

|                                |   |       |                |             |
|--------------------------------|---|-------|----------------|-------------|
| Gene symbol                    | <i>Cacna1a</i> <sup>gry</sup>                   |       |                |             |
| primer                         | Sequence(5'-3')                                 | Size  | target gene    | accession   |
| rCacna1a-14-F<br>rCacna1a-14-R | CGTCCTGAAGTCAATCATGAAG<br>AGTCTCAGCTGCTCTGTGGTT | 256bp | <i>Cacna1a</i> | NM_012918.1 |
| comment                        |   |       |                |             |

|                |     |   |                 |               |                  |              |                        |
|----------------|-----|---|-----------------|---------------|------------------|--------------|------------------------|
| PCR condition  |     |   |                 |               |                  |              |                        |
| Taq polymerase |     | Nova Taq Hot start DNA polymerase   |                 |               |                  |              |                        |
| Thermal cycler |     | PC-808(ASTEC)   |                 |               |                  |              |                        |
| PCR buffer     |     | Ampdirect Plus  |                 |               |                  |              |                        |
|                |     | <b>first denature</b>   | <b>denature</b> | <b>anneal</b> | <b>extension</b> | <b>cycle</b> | <b>final extension</b> |
| PCR            | °C  | 94  | 94              | 60            | 72               | 35           | 72                     |
|                | min | 3   | 0.5             | 0.5           | 1                |              | 3                      |
| comment        |     | FTAカードに採取した血液を未精製のままテンプレートとして用いる。<br>PCR Productを制限酵素 <i>PsiI</i> で処理(37°C、2時間) |                 |               |                  |              |                        |

|   |   |   |         |       |     |   |
|---|---|---|---------|-------|-----|---|
| Electrocataphoresis ;4% agarose gel 300V, 30min |   |   |         |       |     |   |
| 1   | 2 | 3 | gry/gry | gry/+ | +/+ | M |
|   |   |   |         |       |     |   |
| M: (φX174/Hae III digest)                       |   |   |         |       |     |   |

|                           |  |
|---------------------------|--|
| Strain harboring mutation | GRY/Idr, WTC.GRY-Cacna1a <sup>gry</sup> /Kyo |
|---------------------------|--|