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APPENDICES

APPENDIX A

BUFFERS AND REAGENT

1. Lysis Buffer I

Sucrose	109.54	g
1.0 M Tris – HCl (pH 7.5)	10	ml
1.0 M $MgCl_2$	5	ml
Triton X – 100 (pure)	10	ml
Distilled water to	1,000	ml

Sterilize the solution by autoclaving and store in a refrigerator (at 4^0C).

2. Lysis Buffer II

5.0 M NaCl	15	ml
0.5 M EDTA (pH 8.0)	48	ml
Distilled water to	1,000	ml

Sterilize the solution by autoclaving and store at room temperature.

3. 10% SDS solution

Sodium dodecyl sulfate	10	g
Distilled water to	100	ml

Mix the solution and store at room temperature.

4. 20 mg/ml Proteinase K

Proteinase K	2	mg
Distilled water to	1	ml

Mix the solution and store in a refrigerator (at -20^{0}C).

5. 1.0 M Tris – HCl

Tris base	12.11	g

Dissolve in distilled water and adjusted pH to 7.5 with HCl

Distilled water to	100	ml

Sterilize the solution by autoclaving and store at room temperature.

6. 0.5 M EDTA (pH 8.0)

Disodium ethylenediamine tetraacetate.2H_2O	186.6	g

Dissolve in distilled water and adjusted pH to 8.0 with NaOH

Distilled water to	1,000	ml

Sterilize the solution by autoclaving and store at room temperature.

7. 1.0 M $MgCl_2$ solution

Magnesium chloride.6H_2O	20.33	g
Distilled water to	100	ml

Dispense the solution into aliquots and sterilize by autoclaving.

8. 5 M NaCl solution

Sodium chloride	29.25	g
Distilled water to	100	ml

Dispense the solution into aliquot and sterilize by autoclaving.

9. 10X Tris borate buffer (10X TBE buffer)

Tris – base	100	g
Boric acid	55	g
0.5 M EDTA (pH 8.0)	40	ml

Adjust volume to 1,000 ml with distilled water. The solution was mixed and store at room temperature.

10. 6X loading dye

Bromphenol blue	0.25	g
Xylene cyanol	0.25	g
Glycerol	50	ml
1M Tris (pH 8.0)	1	ml
Distilled water until	100	ml

Mixed and stored at 4^0C

11. 7.5 M Ammonium acetate (CH_3COONH_4)

Ammonium acetate	57.81	g
Distilled water	80	ml

Adjust volume to 100 ml with distilled water and sterilize by autoclaving.

12. 25:24:1 (v/v) Phenol-chloroform-isoamyl alcohol

Phenol	25	volume
Chloroform	24	volume
Isoamyl alcohol	1	volume

Mix the reagent and store in a sterile bottle kept in a refrigerator.

13. 2% Agarose gel (w/v)

Agarose	1.6	g
1X TBE	80	ml

Dissolve by heating in microwave oven and occasional mix until no granules of agarose are visible.

14. Ethidium bromide

Ethidium bromide	10	mg
Distilled water	1	ml

Mix the solution and store at 4^0C

APPENDIX B

Primer name	Function	Nucleotide sequence (5'>3')
Ex-1Fy	Forward primer for sequencing exon 1	CCA TCC CTC TTT TCC AGA TG
Ex-1Ry	Reverse primer for sequencing exon 1	GAT CCC AGA GCA GTA ACA CT
Ex-2Fy	Forward primer for sequencing exon 2	CCC AAA GGA GCT TGC AGC TT
Ex-2Ry	Reverse primer for sequencing exon 2	GGG GTG GAG CTT GGA GAG TT
Ex-3Fy	Forward primer for sequencing exon 3	CTG GGC GAC AGA GAG AGA CT
Ex-3Ry	Reverse primer for sequencing exon 3	AAG CCC TCC ATG CAC CTT AG
Ex-4Fy	Forward primer for sequencing exon 4	AAG ACC GCT GTC AAG CTG GG
Ex-4Ry	Reverse primer for sequencing exon 4	TCA GAG GGA GCA GAG CTT GG
Ex-5Fy	Forward primer for sequencing exon 5	CCT TCC ATT CTC TGC TCA GA
Ex-5Ry	Reverse primer for sequencing exon 5	TGC TGG CTG GAG AGG TTT TA
Ex-7Fy	Forward primer for sequencing exon 7	CGA AGC CTT GAA GGT CCC CA
Ex-7Ry	Reverse primer for sequencing exon 7	GAG TGG GGA GCC TTG AAT CC
seq Ex8-F	Forward primer for sequencing exon 8	CGT CGG GCC CCC AGA TCT CC

Primer name	Function	Nucleotide sequence (5'>3')
pIgR Ex8R	Reverse primer for sequencing exon 8	GAC GTT TTT CCT GTG CCG GG
pIgR E9F	Forward primer for sequencing exon 9-10	CCG AGT TTC AAT CAG AAG CTA C
pIgR-Ex10:BR	Reverse primer for sequencing exon 9-10	TTT GTC ACG TAG TGG GGC TT
seq Ex 11-F	Forward primer for sequencing exon 11	GGG GGC TGG GAT GA GAA CA
seq Ex 11-R	Reverse primer for sequencing exon 11	AGG GAG TGG GGT CCC CAG GA
1249 F_A	Forward ARMS primer in set A	GCC CCA CTG TGG TGA AGG GGG TGG CAG GTG
1249 F_B	Forward ARMS primer in set B	GCC CCA CTG TGG TGA AGG GGG TGG CAG GTA
1249 R	Reverse ARMS primer in set A and B	GCT CCT CCA GCA GGG AGA GGC GCC CTC GTA
1895 F_A	Forward ARMS primer in set A	CCG CGA TGT CAG CCT AGC GAA GGC AGA CTT
1895 F_B	Forward ARMS primer in set B	CCG CGA TGT CAG CCT AGC GAA GGC AGA CCC
1895 R	Reverse ARMS primer in set A and B	GTG CTC GGC TCT GGG GTG TTC TCC GAG TGG
2306 R_1	Reverse ARMS primer in set A	TGT GGT GCT CTC AGT GGT GG
2306 R_2	Reverse ARMS primer in set B	TGT GGT GCT CTC AGT GGT GA
2306 F	Forward ARMS primer in set A and B	ACA GTG GAT CCG CCC AGA GA

Primer name	Function	Nucleotide sequence (5'>3')
1122 R_1	Reverse ARMS primer in set A	AAT GGG CTC CAC ACA GGT AT
1122 R_2	Reverse ARMS primer in set B	AAT GGG CTC CAC ACA GGT AG
1122 F	Forward ARMS primer in set A and B	GAG GGC AGG ATC CTG CTC AA
E7-FORSNP-F	Forward primer for PCR-RFLP SNP1739	GGG TCC CGC GAT GTC AGC CTA G
PIGR UTR F	Forward primer for detection 3'UTR polymorphism	TGA GGG TGG CAT GAG GAG GT
PIGR UTR R	Reverse primer for detection 3'UTR polymorphism	TGA TAA GGG TGC AGA GGG GC

APPENDIX C

ARMS Primer Additional Mismatch Selection

Terminal mismatch	Coding strand nucleotide corresponding to penultimate nucleotide in the primer			
	A	G	C	T
AA	A	G	A	G
AG	C	T	A	G
AC	G	A	C	T
TT	C	T	A	G
TG	G	A	T	C or T
TC	C	T	A	G
CC	C	T	A	G
GG	A	G	A	G

The table indicates which base to include at the penultimate position of the ARMS primer. The left-hand column lists the 3' terminal mismatches specified (i.e, the mutation in the coding strand with the normal base in the anti-coding strand) and the top row indicates the target base in the anti-coding strand) and the top row indicates the target base in the coding strand corresponding to the penultimate base in the primer.

APPENDIX D

Estimating Haplotype (EH)

File in this Window package.

1.EH.PAS : Source code of EH program.

2.EH.EXE : Executable code of EH program, which is compiled with a maximum of 30 alleles per locus. Loci, 1000 haplotypes, and 3600 genotypes (product of numbers of genotypes at each locus).

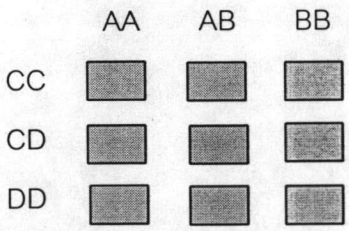
EH.DAT, EH.OUT Sample input and output files.

Protocol for using EH consisted of 2 steps.

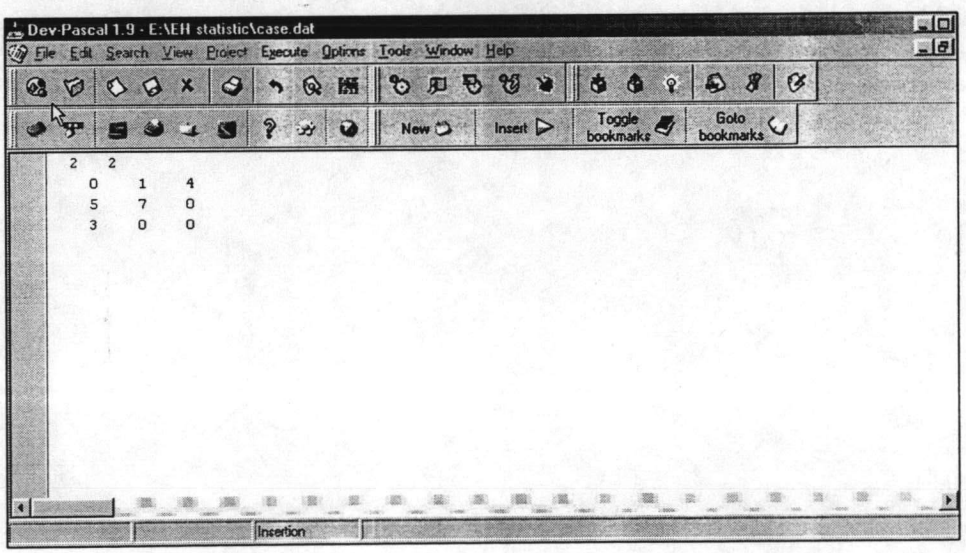
1.Create the data file (.dat)

We created the data file in the Pascal program. The first line was the number of alleles at the first locus, number of alleles at the second locus, and so on. Assuming you have 2 loci, each locus has two alleles A & B and C & D, respectively.

The possible haplotypes.

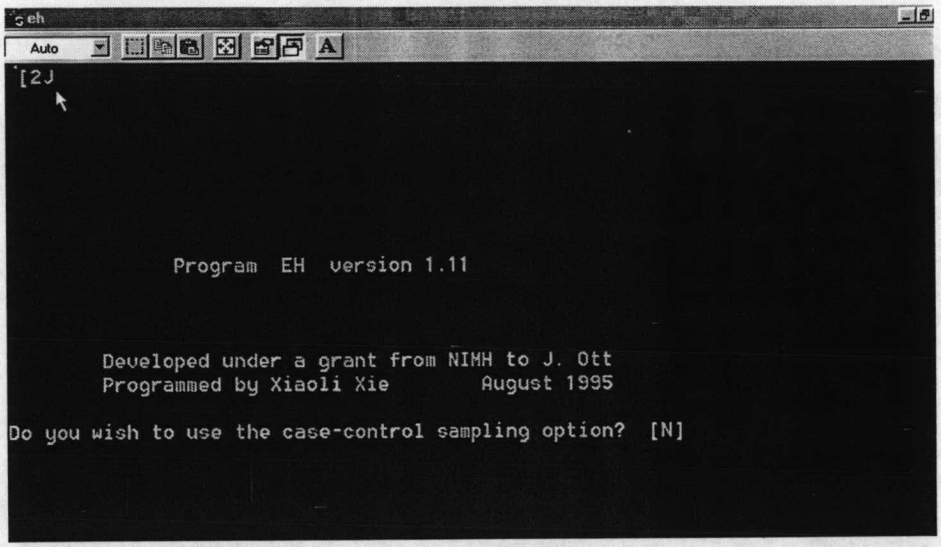


The number of haplotype in the box was filled in Pascal program and save unit as a .dat file.

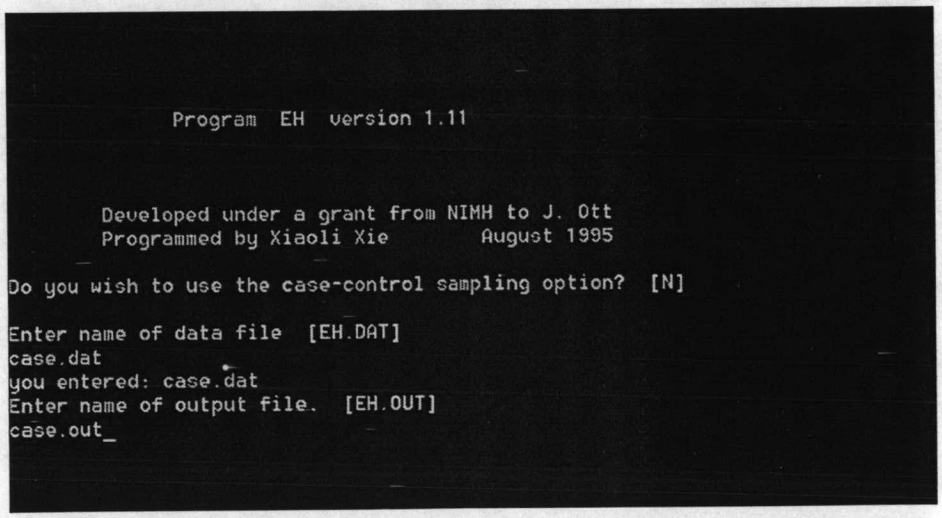


2.Running the EH program

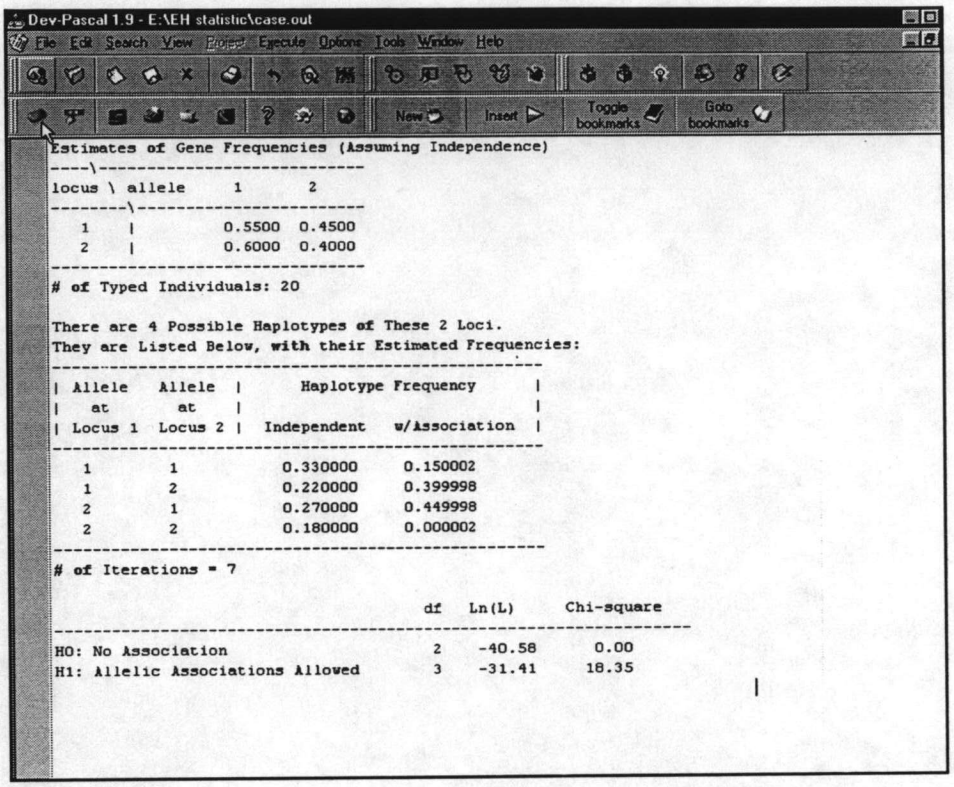
-Running EH program showed the window as below.



-Type your data filename and output filename.



-The output file presented the haplotype frequencies in two kind. "Independent" these are obtained from the allele frequencies at the individual loci. That is, these haplotype frequencies are not estimated but calculated from allele frequencies under the assumption of no association. "w/Association" these are estimated from the data, allowing for association (linkage disequilibrium), assuming Hardy Weinberg equilibrium.



Case-control data.

If you want to test whether haplotype frequencies are significantly different in case and controls, you run EH three times, 1) for cases, 2) for controls, and 3) for cases and controls combined. For a given data set (case.dat, control.dat, provided), results are shown below.

Case.out, control.out, mix.out

	df	Ln(L)	Chi-square
HO: No Association	2	-40.58	0.00
H1: Allelic Associations Allowed	3	-31.41	18.35

	df	Ln(L)	Chi-square
HO: No Association	2	-208.32	0.00
H1: Allelic Associations Allowed	3	-207.69	1.26

	df	Ln(L)	Chi-square
HO: No Association	2	-544.98	0.00
H1: Allelic Associations Allowed	3	-523.19	43.57

The relevant test statistic is T = ln(L,cases) + ln(L,controls) – ln(L,cases + controls together). With a sufficient number of observation, when there is no difference between case and control haplotype frequencies, twice this value has an approximate chi-square distribution with a number of df equal to the number of haplotypes estimated. For the above data, one obtains(-31.41)+(-207.69)-(-523.19) = 284.09 χ^2 = 2*284.09 = 568.18 on 3 df is associationed with an empirical significance level of <0.005

Table: Chi-Square Probabilities

The areas given across the top are the areas to the right of the critical value. To look up an area on the left, subtract it from one, and then look it up (ie: 0.05 on the left is 0.95 on the right)

df	0.995	0.99	0.975	0.95	0.90	0.10	0.05	0.025	0.01	0.005
1	---	---	0.001	0.004	0.016	2.706	3.841	5.024	6.635	7.879
2	0.010	0.020	0.051	0.103	0.211	4.605	5.991	7.378	9.210	10.597
3	0.072	0.115	0.216	0.352	0.584	6.251	7.815	9.348	11.345	12.838
4	0.207	0.297	0.484	0.711	1.064	7.779	9.488	11.143	13.277	14.860
5	0.412	0.554	0.831	1.145	1.610	9.236	11.070	12.833	15.086	16.750
6	0.676	0.872	1.237	1.635	2.204	10.645	12.592	14.449	16.812	18.548
7	0.989	1.239	1.690	2.167	2.833	12.017	14.067	16.013	18.475	20.278
8	1.344	1.646	2.180	2.733	3.490	13.362	15.507	17.535	20.090	21.955
9	1.735	2.088	2.700	3.325	4.168	14.684	16.919	19.023	21.666	23.589
10	2.156	2.558	3.247	3.940	4.865	15.987	18.307	20.483	23.209	25.188

BIOLOGRAPHY

Miss Rungnapa Hirunsatit was born in Bangkok in1976 and transferred to Sampran , Nakornpathom with her family when she was young.In 1999, she graduated from faculty of Science, Chulalongkorn University in Genetics program and then attended to particulate in Medical Science program in Faculty of Medicine for her master degree.