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Welcome to The Genotyping Protocol System

Master Protocol

Strain Name: C57BL/6NJ-*Serpina3n*^{em1J}/J
Stock Number: 027571
Allele: Serpina3n^{em1J}
Protocol Name: Serpina3n^{em1J}
Method: Standard PCR
Created: 24-July -2015 (JKELMEN) **Updated:** 22-October -2015 (ESCHAAB)

Notes

Notes:	This allele from project Serpina3n-6845J-M9384 was generated at The Jackson Laboratory by injecting Cas9 RNA and 3 guides sequences, CTCAGAAGCGGTGTTAACTG, AGTGCCCATGATGAGCATGG and CCTGTTCTGTCCTCAGCCTA which resulted in a 402 bp deletion and an 8 bp insertion (ACAGTGTA) in intron 3 at Chromosome 12 positive strand position 104,411,059 bp (TAACTGAGGAGAAGGTGGAGTCTCTG in GRCm38) and ending after CTGTTCTGTCCTCAGCCTAAGGCC at position 104,411,460 bp in intron 4. This mutation deletes exon 3 and is predicted to cause amino acid sequence changes after residue 212 and early truncation 1 amino acid later.
Expected Results:	Mutant = 488 bp Heterozygote = 232 bp and 488 bp Wild type = 232 bp

Attachments

View 27571.jpg

- View Serpina3n genomic1.gcs
- View Serpina3nem1jMolDesJuly2015[1].docx

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
23480	-	AAC CAA GCC TGG AGA CAA TG	-	Common	-
24414	-	CGT GTC AAG AGG GTC AAA GG	-	Wild type Reverse	-
24415	-	TTA AGT CAG AGC CTG GCA CA	-	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 23480	0.30	.5	-
20 uM 24414	0.30	.5	-
20 uM 24415	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	-
7	60	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			
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