

RBRC 04834

Strain name C57BL/6J-Slc7a11<m1Mtk>

| Primer | primer_name  | primer_seq              | length |           |
|--------|--------------|-------------------------|--------|-----------|
| 1      | 04834_common | CACAGCCCAGCTGTTCCGCAATC | 22     | RIKEN BRC |
| 2      | 04834_wild   | GACGATGGTGATGCTCTTCTCTG | 23     | RIKEN BRC |
| 3      | 04834_mut    | GACGATGGTGATGCTCTTCTCTT | 23     | RIKEN BRC |
|        |              |                         |        |           |
|        |              |                         |        |           |
|        |              |                         |        |           |
|        |              |                         |        |           |
|        |              |                         |        |           |
|        |              |                         |        |           |

**Reaction Components**

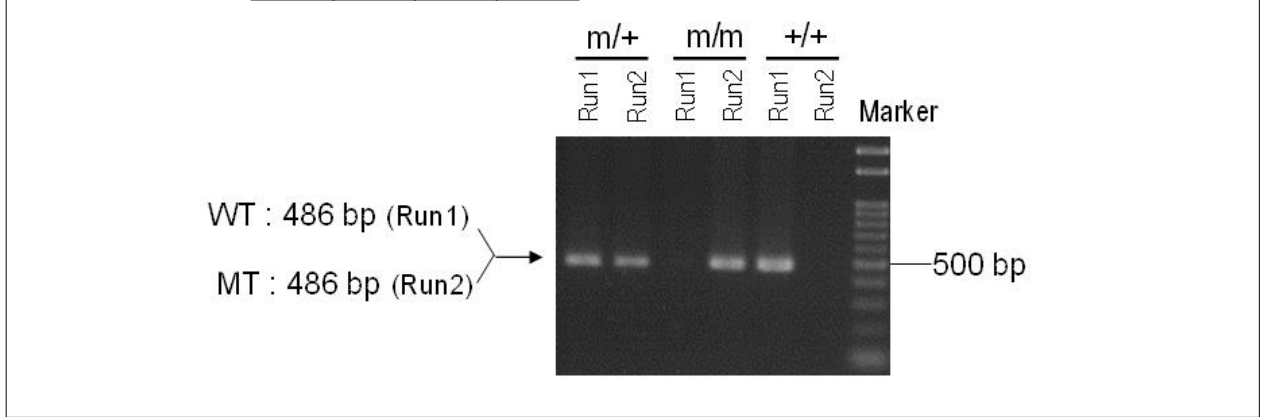
Enzyme QIAGEN Multiplex PCR Kit (Cat. No. 206143)

**Reaction Conditions**

| step | temp (°C) | time (sec) | note                  |
|------|-----------|------------|-----------------------|
| 1    | 95        | 900        |                       |
| 2    | 94        | 30*        |                       |
| 3    | 65        | 90*        |                       |
| 4    | 72        | 60*        | *Repeat for 30 cycles |
| 5    | 72        | 600        |                       |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |

|              | Conc       | Run 1     | Run 2     | Run 3 | Run 4 |
|--------------|------------|-----------|-----------|-------|-------|
| H2O          |            | 2.0       | 2.0       |       |       |
| Master Mix   | 2 X        | 5.0       | 5.0       |       |       |
| primer 1     | 10 pmol/μl | 1.0       | 1.0       |       |       |
| primer 2     | 10 pmol/μl | 1.0       | 0         |       |       |
| primer 3     | 10 pmol/μl | 0         | 1.0       |       |       |
| DNA          |            | 1.0       | 1.0       |       |       |
|              |            |           |           |       |       |
|              |            |           |           |       |       |
|              |            |           |           |       |       |
|              |            |           |           |       |       |
|              |            |           |           |       |       |
|              |            |           |           |       |       |
|              |            |           |           |       |       |
|              |            |           |           |       |       |
|              |            |           |           |       |       |
|              |            |           |           |       |       |
|              |            |           |           |       |       |
| <b>total</b> |            | <b>10</b> | <b>10</b> |       |       |

Allele specific PCR (ASP)



**Marker**  
 Gene Ladder 100 (Wako, Cat. 316-06951)

**Gel**  
 2% agarose in 1 X TAE (100 V, 30 min)

| PCR products      |              |         |  |
|-------------------|--------------|---------|--|
| primer_set        | product_size | product |  |
| primer 1 primer 2 | 486 bp       | WT      |  |
| primer 1 primer 3 | 486 bp       | mutant  |  |
|                   |              |         |  |
|                   |              |         |  |

Comment

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|        |              |                         |        |           |
|        |              |                         |        |           |
|        |              |                         |        |           |
|        |              |                         |        |           |
|        |              |                         |        |           |

**Reaction Components**

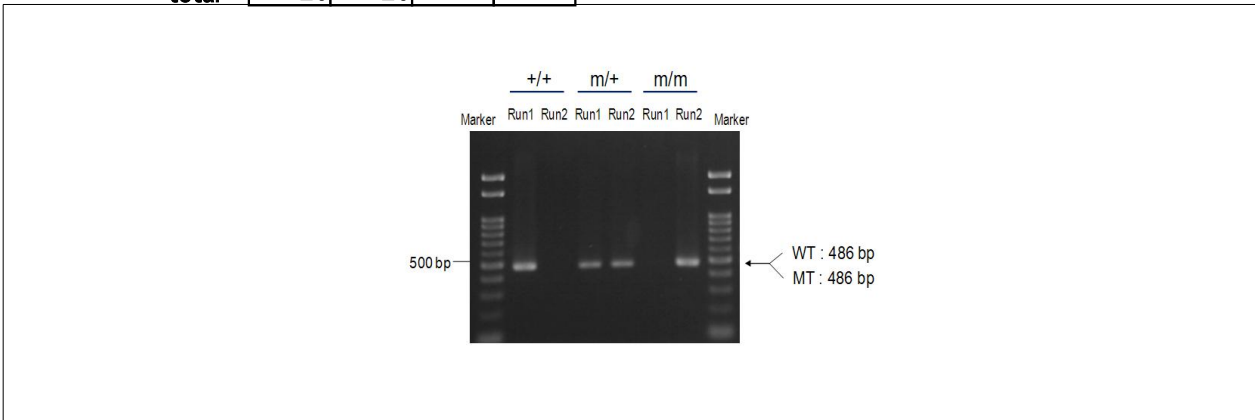
Enzyme Roche Taq (Cat. No. BM1418432)

**Reaction Conditions**

|              | Conc             | Run 1     | Run 2     | Run 3 | Run 4 |
|--------------|------------------|-----------|-----------|-------|-------|
| H2O          |                  | 13.3      | 13.3      |       |       |
| PCR buffer   | 10 X             | 2.0       | 2.0       |       |       |
| dNTP         | 2.5 mM           | 1.6       | 1.6       |       |       |
| primer 1     | 10 pmol/ $\mu$ l | 1.0       | 1.0       |       |       |
| primer 2     | 10 pmol/ $\mu$ l | 1.0       | 0         |       |       |
| primer 3     | 10 pmol/ $\mu$ l | 0         | 1.0       |       |       |
| DNA          |                  | 1.0       | 1.0       |       |       |
| Taq          | 5U/ $\mu$ l      | 0.1       | 0.1       |       |       |
|              |                  |           |           |       |       |
|              |                  |           |           |       |       |
|              |                  |           |           |       |       |
|              |                  |           |           |       |       |
|              |                  |           |           |       |       |
|              |                  |           |           |       |       |
|              |                  |           |           |       |       |
| <b>total</b> |                  | <b>20</b> | <b>20</b> |       |       |

| step | temp (°C) | time (sec) | note                  |
|------|-----------|------------|-----------------------|
| 1    | 94        | 180        |                       |
| 2    | 94        | 30*        |                       |
| 3    | 60        | 30*        |                       |
| 4    | 72        | 60*        | *Repeat for 30 cycles |
| 5    | 72        | 300        |                       |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |

Allele specific PCR (ASP)



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**Gel**  
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| PCR products |              |         |        |
|--------------|--------------|---------|--------|
| primer_set   | product_size | product |        |
| primer 1     | primer 2     | 486 bp  | WT     |
| primer 1     | primer 3     | 486 bp  | mutant |
|              |              |         |        |
|              |              |         |        |
|              |              |         |        |

**Comment**