

## **Appendix 1.0 Chemicals, buffers (for DNA extraction and PCR) and oligonucleotide primers.**

### **A. General solutions**

All chemicals and reagents were purchased from Amresco USA, R&M Marketing U.K., Nascalai Tesque, Japan, Qiagen U.S.A, System ® U.K. and Vivantis Technologies U.S.A.

PCR reagents were purchased from iNtRON Biotechnology Inc., Korea. Agarose gels and electrophoresis solutions were purchased from 1<sup>st</sup> BASE Singapore, Fermentas ThermoFisher Scientific U.S.A and Vivantis Technologies U.S.A.

**Acetic acid,**

**Ammonium acetate, M = 77.08 g/mol**

**Bovine serum albumin (BSA), general grade, pH 5.2**

**Chloroform:Isoamyl alcohol [24:1], biotechnology grade**

**Dimethylsulfoxide (DMSO), ≥ 99.9%, for molecular biology**

**Ethanol absolute (99.8% v/v)**

**Ethanol, 70% (v/v)**

70.0 ml Absolute ethanol

Sterilised distilled water was added to final volume of 100ml and stored at -20 °C.

**Ethylenediaminetetraacetic acid disodium salt (EDTA), 0.5 M [pH 8.0]**

37.22 g EDTA

EDTA was resuspended in 80 ml of sterilized distilled water. The pH of the solution was adjusted to pH 8.0 with 10N NaOH. Sterilised distilled water was further added to a final

## **Appendix 1.0 (continued)**

volume of 100 ml and the solution was sterilized by autoclaving at 121 °C, 27 p.s.i. for 20 minutes. Solution was stored at room temperature.

**Agarose, Molecular biology grade**

**Chloroform:Isoamyl alcohol [24:1], biotechnology grade**

**Glycerol**

**Isopropanol, Biotechnology grade**

**Methylated spirit**

**Polyvinylpyrrolidone (PVP),**

**Silica gel, Self indicating system**

**Sodium chloride (NaCl), 5 M**

29.22 g NaCl

Sterilised distilled water was added to a final volume of 100 ml and the solution was sterilized by autoclaving at 121 °C, 27 p.s.i. for 20 minutes. Solution was stored at room temperature.

**Tris buffer 1X, [pH 8.0], Ultra Pure**

**Tris-EDTA buffer [pH 8.0], (TE)**

10ml 1 M Tris-HCL [pH 8.0]

2ml 0.5 M EDTA [pH 8.0]

## **Appendix 1.0 (continued)**

Sterilised distilled water was added to a final volume of 100 ml and the solution was sterilized by autoclaving at 121 °C, 27 p.s.i. for 20 minutes.

### **Tris-Hydrochloric acid (Tris-HCl), 1 M [pH 8.0]**

12.11 g Ultrapure™ Tris

Tris was resuspended in 80 ml of sterilized water. The pH of the solution was adjusted to pH 8.0 with concentrated HCl. Sterilised distilled water was further added to a final volume of 100 ml and the solution was sterilized by autoclaving at 121 °C, 27 p.s.i. for 20 minutes. Solution was stored at room temperature.

### **β-mercaptoethanol or 2-mercaptoethanol, 55 M**

## **B. DNA extraction solutions and materials**

### **Cetyltrimethyl Ammonium Bromide Polyvinylpyrrolidone (CTAB- PVP) buffer**

100 ml 1 M Tris HCl pH 8.0

280 ml 5 M NaCl

40 ml of 0.5 M EDTA

20 g of CTAB (cetyltrimethyl ammonium bromide)

4 g of PVP (polyvinylpyrrolidone)

Total volume was adjusted to 1 L with sterilized distilled water. The solution was sterilized by autoclaving at 121 °C, 27 p.s.i. for 20 minutes. Solution was stored at room temperature.

### **RNAse A [10 mg/ml], Biotechnology grade**

## Appendix 1.0 (continued)

### C. Oligonucleotide primers

#### a. Oligonucleotide for ITS region

The primers used for PCR amplification of the nuclear ITS regions were ITS4 and ITS5. The primers used for PCR sequencing were similar.

**Table ..** Primers for PCR of ITS

Primer	Sequence	Reference
ITS 4	5'- TCC TCC GCT TAT TGA TAT GC-3'	White <i>et al.</i> , 1990
ITS 5	5'- GGA AGT AAA AGT CGT AAC AAG G-3'	White <i>et al.</i> , 1990

#### b. Oligonucleotide for *matK* region

The primers used for PCR amplification of *matK* region were natKf, natKr, 3F\_KIM\_f and 1R\_KIM\_r. The primers used for PCR sequencing were similar.

**Table ..** Primers for PCR of *matK*

Primer	Sequence	Reference
natKf	5'- ATGGAAGAATTACAARGATA-3'	new, designed for this study
natKr	5'-TAAACTATTTACCRRGTCAT-3'	new, designed for this study
3F_KIM_f	5'-CGTACAGTACTTTTGTGTTTACGAG-3'	CBOL Working Plant Group, 2009
1R_KIM_r	5'-ACCCAGTCCATCTGGAAATCTTGGTTC-3'	CBOL Working Plant Group, 2009

## Appendix 1.0 (continued)

### D. PCR reagents

***i*-Taq™ DNA Polymerase Enzyme [5 U/μl] (iNtRON Biotechnology Inc., Korea)**

***i*-Taq™ DNA Polymerase Reaction buffer [10x] (iNtRON Biotechnology Inc., Korea)**

100 mM Tris-HCl, pH 8.3

500 mM KCl

20 mM MgCl<sub>2</sub>

Enhancer solution

**Deoxyribonucleotide triphosphate mix (dNTP) (iNtRON Biotechnology Inc., Korea)**

### E. Agarose gels and solutions for electrophoresis

**Ethidium bromide [0.5 mg/ml]**

2.5 μg Ethidium bromide

Sterilised distilled water was added to a final volume of 5 ml and the mixture was stored in a dark bottle at room temperature.

**Orange DNA loading dye, 6X**

**Tris-Borate-EDTA (TBE) agarose gel, 2 % (w/v)**

Agarose 1.0 g

5 ml of 10X TBE buffer was added to a final volume of 50 ml, and the mixture was heated until the agarose powder was completely dissolved.

**Tris-Borate-EDTA (TBE) buffer [pH 8.0] 10X, Ultra Pure Grade**

**Tris-Borate-EDTA (TBE) buffer [pH 8.0] 1X, Ultra Pure Grade**

## **Appendix 1.0 (continued)**

**VC 100bp plus DNA ladder, 0.1µg/µl**

**VC 1kb DNA ladder, 0.1µg/µl**

# All agarose gels for this project were viewed with Gel Pro Imager Integrated Solution, Georgia Avenue U.S.A

### **F. Commercial kits**

Megaquickspin™ PCR & Agarose Gel DNA extraction system (iNtRON Biotechnology, Korea)

DNeasy Plant Mini Kit [50 reaction] (Qiagen, U.S.A)