Print Logout

The Jackson Laboratory
Leading the search for tomorrow's cures

Welcome to The Genotyping Protocol System

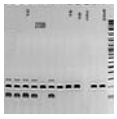
Master Protocol

Strain Name: C57BL/6NJ-*Tmco1*^{em1J}/J
Stock Number: 027200
Allele: Tmco1^{em1J}
Protocol Name: Tmco1^{em1J}
Method: Standard PCR
Created: 17-July -2015 (JKELMEN) **Updated:** 11-September-2015 (ESCHAAB)

Notes

Notes:	This allele from project Tmco1-6660J M-1122 was generated at The Jackson Laboratory by injecting Cas9 RNA and 2 guide sequences, ATAAGACGAAGTGAATATGT and CAACAATAGAGACCTGTCAA which resulted in a 281bp deletion beginning in intron 3 at (TATTCACTTCGTCTTATTGA) Chromosome 1 positive strand position 167316119bp (GRCm38) and ending after (AGTTCTTAAAATGTATTACCT) at position 167316399bp in intron 4. This mutation deletes exon 4 and is predicted to cause amino acid sequence changes after residue 4 and early truncation 11 amino acids later.
Expected Results:	Mutant = 110 bp Heterozygote = 110 bp and 207 bp Wild type = 207 bp

Attachments



View 27200.jpg

- View Tmco1 genomic.gcs
- View Tmco1em1j_6-30-15.docx

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
24325	-	CCA TTG TTT TAG TAT GGA GTA AGC A	-	Common	-
24326	-	CCA TTG ACA GGT CTC TAT TGT TGT	-	Wild type Reverse	-
24327	-	TGA AAT TTA AAT GTG GCA GCA	-	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 MgCl2	0.96	2	-
10 mM dNTP KAPA	0.24	.2	-
20 uM 24325	0.30	.5	-
20 uM 24326	0.30	.5	-
20 uM 24327	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
6	94	15sec	-

Reaction/Components A			
5 mM 10x Loading Dye	1.20	.5	-
2.5 U/ul Kapa 2G HS taq polymerase	0.05	.01	-
DNA	2.00	-	-

Cycling			
7	60	15sec	-
8	72	10sec	-
9	-	-	repeat steps 6-8 for 28 cycles
10	72	2 min	-
11	10	-	hold

Number Of Reactions 1

Version 3.2