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

Decolorizing 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
- MgCl2 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 filly dissolved.

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

Gently mix then pour into gel casting tray and a low to solidify

Preparation of loading dye

25% (w/v) Bromophenol Blue.

30% (v/v) Glycerol.

25% (w/v) xylene Cynol.

In 1% agarose gels, bromoheno1 blue migrates with 300-bp fragments whereas xylene cynol migrates with 4000-bp fragments.

Preparation of running buffer 10 x TBE

Tris base 108g

Boric acid 55 g

Na4 EDTA 9.34G (40m1 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 m1 D.W