



Welcome to The Genotyping Protocol System

Master Protocol

Strain Name: C57BL/6NJ-*Mrpl3^{em1J}*/*J*
Stock Number: 027695
Allele: Mrpl3-6966-KOMP CRISPR
Protocol Name: Mrpl3-6966-KOMP CRISPR
Method: High Resolution Melting
Created: 22-September-2015 (JKELMEN) **Updated:** 24-November -2015 (ESCHAAB)

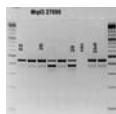
Notes

Notes: This allele from project Mrpl3-6966J F#0101 was generated at The Jackson Laboratory by injecting Cas9 RNA and 4 guide sequences:
 CACTTCTTATAATAACACGG
 , AGTAGGTGCTAGGAATTCCA, ACAAACCACACCATTCCCTG,
 CAAGCCATACTGTAATTAAG, TTATCTGGAAACAAATAAGC, which resulted in a 355 bp deletion beginning in intron 2 at Chromosome 9 positive strand position 105,054,289 (CCCCGTGTTATTATAAGAAGTGT) and ending after (GATGTGACCCCTTAATTAC) at 105054643 bp (GRCm38/mm10). This mutation deletes exon2 and is predicted to cause amino acid sequence changes after residue 31 and early truncation 45 amino acids later.

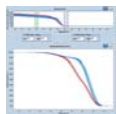
In addition there is a 37 bp deletion after the major 355bp deletion in intron 3 which would not effect the exon deletion results.

Expected Results: Mutant = 391 bp
 Heterozygote = 391 bp and 445 bp
 Wild type = 445 bp

Attachments



View Mrpl3-27695 Gel.jpg



View Mrpl3 HRM.JPG

- View Mrpl3em1j_moldesc_9-18_in progress.docx

- View Mrpl3 genomic.gcs

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
23832	-	CTG TCA AGT ACA GAT TCA TGT GG	-	Common	-
25070	-	TGG TTC CCA GGG ATG TAA AG	-	Wild type Reverse	-
25071	-	TCT GGA ACT ATG CTT CCC AAA C	-	Mutant Reverse	-

Reaction/Components A

Reaction Components	Volume Amt	Final Concentration	Total Volume Amt

Cycling

Step #	Temp °C	Time	Note
1	94	2 min	-

Reaction/Components A				Cycling			
ddH2O	4.85	-	-	2	94	20sec	-
5 X Kapa 2G HS buffer	2.40	1	-	3	65	15sec	-0.5 C per cycle decrease
25 mM MgCl2	0.96	2	-	4	68	10sec	-
10 mM dNTP KAPA	0.24	.2	-	5	-	-	repeat steps 2-4 for 10 cycles
20 uM 23832	0.30	.5	-	6	94	15sec	-
20 uM 25070	0.30	.5	-	7	60	15sec	-
20 uM 25071	0.30	.5	-	8	72	10sec	-
2.5 U/ul Kapa 2G HS taq polymerase	0.05	.01	-	9	-	-	repeat steps 6-8 for 28 cycles
20 X EvaGreen	0.60	1	-	10	72	2 min	-
DNA	2.00	-	-	11	10	-	hold

Number Of Reactions 1

Version 3.2