

## RMRC Genotyping Protocol-PCR

|     |           |             |                                  |
|-----|-----------|-------------|----------------------------------|
| No. | RMRC13342 | Strain Name | C57BL/6-Tg(MMTV-MCTS1)9Hsin/Narl |
|-----|-----------|-------------|----------------------------------|

### A. Primer

|   | Primer name   | Sequence (5' to 3')  |
|---|---------------|--|
| 1 | F-MCT1-ECO    | CCC GAA TTC GCC ACC ATG TTC AAG AAA TTT GAT GAA AAA<br>GAA AAT GTG (48 mer)        |
| 2 | R-MCT1-3F-XBA | CCC TCT AGA TTT ATT TCA GTT ATC TAA TTT GCG GCC GCC TAT<br>TTA TCG TCA TC (53 mer) |

### B. Reaction Conditions

| Component        | Concentration |             | Volume |    | Step | Temperature, °C | Time, sec | Number of Cycles |
|------------------|---------------|-------------|--------|----|------|-----------------|-----------|------------------|
| H <sub>2</sub> O | --            |             | 7.7    | μl | 1    | 95              | 120       | 1                |
| PCR buffer       | 10x           |             | 1      | μl | 2    | 95              | 60        | 30               |
| dNTP             | 25            | mM          | 0.2    | μl | 3    | 58.2            | 60        | 30               |
| Primer 1         | 10            | μM(ρmol/μl) | 0.2    | μl | 4    | 72              | 60        | 30               |
| Primer 2         | 10            | μM(ρmol/μl) | 0.2    | μl | 5    | 72              | 600       | 1                |
| DNA              | --            |             |        | μl | 6    | 12              | ∞         |                  |
| Taq              | 5             | U/μl        | 0.1    | μl | 7    |                 |           |                  |
| Mg <sup>2+</sup> | 20            | mM          |        | μl | 8    |                 |           |                  |
| Total            |               |             | 10     | μl | 9    |                 |           |                  |

### C. PCR Product

|   | Primer set |               | Product size | Target region         |
|---|------------|---------------|--------------|-----------------------|
| 1 | F-MCT1-ECO | R-MCT1-3F-XBA | ~700 bp      | For heterozygous only |

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| Oligo ID | Name          | Sequence(5'to3')  | Size (mer) | GC CONTENT | MELT TEMP (°C) | MOLECULAR WEIGHT (g/mole) | EXTINCTION COEFFICIENT (L/mole.cm) | nmole /OD260 | ug/OD260 | QUANTITY (OD260) | ul to 100uM | Special |
|----------|---------------|---|------------|------------|----------------|---------------------------|------------------------------------|--------------|----------|------------------|-------------|---------|
| 10161455 | F-MCT1-ECO    | CCC GAA TTC GCC ACC ATG TTC AAG AAA TTT<br>GAT GAA AAA GAA AAT GTG        | 48         | 37.5%      | 66.2°C         | 14,800.7                  | 483,300.0                          | 2.07         | 30.62    | 6                | 124         | OPC     |
| 10161456 | R-MCT1-3F-XBA | CCC TCT AGA TTT ATT TCA GTT ATC TAA TTT<br>GCG GCC GCC TAT TTA TCG TCA TC | 53         | 39.6%      | 68.4°C         | 16,115.5                  | 485,300.0                          | 2.06         | 33.21    | 6                | 124         | OPC     |

Forward primer : (位置: 120604236- 120604247)

[Download](#) [GenBank](#) [Graphics](#)

Homo sapiens malignant T-cell amplified sequence 1 (MCTS1), transcript variant 1, mRNA  
Sequence ID: [NM\\_014060.2](#) Length: 9805 Number of Matches: 1

Range 1: 348 to 381 [GenBank](#) [Graphics](#) [Next Match](#) [Previous Match](#)

| Score         | Expect | Identities  | Gaps     | Strand    |
|---------------|--------|-------------|----------|-----------|
| 63.9 bits(34) | 8e-09  | 34/34(100%) | 0/34(0%) | Plus/Plus |

```
Query 15 CATGTTCAAGAAATTTGATGAAAAAGAAAATGTG 48
          |||
Sbjct 348 CATGTTCAAGAAATTTGATGAAAAAGAAAATGTG 381
```

Range 3: 120603889 to 120604247 [GenBank](#) [Graphics](#) [Next Match](#) [Previous Match](#) [First Match](#)

| Score         | Expect | Identities    | Gaps      | Strand    |
|---------------|--------|---------------|-----------|-----------|
| 664 bits(359) | 0.0    | 359/359(100%) | 0/359(0%) | Plus/Plus |

```
Query 1 AAATGCTACTTTGATTGATTCTCCGCCCTCCATTCTTCTCTTCTGCAATTGCTCTAT 60
Sbjct 120603889 AAATGCTACTTTGATTGATTCTCCGCCCTCCATTCTTCTCTTCTGCAATTGCTCTAT 120603948
Query 61 CACTTCTTCCCTCTCAAGTCCCGCCCTTTCAGCTACCTCCAAGTCTGAGGAACCGGTTG 120
Sbjct 120603949 CACTTCTTCCCTCTCAAGTCCCGCCCTTTCAGCTACCTCCAAGTCTGAGGAACCGGTTG 120604008
Query 121 CCTAAAAGGAGCCGGCAAAAAGCGCCTACGTGGAGTCCAGAGGAGCGGAAGTAGTCAGATT 180
Sbjct 120604009 CCTAAAAGGAGCCGGCAAAAAGCGCCTACGTGGAGTCCAGAGGAGCGGAAGTAGTCAGATT 120604068
Query 181 TGACTGAGAGCCGTAAGCGCGGCTGGCTCTCGTTTCCGGATAACGACTACAGCTCCGA 240
Sbjct 120604069 TGACTGAGAGCCGTAAGCGCGGCTGGCTCTCGTTTCCGGATAACGACTACAGCTCCGA 120604128
Query 241 CTGTCAAGTCCCGGCTTCTCGTGTGAGGGGATCTGCCGGACCCCTGCAAATTC AATTC 300
Sbjct 120604129 CTGTCAAGTCCCGGCTTCTCGTGTGAGGGGATCTGCCGGACCCCTGCAAATTC AATTC 120604188
Query 301 TTTCCATTCCGGGCCCTTCCCTATCGTCGCCCCCTCACCTGGATCATGTTCAAGAA 359
Sbjct 120604189 TTTCCATTCCGGGCCCTTCCCTATCGTCGCCCCCTCACCTGGATCATGTTCAAGAA 120604247
```

Reverse Primer (位置: )

抱歉目前找不到正確的位置

D. Gel Photo

gDNAgenotyping\_F5-511-514\_100V1%40min.\_20120709

Exposure Time : 400 mS

1 kb ladder



511

512

513

514

Neg

Pos



F-MCT1-ECO & R-MCT1-3F-XBA Target Site: ~700 bp