

RMRC Genotyping Protocol-PCR

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|------|-----------|------|-------------------------------|
| 種原編號 | RMRC13005 | 品系名稱 | C57BL/6-Tg(Cyp11a1-cre)30Mchu |
| | RMRC13006 | | C57BL/6-Tg(Cyp11a1-cre)16Mchu |

A. Primer

| | Primer name | Sequence |
|---|-------------|-----------------------|
| 1 | iCre-cF18 | gCCCAAAGAAgAAgAggAAAg |
| 2 | iCre-cR468 | TggTCAAAGTCAGTgCgTTC |

B. Reaction Conditions

| Component | Concentration | | Volume | | Step | Tempera- ture, °C | Time, sec | Number of Cycles |
|------------------|---------------|-------------|--------|----|------|----------------------|--------------|---------------------|
| | | | | | | | | |
| H ₂ O | -- | | 5.3 | μl | 1 | 94 | 300 | 1 |
| PCR buffer | 10x | | 1.0 | μl | 2 | 94 | 30 | 30 cycles |
| dNTP | 2.5 | mM | 0.5 | μl | 3 | 56 | 30 | |
| Primer 1 | 1.0 | μM(ρmol/μl) | 1.0 | μl | 4 | 72 | 60 | |
| Primer 2 | 1.0 | μM(ρmol/μl) | 1.0 | μl | 5 | 72 | 420 | 1 |
| DNA | -- | | 1.0 | μl | 6 | 4 | pause | |
| Taq | 2.0 | U/μl | 0.2 | μl | 7 | | | |
| Mg ²⁺ | | mM | | μl | 8 | | | |
| Total | | | 1.0 | μl | 9 | | | |

C. PCR Product

| | Primer set | | Product size | Target region |
|---|------------|------------|--------------|---------------|
| 1 | iCre-cF18 | iCre-cR468 | 450 bp | |
| 2 | | | bp | |

D. Gel Photo

