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APPENDICES

APPENDIX A

Searching Jerusalem artichoke EST databases for resistance gene homologs

Table A1 Searching Jerusalem article EST databases for resistance gene homologs

Accession numbers	NCBI Accession number	GenBank Annotation information (BLASTX)	Source species	E value (BLASTX)	%Similarity (BLASTX)	Class of R gene	Protein size	Conserved domains	SSR motif
Heli_tube.EL434107	AAN73007.1	NBS-LRR resistance protein RS6-8	H. annuus	6.00E-57	44%	NBS-LRR	301	NBS, LRR	none
Heli_tube.EL434458	AAD20950.1	EDS1	A. thaliana	9.00E-22	32%	EDS1	296	none	(ACAAA)2
Heli_tube.EL434999	NP_174038.1	TIR-NBS-LRR class, putative	A. thaliana	9.00E-24	38%	TIR-NBS-LRR	298	LRR	none
Heli_tube.EL435021	AAP82814.1	resistance protein RPP8- like protein	A. thaliana	1.00E-08	29%	CC-NBS-LRR	300	none	none
Heli_tube.EL436736	NP_201491.1	CC-NBS-LRR class, putative	A. thaliana	1.00E-36	37%	CC-NBS-LRR	275	NBS	(TGATG)2, (GTAATT)2
Heli_tube.EL437196	AAT66045.1	EDS1-like protein	H. annuus	3.00E-33	88%	EDS1	299	Lipase3	none
Heli_tube.EL438206	AAL07535.1	resistance gene analog PU3	H. annuus	1.00E-57	46%	TIR-NBS-LRR	273	NBS	none
Heli_tube.EL438367	ABY61371.1	NBS-LRR resistance-like protein SFRGA5	H. annuus	6.00E-63	79%	NBS-LRR	296	NBS	(CTCAA)2
Heli_tube.EL438755	ABQ57619.1	NBS-LRR resistance-like protein RGC304	H. annuus	1.00E-33	66%	NBS-LRR	278	NBS	(TCCATG)2, (AAAAGA)2
Heli_tube.EL438840	AAT08953.1	CC-NBS-LRR	H. annuus	1.00E-10	38%	CC-NBS-LRR	280	none	(ATATC)2
Heli_tube.EL439349	AAP50224.1	resistance candidate RPP8-like protein	A. arenosa	4.00E-32	34%	CC-NBS-LRR	260	NBS	none
Heli_tube.EL439754	AAN73007.1	NBS-LRR resistance protein RS6-8	H. annuus	2.00E-21	47%	NBS-LRR	270	none	(TACCA)2, (TTTAT)2, (AATTG)2
Heli_tube.EL440001	ABQ57618.1	NBS-LRR resistance-like protein RGC303	H. annuus	2.00E-27	45%	NBS-LRR	258	NBS	(GTTAT)2
Heli_tube.EL440241	AAO47085.1	TIR-NBS-LRR disease resistance-like protein	H. annuus	5.00E-06	30%	TIR-NBS-LRR	237	NBS	none
Heli_tube.EL440872	NP_201491.1	CC-NBS-LRR class, putative	A. thaliana	1.00E-31	38%	CC-NBS-LRR	245	LRR	(TCAAT)2
Heli_tube.EL441115	ACF98012.1	NBS-LRR resistance-like protein RGC260	H. annuus	4.00E-88	69%	CC-NBS-LRR	275	LRR	(ATTGG)2
Heli_tube.EL441328	ACY69610.1	TIR-NBS-LRR resistance-like protein RGC151	H. annuus	2.00E-28	35%	TIR-NBS-LRR	261	none	none
Heli_tube.EL441358	ACY69610.1	TIR-NBS-LRR resistance-like protein RGC151	H. annuus	2.00E-19	47%	TIR-NBS-LRR	264	none	(TTTCA)2

Table A1 Searching Jerusalem artichoke EST databases for resistance gene homologs (cont.)

Accession numbers	NCBI Accession number	GenBank Annotation information (BLASTX)	Source species	E value (BLASTX)	%Similarity (BLASTX)	Class of R gene	Protein size	Conserved domains	SSR motif
Heli_tube.EL441364	ACY69610.1	TIR-NBS-LRR resistance-like protein RGC151	H. annuus	4.00E-27	33%	TIR-NBS-LRR	258	none	none
Heli_tube.EL442217	ACY69609.1	CC-NBS-LRR resistance- like protein RGC203	H. annuus	2.00E-64	52%	CC-NBS-LRR	252	none	none
Heli_tube.EL442509	NP_188064.1	NBS-LRR class, putative	A. thaliana	9.00E-21	34%	NBS-LRR	266	LRR	(GTTTG)2
Heli_tube.EL442634	NP_188064.1	NBS-LRR class, putative	A. thaliana	9.00E-21	34%	NBS-LRR	266	LRR	(GTTTG)2
Heli_tube.EL442681	AAN73007.1	NBS-LRR resistance protein RS6-8	H. annuus	1.00E-66	69%	NBS-LRR	304	none	none
Heli_tube.EL444590	NP_190257.1	CC-NBS-LRR class, putative	A. thaliana	2.00E-09	42%	CC-NBS	285	NBS	(AGATGA)3, (GAA)5, (AGATGA)2, (GAA)10, (AGC)4, (AGATGA)2, (GAA)5, (AGATGA)2, (GAA)4
Heli_tube.EL444841	AAN73007.1	NBS-LRR resistance protein RS6-8	H. annuus	2.00E-34	37%	NBS-LRR	259	LRR	(TTGTGA)2, (CTCGT)2
Heli_tube.EL445132	NP_201491.1	CC-NBS-LRR class, putative	A. thaliana	1.00E-21	35%	CC-NBS-LRR	282	NBS	none
Heli_tube.EL445205	AAN73007.1	NBS-LRR resistance protein RS6-8	H. annuus	9.00E-102	73%	NBS-LRR	279	LRR	none
Heli_tube.EL446234	AAN73009.1	NBS-LRR resistance protein RS7-4	H. annuus	1.00E-117	86%	NBS-LRR	321	NBS	none
Heli_tube.EL446443	ACF98012.1	NBS-LRR resistance-like protein RGC260	H. annuus	1.00E-66	67%	CC-NBS-LRR	219	NBS	(GAAAC)2, (TCCCAG)2
Heli_tube.EL447036	AAL07543.1	resistance gene analog NBS8	H. annuus	2.00E-13	36%	TIR-NBS-LRR	158	TIR	(AGCTT)2



Table A1 Searching Jerusalem artichoke EST databases for resistance gene homologs (cont.)

Accession numbers	NCBI Accession number	GenBank Annotation Information (BLASTX)	Source species	E value (BLASTX)	%Similarity (BLASTX)	Class of R gene	Protein size	Conserved domains	SSR motif
Heli_tube.EL447061	ACY69610.1	TIR-NBS-LRR resistance-like protein RGC151	H. annuus	1.00E-54	48%	TIR-NBS-LRR	259	TIR, NBS	(TTAA)3
Heli_tube.EL447295	AAN73007.1	NBS-LRR resistance protein RS6-8	H. annuus	3.00E-65	44%	NBS-LRR	301	LRR	None
Heli_tube.EL447977	NP_001119319.1	TIR-NBS-LRR class, putative	A. thaliana	5.00E-20	28%	TIR-NBS-LRR	262	none	(AAGTT)2
Heli_tube.EL448188	NP_188064.1	NBS-LRR class, putative	A. thaliana	2.00E-20	32%	NBS-LRR	255	LRR	(TGATG)2
Heli_tube.EL448555	NP_192816.1	CC-NBS-LRR class, putative	A. thaliana	3.00E-13	37%	CC-NBS-LRR	252	none	(TGCT)3
Heli_tube.EL448648	NP_201491.1	CC-NBS-LRR class, putative	A. thaliana	3.00E-09	32%	CC-NBS-LRR	236	RPW8	none
Heli_tube.EL449110	AAD20950.1	EDS1	A. thaliana	3.00E-44	40%	EDS1	250	Lipase3	(AAAGC)2
Heli_tube.EL449466	NP_199688.1	TIR-NBS-LRR class, putative	A. thaliana	6.00E-17	42%	TIR-NBS-LRR	269	TIR	(GATACT)2
Heli_tube.EL449960	AAS93911.1	RPPI3-like protein	A. arenosa	6.00E-09	36%	CC-NBS-LRR	308	none	(TTGTG)2, (CGGT)2
Heli_tube.EL450929	AAL07535.1	resistance gene analog PU3	H. annuus	2.00E-42	51%	TIR-NBS-LRR	293	TIR	(ACA)4, (GCTTA)2
Heli_tube.EL451397	NP_175437.1	CC-NBS-LRR class, putative	A. thaliana	1.00E-33	34%	CC-NBS-LRR	323	NBS	none
Heli_tube.EL451666	ACY69609.1	CC-NBS-LRR resistance-like protein RGC203	H. annuus	8.00E-63	55%	CC-NBS-LRR	323	LRR	(TCAGAT)2, (CAAAA)2
Heli_tube.EL452113	AAL07535.1	resistance gene analog PU3	H. annuus	4.00E-47	37%	TIR-NBS-LRR	293	TIR, NBS	none
Heli_tube.EL452280	NP_201491.1	CC-NBS-LRR class, putative	A. thaliana	3.00E-36	34%	CC-NBS-LRR	320	none	(TGATG)2
Heli_tube.EL452935	ACY69609.1	CC-NBS-LRR resistance-like protein RGC203	H. annuus	9.00E-111	72%	CC-NBS-LRR	295	LRR	(CAAAT)2

Table A1 Searching Jerusalem artichoke EST databases for resistance gene homologs (cont.)

Accession numbers	NCBI Accession number	GenBank Annotation information (BLASTX)	Source species	E value (BLASTX)	%Similarity (BLASTX)	Class of R gene	Protein size	Conserved domains	SSR motif
Heli_tube.EL453765	AAL07542.1	resistance gene analog NBS7	H. annuus	2.00E-38	57%	TIR-NBS-LRR	312	TIR	(AAAT)2, (AGAGA)2
Heli_tube.EL454181	AAL07536.1	resistance gene analog NBS1	H. annuus	2.00E-104	87%	TIR-NBS-LRR	294	TIR	none
Heli_tube.EL454311	AAS93910.1	RPPI3-like protein	A. arenosa	7.00E-34	35%	CC-NBS-LRR	289	NBS	none
Heli_tube.EL454403	AAN73007.1	NBS-LRR resistance protein RS6-8	H. annuus	1.00E-48	67%	NBS-LRR	295	LRR	(AAAT)3, (ATGAT)2, (CACCA)2
Heli_tube.EL455330	NP_001119319.1	TIR-NBS-LRR class, putative	A. thaliana	7.00E-25	43%	TIR-NBS-LRR	317	none	(TCCAA)2
Heli_tube.EL455346	NP_201491.1	CC-NBS-LRR class, putative	A. thaliana	3.00E-44	37%	CC-NBS-LRR	326	NBS	(ATGAT)2
Heli_tube.EL455368	AAN73007.1	NBS-LRR resistance protein RS6-8	H. annuus	4.00E-34	43%	NBS-LRR	330	none	(AGGGT)2, (TACTC)2
Heli_tube.EL455612	NP_001119319.1	TIR-NBS-LRR class, putative	A. thaliana	1.00E-14	32%	TIR-NBS-LRR	273	none	(CTTGA)2
Heli_tube.EL455676	ACY69609.1	CC-NBS-LRR resistance-like protein RGC203	H. annuus	4.00E-63	53%	CC-NBS-LRR	268	none	none
Heli_tube.EL456036	NP_201492.1	CC-NBS-LRR class, putative	A. thaliana	3.00E-22	37%	CC-NBS-LRR	317	RPW8	(TTCAT)2, (AATC)4
Heli_tube.EL456436	AAN73007.1	NBS-LRR resistance protein RS6-8	H. annuus	2.00E-99	75%	NBS-LRR	311	LRR	none
Heli_tube.EL456910	NP_201491.1	CC-NBS-LRR class, putative	A. thaliana	3.00E-28	38%	CC-NBS-LRR	285	none	(ATGAT)2
Heli_tube.EL457326	AAN73007.1	NBS-LRR resistance protein RS6-8	H. annuus	6.00E-53	46%	NBS-LRR	296	LRR	(GACAT)2
Heli_tube.EL457580	NP_001119319.1	TIR-NBS-LRR class, putative	A. thaliana	1.00E-23	31%	TIR-NBS-LRR	291	none	(ACCGT)2, (TTTCC)2, (ACTTG)2

Table A1 Searching Jerusalem artichoke EST databases for resistance gene homologs (cont.)

Accession numbers	NCBI Accession number	GenBank Annotation information (BLASTX)	Source species	E value (BLASTX)	%Similarity (BLASTX)	Class of R gene	Protein size	Conserved domains	SSR motif
Heli_tube.EL460845	AAT08953.1	CC-NBS-LRR	H. annuus	6.00E-83	65%	CC-NBS-LRR	271	NBS	none
Heli_tube.EL465866	AAL07535.1	resistance gene analog PU3	H. annuus	6.00E-89	82%	TIR-NBS-LRR	326	TIR	(TATT)2
Heli_tube.EL466370	NP_201492.1	CC-NBS-LRR class, putative	A. thaliana	1.00E-21	38%	CC-NBS-LRR	278	none	(TTTCTC)2, (GTTGCT)2, (ATCTA)2, (AGCTC)2
Heli_tube.EL466818	BAB11239.1	RAR1	A. thaliana	2.00E-86	67%	RAR1	277	CHORD	none
Heli_tube.EL468211	AAN73007.1	NBS-LRR resistance protein RS6-8	H. annuus	6.00E-47	47%	NBS-LRR	288	NBS	none
Heli_tube.EL468882	ACF98012.1	NBS-LRR resistance-like protein RGC260	H. annuus	2.00E-29	68%	CC-NBS-LRR	222	NBS	none
Heli_tube.EL469941	ABQ57533.1	NBS-LRR resistance-like protein RGC49	H. annuus	3.00E-48	60%	NBS-LRR	309	NBS	none
Heli_tube.EL471160	AAL07536.1	resistance gene analog NBS1	H. annuus	7.00E-74	83%	TIR-NBS-LRR	272	TIR	(TATT)2
Heli_tube.EL471862	ACY69610.1	TIR-NBS-LRR resistance-like protein RGC151	H. annuus	4.00E-38	50%	TIR-NBS-LRR	276	TIR	none
Heli_tube.EL472778	ABY61382.1	NBS-LRR resistance-like protein SFRGA16	H. annuus	5.00E-58	72%	NBS-LRR	239	NBS	(TTTGCA)2, (AAGTT)2

APPENDIX B

The dendrogram based on the SRAP and EST-SSR data showing the genetic relationships among 47 *H.tuberosus* genotypes

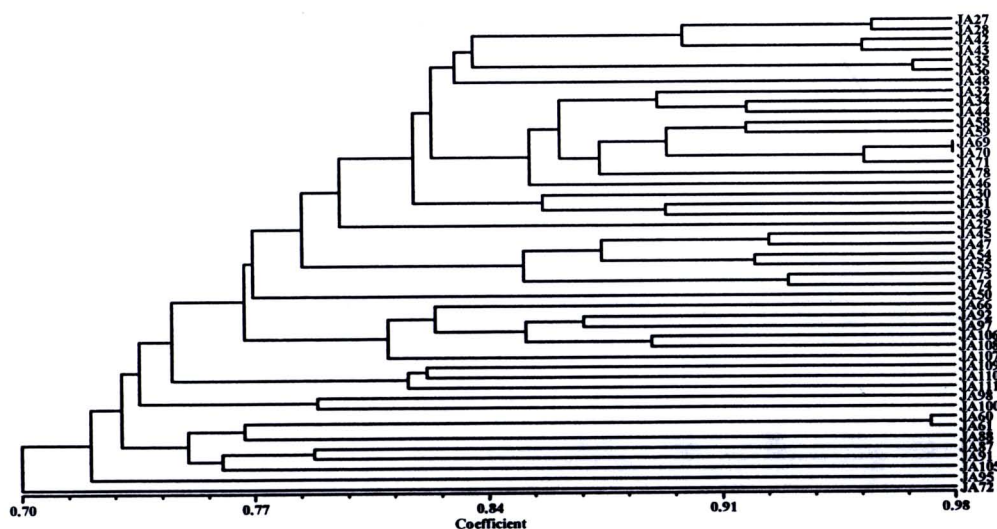


Figure B1 The dendrogram based on the SRAP primer data showing the genetic relationships among 47 *H. tuberosus* genotypes based on the UPGMA clustering for Dice coefficient

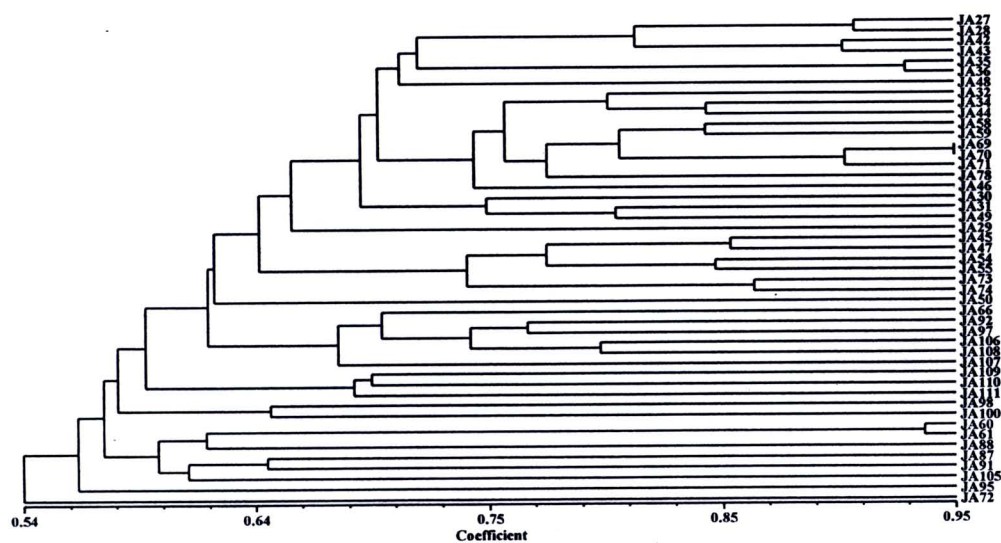


Figure B2 The dendrogram based on the SRAP primer data showing the genetic relationships among 47 *H. tuberosus* genotypes based on the UPGMA clustering for Jaccard coefficient

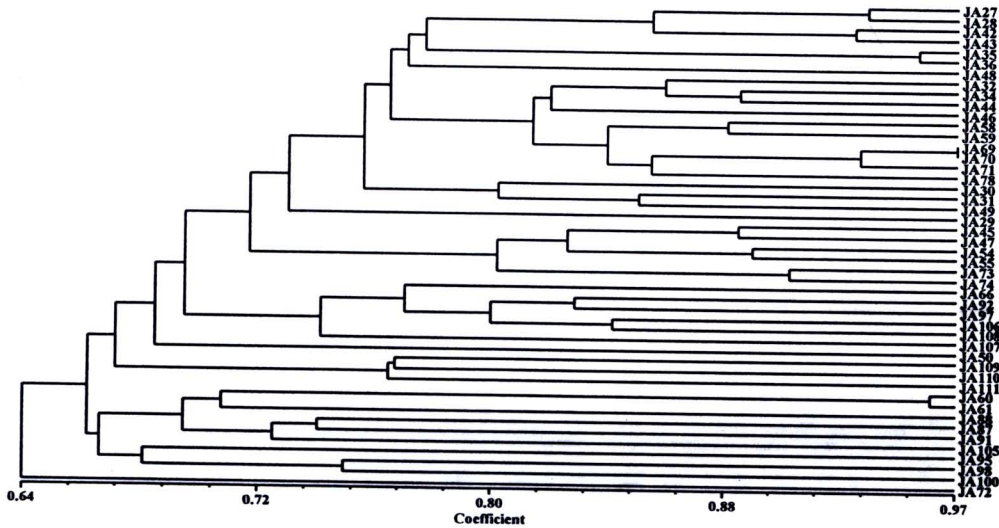


Figure B3 The dendrogram based on the SRAP primer data showing the genetic relationships among 47 *H.tuberosus* genotypes based on the UPGMA clustering for Simple matching coefficient

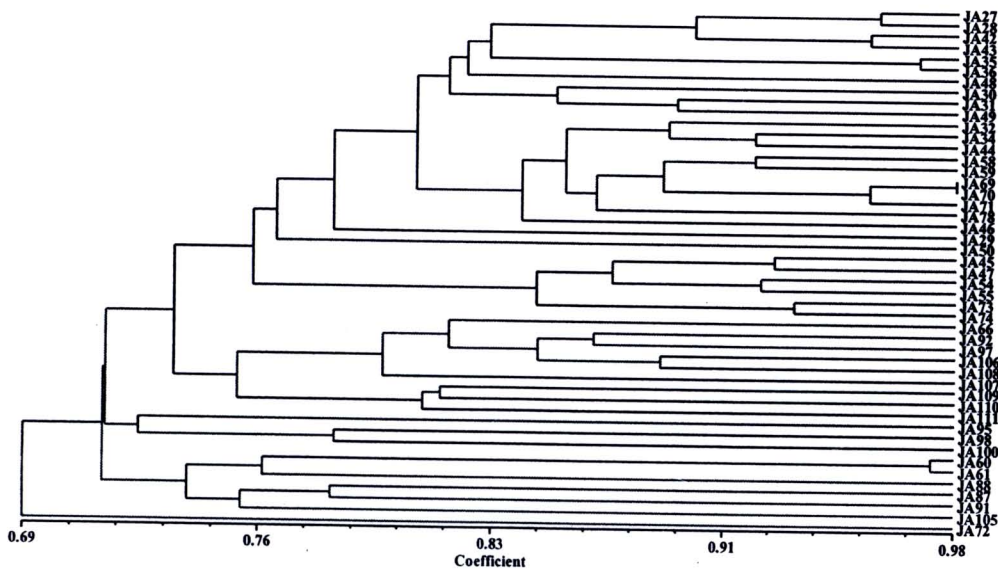


Figure B4 The dendrogram based on the SRAP primer data showing the genetic relationships among 47 *H.tuberosus* genotypes based on the WPGMA clustering for Dice matching coefficient

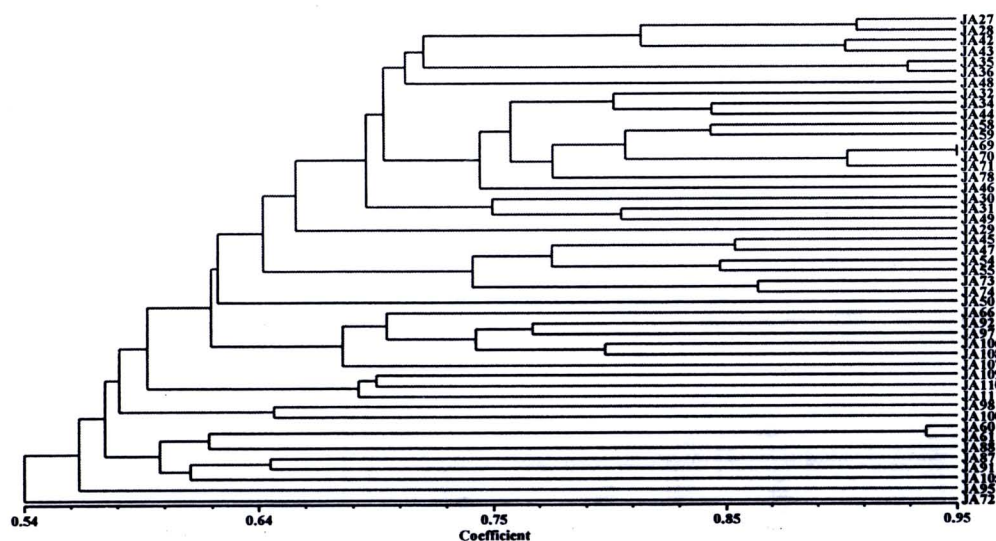


Figure B11 The dendrogram based on the SRAP primer data showing the genetic relationships among 47 *H. tuberosus* genotypes based on the Complete linkage clustering for Jaccard coefficient

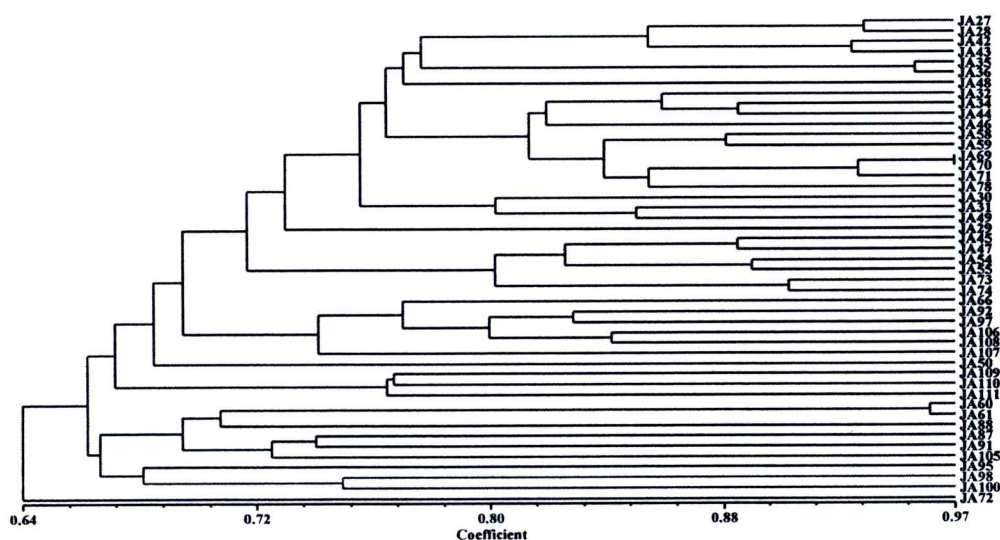


Figure B12 The dendrogram based on the SRAP primer data showing the genetic relationships among 47 *H. tuberosus* genotypes based on the Complete linkage clustering for Simple matching coefficient

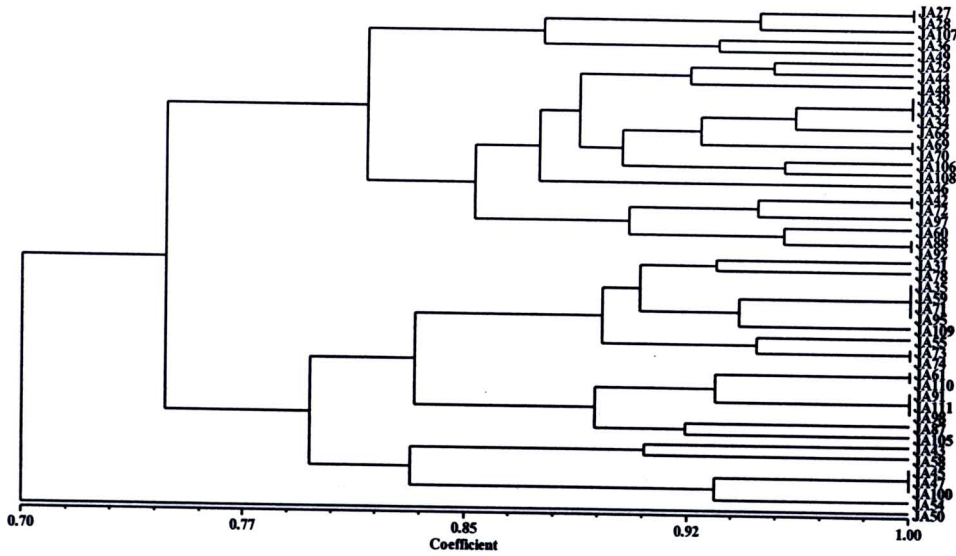


Figure B13 The dendrogram based on EST-SSR data showing the genetic relationships among 47 *H.tuberosus* genotypes based on the UPGMA clustering for Dice coefficient

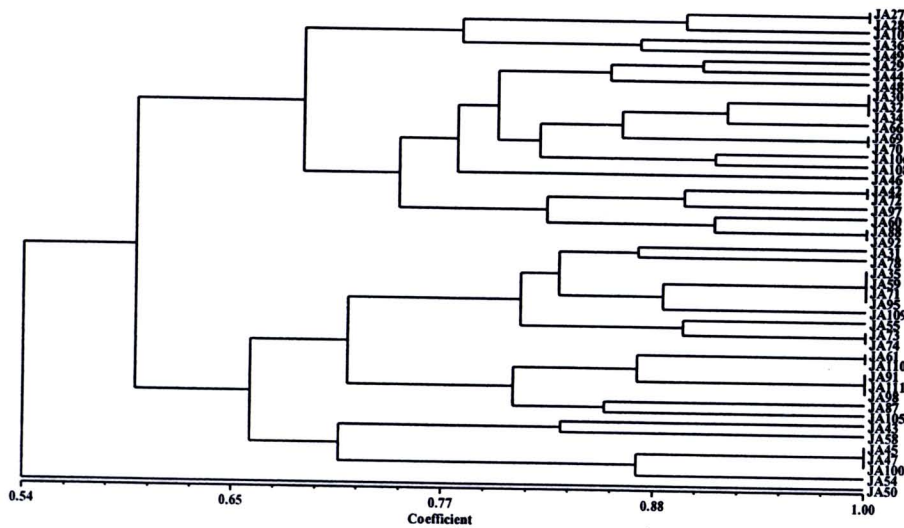


Figure B14 The dendrogram based on EST-SSR data showing the genetic relationships among 47 *H.tuberosus* genotypes based on the UPGMA clustering for Jaccard coefficient

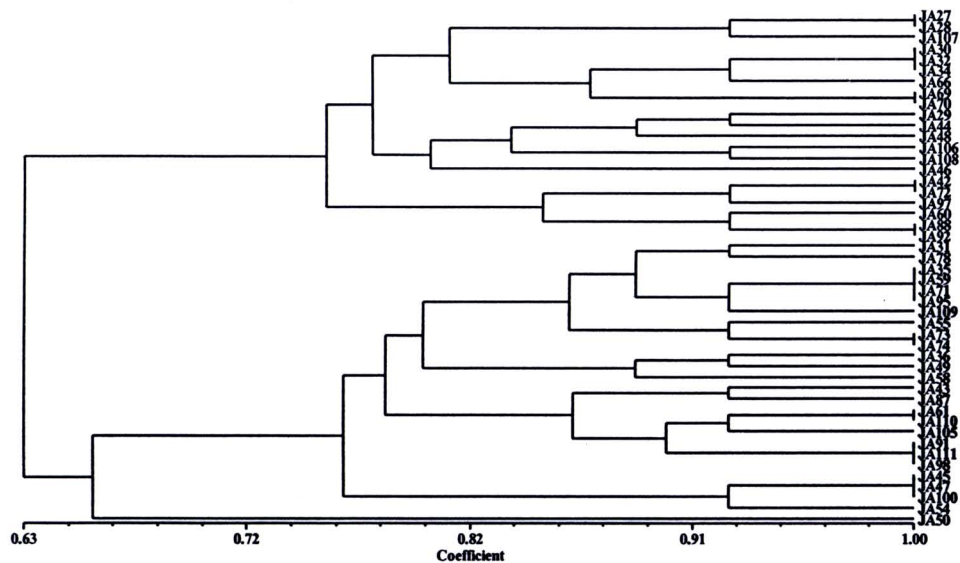


Figure B15 The dendrogram based on EST-SSR data showing the genetic relationships among 47 *H.tuberosus* genotypes based on the UPGMA clustering for Simple matching coefficient

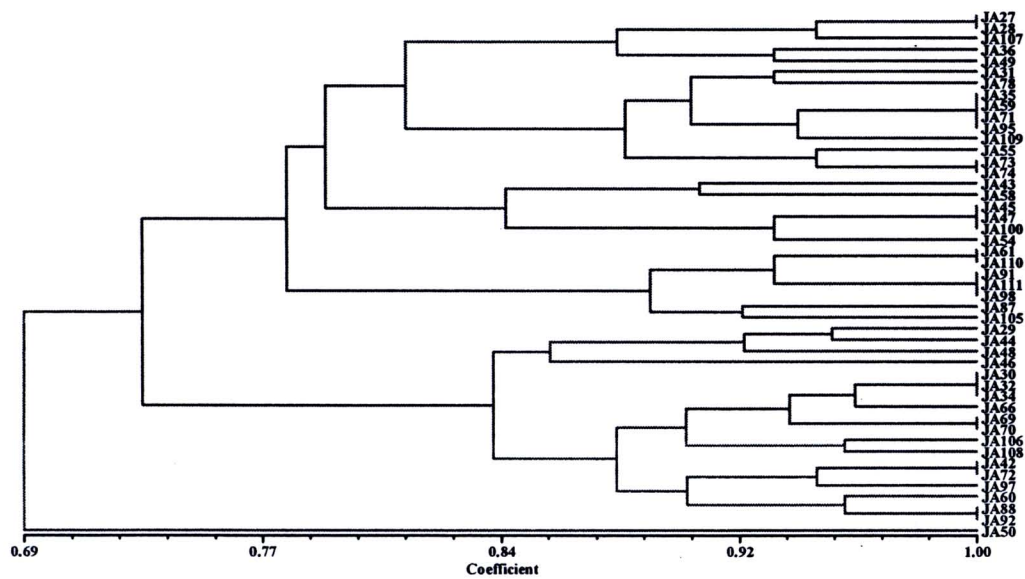


Figure B16 The dendrogram based on EST-SSR data showing the genetic relationships among 47 *H.tuberosus* genotypes based on the WUPGMA clustering for Dice coefficient

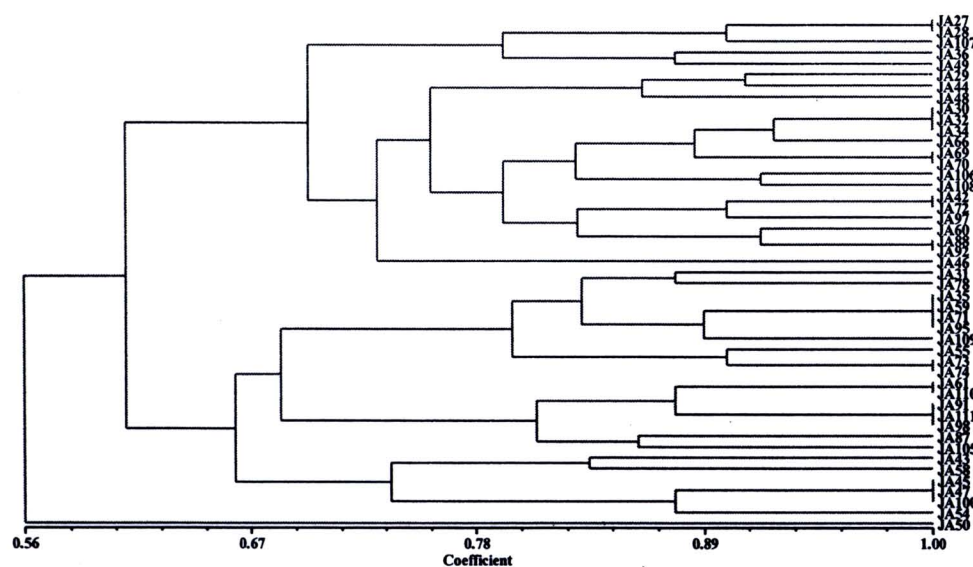


Figure B17 The dendrogram based on EST-SSR data showing the genetic relationships among 47 *H. tuberosus* genotypes based on the WUPGMA clustering for Jaccard coefficient

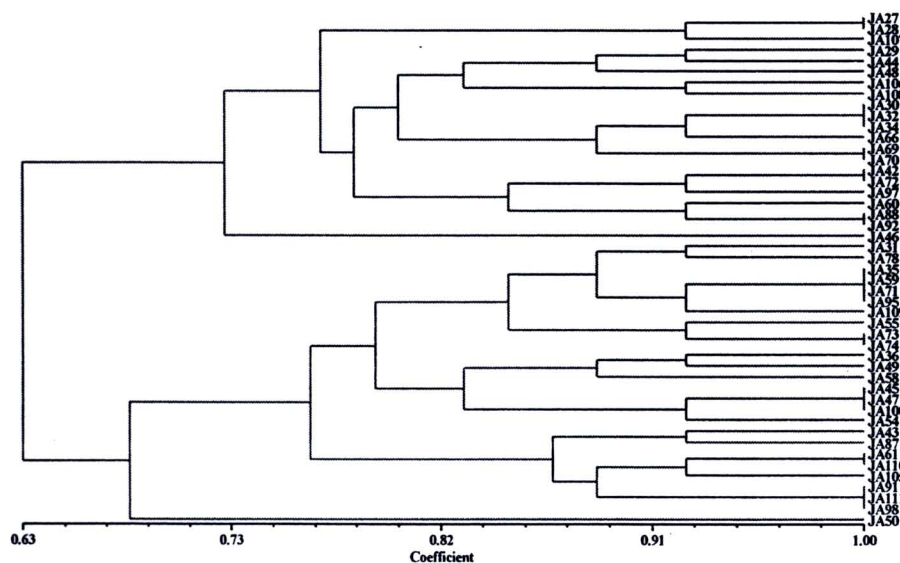


Figure B18 The dendrogram based on EST-SSR data showing the genetic relationships among 47 *H. tuberosus* genotypes based on the WUPGMA clustering for Simple matching coefficient

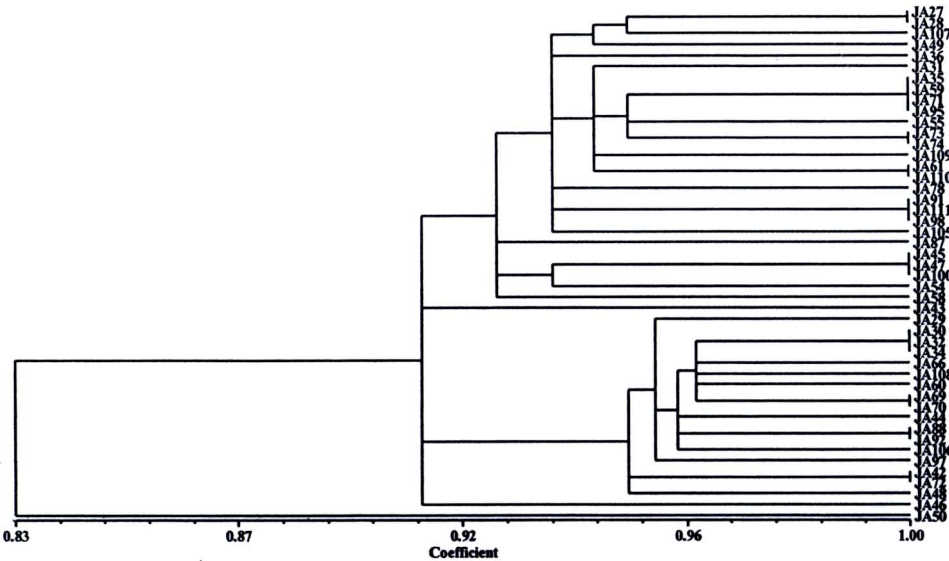


Figure B19 The dendrogram based on EST-SSR data showing the genetic relationships among 47 *H.tuberosus* genotypes based on the Single linkage clustering for Dice coefficient

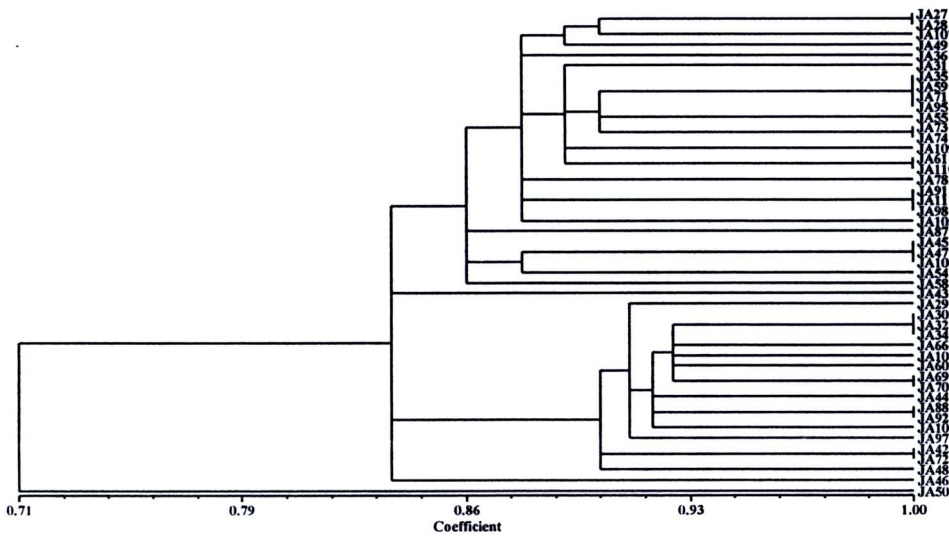


Figure B20 The dendrogram based on EST-SSR data showing the genetic relationships among 47 *H.tuberosus* genotypes based on the Single linkage clustering for Jaccard coefficient

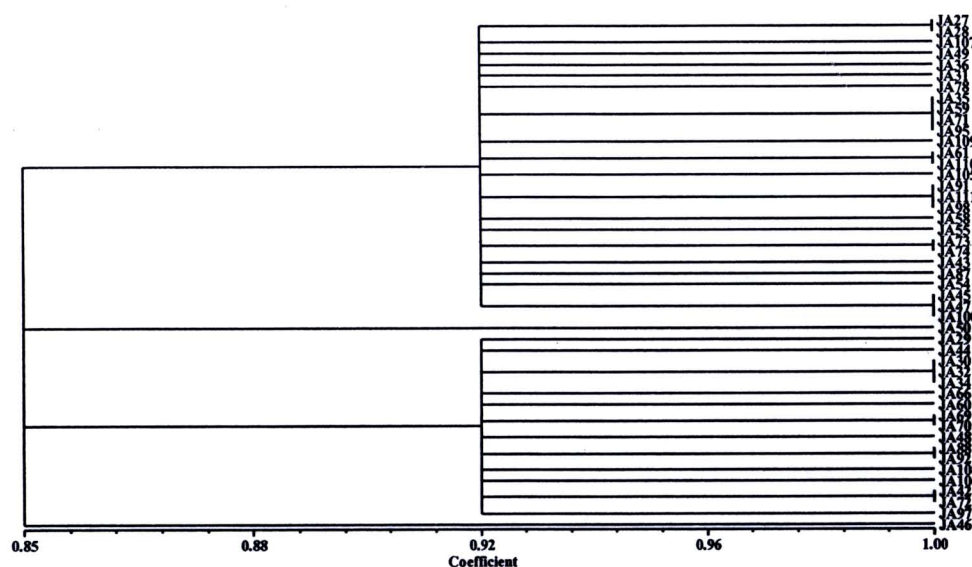


Figure B21 The dendrogram based on EST-SSR data showing the genetic relationships among 47 *H. tuberosus* genotypes based on the Single linkage clustering for Simple matching coefficient

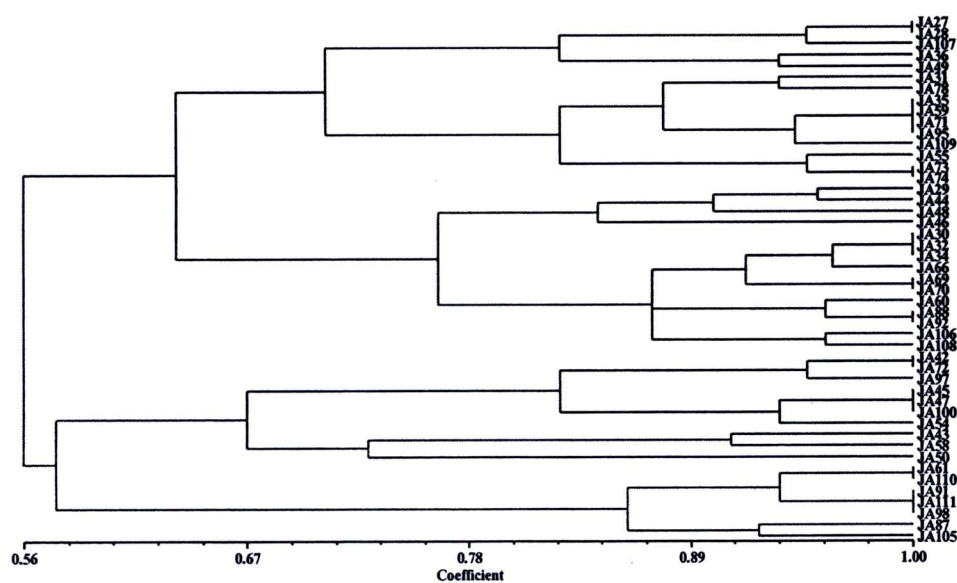
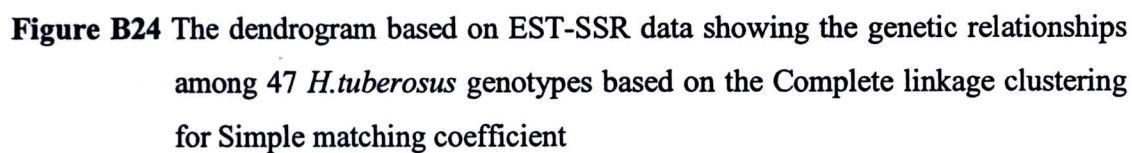
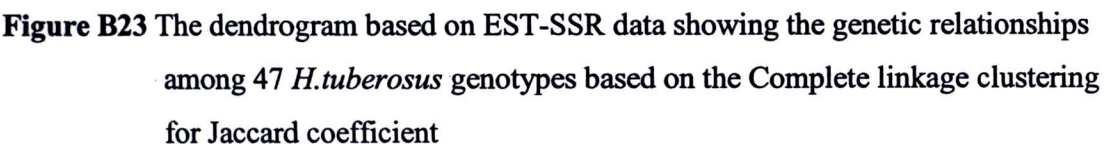


Figure B22 The dendrogram based on EST-SSR data showing the genetic relationships among 47 *H. tuberosus* genotypes based on the Complete linkage clustering for Dice coefficient



APPENDIX C
Chemicals and biochemical reagents

Chemicals and biochemical reagents

1.1 Chemical for DNA extraction

Extraction buffer

- 100 mM Tris-HCl pH 8
- 50 mM EDTA pH 8
- 500 mM NaCl
- 8.3 mM NaOH
- 1.25 % (w/v) SDS
- 0.38 % (w/v) Na bisulfite

T5E

- 50 mM Tris-HCl pH 8
- 10 mM EDTA pH 8

TE buffer

- 10 mM Tris-HCl pH 8
- 1 mM EDTA pH 8

10X TBE

- | | | |
|---------------------|-----|----|
| • Tris-base | 108 | g |
| • Boric acid | 55 | g |
| • 0.5 M EDTA (pH 8) | 40 | ml |

6X loading buffer

- 0.25 % bromophenol blue
- 0.25 % xylene cyanol
- 30 % glycerol

All of chemicals and reagents used in this study were analytical reagent grade. Glacial acetic acid, boric acid, chloroform, ethylenediamine tetraacetic acid (EDTA),

hydrochloric acid, isopropanol, methanol, potassium acetate, potassium chloride, potassium hydroxide, sodium acetate and manganese (II) chloride-4-hydrate were purchased from BDH, England. Acrylamide (electrophoresis grade) was obtained from Amersham, Sweden. α -D-galactoside (X-Gal) was supplied by Amersham, Canada. Ammonium persulfate (electrophoresis grade) and sodium hydroxide were purchased from UNIVAR, Australia. Bacto™ agar, tryptone and yeast extract were supplied by DIFCO Laboratories, U.S.A., α -mercaptoethanol were bought from SIGMA, U.S.A. sodium borate, sodium chloride. Calcium chloride dehydrate and *N,N,N,N*-tetremethylethylenediamine (TEMED) were purchased from Fluka, Germany and Fluka, Switzerland, respectively. *N,N*-bis-methylene-acrylamide and sodium dodecyl sulphate (SDS) were obtained from Bio-Rad, U.S.A. 5-Bromo-4-chloro-3-indolyl-bromophenol blue (X-Gal) and glycerol were supplied by Fisher Chemicals, U.K. Agarose (molecular biology grade) was obtained from Research Organics, U.S.A. Ethanol was bought from Merck, Germany. Isoamyl alcohol, ethidium bromide and MOPS were purchased from BioBasic Inc., Canada. Ampicillin was supplied by A.N.B. Laboratory, Thailand. Equilibrated phenol was bought from USB, U.S.A. GF-1 GEL Recovery Kit was bought from Vivantis, Malaysia. Isopropyl thio-beta-D-galactoside (IPTG) was obtained from Gibco BRL, U.S.A. Tris Ultrapure was supplied by USB, U.S.A.

2. Preparation of 60 ml polyacrylamide for SCI-PLAS (16.5x20cm) apparatus

Electrophoresis

Chemicals reagent	Concentrations	Volume
TBE buffer	5X	12 ml
Polyacrylamide, BIS	45%	8 ml
TEMED	0.008 %	25 μ l
10% Ammonium persulphate (APS)	10 %	600 μ l

2.1 Fix solution: 5% Acetic acid glacial

2.2 Silver solution:

Silver nitrate	00.48 g
5 M NaOH	240 μ l
30% Ammonium solution	1.5 ml
Distilled water	300 ml

2.3 Developer solution:

sodium carbonate	10 g
37% formaldehyde	100 μ l
Distilled water	500 μ l

2.4 Stop solution: 5% Acetic acid glacial

3. Instruments

Instruments used in this study were manufactured by following manufacturers; Auto pipette from Bio-Rad, U.S.A or Gilson, France; centrifuge bench top from Bio-Rad, U.S.A; double distilled water maker from Hamilton, England; 96 well plate reader from Bio-Rad, U.S.A, freezer -20⁰C from Hitachi, Japan; freezer -80⁰C from Harris, U.S.A; gel document from Bio-Rad, U.S.A; GelMate 2000 from Toyobo, Japan; ice maker from Newton, Thailand; 37 ⁰C incubator from Sheldon, U.S.A; incubator shaker from Takigen, Japan; Laminar flow from Woerden, Netherland; Magnetic stirrer hotplate from Framo-Geratetechnik, Germany; Microwave from National, Japan; SCI-PLA(16.5x20cm) apparatus Electrophoresis from Bio-Rad, U.S.A; Minnie-the-GelCiCleTM submarine-agarose Gel Unit HE33, Hoefer Amersham, U.S.A; Oven from Stuart scientific, England; pH meter from Hach, U.S.A; Gradient palm cycle PCR machine from

Corbett Research, Australia; Sorvall Super T21 Centrifuges from DuPont, U.S.A; spectrophotometer (UV-VIS) from Jasco, Japan; spin centrifuge from LabTech, Korea; Speedvacuum from Hitechtrader, England; Electrophoresis Gel apparatus from Vilber Lourmat, Japan; Vortex-2 GENIE from Scientific Industries, U.S.A and Water bath from Heto, U.S.A.

VITAE



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