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The Jackson Laboratory
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Welcome to The Genotyping Protocol System

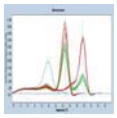
Master Protocol

Strain Name: C57BL/6NJ-*Sebox*^{em1J}/J
Stock Number: 028354
Allele: Sebox^{em1J}
Protocol Name: Sebox^{em1J}
Method: MELT
Created: 21-October -2015 (JKELMEN) **Updated:** 05-August -2016 (LUCASM)

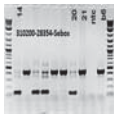
Notes

Notes:	This allele from project Sebox-7066J-M4984 was generated at The Jackson Laboratory by injecting Cas9 RNA and 3 guide sequences, ATCAAGGCTTTGGGGCTGGG, CAACTGCCCGACACTGAAAG, TCTGTCCCACAAGGCTGTGC, which resulted in a 304 bp deletion beginning in intron 2 at Chromosome 11 positive strand position 78,503,682bp, GGGCTGGGTGGAGGCTCTTGC, and ending after TTTCTGTCCCACAAGGCTGTG at 78,503,985bp (GRCm38/mm10) in intron 3. The 304 bp mutation deletes all of exon 2 and is predicted to cause a change in amino acid sequence after amino acid residue 10 and early truncation 80 amino acids later.
Expected Results:	Mutant = 131 bp Heterozygote = 131 bp and 435 bp Wild type = 435 bp

Attachments



View 28354.JPG



View 310200-28354-sebox.jpg

\- View Seboxem1jMolDesOct2015.docx

\- View Sebox genomic.gcc

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
25315	-	GAA GGG AGA GAG TAG AGG TTG C	-	Forward	A
25316	-	CTC CTG GCT TTC TGT CCT TG	-	Reverse	A

Reaction/Components A

Reaction Components	Volume Amt	Final Concentration	Total Volume Amt
ddH2O	4.55	-	-
5 X Kapa 2G HS buffer	2.40	1	-
25 mM MgCl2	0.96	2	-
10 mM dNTPS-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			
20 U Primer 1	0.30	.5	-
20 U Primer 2	0.30	.5	-
5 mM 10x Loading Dye	1.20	.5	-
2.5 U/ul Kapa 2G HS taq polymerase	0.05	.01	-
DNA	2.00	-	-

Cycling			
5	-	-	repeat steps 2-4 for 10 cycles
6	94	15sec	-
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