6 September 2006

Genetic Quality Control
Genetic Quality Monitoring by Biochemical Marker Isoenzymes

Experimental Animal Division, RIKEN BioResource Center

The RIKEN BRC carries out a genetic monitoring program to ensure the genetic quality of mice strains. This monitoring program is useful for confirmation of genetic integrity and uniformity of inbred strains and the genetic background of congenic strains. Isoenzymes are variants of the same protein that exhibit different physical characteristics, such as electrophoretic mobility or enzymatic activity. Strain specific isoenzymes are useful biochemical markers for determining genetic integrity.

1. Materials

Sample Preparation

Blood hemolysates

Red blood cells (RBCs) is prepared from the erythrocyte fraction of heparinized blood by centrifuging at 2,500～3,000 rpm for 10 min at 4℃. The RBCs are washed in saline three times by repeating centrifugation at 2,500～3,000 rpm for 5 min at 4℃. The RBCs are lysed with a fourfold (more than twofold) volume of dH_2O. For iodoacetic acid treatment, RBCs are lysed in a fourfold volume of iodoacetic acid solution instead of water.

Plasma

The plasma fraction separated from the erythrocyte fraction of heparinized blood by centrifuging at 2,500-3,000 rpm for 10 min at 4℃. The supernatant is used.

Kidney homogenates

A kidney at adult surgically is removed and homogenized in a fivefold volume of dH_2O. The homogenates are centrifuged at 10,000-15,000 rpm at 0℃ for 10 min and the supernatant is used.

Urine

Urine from animal is diluted in a twofold volume of dH_2O.

All samples should be used promptly after preparation. Samples can be stored at −80℃ until use. Frequent thawing and freezing causes reduction in enzyme activities or protein degeneration.

Cellulose Acetate Plate Electrophoresis

Membrane plate

Titan III (Helena Laboratories #3900), 25 cellulose acetate plates 94 x 76 mm

Comb

Super Z applicator (Helena Laboratories, #4084), Super Z sample well plate (Helena Laboratories #4085) , Super Z aligning base (Helena Laboratories #4086)

Electrophoresis apparatus

Titan Gel Chamber (Helena Laboratories #4063)

Wicks

Disposable wicks for Zip Zone Chamber (Helena Laboratories #5081)

PAGE Electrophoresis

Gel

Ready Gels J (BioRad #161-J321V), 4% T stacking gel, 10% T resolving gel, 12-well

Ready Gels J (BioRad #161-J341V) , 4% T stacking gel, 15% T resolving gel, 12-well

Tracking dye

Native Sample Buffer (BioRad #161-0738)

Electrophoresis apparatus

Mini-PROTEIN 3 Gel Electrophoresis System (BioRad #165-3301)

Buffer System

EDTA sodium acetate (pH5.6)

17.01 g Sodium acetate trihydrate

2.48 g

Adjust to pH5.6 with HCl or NaOH. Bring up to 1000 ml with dH_2O.

Phosphate (pH6.8)

3.77 g

8.63 g

Adjust to pH6.8 with with HCl or NaOH. Bring up to 1000 ml with dH_2O.

Tris citrate (pH7.6)

12.10 g TRIS

Dissolve in 600 ml of dH_2O. Adjust to pH7.6 with 10% citric acid.

Bring up to 1000 ml with DW.

Tris citrate (pH8.3)

16.64 g TRIS
4.20 g
Adjust to pH8.3 with HCl or NaOH. Bring up to 1000 ml with dH_2O.

Tris EDTA borate (pH8.4)

10.91 g TRIS
3.10 g
0.60 g
Adjust to pH8.6 with HCl or NaOH. Bring up to 1000 ml with dH_2O.

Tris glycine (pH8.3)

1.50 g
7.10 g
Adjust to pH8.3 with HCl or NaOH. Bring up to 1000 ml with dH_2O.

Tris glycine (pH8.5)

3.00 g
14.40 g
Adjust to pH8.5 with HCl or NaOH. Bring up to 1000 ml with dH_2O.

Tris HCl (pH7.0, pH8.0, pH9.0)

24.00 g
Dissolve in 800 ml of dH_2O. Adjust pH with HCl. Bring up to 1000 ml with dH_2O.

Staining Reagents

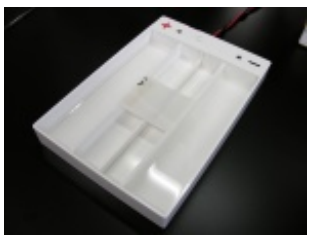
3-(4,5-Dimethyl thiazoly1-2)-2, 5-diphenyl-tetrazolium Bromide (MTT) (Sigma #M2128)
Phenazine Methosulfate (PMS) (Sigma #P9625)
β -Nicotinamide Ademine Dinucleotide Phosphate (NADP) (Sigma #N0505)
DL-Isocitrate (trisodium salt) (Sigma #11252)
L-amino acid oxidase (Sigma #A5147)
Peroxidase (Sigma #P6782)
L-leucyl-alanine (Sigma #L9250)
β -naphthyl-phosphate acid (Sigma #N1132)
Fast blue RR Salt (Wako #069-03712)
$MgCl_2 \cdot 6H_2O$ (Sigma #M2670)
$MnCl_2 \cdot 4H_2O$ (Sigma #M3634)
Ponceau S (Sigma #24-3875-5)
Glucose-6-phosphate (Sigma #G5885)

SYPRO Ruby protein gel stain (Invitrogen #S12001)
Glucose-1-phosphate (Sigma #G7000)
Glucose-1-6-phosphate dehydrogenase (Sigma #G7137)
LD Vis isoenzyme reagent (Helena #J5909)
Fructose-6-phosphate (Sigma #F3627)
Magnesium acetate (Hampton Research #16674-78-5)
Sodium malate (Sigma #28-3290)

2. Experimental Protocol

Cellulose Acetate Electrophoresis

1. Soak the cellulose acetate membrane plate very slowly in the solution for over 5 min.
2. Pour buffer into the electrophoresis chamber. Place fold-up wicks on each support arm, and moisten them by buffer.
3. Bring samples in the slots of the applicator on ice.
4. Place the soaked plate once between paper towels. The plate should not be dry.
5. Stamp the comb into the applicator. One stamp is equivalent to 0.3 μ l.
6. Place the gel on the electrophoresis chamber, and apply it upside down on the paper rows. Place coin on the gel to keep plate flat and ensure an even current through the plate.
7. Running electrophoresis for appropriate time.


Electrophoresis chamber

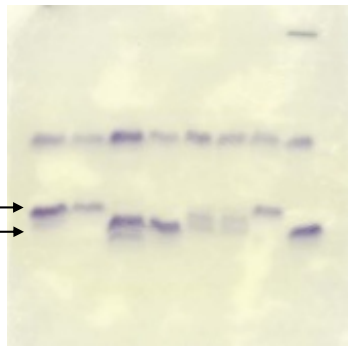

Applicator

PAGE Electrophoresis

1. Place a Gel Cassette Sandwich into the Electrode Assembly with the short plate facing inward.
2. Lift the Gel Cassette Sandwiches into place against the green gaskets and slide into the Clamping Frame.
3. Press down on the Electrode Assembly while closing the two cam levers of the Clamping Frame.
4. Lower the Inner Chamber Assembly into the Mini Tank. Fill the inner chamber with running buffer until the level reaches halfway between the tops of the taller and shorter glass plates of the Gel Cassette Sandwich.
5. Add ~200 ml of running buffer to the mini tank.
6. Place the Lid on the Mini Tank.
7. Transfer samples to the gel, and apply the same amount of tracking dye.
8. Running electrophoresis for appropriate time.

Idh1, isocitrate dehydrogenase-1 (Chr. 1)

Tissue sample:	Kidney in 5 weight dH_2O	
	0.3 μ l	
Buffer system:	Tris citrate; pH8.3	
Supporting media:	Cellulose acetate membrane	
Electrophoresis:	Voltage:	200V
	Time:	45 min
	Temperature:	4℃
	Migration:	Cathode (-) to anode (+)
Stain procedure:	4 mg MTT	
	3 mg PMS	
	4 mg NADP	
	40 mg DL-Isocitrate (trisodium salt)	
	100 μ l $MgCl_2$ (100mg/ml)	
	10 ml dH_2O	
	Fix in 5% acetic acid solution	



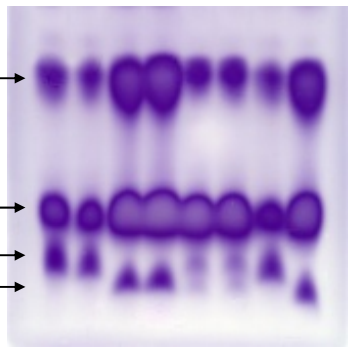
1	2	3	4	5	6	7	8
a	*a*	*b*	*b*	*a/b*	*a/b*	*a*	*b*

1.	C3H/HeN	a	5.	B6D2F1	a/b
2.	C57BL/6J	a	6.	B6D2F1	a/b
3.	AKR/J	b	7.	C57BL/6J	a
4.	DBA/2J	b	8	DBA/2J	b

a = slow *b* = fast

Pep3, peptidase 3 (Chr. 1)

Tissue sample:	Kidney in 5 weight dH_2O	
	1.2 μ l	
Buffer system:	Tris EDTA borate; pH8.4	
Supporting media:	Cellulose acetate membrane	
Electrophoresis:	Voltage:	300V
	Time:	30 min
	Temperature:	4℃
	Migration:	Cathode (-) to anode (+)
Stain procedure:	In agar overlay:	
	10 ml 1% molten agar	
	10 ml phosphate; pH6.8	
	5 mg L-amino acid oxidase	
	5 mg Peroxidase	
	20 mg L-leucyl-alanine	
	4 mg MTT	
	5 mg NADP	
	5 mg PMS	
	Incubate at 37℃ until bands appear (for 30 min.)	
	Fix in 5% acetic acid solution	



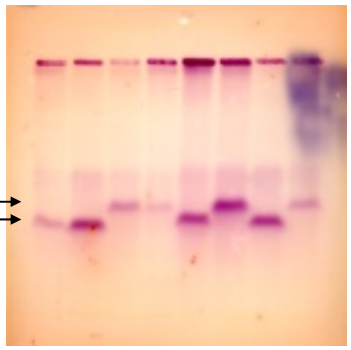
1	2	3	4	5	6	7	8
a	*a*	*b*	*b*	*a/b*	*a/b*	*a*	*b*

1.	C57BL/6J	*a*	5.	B6D2F1	*a/b*
2.	BALB/cA	*a*	6.	B6D2F1	*a/b*
3.	DBA/2J	*b*	7.	C57BL/6J	*a*
4.	A/J	*b*	8.	DBA/2J	*b*

a = slow *b* = middle
c = fast

Akp1, alkaline phosphatase 1 (Chr. 1)

Tissue sample:	Kidney in 5 weight dH_2O	
	0.6 μ l	
Buffer system:	Tris citrate; pH8.3	
Supporting media:	Cellulose acetate membrane	
Electrophoresis:	Voltage:	200V
	Time:	45 min
	Temperature:	4℃
	Migration:	Cathode (-) to anode (+)
Stain procedure:	(a) 10 mg β -naphthyl-phosphate acid	
	5 ml dH_2O	
	(b) 20 mg fast blue RR salt	
	100 μ l $MgCl_2$ (100mg/ml)	
	80 μ l $MnCl_2$ (100mg/ml)	
	5 ml Tris HCl; pH9.0	
	Mixed (a) and (b) just before staining	
	Fix in 5% acetic acid solution	



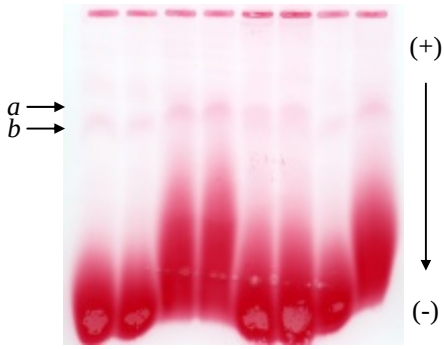
1	2	3	4	5	6	7	8
a	*a*	*b*	*b*	*a*	*b*	*a*	*b*

1. C57BL/6J *a*
2. DBA/2J *a*
3. BALB/cA *b*
4. A/J *b*
5. CBA/J *a*
6. RF/J *b*
7. C57BL/6J *a*
8. BALB/cA *b*

a = fast
b = slow

Car2, carbonic anhydrase 2 (Chr. 3)

Tissue sample:	RBCs in 4 volumes dH_2O	
	1.2 μ l	
Buffer system:	EDTA sodium acetate; pH5.6	
	1 : 4 dilution	
Supporting media:	Cellulose acetate membrane	
Electrophoresis:	Voltage:	200V
	Time:	45 min
	Temperature:	4℃
	Migration:	anode (+) to Cathode (-)
Stain procedure:	Apply Ponceau S	
	Destain in 1% acetic acid	



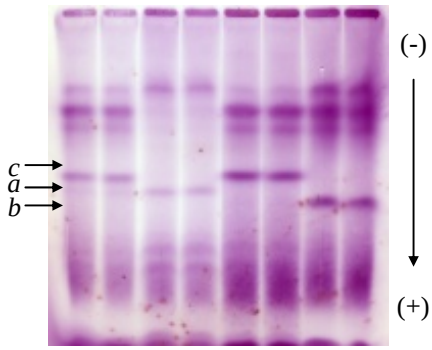
1	2	3	4	5	6	7	8
b	*b*	*a*	*a*	*a/b*	*a/b*	*b*	*a*

1. C3H/HeN *b*
2. DBA/2J *b*
3. C57BL/6J *a*
4. AKR/J *a*
5. B6D2F1 *a/b*
6. B6D2F1 *a/b*
7. DBA/2J *b*
8. C57BL/6J *a*

a = slow *b* = fast

Es3, esterase 3 (Chr. 11)

Tissue sample: Kidney in 5 weight dH_2O
1.2 μ l
Buffer system: Tris borate EDTA; pH8.4
Supporting media: Cellulose acetate membrane
Electrophoresis: Voltage: 350V
Time: 20 min
Temperature: Room temperature
Migration: Cathode (-) to anode (+)
Stain procedure: 10 ml phosphate; pH6.8
0.25 ml 1% β naphtyl acetate
50 mg fast blue RR salt
Incubate at 37℃ until bands appear (for 2 min)
Fix in 5% acetic acid solution



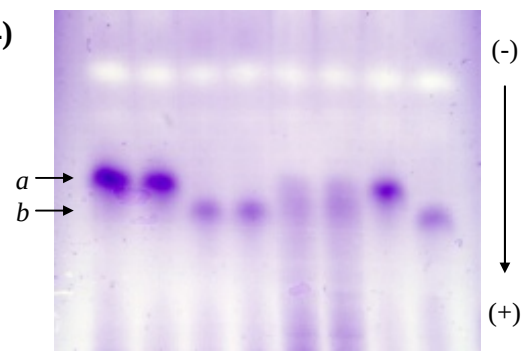
1	2	3	4	5	6	7	8
a/c	*a/c*	*a*	*a*	*c*	*c*	*b*	*b*

1. B6D2F1 *a/c*
2. B6D2F1 *a/c*
3. C57BL/6J *a*
4. BALB/cA *a*
5. DBA/2J *c*
6. A/J *c*
7. RF/J *b*
8. RF/J *b*

a = light band between "c" and "b"
b = fast
c = slow

H6pd (Gpd1), hexose-6-phosphate dehydrogenase (Chr. 4)

Tissue sample: Kidney in 5 weight dH_2O
1.5 μ l
Buffer system: Tris borate EDTA; pH8.4
Supporting media: Cellulose acetate membrane
Electrophoresis: Voltage: 350V
Time: 35 min
Temperature: 4℃
Migration: Cathode (-) to anode (+)
Stain procedure: In agar overlay:
10 ml 1% molten agar
10 ml Tris HCl; pH7.0
20 mg glucose-6-phosphate
4 mg MTT
8 mg NADP
6 gm PMS
Incubate at 37℃ until bands appear
(for 15 min.)
Fix in 5% acetic acid solution



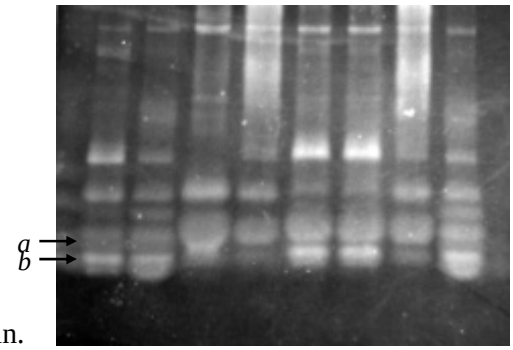
1	2	3	4	5	6	7	8
a	*a*	*b*	*b*	*a/b*	*a/b*	*a*	*b*

1. C57BL/6J a
2. C57BL/10SnJ a
3. DBA/2J b
4. A/J b
5. B6D2F1 a/b
6. B6D2F1 a/b
7. C57BL/6J a
8. DBA/2J b

a = slow *b* = fast

Mup1, major urinary protein 1 (Chr. 4)

Tissue sample:	Urine in same volume dH_2O 1 μ l
Buffer system:	Tris glycine; pH8.5
Supporting media:	Polyacrylamide gel (resoluving gel 15%)
Electrophoresis:	Voltage: 200V Time: 50 min Temperature: 4℃ Migration: Cathode (-) to anode (+)
Stain procedure:	Fix in 10% EtOH, 7% acetic acid for 30 min. Incubate in SYPRO Ruby protein gel stain for 2 hours Wash gel in 10% EtOH, 7% acetic acid for 20 min. Read under UV light



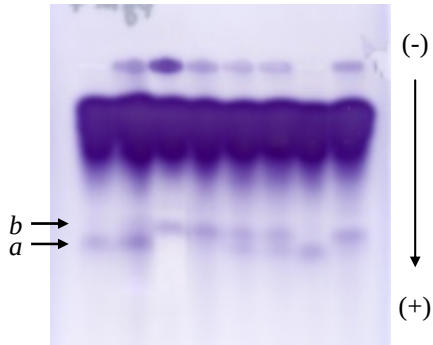
1	2	3	4	5	6	7	8
b	*b*	*a*	*a*	*a/b*	*a/b*	*a*	*b*

1. C57BL/6J *b*
2. NC/Nga *b*
3. DBA/2J *a*
4. A/J *a*
5. B6D2F1 *a/b*
6. B6D2F1 *a/b*
7. DBA/2J *a*
8. C57BL/6J *b*

a = slow *b* = fast

Pgm1, phosphoglucomutase 1 (Chr. 5)

Tissue sample:	Kidney in 5 weight dH_2O 1.2 μ l
Buffer system:	Tris borate EDTA; pH8.4
Supporting media:	Cellulose acetate membrane
Electrophoresis:	Voltage: 150V Time: 60 min Temperature: Room temperature Migration: Cathode (-) to anode (+)
Stain procedure:	In agar overlay: 10 ml 1% molten agar 9.8 ml Tris HCl; pH7.0 5 mg MTT 4 mg PMS 40 mg glucose-1-phosphate 5 mg NADP 0.1 ml glucose-1-6-phosphate (2mg/ml in dH_2O) 0.1 ml glucose-6-phosphate dehydrogenase (2 mg/ml in dH_2O) 400 μ l $MgCl_2$ (100mg/ml) Incubate at 37℃ until bands appear (for 10 min.) Fix in 5% acetic acid solution



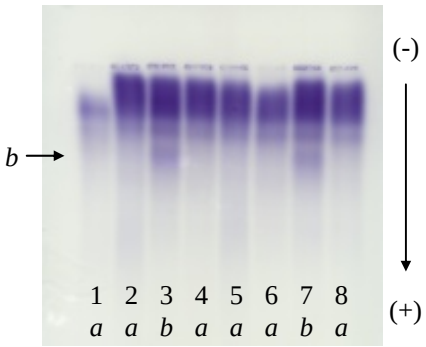
1	2	3	4	5	6	7	8
a	*a*	*b*	*b*	*a/b*	*a/b*	*a*	*b*

1. C57BL/6J *a*
2. A/J *a*
3. DBA/2J *b*
4. C3H/HeN *b*
5. B6D2F1 *a/b*
6. B6D2F1 *a/b*
7. C57BL/6J *a*
8. DBA/2J *b*

a = fast
b = slow

Ldr1, lactate dehydrogenase regulator 1 (Chr. 6)

Tissue sample:	RBCs in 4 volumes dH_2O 0.3 μ l	
Buffer system:	Tris borate EDTA; pH8.4	
Supporting media:	Cellulose acetate membrane	
Electrophoresis:	Voltage:	250V
	Time:	40 min
	Temperature:	4℃
	Migration:	Cathode (-) to anode (+)
Stain procedure:	In agar overlay: 10 ml 1% molten agar LD Vis isoenzyme reagent Incubate at 37℃ until bands appear (for 30 min.) Fix in 5% acetic acid solution	

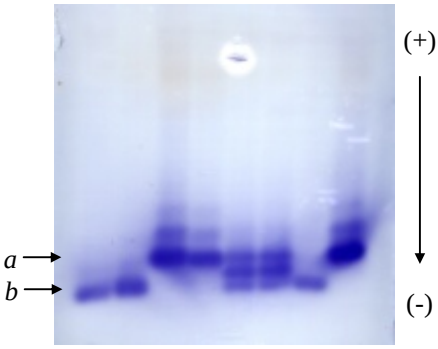


1.	C57BL/6J	*a*	5.	CBA/JMs	*a*
2.	A/J	*a*	6.	CBA/StMs	*a*
3.	CBA/H	*b*	7.	CBA/N	*b*
4.	CBA/J	*a*	8.	DBA/2J	*a*

a = absent *b* = present

Gpi1, glucose phosphate isomerase 1 (Chr. 7)

Tissue sample:	RBCs in 4 volumes dH_2O 0.3 μ l	
Buffer system:	Tris glycine; pH8.5	
Supporting media:	Cellulose acetate membrane	
Electrophoresis:	Voltage:	160V
	Time:	60 min
	Temprature:	Room temprature
	Migration:	Anode (+) to cathode (-)
Stain procedure:	1.0 ml NADP (1mg/ml) 0.1 ml Fluctose-6-Phosphate 0.1 ml magnesium acetate (5.41/100 ml) 0.1 ml MTT (10 mg/ml) 0.1 ml PMS (2.5 mg/ml) 0.2 ml Glucose-6-Phosphate (20 U/ml in dH_2O) Incubate at 37℃ until bands appear for 2 min. Fix in 5% acetic acid solution	

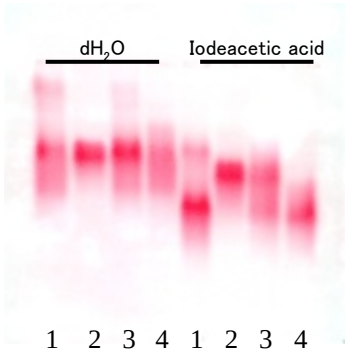


1.	C57BL/6J	*b*
2.	C3H/HeN	*b*
3.	DBA/2J	*a*
4.	A/J	*a*
5.	B6D2F1	*a/b*
6.	B6D2F1	*a/b*
7.	C57BL/6J	*b*
8.	DBA/2J	*a*

a = slow *b* = fast

Hbb, hemoglobin beta chain complex (Chr. 7)

Tissue sample-1:	RBCs in 8 volumes dH_2O	
	0.3 μ l	
Tissue sample-2:	RBCs in 2 volumes iodoacetic acid, mono	
	0.6 μ l	
Buffer system:	Tris EDTA borate; pH8.4	
Supporting media:	Cellulose acetate membrane	
Electrophoresis:	Voltage:	350V
	Time:	40 min
	Temperature:	4℃
	Migration:	Cathode (-) to anode (+)
Stain procedure:	Apply Ponceau S	
	Destain in 5% acetic acid	



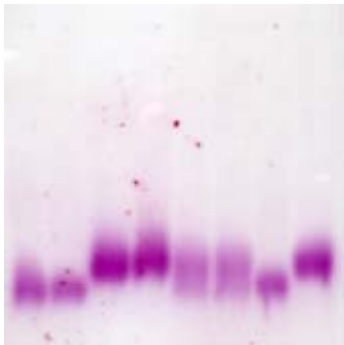
1	2	3	4	1	2	3	4
p	*s*	*p/s*	*d*	*p*	*s*	*p/s*	*d*

1. MSM/Ms *p*
2. C57BL/6J *s*
3. (B6 x MSM)F1 *p/s*
4. C3H/HeN *d*

s = single band *p* = very diffuse
d = diffuse

Es1, esterase 1 (Chr. 8)

Tissue sample-1:	Plasma undiluted	
	0.3 μ l	
Buffer system:	0.01M Phosphate; pH6.8	
Supporting media:	Cellulose acetate membrane	
Electrophoresis:	Voltage:	140V
	Time:	30 min
	Temperature:	Room temperature
	Migration:	Cathode (-) to anode (+)
Stain procedure:	10 ml phosphate; pH6.8	
	0.25 ml 1% β naphtyl acetate	
	50 mg fast blue RR salt	
	Fix in 5% acetic acid solution	



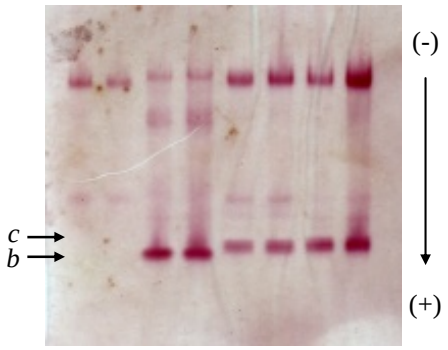
1	2	3	4	5	6	7	8
a	*a*	*b*	*b*	*a/b*	*a/b*	*a*	*b*

1. C57BL/6J *a*
2. C57BL/10SnJ *a*
3. DBA/2J *b*
4. A/J *b*
5. B6D2F1 *a/b*
6. B6D2F1 *a/b*
7. C57BL/6J *a*
8. DBA/2J *b*

a = fast *b* = slow

Es2, esterase 2 (Chr. 8)

Tissue sample:	Kidney in 5 weight dH_2O	
	2.5 μ l	
Buffer system:	Tris glycine; pH8.5	
Supporting media:	Polyacrylamide gel (resoluving gel 10%)	
Electrophoresis:	Voltage:	200V
	Time:	45 min
	Temperature:	4℃
	Migration:	Cathode (-) to anode (+)
Stain procedure:	10 ml phosphate; pH6.8	
	0.25 ml 1% β naphtyl acetate	
	50 mg fast blue RR salt	
	Fix in 5% acetic acid solution	



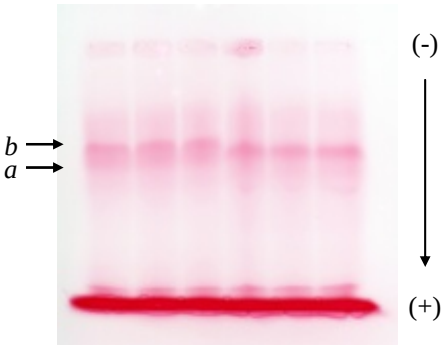
1	2	3	4	5	6	7	8
a	*a*	*b*	*b*	*c*	*c*	*c*	*c*

1. M.spretus *a*
2. M.spretus *a*
3. C57BL/6J *b*
4. C57BL/10J *b*
5. MSM/Ms *c*
6. MSM/Ms *c*
7. PWK/Rp *c*
8. PWK/Rp *c*

a = absent *b* = fast
c = slow

Trf, transferrin (Chr. 9)

Tissue sample:	Plasma undiluted	
	0.6 μ l	
Buffer system:	Tris glycine; pH8.5	
Supporting media:	Cellulose acetate membrane	
Electrophoresis:	Voltage:	150V
	Time:	50 min
	Temperature:	4℃
	Migration:	Cathode (-) to anode (+)
Stain procedure:	Apply Ponceau S	
	Destain in 5% acetic acid	



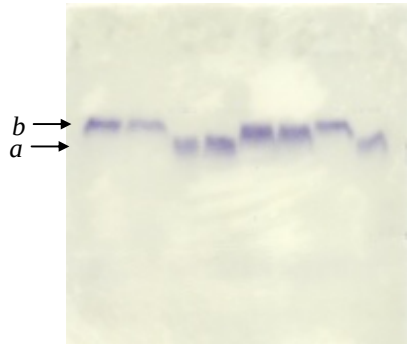
1	2	3	4	5	6
b	*b*	*b*	*a*	*a*	*a*

1. C57BL/6J *b*
2. A/J *b*
3. NC/Nga *b*
4. CBA/J *a*
5. CBA/JBrc *a*
6. CBA/Stm *a*

a = fast *b* = slow

Mod1, Malic enzyme 1 (Chr. 9)

Tissue sample:	Kidney in 5 weight dH_2O	
	0.3 μ l	
Buffer system:	Tris citrate; pH7.6	
Supporting media:	Cellulose acetate membrane	
Electrophoresis:	Voltage:	200V
	Time:	40 min
	Temperature:	4℃
	Migration:	Cathode (-) to anode (+)
Stain procedure:	9 ml Tris HCl; pH8.0	
	1 ml 0.5M Malate (pH8.0)	
	3 mg MTT	
	80 μ l $MnCl_2$ (100 mg/ml)	
	5 mg NADP	
	3 mg PMS	
	Fix in 5% acetic acid solution	



1	2	3	4	5	6	7	8
b	*b*	*a*	*a*	*a/b*	*a/b*	*b*	*a*

1. C57BL/6J *b*
2. AKR/J *b*
3. DBA/2J *a*
4. A/J *a*
5. B6D2F1 *a/b*
6. B6D2F1 *a/b*
7. C57BL/6J *b*
8. DBA/2J *a*

a = fast *b* = slow

References

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