# RMRC Genotyping Protocol-PCR

| No. | RMRC13211 | Strain Name | C57BL/6-Tg(TcraTcrb)1100Mjb Tg(UBC-tdTomato)1Narl/Narl |
|---|---|---|---|

Genotyping Method:

1. Check the red fluorescence for Tg(UBC-tdTomato)1Narl first.

2. Check Tg(Tcra)1100Mjb & Tg(Tcrb)1100Mjb alleles via PCR protocol.

**Tg(UBC-tdTomato)1Narl**

A. Primers

| No. | Primer name | Sequence |
|---|---|---|
| 1 | DsRedF | GTGAAGCACCCCGCCGACAT |
| 2 | DsRedR | CTGCATTACGGGGCCGTCGG |

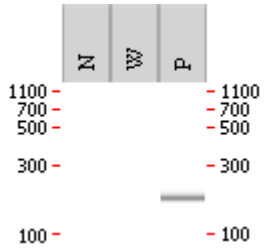
B. Reaction Conditions

| Component | Concentration | Volume | Step | Tempera-ture, °C | Time, sec | Number of cycles |
|---|---|---|---|---|---|---|
| $H_2O$ | – | 15.4 µl | 1 | 94 | 180 | 1 |
| PCR buffer | 10 x | 2 µl | 2 | 94 | 30 | 35 |
| dNTP | 10 mM | 0.4 µl | 3 | 60 | 30 | 35 |
| Primer 1 | 10 µM (ρmol/µl) | 0.5 µl | 4 | 72 | 30 | 35 |
| Primer 2 | 10 µM (ρmol/µl) | 0.5 µl | 5 | 72 | 180 | 1 |
| gDNA | 50-250 ηg/µl | 1 µl | | | | |
| Taq | 5 U/µl | 0.2 µl | | | | |
| $Mg^{2+}$ | mM | µl | | | | |
| Total | | 20 µl | | | | |

C. PCR Product

| No. | Primer set | | Product size | Target region |
|---|---|---|---|---|
| 1 | DsRedF | DsRedR | 195 bp | DsRed |
| 2 | | | bp | |

D. Gel Photo




**Tg(Tcra)1100Mjb & Tg(Tcrb)1100Mjb**

A. Primer

| | Primer name | Sequence |
|---|---|---|
| 1 | oIMR1321 | CAG CAG CAG GTG AGA CAA AGT |
| 2 | oIMR1322 | GGC TTT ATA ATT AGC TTG GTC C |
| 3 | oIMR8744 | CAA ATG TTG CTT GTC TGG TG |
| 4 | oIMR8745 | GTC AGT CGA GTG CAC AGT TT |

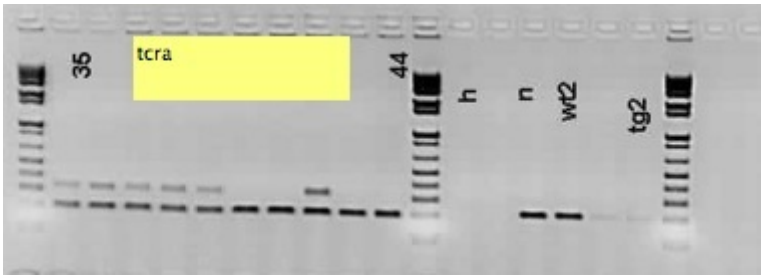
B. Reaction Conditions

| Component | Concentration | | Volume | | Step | Tempera-ture, °C | Time, sec | Number of Cycles |
|---|---|---|---|---|---|---|---|---|
| $H_2O$ | -- | | 4.36 | µl | 1 | 94 | 180 | |
| PCR buffer | 10x | | 1.2 | µl | 2 | 64 | 30 | 35 |
| dNTP | 2.5 | mM | 0.96 | µl | 3 | 62 | 60 | 35 |
| oIMR1321 | 20 | µM(pmol/µl) | 0.2 | µl | 4 | 72 | 60 | 35 |
| oIMR1322 | 20 | µM(pmol/µl) | 0.2 | µl | 5 | 72 | 120 | |
| oIMR8744 | 20 | µM(pmol/µl) | 0.2 | µl | 6 | 10 | ∞ | |
| oIMR8745 | 20 | µM(pmol/µl) | 0.2 | µl | 7 | | | |
| DNA | -- | | 2 | µl | 8 | | | |
| DNA Loading Dye | 5 | mM | 1.66 | µl | 9 | | | |
| Taq | 5 | U/µl | 0.06 | µl | | | | |
| $Mg^{2+}$ | 25 | mM | 0.96 | µl | | | | |
| Total | | | 12 | µl | | | | |

C. PCR Product

| | Primer set | | Product size | Target region |
|---|---|---|---|---|
| 1 | oIMR1321 | oIMR1322 | ~350 bp | Tg(Tcra)1100Mjb |
| 2 | oIMR8744 | oIMR8745 | 200 bp | (Internal Positive Control) |

D. Gel Photo




E. Primer

| | Primer name | Sequence |
|---|---|---|
| 1 | oIMR0675 | AAG GTG GAG AGA GAC AAA GGA TTC |
| 2 | oIMR0676 | TTG AGA GCT GTC TCC |
| 3 | oIMR8744 | CAA ATG TTG CTT GTC TGG TG |
| 4 | oIMR8745 | GTC AGT CGA GTG CAC AGT TT |

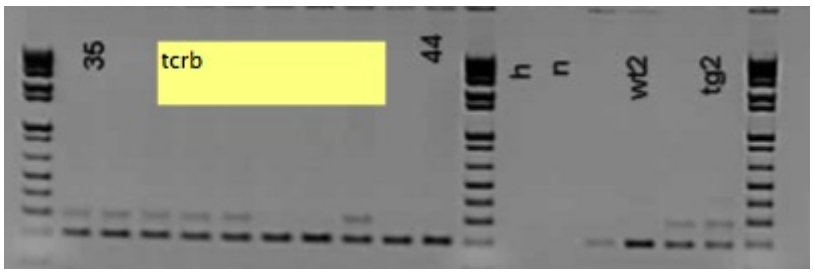
F. Reaction Conditions

| Component | Concentration | | Volume | | Step | Tempera-ture, °C | Time, sec | Number of Cycles |
|---|---|---|---|---|---|---|---|---|
| H2O | -- | | 3.63 | µl | 1 | 94 | 180 | |
| PCR buffer | 10x | | 1.2 | µl | 2 | 94 | 30 | 35 |
| dNTP | 2.5 | mM | 0.96 | µl | 3 | 52 | 45 | 35 |
| oIMR0675 | 20 | µM(pmol/µl) | 0.7 | µl | 4 | 72 | 45 | 35 |
| oIMR0676 | 20 | µM(pmol/µl) | 0.7 | µl | 5 | 72 | 120 | |
| oIMR8744 | 20 | µM(pmol/µl) | 0.04 | µl | 6 | 10 | ∞ | |
| oIMR8745 | 20 | µM(pmol/µl) | 0.04 | µl | 7 | | | |
| DNA | -- | | 2 | µl | 8 | | | |
| DNA Loading Dye | 5 | mM | 1.66 | µl | 9 | | | |
| Taq | 5 | U/µl | 0.12 | µl | | | | |
| $Mg^{2+}$ | 25 | mM | 0.96 | µl | | | | |
| Total | | | 12.01 | µl | | | | |

G. PCR Product

| | Primer set | | Product size | Target region |
|---|---|---|---|---|
| 1 | oIMR0675 | oIMR0676 | 300 bp | Tg(Tcrb)1100Mjb |
| 2 | oIMR8744 | oIMR8745 | 200 bp | (Internal Positive Control) |

H. Gel Photo


35
tcrb
44
h
n
wt2
tg2