



Welcome to The Genotyping Protocol System

Master Protocol

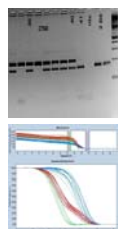
Strain Name: C57BL/6NJ-*Rab11fp4^{em1J}*
Stock Number: 027568
Allele: Rab11fp4^{em1J}
Protocol Name: Rab11fp4^{em1J}
Method: High Resolution Melting
Created: 24-July -2015 (JKELMEN) **Updated:** 23-February -2016 (LUCASM)

Notes

Notes: This allele from project Rab11fp4-6842J-M9341 was generated at The Jackson Laboratory by injecting Cas9 RNA and 3 guides sequences, CGTCTACAAGTGCCTGCA, ATGACATTGGCCCTACCTGA and ACGGGTACTTTCTAGCTCCG, which resulted in a 405bp deletion beginning in intron 4 at CGTGACTCAGCCACCATGAGAG at Chromosome 11 positive strand position 79,680,652 bp (GRCm38) and ending after TTTCTAGCTCCGGGCCACATGCC at position 79,681,056 bp in intron 5. This mutation deletes exon 4 and is predicted to cause amino acid sequence changes after residue 112 and early truncation 28 amino acids later.

Expected Results: Mutant = 219 bp
Heterozygote = 168 bp and 219 bp
Wild type = 168 bp

Attachments



View 27568.jpg

View 27568-GS.JPG

- View Rab11fp4em1jMolDescJuly2015.docx
- View Rab11fp4 genomic1.gcs

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
24397	-	TGC CCA GTC CTC AAG GTA GT	-	Common	-
24398	-	ACC CGT CTA CAA GTG CGT CT	-	Wild type Reverse	-
24399	-	ACA TAC TGA GCC CGT CTT CC	-	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	-

Cycling

Step #	Temp°C	Time	Note
1	94	2 min	-
2	94	20sec	-
3	65	15sec	-0.5 C per cycle decrease

Reaction/Components A				Cycling			
10 mM dNTP KAPA	0.24	.2	-	4	68	10sec	-
20 uM 24397	0.30	.5	-	5	-	-	repeat steps 2-4 for 10 cycles
20 uM 24398	0.30	.5	-	6	94	15sec	-
20 uM 24399	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