



Welcome to The Genotyping Protocol System

Master Protocol

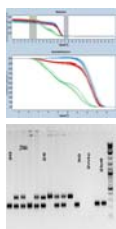
Strain Name: C57BL/6NJ-Klra7^{em1J}/J
Stock Number: 027444
Allele: Klra7^{em1J}
Protocol Name: Klra7^{em1J}alternate1
Method: High Resolution Melting
Created: 20-October -2015 (JKELMEN) **Updated:** 27-October -2015 (ESCHAAB)

Notes

Notes: This allele from project Klra7-6781J-M7991 was generated at The Jackson Laboratory by injecting Cas9 RNA and 3 guide sequences: TCTTGTA CTGTGCATAACC, CAGTCCTCACTAGTTTCTGC and GACATGGACTGACCAAATT which resulted in a 241 bp deletion beginning in 5' upstream sequence at Chromosome 6 negative strand position 130,231,784 bp, beginning TCAGGGTGT TTTATAGCATTAAG, and ending after GTTGCAGAACTAGTGAGGAC in exon 1 at 130,231,544 bp (GRCm38/mm10). This mutation deletes the 5-prime UTR region and part of exon 1 and is predicted to not produce a protein product.

Expected Results: Mutant = 384 bp
Heterozygote = 242 bp and 384 bp
Wild type = 242 bp

Attachments

[View](#) 27444.JPG[View](#) 27444.jpg

- [View](#) Klra7em1jMolDescJuly2015.docx
- [View](#) Klra7 genomic1.gcs

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
23245	-	CAT TAG CTC ATT GGG GCT TC	-	Mutant Forward	-
25288	-	TAG TCA GAC CCC ACC CTT TC	-	Wild type Forward	-
25289	-	AGC TTC TCT GGG CCT TTG AG	-	Common	-

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 23245	0.30	.5	-	5	-	-	repeat steps 2-4 for 10 cycles
20 uM 25288	0.30	.5	-	6	94	15sec	-
20 uM 25289	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