# GENOTYPING BY PCR PROTOCOL FORM

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

Investigator/PI: MMRRC Address: 2795 2nd Street, Suite 400, Davis, CA 95618 Contact: Reneé Araiza
Telephone: 530-754-MMRRC FAX: 530-757-3284 email: mmrrc@ucdavis.edu

**DNA Extraction Method:** NaOH _____ Proteinase K __X___ Other_____

**Protocol:** **NAME OF PCR: C57BL/6J-*Scn10a*$^{m1Btlr}$/Mmcd (*Possum*), MMRRC #030627-UCD**

| Reagent/ Constituent | Volume (uL) |
|---|---|
| DNA Sample | 1 |
| 10x Buffer (contains 15mM MgCl2) | 5 |
| dNTPs (stock concentration is 25mM) | 0.4 |
| Primer **1** (stock concentration is 20 uM) | 1 |
| Primer **2** (stock concentration is 20 uM) | 1 |
| Primer **3** (stock concentration is 20 uM) | |
| Primer **4** (stock concentration is 20 uM) | |
| Taq Polymerase | 2.5 |
| water | 39.1 |
| **TOTAL VOLUME OF REACTION:** | **50 ul** |

***Comments on protocol*** *(e.g.,* different concentration of MgCl2, etc): *Possum* genotyping is performed by amplifying the region containing the mutation using PCR, followed by sequencing of the amplified region to detect the single nucleotide transversion. Use primers 1 & 2 for amplification and 3 & 4 for sequencing.

**Strategy:**

| Steps | Temp ($^{o}$C ) | Time (min) | # of Cycles |
|---|---|---|---|
| 1. Initiation/Melting HOT START?..CHECK HERE [ ] | 94 | 2 | **1** |
| 2. Denaturation | 94 | 30 seconds | |
| 3. Annealing } steps 2-3-4 will cycle in sequence | 60 | 30 seconds | **30x** |
| 4. Elongation | 72 | 1 min | |
| 5. Amplification (i.e., 72$^{o}$C, 10 min) | 72 | 7 | **1** |
| 6. Finish (i.e., 4$^{o}$C, indefinite) | 4 | n/a | n/a |

**Primers:**

| Primer Name | Nucleotide Sequence (5$^{-}$ - 3$^{-}$) |
|---|---|
| **1:**Scn10a_PossumF | GCCTAGCACCATCACTGTCAAGAAG |
| **2:**Scn10a_PossumR | TGAAACAGATGGCAGACAAGCCTC |
| **3:** Possum_seqF | AGATGTAGTCCTGGCTGACCTC |
| **4:** Possum_seqR | TCAATTTAGCGACCACTGGAAG |

**Electrophoresis Protocol:**

**% Agarose:** _____ **V :** _____

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

| Primer combination | Band (bp) | genotype |
|---|---|---|
| **(i.e. 1&2)** | **602** | |
| **(i.e. 3&4)** | | |
| **(i.e. 1&2&3)** | | |

**The flanking sequences of mutation sites:**

**Wt** AACCTG**A**CCTTCA
***Possum*** AACCTG**G**CCTTCA