# GENOTYPING BY PCR PROTOCOL FORM

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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**DNA Extraction Method:** NaOH _____ Proteinase K _____ Other QiagenGenomic DNA Isolation Kit

**Protocol: NAME OF PCR: B6;FVB-Tg(CMV-ENPP1 )#Xxx/Mmcd, MMRRC #032878-UCD;**

| Reagent/ Constituent | Volume (uL) |
|---|---|
| DNA Sample | 5 |
| 10x Buffer (contains 15mM MgCl2) | 2.5 |
| 25 mM $MgCl_2$ | 2.5 |
| dNTPs (stock concentration is 25mM) | .2 |
| Primer **1** (stock concentration is 10 uM) | .5 |
| Primer **2** (stock concentration is 10 uM) | .5 |
| Primer **3** (stock concentration is 20 uM) | |
| Primer **4** (stock concentration is 20 uM) | |
| Taq Polymerase | 0.2 |
| Additives if applicable: $dH_2O$ | 13.6 |
| **TOTAL VOLUME OF REACTION:** | **25 ul** |

***Comments on protocol*** *(e.g.,* different concentration of MgCl2, etc): _____

_____

**Strategy:**

| Steps | Temp ($^oC$ ) | Time (min) | # of Cycles |
|---|---|---|---|
| 1. Initiation/Melting HOT START?..CHECK HERE [ ] | 94 | 2 | **1** |
| 2. Denaturation | 94 | 15 sec | |
| 3. Annealing } steps 2-3-4 will cycle in sequence | 55 | 30 sec | } 25 |
| 4. Elongation | 72 | 45 sec | |
| 5. Amplification (i.e., 72$^o$C, 10 min) | 72 | 5 | **1** |
| 6. Finish (i.e., 4$^o$C, indefinite) | | n/a | n/a |

**Primers:**

| Primer Name | Nucleotide Sequence ($5^-$ - $3^-$) |
|---|---|
| **1: PC1EcoR1/L** | **TTT GCC GAT TGA GGA TTT TC** |
| **2: PC1EcoR1/R** | **AGT GCA AAG GCG TCT GAG AT** |
| **3:** | |
| **4:** | |

**Electrophoresis Protocol:**

**% Agarose: 2 V : 100**

**Estimated Running Time (min): 30**

| Primer combination | Band (kB) | genotype |
|---|---|---|
| **(i.e. 1&2)** | ~.643 | * |
| **(i.e. 3&4)** | | |
| **(i.e. 1&2&3)** | | |

1 2 3 4 5 6 7 8 9 10 11 12 13

Lanes:
1. DNA ladder (AllStar)
2,3, 7 9 -11 ENPP1 pos Tg
4-6,8. ENPP1 neg Tg
12. ENPP1 positive control
13. $dH_2O$