

# GENOTYPING BY PCR PROTOCOL

## MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

[mmrrc@ucdavis.edu](mailto:mmrrc@ucdavis.edu)

530-754-MMRRC

NAME OF PCR: C57BL/6J Tg(Ttr-HfeMyc)1Nca/Mmucd MMRRC # 036292-UCD

Protocol: *(PCR protocol provided by Donating Investigator)*

| Reagent/ Constituent                                                                                                                                                    | Volume (μL) |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------|
| Water                                                                                                                                                                   | 13.5        |
| 10x Buffer (20mM MgCl <sub>2</sub> )                                                                                                                                    | 2.5         |
| dNTPs (stock concentration is 10 mM)                                                                                                                                    | 0.5         |
| Primer 1 (stock concentration is 10 μM)                                                                                                                                 | 2.0         |
| Primer 2 (stock concentration is 10 μM)                                                                                                                                 | 2.0         |
| Taq Polymerase                                                                                                                                                          | 0.5         |
| DNA sample extracted <input type="checkbox"/> NaOH <input checked="" type="checkbox"/> Proteinase K <input checked="" type="checkbox"/> Invitrogen Purelink genomic DNA | 4.0         |
| <b>TOTAL VOLUME OF REACTION:</b>                                                                                                                                        | <b>25μL</b> |

Comments on protocol:

Strategy:

| Steps                                                     | Temp (°C) | Time (m:ss) | # of Cycles |
|-----------------------------------------------------------|-----------|-------------|-------------|
| 1. Initiation/Melting HOT START? <input type="checkbox"/> | 94        | 3:00 min    | 1           |
| 2. Denaturation                                           | 94        | 0:20 sec    | } 35        |
| 3. Annealing } steps 2-3-4 will cycle in sequence         | 61        | 0:30 sec    |             |
| 4. Elongation                                             | 72        | 0:30 sec    |             |
| 5. Amplification                                          | 72        | 2:00 min    | 1           |
| 6. Finish                                                 | 4         | n/a         | n/a         |

Primers:

| Name     | Nucleotide Sequence (5' - 3') |
|----------|-------------------------------|
| 1: PS133 | GGAATGGGACGAGCAC              |
| 2: PS134 | GATGGCACAGATGGTG              |

Electrophoresis Protocol:

Agarose: 2% V: 180 Estimated Running Time: Depends on size of gel min

| Primer Combination | Band   | Genotype |
|--------------------|--------|----------|
| PS133+PS134        | 450 bp | Tg+      |

