

RMRC Genotyping Protocol-PCR

| | | | |
|-----|------------|-------------|--|
| No. | RMRC 13411 | Strain Name | C57BL/6- <i>Irf6</i> ^{tm1CGCFC} /Narl |
|-----|------------|-------------|--|

A. Primer

| | Primer name | Sequence |
|---|-------------|--------------------------------------|
| 1 | CU | 5'-ATAGCGGCCGCTTGGGATGGAGGAGTAACA-3' |
| 2 | FD | 5'-GAAGTCGACGAGTCTGTACTCAAGGTCTC-3' |
| 3 | GU | 5'-ATAGCGGCCGCATCCACTGACCTATGTGT-3' |
| 4 | JD | 5'-GAAGTCGACTGATGAAGAAGCTCTGAGGC-3' |

B. Genomic extraction :

- Phenol-Chloroform extraction commercial extraction kit _____
 Lysis buffer _____ other Hot-Alkaline DNA extraction method with 300ul 50mM NaOH in 105°C dry bath for at least 30 min, and neutralise with 50 ul 1M Tris-HCl

C. Reaction Conditions

| Component | Concentration | | Volume | | Step | Tempera- -ture, °C | Time, min | Number of Cycles |
|------------------|---------------|-------------|--------|----|------|-----------------------|--------------|---------------------|
| | | | | | | | | |
| H ₂ O | -- | | 13.3 | μl | 1 | 95 | 5 | |
| PCR buffer | 10x | | 2 | μl | 2 | 95 | 1 | 30 |
| dNTP | 10 | mM | 0.5 | μl | 3 | 60 | 1 | 40 |
| Primer 1 | 10 | μM(ρmol/μl) | 1 | μl | 4 | 72 | 1 | 30 |
| Primer 2 | 10 | μM(ρmol/μl) | 1 | μl | 5 | 72 | 7 | |
| DNA | -- | | 2 | μl | 6 | 4 | Hold | |
| Taq | | U/μl | 0.2 | μl | 7 | | | |
| Mg ²⁺ | | mM | | μl | 8 | | | |
| Total | | | 20 | μl | 9 | | | |

The information of Taq, including company name and catalog number :

D. PCR Product

| | Primer set | | Product size | Target region |
|---|------------|----|----------------------------|---------------|
| 1 | CU | FD | WT 482 bp Target 580 bp | 5'LoxP site |
| 2 | GU | JD | WT 495 bp Target 593 bp | 3'LoxP site |

E. Gel Photo

