

Appendix

APPENDIX I

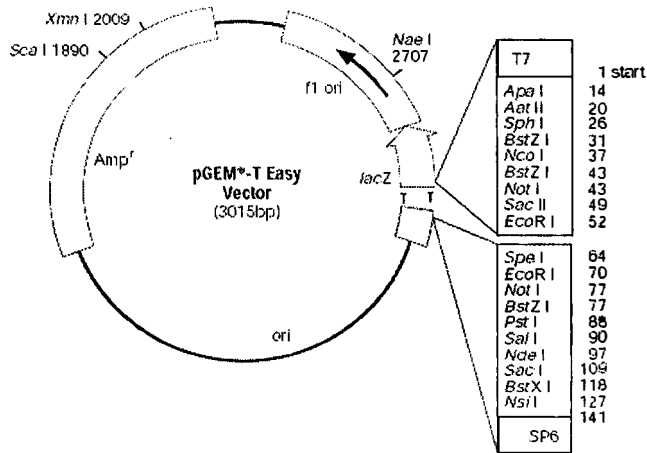
CHEMICALS AND BIOCHEMICALS

Applied Biosystems, USA	Ampli Taq Gold, LIZ-600 size standard, Hi-Di formamide
Ambion Inc. (USA).	Nuclease free water
Amersham Pharmacia (GE Healthcare, USA)	Hybond N+ nylon membrane
Borosil or Schot-Duran	All the glasswares
Hi-media	Glucose (Dextrose), Bacteriological gar, Tryptone, Di-sodium hydrogen phosphate, Sodium dihydrogen phosphate
Invitrogen (USA).	Agarose, Tris, Acrylamide, N, N'-Methylene bis-acrylamide, Ammonium per sulphate (APS), Trizol TM reagent,
Les Alcohols De Commerce Inc., Ontario.	analytical grade absolute alcohol
New England Biolabs Inc. (USA)	All the restriction endonucleases, the size markers like 100 bp ladder and 1kb ladder, T4 DNA ligase
Promega Corp. (Madison, Wisconsin, USA)	T4 polynucleotide kinase, Taq DNA polymerase, dNTPs, and Nuclease free water, Low melting point (LMP) agarose, oligo dT ₁₈ primer
Qualigens	Hydrochloric acid, Glacial acetic acid, Sodium chloride, Chloroform, Isoamylalcohol, Isopropanol, Phenol, Sodium hydroxide, Di-potassium hydrogen phosphate, Potassium chloride, Magnesium sulphate, Ammonium chloride, Potassium di-hydrogen phosphate
Sigma, (USA).	Ampicillin, Cetyl trimethyl ammonium bromide (CTAB), Ethidium bromide, Ethylene diamine tetra acetic acid (EDTA), Boric acid, Calcium chloride, Salmon sperm DNA, Sodium dodecyl sulphate (SDS), Sodium acetate, Tween 20, RNase zap, RNase A, Urea
Tarson or Axygen.	the plasticwares
US Biochemicals (USB, USA).	Bovine serum albumin (BSA), Diethyl pyrocarbonate (DEPC), Bromophenol blue (BPB), Xylene cyanol FF, Glycerol, Isopropyl- β -D-1-thiogalactoside (IPTG), 5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside (X-Gal), β -mercaptoethanol, N,N,N',N'-tetramethylethylenediamine (TEMED)
Whatman	Filter papers of Grade 2 and 3

KITS USED IN THE STUDY

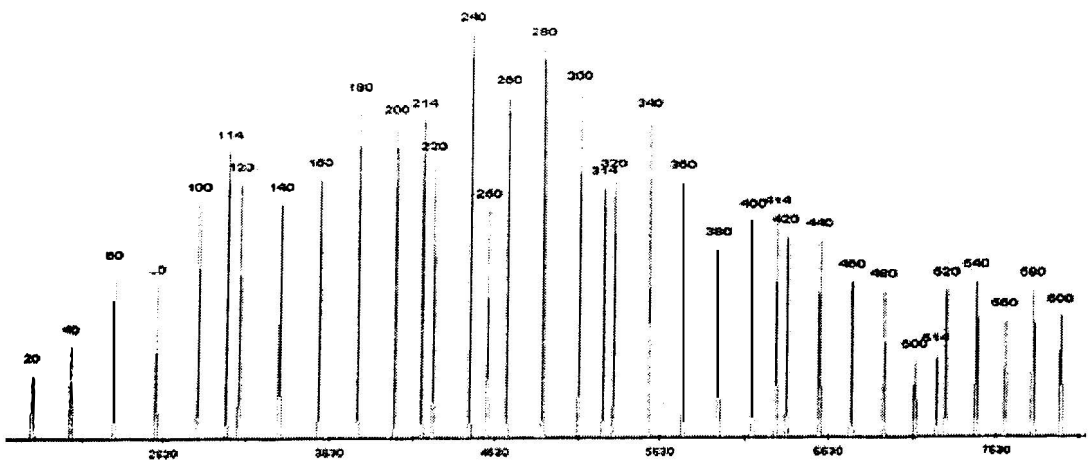
Amersham Pharmacia (GE Healthcare, USA)	a) GFX TM PCR DNA and Gel Band Purification Kit b) Nucleon Phytopure Plant DNA extraction kit
Applied Biosystems	Big Dye TM Terminator Cycle Sequencing Ready Reaction Kit Version.3.1
BD Biosciences/ Clontech	a) BD Genome Walker TM Universal kit b) SMART cDNA library construction kit
GeneX	qPCR Mastermix Plus for SYBR Green I dNTP sample
Invitrogen	AFLP Analysis System I AFLP starter Primer kit
Promega	a) pGEM-T Easy vector system b) Poly A Tract mRNA isolation system III

- **Radioactive labeled dATP** used in the study was obtained from Board of Radiation and Isotope Technology (BRIT), Anushakti Nagar, Mumbai.
- **Bacterial strain used:** JM 109 : (*endA1*, *recA1*, *gyrA96*, *thi*, *hsdR17* (r_K^- , m_K^+), *relA1*, *supE44*, $\Delta(lac-proAB)$, [F' , *tra D36*, *proAB*, *lacI*^q Δ M15])
- **Plasmid vector used:** pGEM-T Easy vector system from Promega



- **GENE SCAN™ -600 LIZ SIZE STANDARD (APPLIED BIOSYSTEMS):** Gene Scan 600 LIZ is designed for sizing DNA fragments in the 20 to 600 bp range and produces 36 single stranded labeled fragments as shown below.

Electropherogram of the GeneScan™ - 600 LIZ® Size Standard



APPENDIX II

BUFFERS AND REAGENTS

BACTERIOLOGY

50% Glycerol	50% in water, autoclaved
1M CaCl ₂	1M in water, autoclaved
IPTG	1M in sterile water
X-gal	40mg/mL in Dimethyl formamide
Ampicillin	100mg/mL in sterile water
LB agar, 1 litre	NaCl (10g), Tryptone(10g),Yeast extract(5g), pH adjusted to 7.0 with NaOH and added 15 g of bacteriological agar and autoclaved
LB broth, 1 litre	NaCl (10g), Tryptone (10g), Yeast extract (5g), pH adjusted to 7.0 with NaOH and autoclaved

MOLECULAR BIOLOGY

Chloroform isoamyl alcohol	24:1
Phenol: chloroform: isoamylalcohol	25:24:1
EDTA, pH 8	0.5 M, pH adjusted with NaOH
Ethidium bromide	10mg/mL in sterile water
Glucose	1M in water, autoclaved
Magnesium chloride	1M in water, autoclaved
Tris saturated Phenol	Neutral Phenol saturated with Tris-HCl (pH 8)
RNase A	10mg/mL in sterile water
SDS	10% in sterile water
Sodium acetate	3M in water, pH adjusted with glacial acetic acid to 5.2, autoclaved
Sodium chloride	5M in water, autoclaved
Sodium hydroxide	2N in water
Sodium phosphate	1M in sterile water
Tris -HCl	1M Tris, pH adjusted to 6.8, 7.5 and 8 with concentrated HCl, autoclaved
6X Gel Loading Dye	0.25 % (w/v) of Bromophenol blue, 30% glycerol in water (v/v), 0.25 % (w/v) of Xylene cyanol FF
5X TBE buffer, 1 litre	20mL of 0.5M EDTA (pH 8), 27.5 g of Boric acid, 54 g of Tris
TE buffer	1mM of EDTA, pH 8, 10mM of Tris-HCl, pH 8
DNA ISOLATION : CTAB METHOD (<i>all reagents autoclaved</i>)	
2XCTAB buffer	2% (w/v) of CTAB, 20mMEDTA (pH 8), 1.4M NaCl, 1% PVP(M,40,000), 100mM Tris-HCl(pH 8)
5% CTAB solution	5% (w/v) CTAB,0.7M NaCl
CTAB precipitation solution	1% CTAB, 10mM EDTA (pH 8), 50mM Tris -HCl (pH 8)
High-salt TE	1mM EDTA (pH 8), 1 M NaCl, 10mM Tris -HCl (pH 8)

CTAB Isolation buffer (850 μ L)	100 μ L of β -mercaptoethanol, 750 μ L of 2X CTAB buffer
STEPHEN DELLAPORTA METHOD (<i>all reagents autoclaved</i>)	
Extraction buffer	50mM Tris, pH 8.0, 10mM EDTA, pH 8.0, 100mM Sodium Chloride, 1% SDS, 10mM β -mercaptoethanol
5M potassium acetate	49.75 g in 100mL, autoclaved
3M Sodium acetate	24.6g in 100mL, autoclaved
PLASMID ISOLATION	
SET buffer (Cell washing buffer)	1mM EDTA, pH 8, 0.1 M NaCl, 10mM of Tris-HCl, pH 8
Solution I (Cell resuspension solution)	10mM of EDTA, pH 8, 50mM Glucose, 25mM Tris-HCl, pH 8
Solution II (Cell lysis solution)	0.2 N NaOH, 1% SDS
Solution III	60 mL of 5M potassium acetate, 11.5mL of Glacial acetic acid, 28.5mL of water
RNA ISOLATION	
Sodium acetate	3M in DEPC treated water, autoclaved
Tris-HCl, pH 8	1M in Tris-HCl in autoclaved DEPC water
RNAse free water	0.01 % (v/v) of diethyl pyrocarbonate (DEPC) in RNAse free glass bottles. This was allowed to stand overnight and later autoclaved and used.
ENRICHMENT STUDIES	
2XBW (Binding and Washing buffer) (1mL)	10mM Tris HCl pH 7.5, 1mM EDTA, 2mM NaCl, water (987.6 μ L)
20X SSC (1litre), pH 7.0, stored at room temperature	3M Sodium Chloride, 0.3M Sodium citrate and 880.55mL water
20X SSPE (1mL)	3.6 M NaCl, 0.2 M Sodium phosphate, 20mM EDTA, water (40 μ L)
SLOT BLOT HYBRIDISATION	
Hybridization buffer	1.4g SDS in 9mL water, 200 μ L of BSA (200mg/mL), 40 μ L of 0.5M EDTA, 10mL of phosphate buffer

APPENDIX III

EQUIPMENTS USED IN THE STUDY

-20° C freezer	Vest Frost
-70° C deep freezer	New Brunswick Scientific
Autoclave	Stericylindrical Horizontal sterilizer, Yorco Scientific Udyog
Automated Sequencer	ABI PRISM TM 3730 Genetic Analyzer, Perkin-Elmer
Real Time	ABI 7500 Real Time PCR machine
Centrifuges	12C MicroCentrifuge, REMI, Universal 16R, Hettich(Refrigerated), himacCR21E, Hitachi (Refrigerated)
Dri-Bath	Type 17600, Thermolyne
Gel Drier	Model 583, Biorad
GM counter	Model 2241 Survey meter, Ludlum Measurements
Heating plate	Delite, Johnson
Horizontal Electrophoresis Apparatus	Broviga
Hotair Oven	Beston
Hybridization oven	Hofer, Single barrel
Hofer SQ3 Sequencer' Apparatus	Amersham Pharmacia Biotech
Ice maker	Simag-SP 255
Incubator	R1-10S Bacteriological incubator, REMI
Laminar Air Flow	Alpha Linear, LabLine
Magnetic Stirrer	2MLH, REMI
Micropipettes	Eppendorf, Eppendorf research, Finpipette, Thermolab sytems
Microwave oven	BPL
Multimager	Flour-S TM Multimager, Biorad
PCR machines	Master cycler gradient and Master cylder Eppgradient from Eppendorf. BioRad iCycler
pH meters	μ pH System 361, Systronics
Phosphor Imager	Personal Molecular Imager FX, Bio-Rad
Power packs	Powerpack200-Bio-Rad/Broviga
Refrigerator	Ultra, Godrej
Rocker	Red Rotor, Hofer
Scanner	ScanJet 6200C, Hewlett Packard
Shaker	Lab-Therm, Kuhner
Spectrophotometer	Biospec-1601, DNA/Protein/Enzymes Analyzer, Shimadzu
UV Crosslinker	UVC 500, Hofer
UV illuminator	UVT-40M. Herolab
Vacuum blotting apparatus	BioRad
Vortexer	Cyclomixer CM101, REMI
Water purification System	Milli Q-Plus, Millipore
Waterbath	20B with F200 cooler, Julabo(refrigerated)VPC, Julabo
Weighing balance	BL 600, BP 221S, Sartorius

APPENDIX IV
PRIMERS AND ADAPTORS USED IN THE STUDY

Name	Sequence (5' to 3')	Length((bp)
Tsp A ₂	5' GGTCTACTGGACTCACT 3'	17
Tsp A ₃	5' 3 AATTAGTGAGTCCAGTAGACC 3'	21
Tsp P	5' GGTCTACTGGACTCAC 3'	16
Biotynylated (AC) ₁₃	5' 7 CACACACACACACACACACACACA 3'	26
Biotynylated (TC) ₁₃	5' 7 TCTCTCTCTCTCTCTCTCTCTCTC 3'	26
T7	5' TAATACGACTCACTATAGGG 3'	20
SP6	5' TATTTAGGTGACACTATAG 3'	19
(TC) ₂₀	5' TC 3'	40
GW AP	5'GTAATACGACTCACTATAGGGCACGCGTGGTCGACGGCCCGGGCTGGT-3' 3'- ² H ₂ N-CCCCACCA-PO ₄ -5'	
GW AP1	5'-GTAATACGACTCACTATAGGGC-3'	22
GW AP2	5'-ACTATAGGGCACGCGTGGT-3'	19
(CA) ₂₀	5'CA3'	40
(AT) ₂₀	5'AT 3'	40
(CTAT) ₇	5'-CTATCTATCTATCTATCTATCTATCTAT-3'	28
(CTT) ₈	5'-K CTTCTTCTTCTTCTTCTTCTTCTT-3'	24
PnCA25F	5' GTGTGTTATTTGTCTCTGGGTTTTCC 3'	27
PnCA25R	5' CTTGGGGCACTCTAACCATCGTCTG 3'	26
PnCA88F	5' CACATATTTTCTTACATTGCG 3'	22
PnCA88R	5' GATTATGGGCTGCCGGATT 3'	20
PnGT119F	5' CCCAACTTCAGAATGATTATACAGC 3'	25
PnGT119R	5' CTGGGCAGTAAGCAAACATA 3'	20
PnAG30F	5' ACTAAGGCTAATGTGATAACCTGAGGA 3'	27
PnAG30R	5' ATCCCTGGATGGAAATTTGAAGGCTTGC 3'	28
PnGT2F	5' CTAGAGAGTAACAGTTATCACTTCACAGC 3'	29
PnGT2R	5' CTAGCAAATTTGTTCTCTAATTCACATGT 3'	29
PnGATA10F	5' CTCCCAGTTATACAACATCACAACCTAGCAC 3'	31
PnGATA10R	5' AGAGGCTTGTCTTAGTTGTGCTCGGGA 3'	27
PnCA9F	5' TCATCAATCACACCTAAAAGAAGGCTATCC 3'	30
PnCA9R	5' ATGTGGCTATGGGGAACGGTCAGGGGT 3'	27
<i>Fluorescent labeled microsatellite primers</i>		
PNAG30F	5' (6-FAM) ACTAAGGCTAATGTGATAACCTGAGGA3'	27
PnGT2F	5' (6-FAM) CTAGAGAGTAACAGTTATCACTTCACAGC3'	29
PnGATA10F	5' (HEX) CTCCCAGTTATACAACATCACAACCTAGCAC3	31
PnCA9F	5' (HEX) TCATCAATCACACCTAAAAGAAGGCTATCC3'	30
SMARTIV Oligonucleotide	5' AAGCAGTGGTATCAACGCAGAGTGGCCATTACGGCCGGG 3'	39
CDSIII/3' PCR Primer	5'-ATTCTAGAGGCCGAGGCGCCGACATG-d(T) ₃₀ N ₋₁ N-3'	58
5' PCR Primer	5' AAGCAGTGGTATCAACGCAGAGT 3'	23
3PAG1F1	5' CACACTATAACATGACATCCCACGCCCTC 3'	28
3PAG1F2	5' CTTTATCAAGATGGCCCCATTCTACAA 3'	28
3PAG2F1	5' CCACATCGCAACATAAAACAACAGGT 3'	25
3PAG2F2	5' GGTTATACGCTAATCTCTGGCTTGCTTTG 3'	29
3PAG3F1	5' CCAGAGATTAATATGGGAGTTCGATGC 3'	27
3PAG3F2	5' TCAGGCAATAAAACAATAAGCGAGTC 3'	25

Note: All the primers and oligos used in the study were custom synthesized by **Genosys, Sigma** except for the microsatellite repeat oligos (TC)₂₀, (CA)₂₀ and (AT)₂₀ which were kindly gifted by Dr.Saha, Rubber Research Institute of India, Kottayam. 3 = 5' phosphorylation, 7 = biotinylation, M = A + C, K = G + T