hotplate, JENWAY Ltd, UK

## Appendix III

### Preparation agarose gel

Agarose                    1g  
0.5 x TBE                100ml

Heat till fully dissolved.

Cool to approximately 50 °C before adding 5 µl of ethidium bromide solution (10mg / ml).

Gently mix then pour into gel casting tray and allow to solidify

### Preparation of loading dye

25% (w/v) Bromophenol Blue.

30% (v/v) Glycerol.

25% (w/v) xylene Cyanol.

In 1% agarose gels, bromophenol blue migrates with 300-bp fragments whereas xylene cyanol migrates with 4000-bp fragments.

### Preparation of running buffer 10 x TBE

Tris base    108g

Boric acid   55 g

Na<sub>4</sub> EDTA 9.34G (40ml of 0.5 M EDTA)

Add 1-120 to give a final volume of 1 liter

pH: is 8.3

### Preparation of 1 x TBE

10 ml            10 x TBE

90 ml           D.W