APPENDICES

Appendix 1

Electrophoretic Conditions and Staining Methods for Various Systems Analysed

1.1 Plasma Amylase (E.C. No. 3.2.1.1)

Electrophoretic Conditions

Buffer	:	Tris Barbitol - sodium Barbitol pH 8.8
Samples	:	4 applications
Run	:	60 minutes, 210V, 8mA

Staining Method

Step 1

3ml	0.1M Sodium acetate acetic acid pH 5.6
3ml	Maltose
35µl	Glucose oxidase
8µl	Peroxidase

Step 2

Dissolve 10mg 3-amino-9-ethyl carbozole in ml N,N,dimethyl formamide. Add this to the step 1 mixture.

Step 3

Add 2ml of 2% agarose as an overlay in the final mixture. Incubate the gel strip at 37°C until pink bands appear

1.2 Acid Phosphatase (E.C. No. 3.1.3.2)

Electrophoretic Conditions

Buffer	:	0.1M Phosphate pH 7.4 with 10% Photo Flo 600
Samples	:	2 applications
Run	:	60 minutes, 210V, 40mA

Staining Method

3ml	0.05 Citric acid pH 4.0
2mg	Methylumbelliferyl phosphate
2ml	2% agarose

1.3 Alkaline Phosphatase

(E.C. No. 3.1.3.1)

Electrophoretic Conditions

Buffer	:	Gelman's high Resoltion Buffer pH 8.8
Samples	:	Plasma 5 applications
Run	:	60 minutes, 200V

Staining Method

10mg	ß-Napthyl acid phosphate
8mg	Fast Blue BB salt
2ml	0.1M Tris-HCL pH 8.6
200µl	$MgCl_2$

1.4 Albumins

Electrophoretic Conditions:

Buffer	:	Gelman's high Resolution Buffer pH 8.8
Samples	:	Plasma 1 applications
Run	:	60 minutes, 200V

Staining Method

Step 1

Staining Solution

Prepare 250mg Coommasie Brilliant Blue R-250 of stain solution dissolved in 93ml of distilled water and 7ml of glacial acetic acid.

Destaining Solution

Prepare a solution of methonal: distilled water: glacial acetic acid in the ratio of 5:5:1.

Step 2

After the run is completed, gently blot the gel strips in a blotting paper. Dip the gel with the unpolished surface facing upwards into the staining solution for two minutes. Remove the gel from the staining solution and washed it gently with running tap water and then dip it into the tank of destaining solution with gentle shaking of the tank.

1.5 Adenylate Kinase (E.C. No. 2.7.4.3)

Electrophoretic Conditions:

Buffer	:	0.02 Citrate phosphate pH 6.4 with Photo Flo (Modified from Richardson et al., 1986)
Samples	:	4 applications
Run	:	60 minutes, 200V

Staining Method

2ml	0.1M Tris-HCL pH 8.0
6mg	ADP
2mg	Glucose
200µl	NADP, 0.1M $MgCl_2$, MTT, PMS
10µl	Hexokinase
10µl	G6PD
2ml	2% agarose

1.6 : Billiverdin Reductase (E.C. No. 1.3.1.24)

Electrophoretic Conditions

Buffer	:	0.1M Phosphate pH 7.0
Samples	:	4 applications
Run	:	100V, 25mA,4°C for 2 hours

Staining Method

Modified from Meera Khan et al.' 1985

2.5ml	0.1M Tris-HCL pH 8.0
1mg	Billiverdin
2mg	NADPH
0.2ml	1M $MgCl_2$

Dissolve Billiverdin in 1.5ml staining buffer and dissolve NADPH in 1 ml staining buffer and $MgC1_2$. Mix the two solutions just before staining.

1.7 Carbonic Anhydrase (E.C. No. 4.2.1.1)

Electrophoretic Conditions:

Buffer	:	0.2M Citrate Phosphate pH 6.4
Samples	:	2 applications
Run	:	60 minutes, 200V

Staining Method

Modified from Meera Khan et al., 1982

Dissolve 4mg of florescent diacetate in 100µl of acetone.

2ml	0.1M Phosphate buffer pH 4.0
2ml	2% agarose to the mixture

1.8 NADH-Diaphorase 1 (Zone 1) (E.C. No. 1.6.2.2)

Electrophoretic Conditions:

Buffer	:	0.02M Citrate Phosphate pH 6.4 with Photo Flo
Samples	:	4 applications
Run	:	60 minutes, 200V

Staining Method

4mg	NADH
2ml	0.1M Tris-HCL pH 8.6
200µl	DCIP, MTT
2ml	2% agarose

1.9 NADH-Diaphorase 1 (Zone 2) (E.C. No. 1.6.2.2)

Electrophoretic Conditions:

Buffer	:	0.02M Citrate Phosphate pH 6.4
Samples	:	4 applications
Run	:	60 minutes, 200V

Staining Method

4mg	NADH
2ml	0.1M Tris-HCL pH 8.6
200µl	DCIP, MTT
2ml	2% agarose

1.10 NADH-Diaphorase -2

Electrophoretic Conditions:

Buffer	:	0.001M Tris / o.o83 Citric acid) pH 7.0
	:	0.1M Citrate phosphate ph 6.4 (gel buffer) (Modified from Richardson et al., 1986)
Samples	:	6 applications
Run	:	100V,180 minutes

Staining Method

2ml	1M Tris-HCL-Na_2EDTA pH 8.6
200µl	Glucose-6-phosphate
200µl	NADP, Methylene blue, MTT
5µl	Glucose-6-phosphate dehydrogenase

Incubate for 15-30 minutes at 37°C

(Staining method used ia as in Meera Khan et al., 1982)

1.11 Diphosphoglyceromutase (E.C. No. 2.7.5.4)

Electrophoretic Conditions:

Buffer	:	0.1M Tris,).1M NaH_2PO_4, pH 7.4 (unadjusted)
Samples	:	6 applications
Run	:	200V, 60 minutes

Staining Method

5ml	0.1M Tris-HCL pH 8.0
10ml	α-Glycerophosphate
2mg	K_2HPO_4
100µl	NAD
500µl	Glyceraldehyde-3-phosphate (Richardson et al., 1974)
2IU	GAPDH

(Receipe modified from Harrison and Hopkinson,1974)

1.12 Fructokinase (E.C. No. 2.7.1.4)

Electrophoretic Conditions:

Buffer	:	0.04M Citrate Phosphate pH 6.4 with Photo Flo (Modified from Richardson et al., [1986])
Samples	:	6 applications
Run	:	70 minutes, 200V

Staining Method

Meera Khan et al., 1982)

0.5ml	0.002M Cystine-HCL pH 8.0
0.2ml	0.054 EDTA pH 7.0
0.5ml	0.1M Tris-HCL-EDTA pH 8.6
100µl	1M $MgCl_2$
200µl	NADP, MTT, PMS
7mg	ATP
10mg	Fructose
5µl	Phosphoglucose Isomerse
2ml	2% agarose

1.13 Fructose-1, 6-Diphosphate (E.C. No. 3.1.3.11)

Electrophoretic Conditions:

Buffer	:	0.1M Citrate phosphate pH 6.4 with Photo Flo
Samples	:	6 applications
Run	:	90 minutes, 200V

Staining Method

(From Richardson et al., 1986)

2.5ml	0.1M Tris-HCl pH 8.0
10mg	Fructose-1, 6-diphosphate
200ul	NADP, $MgCl_2$, MTT, PMS
20ul	Glucose-6-Phosphate dehydrogenase
10ul	Glucose phosphate Isomerase
2ml	2% agarose

1.14 Fumarase or Fumarate Hydratase (E.C. No. 4.2.1.2)

Electrophoretic Conditions:

Buffer	:	0.05M Citrate phosphate pH 6.4 with Photo Flo (Modified from Richardson et al., 1986)

Samples	:	6 applications
Run	:	60 minutes, 200V

Staining Method

(From Richardson et al., 1986)

2ml	0.1M Tris-HCl pH 8.0
200ul	Fumarate
200ul	NAD, MTT, PMS
41.U.	Malate dehydogenase
2ml	2% agarose

1.15 Glucose Dehydrogenase (E.C. No. 1.1.1.47)

Electrophoretic Conditions:

Buffer	:	0.1M Citrate phosphate pH 7.4 with Photo Flo
Samples	:	6 applications
Run	:	60 minutes, 200V

Staining Method

500mg	Glucose
2.0ml	0.1M Tris-HCL pH 8.6
200ul	NAD, MTT, PMS
2ml	2% agarose

1.16 Esterase-2 (E.C. No. 3.1.1)

Electrophoretic Conditions:

Buffer	:	0.1M Tris-Maleate pH 7.8
Samples	:	4 applications
Run	:	200V, 60 minutes

Staining Method

2.5ml	0.1M Tris-Maleate pH 6.5
10mg	α-Napthyl acetate
6mg	Fast Garnet GBC salt
2ml	2% agarose

1.17 Glucosephosphate Isomerase (E.C. No. 5.3.1.9)

Electrophoretic Conditions:

Buffer	:	0.02M Citrate phosphate pH 6.4 with Photo Flo
Samples	:	1 application
Run	:	90 minutes, 200V

Staining Method

2ml	0.1M Tris-HCL pH 8.0
4mg	Fructose-1-Phosphate
200ul	1M $MgCl_2$,NADP, MTT, PMS
8 I.U.	G6PD
2ml	2% agarose

1.18 Glutamate Pyruvate Transaminase (E.C. No.2.6.1.2)

Electrophoretic Conditions:

Buffer	:	0.025 Phosphate pH 7.0
Samples	:	6 applications
Run	:	60 minutes , 200V

Staining Method

2.5ml	0.1M Tris-HCl pH 7.0
10mg	L-alanine
3mg	NADH
200ul	α-Ketoglutarate
10ul	Lactate dehydrogenase
2ml	2% agarose

(Richardson et al., 1986)

After 30-45 minutes of incubation view under UV light. When bands (dark area in a fluorescent background) are distinct, stain by pouring aver the initial agarose overlay with this positive stain mixture:

1ml	0.1M Tris-HCl pH 8.0
100ul	MTT, PMS

(Modified from Meea Khan et al., 1982)

1.19 Glutamate Oxaloacetate Transaminase (E.C. No.2.6.1.1)

Electrophoretic Conditions:

Buffer	:	0.1M Phosphate pH 7.0 (Modified from Richardson et al., 1986)
Samples	:	6 applications
Run	:	60 minutes , 200V

Staining Method

2ml	0.1M Tris-HCl pH 8.0
3mg	Fast Blue BB Salt
200ul	α-Ketoglutarate aicd
200ul	L-aspartic acid
2ml	2% agarose

1.20 Glyceraldehyde-3-Phosphate Dehydrogenase (E.C. No. 1.2.1.12)

Electrophoretic Conditions:

Buffer	:	0.13M Tris-EDTA-borate pH 8.9 (Tan et a., 1991)
Samples	:	6 applications
Run	:	90 minutes , 200V

Staining Method

	L-glycerol-3-phosphate
2ml	0.1M Tris-HCl pH 8.0
200ul	NAD
200ul	MTT
200ul	PMS

1.21 Glutathione Reductase (E.C. No. 1.6.4.2)

Electrophoretic Conditions:

Buffer	:	0.13M Tris-EDTA-borate pH 8.9 (Richardson et al., 1986)
Samples	:	4 applications
Run	:	120 minutes , 200V

Staining Method

2mg	NADPH
2ml	0.1M Tris-HCL pH 8.6
200ul	DCIPT
200ul	MTT
10mg	Oxidased glutathione

Counterstain with 3ml of 1M HC1 to remove the blue background when the isozymes have a satisfactory intensity, hence to remove the blue background.

1.22 Hexokinase (E.C. No. 2.7.1.1)

Electrophoretic Conditions:

Buffer	:	0.1M Phosphate pH 7.0 with Photo Flo (Modified from Richardson et al., 1986)
Samples	:	6 applications
Run	:	60 minutes , 200V

Staining Method

2ml	0.1M Tris-HCl pH 8.6
2mg	Glucose
6mg	ATP
200ul	NADP, $MgC1_2$,MTT, PMS
12ul	G6PD
2ml	2% agarose

1.23 Haemoglobin

Electrophoretic Conditions:

Buffer	:	Gelman's buffer pH 9.2
Samples	:	1 application (1 part of red cell into 4 parts of water)

Staining Method

No staining required, the gel is fixed in 7% acetic acid.

1.24 Isocitrate Dehydrogenase (E.C. No. 1.1.1.42)

Electrophoretic Conditions:

Buffer	:	0.015M Tris-Citrate pH 7.4 with Photo Flo
Samples	:	6 applications
Run	:	60 minutes , 200V

Staining Method

2ml	0.1M Tris-HCl pH 8.0
10mg	Dl-Isocitracte acid
200ul	$MgCl_2$,NADP, MTT, PMS
2ml	2% agarose

1.25 Lactate Dehydrogenase (E.C. No. 1.1.1.27)

Electrophoretic Conditions:

Buffer	:	0.02M Citrate Phosphate pH 6.4 with Photo Flo
Samples	:	one application
Run	:	70 minutes , 200V

Staining Method

2ml	0.1M Tris-HCl pH 8.0
400ul	Na-Lactate
200ul	NAD, MTT, PMS
2ml	2% agarose

1.26 Malate Dehydrogenase (E.C. No. 1.1.1.37)

Electrophoretic Conditions:

Buffer	:	0.02M Citrate Phosphate pH 6.4 with Photo Flo
Samples	:	4 applications
Run	:	60 minutes , 210V

Staining Method

2ml	0.1M Tris-HCl pH 8.0
400ul	Na-Malate
200ul	NAD, MTT, PMS
2ml	2% agarose

1.27 Malic Enzyme (E.C. No. 1.1.1.40)

Electrophoretic Conditions:

Buffer	:	0.02M Citrate Phosphate pH 6.4 with Photo Flo
Samples	:	6 applications
Run	:	60 minutes , 210V

Staining Method

2ml	0.1M Tris-HCl pH 7.4
400ul	Na-Malate
200ul	NADP, $MgCl_2$,MTT, PMS
2ml	2% agarose

1.28 Mannose Phosphate Isomerase (E.C. No. 5.3.1.8)

Electrophoretic Conditions:

Buffer	:	0.01M Phosphate pH 7.0
Samples	:	6 applications
Run	:	60 minutes , 200V

Staining Method

2ml	0.1M Tris-HCl pH 8.0
8mg	Mannose-6-Phosphate
200ul	NADP, $MgCl_2$,MTT, PMS
0.3ul	GPI
6ul	G6PD
2ml	2% agarose

1.29 Peptidase A (E.C. No. 3.4.11/13)

Electrophoretic Conditions:

Buffer	:	0.1M Citrate Phosphate pH 6.4
Samples	:	4 applications
Run	:	60 minutes , 210V

Staining Method

2ml	0.1M Tris-HCl pH 8.0
2mg	L-amino acid oxidase
2mg	Peroxidase
8mg	O-Dianisidine di-HCl
10mg	Valine-leucine
200ul	$MgCl_2$
2ml	2% agarose

1.30 Peptidase B (E.C. No. 4.4.11/13)

Electrophoretic Conditions:

Buffer	:	0.01M Tris-NAH_2PO_4 pH 7.4
Samples	:	4 applications
Run	:	60 minutes , 200V

Staining Method

2ml	0.1M Tris-HCl pH 8.0
2mg	L-amino acid oxidase
2mg	Peroxidase
8mg	O-Diansidine di-HCl
10mg	Leucine-glycine-glycine
200ul	$MgCl_2$
2ml	2% agarose

1.31 Peptidase D (E.C. No. 3.4.11/14)

Electrophoretic Conditions:

Buffer	:	0.02M Tris-NAH_2PO_4 pH 7.4
Samples	:	4 applications
Run	:	60 minutes , 200V

Staining Method

2ml	0.1M Tris-HCl pH 8.0
2mg	L-amino acid oxidase
2mg	Peroxidase
8mg	O-Dianisidine di-HCl
15mg	Lysine-leucine/ Leu-gly-glycine
2ml	2% agarose

1.32 Peptidase D (E.C. No. 3.4.11/13)

Electrophoretic Conditions:

Buffer	:	0.01M Citrate phosphate pH 6.4
Samples	:	4 applications
Run	:	60 minutes , 210V

Staining Method

10mg	Phenylalanine-proline
8mg	O-Diansidine di-HCl
2ml	0.1M Tris-HCl pH 8.0
2mg	L-amino acid oxidase
2mg	Peroxidase
200ul	$MgCl_2$

1.33 Phosphoglucomutase (E.C. No. 2.7.5.1)

Electrophoretic Conditions:

Buffer	:	0.025M Phosphate pH 7.0 with Photo Flo
Samples	:	6 applications
Run	:	70 minutes , 210V

Staining Method

2ml	0.1M Tris-HCl pH 8.0
10mg	Glucose-1-Phosphate
200ul	NADP, 1M $MgCl_2$, MTT, PMS
0.2mg	Glucose-1, ^-diphosphate
10ul	G6PD
2ml	2% agarose

1.34 6-Phosphogluconate Dehydrogenase (E.C. No. 1.1.1.44)

Electrophoretic Conditions:

Buffer	:	0.025M Phosphate pH 7.0 with Photo Flo
Samples	:	4 applications
Run	:	60 minutes , 210V

Staining Method

2ml	0.1M Tris-HCl pH 8.0
5mg	6-Phosphogluconic acid
200ul	NADP, $MgCl_2$, MTT, PMS
2ml	2% agarose

1.35 Purine Nucleoside Phosphorylase (E.C. No. 2.4.2.1)

Electrophoretic Conditions:

Buffer	:	0.01M Phosphate pH 7.4
Samples	:	1 application (1 part of Red cell into 4 parts of Distilled water)
Run	:	60 minutes , 200V

Staining Method

2.5ml	0.1M Tris-HCl pH 8.0
8mg	Inosine
0,1ml	Arsenate
200ul	MTT, $MgCl_2$, PMS
10ul	Xanthine axidase
2ml	2% agarose

1.36 Pyruvate Kinase (E.C. No. 2.7.1.40)

Electrophoretic Conditions:

Buffer	:	0.05M Citrate Phosphate pH 6.4
Samples	:	4 applications
Run	:	70 minutes , 200V

Staining Method

2ml	0.1M Tris-HCl pH 8.0
2mg	Phosphophenolpyruvate (PEP)
4mg	ADP
2mg	Glucose
200ul	KCl, NADP, $MgCl_2$, MTT, PMS
10ul	Hexokinase
10ul	G6PD
2ml	2% agarose

On prolonged incubation another zone of PK activity which consists of 1 band per sample appears between the strong AK band and the strong PK zones mentioned above.

1.37 Superoxide Dismutase (E.C. No. 1.15.1.1)

Electrophoretic Conditions:

Buffer	:	0.1M Citrate Phosphate pH 6.4
Samples	:	4 applications
Run	:	60 minutes , 200V

Staining Method

2ml	0.1M Tris-HCl pH 8.0
200ul	MTT, PMS
2ml	2% agarose

1.38 Sorbitol Dehydrogenase (E.C. No. 1.1.14)

Electrophoretic Conditions:

Buffer	:	0.13 Tris-EDTA-borate pH 8.9
Samples	:	5 applications
Run	:	100 minutes , 200V

Staining Method

2ml	0.1M Tris-HCl pH 8.0
15mg	Sarbitol
200ul	NAD
200ul	MTT
200ul	PMS

1.39 Transferrin

Electrophoretic Conditions:

Buffer	:	Gelman HB buffer pH 9.2 (Tris-EDTA-Glycine)
Samples	:	Plasma 2 applications
Run	:	60 minutes , 210V

Staining Method

Same as in the case of staining Albumins.

1.40 X-Protein

Electrophoretic Conditions: (Starch Gel Electrophoresis)

Buffer gel	:	21ml 1.05% citric acid + 19 ml 2.30% Tris + 210 H_2O
Bridge	:	1.85% boric acid + 0.4% NaoH pH 8.7
Whatman Paper	:	No.17
Gel Concentration	:	12% starch
Run	:	150V for 15 minutes, 300V for 5 hours

Staining Method

The gel was sliced horizontally into 2 pieces and each slice vas stained with commossie blue in 7% acetic acid on its cuts surface.

Ten minutes later, the gel is raised under running water. The gel was then immersed in the destaining solution (5 distilled water: 5 methanol: 1 glacial acetic acid) for 24 hours to fix it for storage.

Appendix 3. Details of substrates referred in this study

Substrates	Enzyme to be stained	Receipe
Fumarate	FUM	50 mg/ml Fumaric acid pH 8.0
Aspartate	GOT	50 mg/ml L -aspartic acid pH 8.0
α-Ketoglutarate	GOT, GPT	50 mg/ml α-ketolutaric acid pH 8.0
Lactate	LDH	50 mg/ml L -lactic acid pH 8.0
Malate	MDH, ME	50 mg/ml L -malic acid pH 8.0

Appendix 2. Details of the reagents referred in this study

	Compound	Product	Comments
1.	Acetaldehde	BDH	
2.	Acetic Acid	BDH	destaining reagent-glacial
3.	Acetone	BDH	highly inflammable
4.	Adenosine 5'-diphosphate (ADP)	SIGMA	di-sodium salt
5.	L-Alanine	SIGMA	
6.	L-Amino acid oxidase	SIGMA	linking enzyme
7.	3-Amino-9-ethyl-carbozole	SIGMA	
8.	Arsenate (arsenic acid)	SIGMA	sodium salt
9.	L-Aspartate (L-aspartic acid)	SIGMA	sodium salt
10.	Adenosine 5'-triphosphate (ATP)	SIGMA	di-sodium salt
11.	Boric acid	BDH	buffer reagent
12.	Brilliant Blue R	SIGMA	
13.	Calcium chloride ($CaCl_2$)	BDH	
14.	Citric acid	BDH	buffer reagent
15.	2,6 dichlorophenol indophenol (DCIP)	SIGMA	
16.	O-Dianisidine diHCL	SIGMA	possible carcinogen
17.	Ethylenediamine-tetra acetic acid acid (EDTA)	BDH	di-sodium salt
18.	Fast Blue BB salt	SIGMA	possible carcinogen
19.	Fast Blue RR salt	SIGMA	possible carcinogen
20.	Fast Garnet GBC salt	SIGMA	possible carcinogen
21.	Fructose-1, 6-diphosphate	SIGMA	tri-sodium salt
22.	Fructose-6-phosphate	SIGMA	disodium salt
23.	Fumarate (Fumaric acid)	SIGMA	disodium salt

(Contd. Appendix 2.)

	Compound	Product	Comments
24.	Glucose	BDH	
25.	Glucose-1-phosphate	SIGMA	contains 1% glucose-1,6-diphosphate
26.	Glucose-6-phosphate	SIGMA	sodium salt
27.	Glucose-6-phosphate dehydrogenase (G6PD)	SIGMA	linking enzyme
28.	Glucose phosphate isomerase (GPI)	SIGMA	linking enzyme
29.	Glutathione-reduced form (GSH)	SIGMA	
30.	Glyceraldehyde-3-phosphate dehydrogenase (GAPD)	SIGMA	linking enzyme
31.	DL-α - Glycerophosphate	SIGMA	disodium salt
32.	ß - Glycerophosphate dehydrogenase	SIGMA	linking salt
33.	Glycine	SIGMA	buffer reagent
34.	Hydrochloric acid (HCL)	SIGMA	
35.	Hexokinase (HK)	SIGMA	linking enzyme
36.	Hypoxanthine	SIGMA	
37.	Inosine	SIGMA	
38.	DL - Isocitric acid	SIGMA	trisodium salt
39.	Potassium chloride (KCL)	BDH	
40.	α - ketoglurate (KCL)	SIGMA	sodium salt
41.	DL-Lactate (DL-lactic acid)	SIGMA	
42.	Lactate dehydrogenase	SIGMA	linking enzyme

(Contd. Appendix 2.)

	Compound	Product	Comments
43.	L-leucine-L-alainine (leu-ala)	SIGMA	
44.	L-leucine-glycine-glycine (leu-gly-gly)	SIGMA	
45.	L-lysine-Lleucine (lys-leu)	SIGMA	
46.	L-Malate (L-malic acid)	SIGMA	disodium salt
47.	Malate dehydrogenase (MDH)	SIGMA	linking enzyme
48.	Maleic acid	UNILAB	buffer reagent
49.	Maltose	SIGMA	
50.	Mannose-6-phosphate	SIGMA	barium salt
51.	Methanol	BDH	
52.	4-Methylumbelliferyl acetate	SIGMA	
53.	4-Methylumbelliferyl phosphate	SIGMA	
54.	Magnesium chloride	BDH	
55.	Mangannese chloride	BDH	
56.	Methylthiazolyl blue (MTT)	SIGMA	carcinogen-protect from light
57.	Nicotinamide adenine dinucleotide (NAD)	SIGMA	
58.	Nicotinamide adenine dinucleotide-reduced form (NADH)	SIGMA	disodium salt
59.	Nicotinamide adenine dinucleotide phosphate (NADP)	SIGMA	
60.	Nicotinamide adenine dinucleotide phosphate-reduced form (NADPH)	SIGMA	tetrasodium salt
61.	Disodium hydrogen orthophosphate (NaH_2PO_4)	BDH	buffer reagent
62.	Sodium duhydrogen orthophosphate (NaH_2PO_4)	BDH	

(Contd. Appendix 2.)

	Compound	Product	Comments
63.	Sodium hydroxide (NaOH)	BDH	
64.	α - Napthyl acetate	SIGMA	disodium salt
65.	ß - Naptyl acid phosphate	SIGMA	disodium salt
66.	Nucleoside phosphorylase (NP)	SIGMA	linking enzyme
67.	Peroxidase	SIGMA	linking enzyme
68.	2 -Phosphoglyceric acid (2 PGA)	UNILAB	buffer reagent
69.	3 - Phosphoglyceric acid (3 PGA)	SIGMA	trisodium salt
70.	6 -Phosphoglyconic acid (6PGA)	SIGMA	trisodium salt
71.	L-Phenylalanine-L-proline (phe-pro)	BDH	trisodium salt
72.	Phenazine methasulphate (PMS)	SIGMA	
73.	Pyruvate (Pyruvic acid)	SIGMA	
74.	Pyruvate kinase (PK)	BDH	disodium salt
75.	Sorbitol	BDH	
76.	Tris-hydroxymethyl aminomethane (TRIS)	SIGMA	
77.	L-valine-L-teucine (Val-leu)	SIGMA	
78.	Xanthine oxidase (XO)	SIGMA	linking enzyme

APPENDIX 4. Details of the stock referred in this study.

Reagent	Molarity	Quantity	Comments
Commonly used			
MTT	14.5 mM	6 mg/ml	protect from light
PMS	6.5 mM	2 mg/ml	protect from light
NADP	25.0 mM	20 mg/ml	
NAD	40.0 mM	40 mg/ml	
$MgCl_2$	0.2 mM	19 mg/ml	anhydrous form
1M $MgCl_2$	1.0 mM	95 mg/ml	anhydrous form
Used in several stain			
Arsenate	20.0 mM	6 mg/ml	disodium salt
KCl	0.1 mM	75 mg/ml	
DCIP	3.5 mM	1 mg/ml	
$MnCl_2$	0.2 mM	39 mg/ml	
Used only for single stain			
EDTA	0.1 M	37 mg/ml	disodium salt (PGK)
Amino-ethyl-carbazole		50 mg/ml	in acetone (Amylase)
Commosssie Blue		2.5 mg/ml	in 7% acetic solution (Tf and ALB)
Methylene Blue		1 mg/ml	DIA-2

APPENDIX 5. Genotype data input for BIOSYS-1 analysis.

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GENOTYPE PHD GOATS INPUT
NOTU=13,NLOC=9,NALL=6,CRT;
(9(1X,A5))
 ALB   CA    DIA1  DIA2  HB    MDH   ME    TF    XP

STEP DATA:
DATYP=2,NCOL=6,ALPHA;
(6X,6(1X,2A1,1X,I2))
G1   MARDI/IPT,M'SIA          1
ALB    AA:47 AB:08
CA     BB:47 CC:06
DIA1   BB:55
DIA2   AB:02 BB:52 BC:01
HB     AA:43 AB:05 AC:07
MDH    AA:39 AB:05 BB:11
ME     BB:03 BC:07 CC:45
TF     AA:12 AB:24 AD:01 BB:14 BC:02 BD:02
XP     AA:06 AB:16 BB:27 CC:06
G2   SABAH,MALAYSIA           1
ALB    AA:31 AB:20
CA     BB:45 BC:02 CC:03
DIA1   BB:46 BC:04 CC:01
DIA2   AA:07 AB:08 BB:34
HB     AA:38 AB:06 AC:07
MDH    AA:31 AB:04 BB:16
ME     BB:06 BC:07 CC:34 CD:01 DD:03
TF     AA:28 AB:22 BB:01
XP     AA:08 AB:07 BB:31 CC:05
G3   SARAWAK,M'SIA            1
ALB    AA:42 AB:27 BB:02
CA     BB:61 BC:03
DIA1   BB:58 BC:06 CC:06
DIA2   AA:14 AB:09 BB:39
HB     AA:60 AC:11
MDH    AA:48 AB:08 BB:15
ME     BB:03 BC:06 CC:54 CD:03 DD:05
TF     AA:24 AB:29 AC:02 BB:15 BD:01
XP     AA:20 AB:05 BB:34 CC:05
G4   BOGOR,INDONESIA          2
ALB    AA:26 AB:24
CA     BB:21 BC:07 CC:18
DIA1   BB:50
DIA2   AA:09 AB:10 BB:31
HB     AA:46 AB:04
MDH    AA:38 AB:03 BB:09
ME     BB:05 BC:03 CC:38 DD:04
TF     AA:23 AB:22 BB:02 BC:03
XP     AA:08 AB:05 BB:34 CC:03
```

```
G5    SULAWESI,IND'SIA        2
ALB    AA:32 AB:05 BB:11
CA     BB:24 BC:04 CC:20
DIA1   BB:48
DIA2   AA:04 AB:05 BB:39
HB     AA:35 AB:08 BB:05
MDH    AA:25 AB:13 BB:10
ME     BB:05 BC:06 CC:29 CD:03 DD:05
TF     AA:20 AB:18 BB:06 BC:01 CD:03
XP     AA:07 AB:06 BB:29 CC:06
G6    MEDAN,INDONESIA         2
ALB    AA:29 AB:21
CA     BB:44 CC:06
DIA1   BB:50
DIA2   AA:04 AB:02 BB:44
HB     AA:42 AB:06 BB:02
MDH    AA:34 AB:09 BB:07
ME     BB:02 BC:04 CC:34 CD:03 DD:07
TF     AA:27 AB:19 AC:01 AD:01 BB:02
XP     AA:03 AB:08 BB:33 CC:06
G7    MUSUAN,PHI'PINES        3
ALB    AA:19 AB:30 BB:02
CA     BB:40 BC:07 CC:04
DIA1   AA:02 AB:03 BB:31 BC:02 CC:11
DIA2   AA:07 AB:08 BB:34
HB     AA:38 AB:08 BB:05
MDH    AA:31 AB:13 BB:07
ME     BB:09 BC:01 CC:36 CD:01 DD:04
TF     AA:17 AB:26 AC:03 BB:04 BC:01
XP     AA:06 AB:12 BB:31 CC:02
G8    CHENGMAI,TH'LAND        4
ALB    AA:27 AB:21 BB:02
CA     BB:38 BC:05 CC:02
DIA1   BB:42 BC:03 CC:05
DIA2   AA:05 AB:07 BB:38
HB     AA:39 AB:06 BB:05
MDH    AA:31 AB:10 BB:09
ME     BB:05 BC:09 CC:29 CD:01 DD:06
TF     AA:20 AB:21 BB:07 BC:02
XP     AA:09 AB:08 BB:26 CC:07
G9    HATYAI,THAILAND         4
ALB    AA:12 AB:24 BB:03
CA     BB:38 BC:05 CC:02
DIA1   BB:33 CC:06
DIA2   AA:01 AB:07 BB:22 BC:05 CC:02
HB     AA:33 AB:04 BB:02
MDH    AA:15 AB:11 BB:13
ME     BB:03 BC:05 CC:25 CD:03 DD:03
TF     AA:08 AB:22 BB:09
XP     AA:11 AB:05 BB:23
```

```
G10  HAMOTA,S.LANKA             5
ALB   AA:03 AB:07
CA    AA:01 BB:02 BC:07
DIA1  BB:10
DIA2  BB:08
HB    AB:03 BB:07
MDH   AA:01 AB:07 BB:02
ME    BB:03 BC:01 CC:03 DD:03
TF    AA:11
XP    AA:03 AB:03 BB:03 CC:01
G11  WERA,S.LANKA               5
ALB   AA:30 AB:07
CA    AB:02 BB:32 BC:02
DIA1  BB:36
DIA2  AB:02 BB:23 BC:08 CC:02
HB    AA:06 AB:08 BB:22
MDH   AA:14 AB:11 BB:11
ME    BB:04 CC:19 CD:03 DD:10
TF    AA:37
XP    AA:10 AB:02 BB:20 CC:04
G12  THAMB,S.LANKA              5
ALB   AA:28 AB:03
CA    BB:29 CC:02
DIA1  BB:31
DIA2  AA:13 AB:04 BB:14
HB    AB:07 BB:24
MDH   AA:28 BB:03
ME    BB:08 BC:08 CC:13 CD:01 DD:01
TF    AA:28 AB:03
XP    AA:08 AB:07 BB:13 CC:03
G13  NSW,AUSTRALIA              6
ALB   AA:41 AB:11
CA    BB:44 BC:02
DIA1  BB:52
DIA2  AA:32 AB:11 BB:09
HB    AA:05 AB:14 BB:33
MDH   AA:42 AB:02 BB:08
ME    AA:02 BB:23 BC:05 CC:20 CD:01 DD:01
TF    AA:36 AB:12 BB:04
XP    AA:36 AB:12 BB:04
END;


STEP VARIAB:
FULLOUT,HYTYPE=2,PCRIT=2;
END;

STEP HDYWBG:
LEVENE,EXACTP;
END;
```

```
STEP SIMDIS:
NEI,SINGLE=1;
END;

STEP COEFOUT:
ABOVE=1,BELOW=2;
END;


STEP HIERARCHY:
NLEVEL=1;
COUNTRY
LOCALITY
MALAYSIA
INDONESIA
PHILIPIINES
THAILAND
SRI LANKA
AUSTRALIA
END;

STEP FSTAT:
OUTPUT=3;
END;

STEP WRIGHT78:
END;
```

APPENDIX 6. Allele data input for BIOSYS-1 analysis.

```
PHD ALLELE FREQUENCY OS INPUT
NOTU=13,NLOC=40,NALL=5,CRT;
(10(1X,A5)/10(1X,A5)/10(1X,A5)/10(1X,A5))
 ACP   ALB   AK    AMY   AP    BLVR  CA    DPGM  FK    FDP
 FUM   GLDH  EST-2 GPI   GOT   GPT   GPDH  GSR   HB    HK
 IDH   LDH   ME    MDH   MPI   NP    DIA1  DIA2  DI2   PEPA
 PEPB  PEPC  PEPD  PGM2  PK    6PGD  SORDH SOD   TF    XP

STEP DATA:
DATYP=3,NCOL=4,ALPHA,CHECKSUM;
(5X,I4,4(2X,A1,1X,F4.3))
G1    MARDI/IPT              1
ACP    25  A:1000
ALB    55  A: 927  B: 073
AK     25  A:1000
AMY    55  A: 409  B: 591
AP     55  A: 464  B: 536
BLVR   25  A:1000
CA     53  B: 887  C: 113
DPGM   25  A:1000
FK     25  A:1000
FDP    25  A:1000
FUM    25  A:1000
GLDH   25  A:1000
EST-2  25  A:1000
GPI    25  A:1000
GOT    25  A:1000
GPT    25  A:1000
GPDH   25  A:1000
GSR    25  A:1000
HB     55  A: 891  B: 045  C: 064
HK     25  A:1000
IDH    25  A:1000
LDH    25  A:1000
ME     55  B: 118  C: 882
MDH    55  A: 755  B: 245
MPI    25  A:1000
NP     55  A: 364  B: 636
DIA1   55  B:1000
DIA2   55  A: 018  B: 973  C: 009
DI2    25  A:1000
PEPA   25  A:1000
PEPB   25  A:1000
PEPC   25  A:1000
PEPD   25  A:1000
PGM2   25  A:1000
PK     25  A:1000
6PGD   25  A:1000
SORDH  25  A:1000
```

```
SOD     25  A:1000
TF      55  A: 445  B: 509  C: 018  D: 027
XP      49  A: 286  B: 714

G2   SABAH,MALAYSIA            1
ACP     25  A:1000
ALB     51  A: 804  B: 196
AK      25  A:1000
AMY     51  A: 520  B: 480
AP      51  A: 422  B: 578
BLVR    25  A:1000
CA      50  B: 920  C: 080
DPGM    25  A:1000
FK      25  A:1000
FDP     25  A:1000
FUM     25  A:1000
GLDH    25  A:1000
EST-2   25  A:1000
GPI     25  A:1000
GOT     25  A:1000
GPT     25  A:1000
GPDH    25  A:1000
GSR     25  A:1000
HB      51  A: 873  B: 059  C: 069
HK      25  A:1000
IDH     25  A:1000
LDH     25  A:1000
ME      51  B: 186  C: 745  D: 069
MDH     51  A: 647  B: 353
MPI     25  A:1000
NP      51  A: 480  B: 520
DIA1    51  B: 941  C: 059
DIA2    49  A: 224  B: 776
DI2     25  A:1000
PEPA    25  A:1000
PEPB    25  A:1000
PEPC    25  A:1000
PEPD    25  A:1000
PGM2    25  A:1000
PK      25  A:1000
6PGD    25  A:1000
SORDH   25  A:1000
SOD     25  A:1000
TF      51  A: 765  B: 235
XP      46  A: 250  B: 750
G3   SARAWAK,M'SIA             1
ACP     25  A:1000
ALB     71  A: 782  B: 218
AK      25  A:1000
AMY     71  A: 394  B: 606
AP      71  A: 289  B: 711
BLVR    25  A:1000
```

CA	64	B: 977	C: 023		
DPGM	25	A:1000			
FK	25	A:1000			
FDP	25	A:1000			
FUM	25	A:1000			
GLDH	25	A:1000			
EST-2	25	A:1000			
GPI	25	A:1000			
GOT	25	A:1000			
GPT	25	A:1000			
GPDH	25	A:1000			
GSR	25	A:1000			
HB	71	A: 923	C: 077		
HK	25	A:1000			
IDH	25	A:1000			
LDH	25	A:1000			
ME	71	B: 084	C: 824	D: 092	
MDH	71	A: 732	B: 268		
MPI	25	A:1000			
NP	71	A: 528	B: 472		
DIA1	70	B: 871	C: 129		
DIA2	62	A: 298	B: 702		
DI2	25	A:1000			
PEPA	25	A:1000			
PEPB	25	A:1000			
PEPC	25	A:1000			
PEPD	25	A:1000			
PGM2	25	A:1000			
PK	25	A:1000			
6PGD	25	A:1000			
SORDH	25	A:1000			
SOD	25	A:1000			
TF	71	A: 556	B: 423	C: 014	D: 007
XP	59	A: 381	B: 619		
G4	BOGOR,INDONESIA		2		
ACP	25	A:1000			
ALB	50	A: 760	B: 240		
AK	25	A:1000			
AMY	50	A: 320	B: 680		
AP	50	A: 470	B: 530		
BLVR	25	A:1000			
CA	46	B: 533	C: 467		
DPGM	25	A:1000			
FK	25	A:1000			
FDP	25	A:1000			
FUM	25	A:1000			
GLDH	25	A:1000			
EST-2	25	A:1000			
GPI	25	A:1000			
GOT	25	A:1000			
GPT	25	A:1000			
GPDH	25	A:1000			

GSR	25	A:1000		
HB	50	A: 960	B: 040	
HK	25	A:1000		
IDH	25	A:1000		
LDH	25	A:1000		
ME	50	B: 130	C: 790	D: 080
MDH	50	A: 790	B: 210	
MPI	25	A:1000		
NP	50	A: 530	B: 470	
DIA1	50	B:1000		
DIA2	50	A: 280	B: 720	
DI2	25	A:1000		
PEPA	25	A:1000		
PEPB	25	A:1000		
PEPC	25	A:1000		
PEPD	25	A:1000		
PGM2	25	A:1000		
PK	25	A:1000		
6PGD	25	A:1000		
SORDH	25	A:1000		
SOD	25	A:1000		
TF	50	A: 680	B: 290	C: 030
XP	47	A: 223	B: 777	
G5	SULAWESI,IND,SIA		2	
ACP	25	A:1000		
ALB	48	A: 760	B: 240	
AK	25	A:1000		
AMY	48	A: 427	B: 573	
AP	48	A: 250	B: 750	
BLVR	25	A:1000		
CA	46	B: 533	C: 467	
DPGM	25	A:1000		
FK	25	A:1000		
FDP	25	A:1000		
FUM	25	A:1000		
GLDH	25	A:1000		
EST-2	25	A:1000		
GPI	25	A:1000		
GOT	25	A:1000		
GPT	25	A:1000		
GPDH	25	A:1000		
GSR	25	A:1000		
HB	48	A: 812	B: 188	
HK	25	A:1000		
IDH	25	A:1000		
LDH	25	A:1000		
ME	48	B: 167	C: 698	D: 135
MDH	48	A: 656	B: 344	
MPI	25	A:1000		
NP	48	A: 385	B: 615	
DIA1	48	B:1000		
DIA2	48	A: 135	B: 865	

DI2	25	A:1000			
PEPA	25	A:1000			
PEPB	25	A:1000			
PEPC	25	A:1000			
PEPD	25	A:1000			
PGM2	25	A:1000			
PK	25	A:1000			
6PGD	25	A:1000			
SORDH	25	A:1000			
SOD	25	A:1000			
TF	48	A: 604	B: 323	C: 042	D: 031
XP	42	A: 238	B: 762		
G6	MEDAN, INDONESIA		2		
ACP	25	A:1000			
ALB	50	A: 790	B: 210		
AK	25	A:1000			
AMY	50	A: 380	B: 620		
AP	50	A: 310	B: 690		
BLVR	25	A:1000			
CA	50		B: 880	C: 120	
DPGM	25	A:1000			
FK	25	A:1000			
FDP	25	A:1000			
FUM	25	A:1000			
GLDH	25	A:1000			
EST-2	25	A:1000			
GPI	25	A:1000			
GOT	25	A:1000			
GPT	25	A:1000			
GPDH	25	A:1000			
GSR	25	A:1000			
HB	50	A: 900	B: 100		
HK	25	A:1000			
IDH	25	A:1000			
LDH	25	A:1000			
ME	50		B: 080	C: 750	D: 170
MDH	50	A: 770	B: 230		
MPI	25	A:1000			
NP	50	A: 550	B: 450		
DIA1	50		B:1000		
DIA2	50	A: 100	B: 900		
DI2	25	A:1000			
PEPA	25	A:1000			
PEPB	25	A:1000			
PEPC	25	A:1000			
PEPD	25	A:1000			
PGM2	25	A:1000			
PK	25	A:1000			
6PGD	25	A:1000			
SORDH	25	A:1000			
SOD	25	A:1000			
TF	50	A: 750	B: 230	C: 010	D: 010

XP	44	A: 159	B: 841	
G7	MUSUAN,PHI'PINES			3
ACP	25	A:1000		
ALB	51	A: 667	B: 333	
AK	25	A:1000		
AMY	51	A: 333	B: 667	
AP	51	A: 255	B: 745	
BLVR	25	A:1000		
CA	53	B: 853	C: 147	
DPGM	25	A:1000		
FK	25	A:1000		
FDP	25	A:1000		
FUM	25	A:1000		
GLDH	25	A:1000		
EST-2	25	A:1000		
GPI	25	A:1000		
GOT	25	A:1000		
GPT	25	A:1000		
GPDH	25	A:1000		
GSR	25	A:1000		
HB	51	A: 824	B: 176	
HK	25	A:1000		
IDH	25	A:1000		
LDH	25	A:1000		
ME	51	B: 186	C: 726	D: 088
MDH	51	A: 735	B: 265	
MPI	25	A:1000		
NP	51	A: 333	B: 667	
DIA1	49	A: 071	B: 684	C: 245
DIA2	49	A: 224	B: 776	
DI2	25	A:1000		
PEPA	25	A:1000		
PEPB	25	A:1000		
PEPC	25	A:1000		
PEPD	25	A:1000		
PGM2	25	A:1000		
PK	25	A:1000		
6PGD	25	A:1000		
SORDH	25	A:1000		
SOD	25	A:1000		
TF	51	A: 618	B: 343	C: 039
XP	49	A: 245	B: 755	
G8	CHENGMAI,TH'LAND			4
ACP	25	A:1000		
ALB	50	A: 750	B: 250	
AK	25	A:1000		
AMY	50	A: 420	B: 580	
AP	50	A: 450	B: 550	
BLVR	25	A:1000		
CA	45	B: 900	C: 100	
DPGM	25	A:1000		
FK	25	A:1000		

FDP	25	A:1000		
FUM	25	A:1000		
GLDH	25	A:1000		
EST-2	25	A:1000		
GPI	25	A:1000		
GOT	25	A:1000		
GPT	25	A:1000		
GPDH	25	A:1000		
GSR	25	A:1000		
HB	50	A: 840	B: 160	
HK	25	A:1000		
IDH	25	A:1000		
LDH	25	A:1000		
ME	50	B: 190	C: 680	D: 130
MDH	50	A: 720	B: 280	
MPI	25	A:1000		
NP	50	A: 430	B: 570	
DIA1	50	B: 870	C: 130	
DIA2	50	A: 170	B: 830	
DI2	25	A:1000		
PEPA	25	A:1000		
PEPB	25	A:1000		
PEPC	25	A:1000		
PEPD	25	A:1000		
PGM2	25	A:1000		
PK	25	A:1000		
6PGD	25	A:1000		
SORDH	25	A:1000		
SOD	25	A:1000		
TF	50	A: 610	B: 370	C: 020
XP	43	A: 302	B: 698	
G9	HATYAI,THAILAND			4
ACP	25	A:1000		
ALB	39	A: 615	B: 385	
AK	25	A:1000		
AMY	39	A: 397	B: 603	
AP	39	A: 526	B: 474	
BLVR	25	A:1000		
CA	39	B: 974	C: 026	
DPGM	25	A:1000		
FK	25	A:1000		
FDP	25	A:1000		
FUM	25	A:1000		
GLDH	25	A:1000		
EST-2	25	A:1000		
GPI	25	A:1000		
GOT	25	A:1000		
GPT	25	A:1000		
GPDH	25	A:1000		
GSR	25	A:1000		
HB	39	A: 897	B: 103	
HK	25	A:1000		

IDH	25	A:1000		
LDH	25	A:1000		
ME	39	B: 141	C: 744	D: 115
MDH	39	A: 526	B: 474	
MPI	25	A:1000		
NP	39	A: 500	B: 500	
DIA1	39	B: 846	C: 154	
DIA2	37	A: 122	B: 757	C: 122
DI2	25	A:1000		
PEPA	25	A:1000		
PEPB	25	A:1000		
PEPC	25	A:1000		
PEPD	25	A:1000		
PGM2	25	A:1000		
PK	25	A:1000		
6PGD	25	A:1000		
SORDH	25	A:1000		
SOD	25	A:1000		
TF	39	A: 487	B: 513	
XP	39	A: 346	B: 654	
G10	HAMOTA,S.LANKA		5	
ACP	10	A:1000		
ALB	10	A: 650	B: 350	
AK	10	A:1000		
AMY	10	A: 500	B: 500	
AP	10	A: 450	B: 550	
BLVR	10	A:1000		
CA	10	A: 100	B: 550	C: 350
DPGM	10	A:1000		
FK	10	A:1000		
FDP	10	A:1000		
FUM	10	A:1000		
GLDH	10	A:1000		
EST-2	10	A:1000		
GPI	10	A:1000		
GOT	10	A:1000		
GPT	10	A:1000		
GPDH	10	A:1000		
GSR	10	A:1000		
HB	10	A: 150	B: 850	
HK	10	A:1000		
IDH	10	A:1000		
LDH	10	A:1000		
ME	10	B: 350	C: 350	D: 300
MDH	10	A: 450	B: 550	
MPI	10	A:1000		
NP	10	A: 450	B: 550	
DIA1	10	B:1000		
DIA2	8	B:1000		
DI2	10	A:1000		
PEPA	10	A:1000		
PEPB	10	A:1000		

PEPC	10	A:1000			
PEPD	10	A:1000			
PGM2	10	A:1000			
PK	10	A:1000			
6PGD	10	A:1000			
SORDH	10	A:1000			
SOD	10	A:1000			
TF	11	A:1000			
XP	9	A: 500	B: 500		
G11	WERA,S.LANKA			5	
ACP	25	A:1000			
ALB	37	A: 905	B: 095		
AK	25	A:1000			
AMY	37	A: 378	B: 622		
AP	37	A: 514	B: 486		
BLVR	25	A:1000			
CA	36	A: 028	B: 944	C: 028	
DPGM	25	A:1000			
FK	25	A:1000			
FDP	25	A:1000			
FUM	25	A:1000			
GLDH	25	A:1000			
EST-2	25	A:1000			
GPI	25	A:1000			
GOT	25	A:1000			
GPT	25	A:1000			
GPDH	25	A:1000			
GSR	25	A:1000			
HB	36	A: 278	B: 722		
HK	25	A:1000			
IDH	25	A:1000			
LDH	25	A:1000			
ME	36	B: 111	C: 569	D: 319	
MDH	36	A: 542	B: 458		
MPI	25	A:1000			
NP	36	A: 500	B: 500		
DIA1	36	B:1000			
DIA2	35	A:	B:	C:	D:
DI2	25	A:1000			
PEPA	25	A:1000			
PEPB	25	A:1000			
PEPC	25	A:1000			
PEPD	25	A:1000			
PGM2	25	A:1000			
PK	25	A:1000			
6PGD	25	A:1000			
SORDH	25	A:1000			
SOD	25	A:1000			
TF	37	A:1000			
XP	32	A: 344	B: 656		
G12	THAM,S.LANKA			5	
ACP	25	A:1000			

ALB	31	A: 952	B: 048	
AK	25	A:1000		
AMY	31	A: 435	B: 565	
AP	31	A: 435	B: 565	
BLVR	25	A:1000		
CA	31	B: 935	C: 065	
DPGM	25	A:1000		
FK	25	A:1000		
FDP	25	A:1000		
FUM	25	A:1000		
GLDH	25	A:1000		
EST-2	25	A:1000		
GPI	25	A:1000		
GOT	25	A:1000		
GPT	25	A:1000		
GPDH	25	A:1000		
GSR	25	A:1000		
HB	31	A: 113	B: 887	
HK	25	A:1000		
IDH	25	A:1000		
LDH	25	A:1000		
ME	31	B: 387	C: 565	D: 048
MDH	31	A: 903	B: 097	
MPI	25	A:1000		
NP	31	A: 565	B: 435	
DIA1	31	B:1000		
DIA2	31	A: 484	B: 516	
DI2	25	A:1000		
PEPA	25	A:1000		
PEPB	25	A:1000		
PEPC	25	A:1000		
PEPD	25	A:1000		
PGM2	25	A:1000		
PK	25	A:1000		
6PGD	25	A:1000		
SORDH	25	A:1000		
SOD	25	A:1000		
TF	31	A: 952	B: 048	
XP	28	A: 411	B: 589	
G13	NSW,AUSTRALIA		6	1
ACP	25	A:1000		
ALB	52	A: 894	B: 106	
AK	25	A:1000		
AMY	52	A: 558	B: 442	
AP	52	A: 462	B: 538	
BLVR	25	A:1000		
CA	46	B: 978	C: 022	
DPGM	25	A:1000		
FK	25	A:1000		
FDP	25	A:1000		
FUM	25	A:1000		
GLDH	25	A:1000		

```
EST-2  25  A:1000
GPI    25  A:1000
GOT    25  A:1000
GPT    25  A:1000
GPDH   25  A:1000
GSR    25  A:1000
HB     52  A: 231  B: 769
HK     25  A:1000
IDH    25  A:1000
LDH    25  A:1000
ME     52  A: 038  B: 490  C: 442  D: 029
MDH    52  A: 827  B: 173
MPI    25  A:1000
NP     52  A: 288  B: 712
DIA1   52  B:1000
DIA2   52  A: 721  B: 279
DI2    25  A:1000
PEPA   25  A:1000
PEPB   25  A:1000
PEPC   25  A:1000
PEPD   25  A:1000
PGM2   25  A:1000
PK     25  A:1000
6PGD   25  A:1000
SORDH  25  A:1000
SOD    25  A:1000
TF     52  A: 808  B: 192
XP     52  A: 808  B: 192
END;


STEP VARIAB:
FULLOUT,HTYPE=1,PCRIT=2;
END;

STEP HDYWBG:
LEVENE,EXACTP;
END;

STEP SIMDIS:
NEI,SINGLE=1;
END;

STEP SINGLE:
COEF=1;
END;

STEP COEFOUT:
ABOVE=1,BELOW=2;
END;
```

```
STEP HIERARCHY:
NLEVEL=1;
COUNTRY
LOCALITY
MALAYSIA
INDONESIA
PHILIPPINES
THAILAND
SRI LANKA
AUSTRALIA


STEP SIMAVE:
COEF=1, LEVEL=1;
END;

STEP FSTAT:
OUTPUT=1;
END;

STEP HETXSQ:
SUBDIV=1, CONTAB;
END;

STEP CLUSTER:
COEF=2,ALGOR=1;
COEF=2,ALGOR=3;
END;
```

Appendix 7. Phenotype frequencies with the null alleles of the goat populations examined.

ENZYME	GENOTYPE	MALAYSIA			INDONESIA			PHILIPPINES	THAILAND		SRI LANKA			AUSTRALIA
		MARDI/IPT (55)	SABAH (51)	SARAWAK (71)	BOGOR (50)	SULUWESI (48)	MEDAN (50)	MUSUAN (51)	CHENG MAI (50)	HAD YAI (39)	S1 (10)	S2 (37)	S3 (31)	N.S.W. (52)
Albumin														
ALB	(N)	55	51	71	50	48	50	51	50	39	10	37	31	52
	A	47	31	42	26	32	29	19	27	12	3	30	28	41
	AB	8	20	27	24	5	21	30	21	24	7	7	3	11
	B	0	-	2	-	11	-	2	2	3	-	-	-	-
Plasma Amylase														
AMY	(N)	55	51	71	50	48	50	51	50	39	11	37	31	52
	H	36	39	45	27	32	31	28	33	25	8	23	21	42
	L	19	12	26	23	16	19	23	17	14	3	14	10	10
Alkaline phosphatase														
AP	(N)	55	51	71	50	48	50	51	50	39	10	37	31	52
	FF	39	34	35	46	21	26	23	34	30	7	28	21	37
	00	16	17	36	4	27	24	28	16	9	3	9	10	15
Carbonic anhydrase														
CA	(N)	51	50	64	46	48	46	51	45					
	102	-	-	-	-	-	-	-	-	-	1	0	0	0
	102/100	-	-	-	-	-	-	-	-	-	0	2	0	0
	100	47	45	61	21	24	44	40		37	2	32	29	44
	100/98	-	2	3	7	4	-	7	5	2	7	2	0	2
	98	6	3	-	18	20	6	4	2	-	0	0	2	0
	00	2	1	7	4	-	-	-	5	-	0	0	0	6

(Contd.)

Appendix 7. Phenotype frequencies with the null alleles of the goat populations examined.

ENZYME	GENOTYPE	MALAYSIA			INDONESIA			PHILIPPINES	THAILAND		SRI LANKA			AUSTRALIA
		MARDI/IPT (55)	SABAH (51)	SARAWAK (71)	BOGOR (50)	SULUWESI (48)	MEDAN (50)	MUSUAN (51)	CHENG MAI (50)	HAD YAI (39)	S1 (10)	S2 (37)	S3 (31)	N.S.W. (52)
NADH Diaphorase 1 Zone 1														
DIA 1	102	-	-	-	-	-	-	2	-	-	0	0	0	0
	102/100	-	-	-	-	-	-	3	-	-	0	0	0	0
	100	55	46	58	50	48	50	31	42	33	10	36	31	52
	100/98	-	4	6	-	-	-	2	3	-	0	0	0	0
	98	-	1	6	-	-	-	11	5	6	0	0	0	0
	00	-	-	1	-	-	-	2	-	-	0	0	0	0
NADH-Diaphorase 1 zone 2														
DIA 2	102	-	7	14	9	4	4	7	5	1	0	0	13	32
	102/100	2	8	9	10	5	2	8	7	7	0	2	4	11
	100	52	34	39	31	39	44	34	38	22	8	23	14	9
	100/98	1	-	-	-	-	-	-	-	5	0	8	0	0
	98	-	-	-	-	-	-	-	-	2	0	2	0	0
	00	-	2	9	-	-	-	2	-	2	2	1	0	0
Haemoglobin														
HB	A	43	38	60	46	35	42	38	39	33	0	6	0	5
	AB	5	6	-	4	8	6	8	6	4	3	8	7	14
	AX	7	7	11	-	-	-	-	-	-	0	0	0	0
	B	-	-	-	-	5	2	5	5	2	7	22	24	33

(Contd.)

Appendix 7. Phenotype frequencies with the null alleles of the goat populations examined.

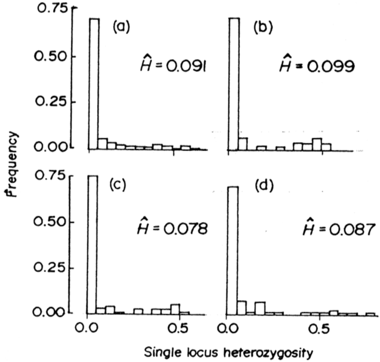
ENZYME	GENOTYPE	MALAYSIA			INDONESIA			PHILIPPINES	THAILAND		SRI LANKA			AUSTRALIA
		MARDI/IPT (55)	SABAH (51)	SARAWAK (71)	BOGOR (50)	SULUWESI (48)	MEDAN (50)	MUSUAN (51)	CHENG MAI (50)	HAD YAI (39)	S1 (11)	S2 (37)	S3 (31)	N.S.W. (52)
Malate dehydrogenase														
MDH	100	39	31	48	38	25	34	31	31	15	1	14	28	42
	100/98	5	4	8	3	13	9	13	10	11	7	11	0	2
	98	11	16	15	9	10	7	7	9	13	2	11	3	8
Malic enzyme														
ME	104	-	-	-	-	-	-	-	-	-	-	-	-	2
	102	3	6	3	5	5	2	9	5	3	3	4	8	23
	102/100	7	7	6	3	6	4	1	9	5	1	0	8	5
	100	45	34	54	38	29	34	36	29	25	3	19	13	20
	100/98	-	1	3	-	3	3	1	1	3	0	3	1	1
	98	-	3	5	4	5	7	4	6	3	3	10	1	1
Nucleoside phosphorylase														
NP	H	33	37	55	39	30	40	28	34	29	7	27	25	26
	L	22	14	16	11	18	10	23	16	10	3	9	6	26

(Contd.)

Appendix 7. Phenotype frequencies with the null alleles of the goat populations examined.

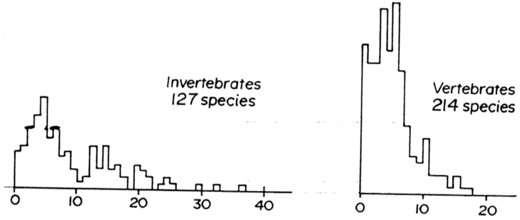
ENZYME	GENOTYPE	MALAYSIA			INDONESIA			PHILIPPINES	THAILAND		SRI LANKA			AUSTRALIA
		MARDI/IPT (55)	SABAH (51)	SARAWAK (71)	BOGOR (50)	SULUWESI (48)	MEDAN (50)	MUSUAN (51)	CHENG MAI (50)	HAD YAI (39)	S1 (11)	S2 (37)	S3 (31)	N.S.W. (52)
Transferrin														
TF	A	12	28	24	23	20	27	17	20	8	11	37	28	36
	AB	24	22	29	22	18	19	26	21	22	-	-	3	12
	AC	-	-	2	-	-	1	3	-	-	-	-	-	-
	AD	1	-	-	-	-	1	-	-	-	-	-	-	-
	B	14	1	15	2	6	2	4	7	9	-	-	-	4
	BC	2	-	-	3	1	-	1	2	-	-	-	-	-
	BD	2	-	1	-	-	-	-	-	-	-	-	-	-
	CD	-	-	-	-	3	-	-	-	-	-	-	-	-
X-Protein														
XP	X-1	6	8	20	8	7	3	6	9	11	3	10	8	36
	X 1-2	16	7	5	5	6	8	12	8	5	3	2	7	12
	X-2	27	31	34	34	29	33	31	26	23	3	20	13	4
	OO	6	5	5	3	6	6	2	7	-	1	4	3	-

Appendix 8



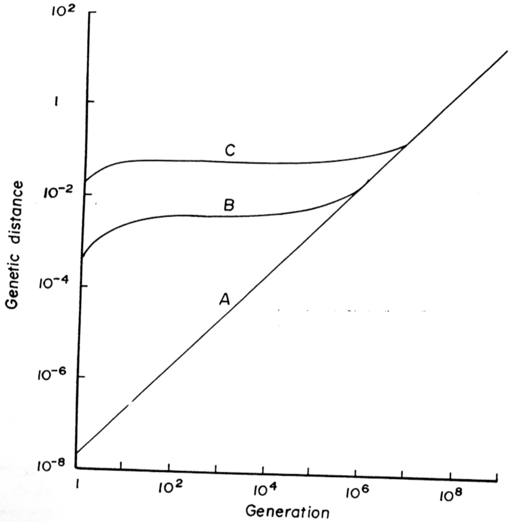
Distributions of single-locus heterozygosities for (b) man, (c) the horse mouse (*Mus musculus*), and (d) the *Drosophila mulleri* species group. The theoretical distribution (a) was obtained by computer simulation with the infinite-allele model. $\hat{H}$ stands for average heterozygosity. From Nei et al. (1976b).

Appendix 9



Distributions of average gene diversity (heterozygosity) for species of invertebrates and vertebrates. Only species in which 20 or more loci were examined are included. From Nei and Graur (1984).

Appendix 10



Bottleneck effects on the genetic distance. Computations have been made under the assumption that one isolated population (or species) is established through a bottleneck of size N_o and thereafter population size increases to the level of the parental population following the sigmoid curve. The genetic distance in the ordinate represents the distance between this population and its parental population which has undergone independent evolution. The infinite-allele model is used. A: no bottlenecks. B: $N_o = 100$. C: $N_o = 10$. From Chakraborty and Nei (1977).

APPENDIX 11. Sample performa sheet used for electrophoretic data scoring

AN ACIAR PROJECT (SOUTH EAST ASIA)
PROJECT NO: 30286
INSTITUTE OF ADVANCED STUDIES.
UNIVERSITY OF MALAYA.KUALA LUMPUR.

A.

Species: goats. (C.h) Tissue samples: Red cell
Location/Area: Medan Code No: G5

B.

Enzyme: malic enzyme Time : 60 minutes
Buffer : 0.02 citrate Phosphate Voltage : 200V
pH : 6.4 Temperature : 4°C
Date : 15-4-95 Current : 4 mA

C.

No	Identification	Phenotype	Descriptions	Origin
1	G5 33	102		
2	G5 35	102		
3	G5 37	98		
4	G5 36	102		
5	G5 32	100		
6	G5 29	98		
7	G5 14	102/100		
8	Control (Me 100)	100		

D.

Notes/Remarks

- Buffer was mixed with 10% Photoflo solution.
- Run for 60 minutes.