Gene: Odaph ENSMUSG00000096035, Gm1045, LOC381651

**Genotyping protocol for Odaph-C41X**

NM\_001177577.1:c.123C>A

First, perform PCR using the following primer set

Odaph-P2-F: ATTCCTCCATAAAATCACATTTGTGCTGA
Odaph-P2-R: AATGTGTAATCCAAACTCCTTGTTGTTGA; RC: TCAACAACAAGGAGTTTGGATTACACATT

Amplicon size: 996 bp

Each PCR reaction contained 10 µL of Platinum Hot Start PCR Master Mix (2x) (Invitrogen, Carlsbad, CA, USA), 1 µL of 10 µM primer mix, 2 µL of DNA template (final conc. <500 ng/rxn) and raised to 20 µL with distilled water. The reactions were run using a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA, USA) Thermocycler.

The reaction conditions were: template denaturation @ 94 °C for 2 min, then [30 cycles of 94 °C for 30 s (template denaturation) then 59 °C for 30 s (primer annealing) followed by 72 °C for 60 s (primer extension)], 72 °C for 2 min and then hold at 4 °C.

>*Odaph*-WT

ATTCCTCCATAAAATCACATTTGTGCTGATCCTCATTCTGACTTCTTACCTTAGCTCCACTTTGCTTATTCATGAACACTGTACACCTTTTTGTGTTTAATTTCCATTGTTTATTGTAGCTTGGATTCATCTGGGTGTCTAGGGCCTGGCTGGTTTTGCTTTTCATTCTGGGGACTGTTTCATTGTATGAAGTATATGGTTTATTTAGCTATCCTCCTATTGATGGGGGCATTTGGATTGTTTAGGTTACTGTAACCTATTTAGTCTTCTAGTGTCTTTGATAAATATGTATTTATTTCTTTTGGACCAGTTCCCCAGATAAATATGTGGATCGTTAGCTGTGTCCATGCATCATCTCCTTGAGTCACTGCAGTTCAGTCATGCTTTAAAATGTCTCTGTTTGATTCTTATCTCTCTCCAAGCCTCACCCGGAGGGATCTAACTGTCTTTTGTTTCTGCTGCTAGGCCCATTTCCTGACCTACATTTTCATTTCCACAGGACAAGATGTAGTCACCCCTCCTGGCGGCTCACAAAATAACGCAAAGCCTACAGACTGCCABglIIGATCTTCACACTCACTCCTCCGCCCACCACAAGGAATCTGGTAACAAGGGCCCAGCCCATCCCAAGGACACCCACGTTTTCTTTTCCACCAAGGGGGCCGGGCTTCTCCCCGAGGTTCCCTTTCTTCCTTCCAAACAACCGCCGCTTCCAGTTCTGGCCATTCTACAGGCCGCGAGGTAGACTGATCCCTTGGCGACTCATCCTTAGAAGACAGCAGCAGAGCGGAAGCTCATCTGAGGAGAGCAGGGAGAACTGAGAAGCGCAGACTTGTTGAAGCAAAAGAATCGGCGCCTCTGGAAAGGGTGATGAAACTGTTTCGCTCTCCAATCTAAAATCCCTGCGTTGGAAGATTAAGTACCCTCAATGACAGTGACGAGATTTTTTTTCTCTTTCTTTCTTTTTTTTAATCAACAACAAGGAGTTTGGATTACACATT

>*Odaph*-C41X

ATTCCTCCATAAAATCACATTTGTGCTGATCCTCATTCTGACTTCTTACCTTAGCTCCACTTTGCTTATTCATGAACACTGTACACCTTTTTGTGTTTAATTTCCATTGTTTATTGTAGCTTGGATTCATCTGGGTGTCTAGGGCCTGGCTGGTTTTGCTTTTCATTCTGGGGACTGTTTCATTGTATGAAGTATATGGTTTATTTAGCTATCCTCCTATTGATGGGGGCATTTGGATTGTTTAGGTTACTGTAACCTATTTAGTCTTCTAGTGTCTTTGATAAATATGTATTTATTTCTTTTGGACCAGTTCCCCAGATAAATATGTGGATCGTTAGCTGTGTCCATGCATCATCTCCTTGAGTCACTGCAGTTCAGTCATGCTTTAAAATGTCTCTGTTTGATTCTTATCTCTCTCCAAGCCTCACCCGGAGGGATCTAACTGTCTTTTGTTTCTGCTGCTAGGCCCATTTCCTGACCTACATTTTCATTTCCACAGGACAAGATGTAGTCACCCCTCCTGGCGGCTCACAAAATAACGCAAAGCCTACcGAtTGaCAGATtTTtACcCTgACTCCTCCGCCCACCACAAGGAATCTGGTAACAAGGGCCCAGCCCATCCCAAGGACACCCACGTTTTCTTTTCCACCAAGGGGGCCGGGCTTCTCCCCGAGGTTCCCTTTCTTCCTTCCAAACAACCGCCGCTTCCAGTTCTGGCCATTCTACAGGCCGCGAGGTAGACTGATCCCTTGGCGACTCATCCTTAGAAGACAGCAGCAGAGCGGAAGCTCATCTGAGGAGAGCAGGGAGAACTGAGAAGCGCAGACTTGTTGAAGCAAAAGAATCGGCGCCTCTGGAAAGGGTGATGAAACTGTTTCGCTCTCCAATCTAAAATCCCTGCGTTGGAAGATTAAGTACCCTCAATGACAGTGACGAGATTTTTTTTCTCTTTCTTTCTTTTTTTTAATCAACAACAAGGAGTTTGGATTACACATT

Then, perform enzyme digestion usingBglII  (NEB, Ipswich, MA, USA)

Enzyme digestion at 37⁰C for 30 mins in a GeneAmp PCR System Thermocycler

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| BglII Enzyme 0.6 ul10xNEBuffer (3.1) 2.5 ulPCR product 10 ulddH2O 11.9 ulTotal 25 ul |
|

WT band=436 and 560 bp

MU (C41X) band=996 bp



DNA ladder: 1 Kb Plus DNA Ladder (Invitrogen, Carlsbad, CA, USA)