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 dH₂O. 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 dH₂O. 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 dH₂O.

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 dH2O.

Phosphate (pH6.8)
3.77 g
8.63 g
Adjust to pH6.8 with with HCl or NaOH. Bring up to 1000 ml with dH2O.

Tris citrate (pH7.6)
12.10 g TRIS
Dissolve in 600 ml of dH2O. 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 dH2O.

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 dH2O.

Tris glycine (pH8.3)
1.50 g
7.10 g
Adjust to pH8.3 with HCl or NaOH. Bring up to 1000 ml with dH2O.

Tris glycine (pH8.5)
3.00 g
14.40 g
Adjust to pH8.5 with HCl or NaOH. Bring up to 1000 ml with dH2O.

Tris HCl (pH7.0, pH8.0, pH9.0)
24.00 g
Dissolve in 800 ml of dH2O. Adjust pH with HCl. Bring up to 1000 ml with dH2O.

Staining Reagents
3-(4,5-Dimethyl thiazolyl-2)-2', 5-diphenyl-tetrazolium Bromide (MTT) (Sigma #M2128)
Phenazine Methosulfate (PMS) (Sigma #P9625)
\(\beta\)-Nicotinamide Adenine 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)
\(\beta\)-naphthyl-phosphate acid (Sigma #N1132)
Fast blue RR Salt (Wako #069-03712)
MgCl\(_2\)・6H\(_2\)O (Sigma #M2670)
MnCl\(_2\)・4H\(_2\)O (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 μl.
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.

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 dH2O
0.3 μl
Buffer system: Tris citrate; pH8.3
Supporting media: Cellulose acetate membrane
Electrophoresis:
- Voltage: 200V
- Time: 45 min
- Temperature: 4°C
- Migration: Cathode (-) to anode (+)
Stain procedure:
- 4 mg MTT
- 3 mg PMS
- 4 mg NADP
- 40 mg DL-Isocitrate (trisodium salt)
- 100 μl MgCl₂ (100mg/ml)
- 10 ml dH₂O
Fix in 5% acetic acid solution

**Pep3, peptidase 3 (Chr. 1)**

Tissue sample: Kidney in 5 weight dH₂O
1.2 μl
Buffer system: Tris EDTA borate; pH8.4
Supporting media: Cellulose acetate membrane
Electrophoresis:
- Voltage: 300V
- Time: 30 min
- Temperature: 4°C
- 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°C until bands appear (for 30 min.)
Fix in 5% acetic acid solution
**Akp1, alkaline phosphatase 1 (Chr. 1)**

Tissue sample: Kidney in 5 weight dH2O

Buffer system: Tris citrate; pH8.3

Supporting media: Cellulose acetate membrane

Electrophoresis:
- Voltage: 200V
- Time: 45 min
- Temperature: 4°C
- Migration: Cathode (-) to anode (+)

Stain procedure:
(a) 10 mg β-naphthyl-phosphate acid
   5 ml dH2O
(b) 20 mg fast blue RR salt
   100 μl MgCl₂ (100mg/ml)
   80 μl MnCl₂ (100mg/ml)
   5 ml Tris HCl; pH9.0
Mixed (a) and (b) just before staining
Fix in 5% acetic acid solution

---

**Car2, carbonic anhydrase 2 (Chr. 3)**

Tissue sample: RBCs in 4 volumes dH2O

Buffer system: EDTA sodium acetate; pH5.6

Supporting media: Cellulose acetate membrane

Electrophoresis:
- Voltage: 200V
- Time: 45 min
- Temperature: 4°C
- Migration: anode (+) to Cathode (-)

Stain procedure:
Apply Ponceau S
Destain in 1% acetic acid

---
**Es3, esterase 3 (Chr. 11)**

Tissue sample: Kidney in 5 weight dH2O
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°C until bands appear (for 2 min)
- Fix in 5% acetic acid solution

<table>
<thead>
<tr>
<th>Lanes</th>
<th>Genotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>B6D2F1 a/c</td>
</tr>
<tr>
<td>2</td>
<td>B6D2F1 a/c</td>
</tr>
<tr>
<td>3</td>
<td>C57BL/6J a</td>
</tr>
<tr>
<td>4</td>
<td>BALB/cA a</td>
</tr>
<tr>
<td>5</td>
<td>DBA/2J c</td>
</tr>
<tr>
<td>6</td>
<td>A/J c</td>
</tr>
<tr>
<td>7</td>
<td>RF/J b</td>
</tr>
<tr>
<td>8</td>
<td>RF/J b</td>
</tr>
</tbody>
</table>

**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 dH2O
Buffer system: Tris borate EDTA; pH8.4
Supporting media: Cellulose acetate membrane
Electrophoresis:
- Voltage: 350V
- Time: 35 min
- Temperature: 4°C
- 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°C until bands appear (for 15 min.)
- Fix in 5% acetic acid solution

<table>
<thead>
<tr>
<th>Lanes</th>
<th>Genotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C57BL/6J a</td>
</tr>
<tr>
<td>2</td>
<td>C57BL/10SnJ a</td>
</tr>
<tr>
<td>3</td>
<td>DBA/2J b</td>
</tr>
<tr>
<td>4</td>
<td>A/J b</td>
</tr>
<tr>
<td>5</td>
<td>B6D2F1 a/b</td>
</tr>
<tr>
<td>6</td>
<td>B6D2F1 a/b</td>
</tr>
<tr>
<td>7</td>
<td>C57BL/6J a</td>
</tr>
<tr>
<td>8</td>
<td>DBA/2J b</td>
</tr>
</tbody>
</table>

**a** = slow
**b** = fast
**Mup1, major urinary protein 1 (Chr. 4)**

Tissue sample: Urine in same volume dH2O
Buffer system: Tris glycine; pH8.5
Supporting media: Polyacrylamide gel (resolving gel 15%)
Electrophoresis:
Voltage: 200V
Time: 50 min
Temperature: Room temperature
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

---

**Pgm1, phosphoglucomutase 1 (Chr. 5)**

Tissue sample: Kidney in 5 weight dH2O
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 dH2O)
0.1 ml glucose-6-phosphate dehydrogenase
(2 mg/ml in dH2O)
400 μl MgCl₂ (100mg/ml)
Incubate at 37°C until bands appear (for 10 min.)
Fix in 5% acetic acid solution

---
**Ldst1, lactate dehydrogenase regulator 1 (Chr. 6)**

- **Tissue sample:** RBCs in 4 volumes dH2O
- **Buffer system:** Tris borate EDTA; pH 8.4
- **Supporting media:** Cellulose acetate membrane
- **Electrophoresis:**
  - **Voltage:** 250V
  - **Time:** 40 min
  - **Temperature:** 4°C
  - **Migration:** Cathode (-) to anode (+)
- **Stain procedure:** In agar overlay:
  - 10 ml 1% molten agar
  - LD Vis isoenzyme reagent
  - Incubate at 37°C until bands appear (for 30 min.)
  - Fix in 5% acetic acid solution

<table>
<thead>
<tr>
<th>Ldst1 Allele</th>
<th>C57BL/6J</th>
<th>CBA/JMs</th>
<th>A/J</th>
<th>CBA/StMs</th>
<th>CBA/H</th>
<th>CBA/N</th>
<th>CBA/J</th>
<th>DBA/2J</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>b</td>
<td>a</td>
<td>b</td>
<td>a</td>
</tr>
<tr>
<td>b</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Gpil, glucose phosphate isomerase 1 (Chr. 7)**

- **Tissue sample:** RBCs in 4 volumes dH2O
- **Buffer system:** Tris glycine; pH 8.5
- **Supporting media:** Cellulose acetate membrane
- **Electrophoresis:**
  - **Voltage:** 160V
  - **Time:** 60 min
  - **Temperature:** Room temperature
  - **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 dH2O)
  - Incubate at 37°C until bands appear for 2 min.
  - Fix in 5% acetic acid solution

<table>
<thead>
<tr>
<th>Gpil Allele</th>
<th>C57BL/6J</th>
<th>C3H/HeN</th>
<th>DBA/2J</th>
<th>A/J</th>
<th>B6D2F1</th>
<th>B6D2F1</th>
<th>C57BL/6J</th>
<th>DBA/2J</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a/b</td>
<td>a/b</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td>b</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\[ a = slow \quad b = fast \]
**Hbb, hemoglobin beta chain complex (Chr. 7)**

Tissue sample-1: RBCs in 8 volumes dH₂O
0.3 μl

Tissue sample-2: RBCs in 2 volumes iodoacetic acid, mono
0.6 μl

Buffer system: Tris EDTA borate; pH8.4

Supporting media: Cellulose acetate membrane

Electrophoresis:
- Voltage: 350V
- Time: 40 min
- Temperature: 4°C
- Migration: Cathode (-) to anode (+)

Stain procedure: Apply Ponceau S
Destain in 5% acetic acid

---

**Es1, esterase 1 (Chr. 8)**

Tissue sample-1: Plasma undiluted
0.3 μl

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
**Es2, esterase 2 (Chr. 8)**

Tissue sample: Kidney in 5 weight dH2O
2.5 μl

Buffer system: Tris glycine; pH8.5

Supporting media: Polyacrylamide gel (resolving gel 10%)

Electrophoresis: Voltage: 200V
Time: 45 min
Temperature: 4°C
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

---

**Trf, transferrin (Chr. 9)**

Tissue sample: Plasma undiluted
0.6 μl

Buffer system: Tris glycine; pH8.5

Supporting media: Cellulose acetate membrane

Electrophoresis: Voltage: 150V
Time: 50 min
Temperature: 4°C
Migration: Cathode (-) to anode (+)

Stain procedure: Apply Ponceau S
Destain in 5% acetic acid

---
**Mod1, Malic enzyme 1 (Chr. 9)**

Tissue sample: Kidney in 5 weight dH2O
Buffer system: Tris citrate; pH7.6
Supporting media: Cellulose acetate membrane
Electrophoresis: Voltage: 200V
Time: 40 min
Temperature: 4°C
Migration: Cathode (−) to anode (+)

Stain procedure: 9 ml Tris HCl; pH8.0
1 ml 0.5M Malate (pH8.0)
3 mg MTT
80 μl MnCl₂ (100 mg/ml)
5 mg NADP
3 mg PMS
Fix in 5% acetic acid solution

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

\[ \text{a} = \text{fast} \quad \text{b} = \text{slow} \]

**References**

