

RBRC 11284

Strain name C57BL/6J-Cant1<em1Iwt>

| Primer | primer_name | primer_seq              | length | Reference |
|--------|-------------|-------------------------|--------|-----------|
| 1      | WT          | ACAGAGGACTCCAGGTGGGATTC | 23     | RIKEN BRC |
| 2      | MT          | ACAGAGGACTCCAGGTACCGAA  | 22     | RIKEN BRC |
| 3      | exon 2      | ACTCCACGCTGACCCTGTCC    | 20     | RIKEN BRC |
|        |             |                         |        |           |
|        |             |                         |        |           |
|        |             |                         |        |           |
|        |             |                         |        |           |
|        |             |                         |        |           |
|        |             |                         |        |           |
|        |             |                         |        |           |
|        |             |                         |        |           |

## Reaction Components

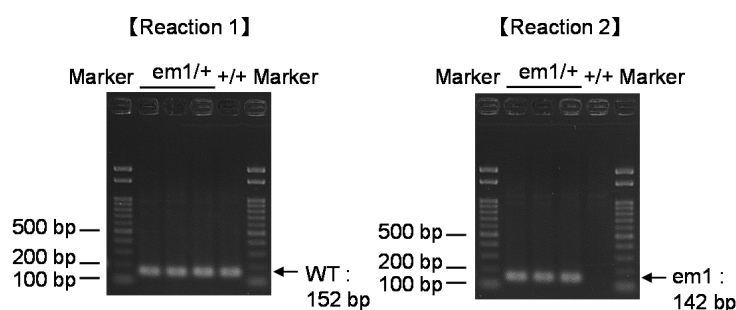
|        |                                   |
|--------|-----------------------------------|
| Enzyme | TOYOBO KOD FX (Code.No : KFX-101) |
|--------|-----------------------------------|

## Reaction Conditions

| step | temp (°C) | time (sec) | note                  |
|------|-----------|------------|-----------------------|
| 1    | 94        | 120        |                       |
| 2    | 98        | 10*        |                       |
| 3    | 60        | 30*        |                       |
| 4    | 68        | 60*        | *Repeat for 30 cycles |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |

| Reagent    | Conc       | Reaction 1 | 2    | 3 | 4 |
|------------|------------|------------|------|---|---|
| H2O        |            | 2.8        | 2.8  |   |   |
| PCR buffer | 2 X        | 10.0       | 10.0 |   |   |
| dNTP       | 2 mM       | 4.0        | 4.0  |   |   |
| primer 1   | 10 pmol/μl | 1.0        | 0    |   |   |
| primer 2   | 10 pmol/μl | 0          | 1.0  |   |   |
| primer 3   | 10 pmol/μl | 1.0        | 1.0  |   |   |
| DNA        |            | 1.0        | 1.0  |   |   |
| Taq        | 1 U/μl     | 0.2        | 0.2  |   |   |
|            |            |            |      |   |   |
|            |            |            |      |   |   |
|            |            |            |      |   |   |
|            |            |            |      |   |   |
|            |            |            |      |   |   |
| total      |            | 20         | 20   |   |   |

|        |                                        |
|--------|----------------------------------------|
| Marker | Gene Ladder 100 (Wako, Cat. 316-06951) |
| Gel    | 2% agarose in 1 X TAE (100 V, 30 min)  |



| primer_set | product_size | product (allele type) |
|------------|--------------|-----------------------|
| primer 1   | primer 3     | 152 bp                |
| primer 2   | primer 3     | 142 bp                |
|            |              |                       |
|            |              |                       |
|            |              |                       |
|            |              |                       |

Comment Allele specific PCR (ASP)

Novagen KOD Xtreme™ Hot Start DNA Polymerase can be purchased from EMD.