



Welcome to The Genotyping Protocol System

Master Protocol

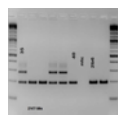
Strain Name: C57BL/6NJ-*Olfml2b*^{em1J/J}
Stock Number: 027477
Allele: *Olfml2b*^{em1J}
Protocol Name: *Olfml2b*^{em1J}
Method: Standard PCR
Created: 24-July -2015 (JKELMEN) **Updated:** 03-November -2015 (ESCHAAB)

Notes

Notes: This allele from project *Olfml2b*-6834J-F3656 was generated at The Jackson Laboratory by injecting Cas9 RNA and 3 guides sequences, ATACAGTCGCACTTCCCAAG, AATCACCTACTATAAAGCCA and GGGATTTTCATCCTTAGTGCA which resulted in a 390bp deletion beginning in intron 5 at GGCCCTCTTGGGAAGTGC GACTGTAT at Chromosome 1 positive strand position 170,666,451 bp (GRCm38) and ending after TCAGGGATTTTCATCCTTAGTG CAG at position 170,666,840 bp in intron 6. This mutation deletes exon 5 and is predicted to cause amino acid sequence changes after residue 241 and early truncation 13 amino acids later.

Expected Results: Mutant = 280 bp
Heterozygote = 193 bp and 280 bp
Wild type = 193 bp

Attachments



View [Olf-27477 Gel.jpg](#)

- View [Olfml2bem1jMolDesc.docx](#)

- View [Olfml2b genomic.gcs](#)

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
23448	-	CCA GGC TGG ATC CTA AAG GT	-	Common	-
24395	-	CAG GAC GAA CAG TGA TGG AG	-	Wild type Reverse	-
24396	-	AGC CTT GGT TTC CCT ATC TGT	-	Mutant Reverse	-

Reaction/Components A

Reaction Components	Volume Amt	Final Concentration	Total Volume Amt
ddH2O	4.25	-	-
5 X Kapa 2G HS buffer	2.40	1	-
25 mM MgCl ₂	0.96	2	-
10 mM dNTP KAPA	0.24	.2	-
20 uM 23448	0.30	.5	-
20 uM 24395	0.30	.5	-

Cycling

Step #	Temp°C	Time	Note
1	94	2 min	-
2	94	20sec	-
3	65	15sec	-0.5 C per cycle decrease
4	68	10sec	-
5	-	-	repeat steps 2-4 for 10 cycles

Reaction/Components A				Cycling			
20 uM 24396	0.30	.5	-	6	94	15sec	-
5 mM 10x Loading Dye	1.20	.5	-	7	60	15sec	-
2.5 U/ul Kapa 2G HS taq polymerase	0.05	.01	-	8	72	10sec	-
DNA	2.00	-	-	9	-	-	repeat steps 6-8 for 28 cycles
				10	72	2 min	-
				11	10	-	hold

Number Of Reactions 1

Version 3.2