Gene: Odaph ENSMUSG00000096035, Gm1045, LOC381651

**Genotyping protocol for Odaph-Ins** (NM\_001177577.1:c.117\_118insA and NM\_001177577.1:c.127\_128delinsTGA)

First, use the following primer set for PCR amplification

Odaph-Int1 -F: GTTCCCCAGATAAATATGTGGATCGT

Odaph-Ex2-R: GTTGTTTGGAAGGAAGAAAGGGAAC; RC: GTTCCCTTTCTTCCTTCCAAACAAC

Amplicon size: WT=390 bp; MU=392 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 30 s (primer extension)], 72 °C for 2 min and then hold at 4 °C.

WT: TACA GACTGCCAGAT CTTCACACTC

MU: TACAAGACTGCCAGTGACTTCACACTC

>Odaph-WT

GTTCCCCAGATAAATATGTGGATCGTTAGCTGTGTCCATGCATCATCTCCTTGAGTCACTGCAGTTCAGTCATGCTTTAAAATGTCTCTGTTTGATTCTTATCTCTCTCCAAGCCTCACCCGGAGGGATCTAACTGTCTTTTGTTTCTGCTGCTAGGCCCATTTCCTGACCTACATTTTCATTTCCACAGGACAAGATGTAGTCACCCCTCCTGGCGGCTCACAAAATAACGCAAAGCCTACAGACTGCCAGATCTTCACACTCACTCCTCCGCCCACCACAAGGAATCTGGTAACAAGGGCCCAGCCCATCCCAAGGACACCCACGTTTTCTTTTCCACCAAGGGGGCCGGGCTTCTCCCCGAGGTTCCCTTTCTTCCTTCCAAACAAC

>Odaph-Ins

GTTCCCCAGATAAATATGTGGATCGTTAGCTGTGTCCATGCATCATCTCCTTGAGTCACTGCAGTTCAGTCATGCTTTAAAATGTCTCTGTTTGATTCTTATCTCTCTCCAAGCCTCACCCGGAGGGATCTAACTGTCTTTTGTTTCTGCTGCTAGGCCCATTTCCTGACCTACATTTTCATTTCCACAGGACAAGATGTAGTCACCCCTCCTGGCGGCTCACAAAATAACGCAAAGCCTACAAGACTGCBsrICAGTGACTTCACACTCACTCCTCCGCCCACCACAAGGAATCTGGTAACAAGGGCCCAGCCCATCCCAAGGACACCCACGTTTTCTTTTCCACCAAGGGGGCCGGGCTTCTCCCCGAGGTTCCCTTTCTTCCTTCCAAACAAC

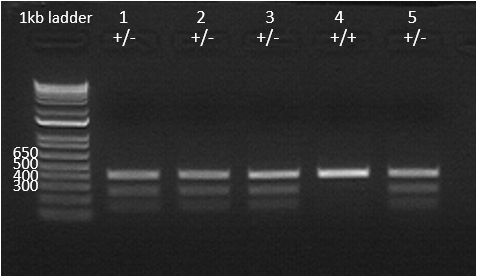
Then, perform enzyme digestion using Enzyme digestion BsrI Bsr-I-cutsite_1 (NEB, Ipswich, MA, USA)

Enzyme digestion at 65⁰C for 30 mins in a GeneAmp PCR System Thermocycler

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| --- |
| BsrI Enzyme 0.6 ul 10xNEBuffer (3.1) 2.5 ul PCR product 10 ul ddH2O 11.9 ul Total 25 ul |
|

WT band=390 bp

MU band=142 and 250 bp



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