



Welcome to The Genotyping Protocol System

Master Protocol

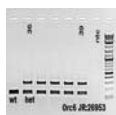
Strain Name: C57BL/6NJ-*Orc6*^{em1J/J}
Stock Number: 026953
Allele: *Orc6*^{em1J}
Protocol Name: *Orc6*^{em1J}
Method: MELT
Created: 17-July -2015 (JKELMEN) **Updated:** 18-August -2015 (ESCHAAB)

Notes

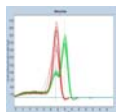
Notes: This allele from project *Orc6*-6540J M-8033 was generated at The Jackson Laboratory by injecting Cas9 RNA and 3 guides sequences, CATCTGTTCCAGCAGTAAATG, AACTGCATCCGGTGTGAAAA and TACTAAACCACTTTATTTCGC which resulted in a 435bp deletion beginning in intron 4 at (CTCATTTACTGCTGAACAGATGAA) Chromosome 8 positive strand position 85304977bp (GRCm38) and ending after (CTGCGAATAAAGTGGTTTAGTAC) at position 85305411bp in intron 5. This mutation deletes exon 5 and is predicted to cause amino acid sequence changes after residue 150 and early truncation 6 amino acids later.

Expected Results: Mutant = 295 bp
Heterozygote = 151 bp and 295 bp
Wild type = 151 bp

Attachments



View [Orc6-26953 Gel.jpg](#)



View [Orc6 Mplex Melt.jpg](#)

- View [Orc6_em1j__6-30-15\[1\].docx](#)

- View [Orc6 genomic.gcs](#)

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
22454	-	TCC ATT CAG CAA ATT GGT AAC	-	Common	-
24314	-	GAA GCT TGC AGA TTT GCT CTC	-	Wild type Reverse	-
24315	-	AGG GCC TAG TCT TGG GTT CT	-	Mutant Reverse	-

Reaction/Components A

Reaction Components	Volume Amt	Final Concentration	Total Volume Amt
ddH2O	4.85	-	-
5 X Kapa 2G HS buffer	2.40	1	-
25 mM MgCl2	0.96	2	-
10 mM dNTP KAPA	0.24	.2	-

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	-

Reaction/Components A				Cycling			
20 uM 22454	0.30	.5	-	5	-	-	repeat steps 2-4 for 10 cycles
20 uM 24314	0.30	.5	-	6	94	15sec	-
20 uM 24315	0.30	.5	-	7	60	15sec	-
2.5 U/ul Kapa 2G HS taq polymerase	0.05	.01	-	8	72	10sec	-
20 X EvaGreen	0.60	1	-	9	-	-	repeat steps 6-8 for 28 cycles
DNA	2.00	-	-	10	72	2 min	-
				11	10	-	hold

Number Of Reactions 1

Version 3.2