

Chapter 2

2.1 CHEMICALS

All chemicals and reagents used were of analytical grade and were obtained from the following sources :

GIBCO BRL, USA

Acrylamide

Bis-acrylamide

Ammonium persulphate

Urea

Terminal Deoxynucleotidyl Transferase, Recombinant (rTdT) + 5x buffer

SIGMA CHEMICALS COMPANY, ST. LOUIS, USA

Sodium citrate

X-gal

Trizma base

Boric acid

Sodium chloride

Ethylenediamine tetraacetic acid (EDTA)

Sodium dodecyl sulphate (SDS)

Potassium acetate

PRONADISA, MADRID, SPAIN

Luria-Bertani Agar

Luria-Bertani Broth

QIAGEN, DENMARK

Proteinase K

RNase A

NEN, USA

α^{33} pATP

BIOWHITTAKER MOLECULAR APPLICATIONS, USA

20 bp ladder

AMRESCO, USA

Denhart's solution

Phenol : Chloroform : Isoamyl alcohol (25:24:1)

Ampicillin

FERMENTAS, LITHUANIA

MassRuler™ DNA Ladder, High Range

PROMEGA, USA

100bp DNA Ladder + 6x Loading buffer

λ DNA / *Hind* III markers + 6x Loading buffer

Agarose

Taq DNA Polymerase in Storage Buffer B + 10x PCR Buffer and 25mM MgCl₂

Set of dATP, dCTP, dGTP, dTTP

MERCK, GERMANY

Absolute ethanol

Acetic acid

ALPHA DNA, CANADA

RAMS Primer, VJI : N₇KKVRVRV(CT)₁₀

Microsatellite Primers :

Sequence name : K10

Forward GCA GGT AAC TGA AGA GCA TT

Reverse AAA ATT ACC TGC TTG TGT GC

Sequence name : K13

Forward GCA CTG TTA AGT TCT GGT GTC

Reverse GAT ACG CAT GAC ATT CTG TG

Sequence name : K16

Forward CAG TGG TTG CAC ACT TAC AG

Reverse AAA GTC GGC ATG ATG AAA TA

Sequence name : K17

Forward ATA TTT CAT CAT GCC GAC TT

Reverse TGG TAT TTT CTC GTG CAT TA

Sequence name : K20

Forward AGC TGA CAC TTT GAA GCA CT

Reverse GTG CTA ATT CAG CGA CTC TT

Sequence name : K27

Forward CCA TTA ACC CCT TGT CCT CA

Reverse AAG GAT GCA GGA GAG CAA AA

Sequence name : K29

Forward CCC AGT GGT TGC ACA CTT AC

Reverse TGA AAG GAA TTT TCA AGG GTT T

Sequence name : K37

Forward CCA TTA GCA AAC CCA TGC TT

Reverse TGG AAA TGT GTC ATC CTT CAG

2.2 KITS

2.2.1 BST TECH LAB, PURE-GENE™ PLASMID MINIPREP KIT

HiBind Miniprep Columns (II) 200

2ml Collection Tubes 200

Solution I 120 ml

Solution II 120 ml

Solution III 2 x 80 ml

Buffer HB 120 ml

Wash buffer concentrate 3 x 40 ml

RNase A, concentrate 400 µl

2.2.2 PROMEGA SILVER SEQUENCE™ DNA STAINING REAGENTS

Bind silane (Q421A)	500 µl
Silver nitrate (Q426A)	10 x 2 g
Formaldehyde, 37% (Q427A)	20 x 3 ml
Sodium thiosulphate (Q428A)	10 x 1 ml
Sodium carbonate	600 g

2.2.3 INVITROGEN, TOPO TA CLONING KIT

TOPO TA Cloning® Reagents :

pRC®2.1-TOPO® plasmid DNA	20 µl
10x PCR buffer	100 µl
Salt solution	50 µl
dNTP mix	10 µl
M13 Forward (-20) primer	20 µl
M13 reverse primer	20 µl
Control template	10 µl
Control PCR primers	10 µl
Sterile water	1 ml

One Shot® Reagents :

SOC Medium	6 ml
TOP 10 cells (chemically competent)	21 x 50 µl