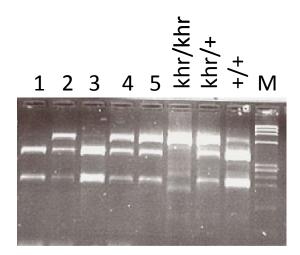
| Gene symbol Hr <sup>khr</sup> |  |
|-------------------------------|--|
|-------------------------------|--|

| primer  | Sequence(5'-3')      | Size | target gene | accession   |
|---------|----------------------|------|-------------|-------------|
| rHr-05  | GCAGGCAGCAGAATCTTTG  |      | Hr          | NW_024364.1 |
| rHr-06  | TCCTGTGGATGTCTCTGGTG |      | ///         |             |
|         |                      |      |             |             |
| comment |                      |      |             |             |
|         |                      |      |             |             |
|         |                      |      |             |             |

| PCR condition  | 1   |                |          |        |           |       |                 |
|--|-----|----------------|----------|--------|-----------|-------|-----------------|
| Taq polymerase BIOTAQTM HS DNA Polymerase (Bioline Reagents Ltd., UK)  |     |                |          |        |           |       |                 |
| Thermal cycler PC-808(ASTEC)   |     |                |          |        |           |       |                 |
| PCR buffer Ampdirect Plus  |     |                |          |        |           |       |                 |
|  |     | first denature | denature | anneal | extension | cycle | final extension |
| PCR  | °C  | 94             | 94       | 60     | 72        | 40    | 72              |
|  | min | 3              | 0.5      | 1      | 0.75      |       | 3               |
| ①PCR method; Blood was applied to FTA®card (GE Healthcare UK Ltd.,UK) and dried. 1.5mm FTA disc was removed from the bloody-stanined region on FTA®card. Untreated sample discs were placed directly in 15 μ L PCR mixture containig 1 × Ampdirect®Plus, 0.2 μ M each primer and 0.4 units of BIOTAQTM HS DNA Polymerase. ②RFLP of PCR products; <i>Hpy188I</i> cutting site in the Gja8 +/+ genome. |     |                |          |        |           |       |                 |

Electrocataphoresis ;4% agarose gel 300V, 30min



M: (φX174/Hae III digest)

Strain harboring mutation

F344-*Hr*<sup>khr</sup>/Kyo