



Welcome to The Genotyping Protocol System

Master Protocol

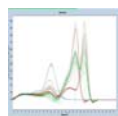
Strain Name: C57BL/6NJ-Krt80^{em1J/J}
Stock Number: 027793
Allele: Krt80-6977-KOMP CRISPR
Protocol Name: Krt80-6977-KOMP CRISPR
Method: MELT
Created: 22-September-2015 (JKELMEN) **Updated:** 17-November -2015 (ESCHAAB)

Notes

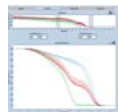
Notes: This allele from project Krt80-6977J M#3128 was generated at The Jackson Laboratory by injecting Cas9 RNA and 4 guide sequences:
 ACTTCCTCTTTCTCCACCTT,CACCCCACCAGAGTACAGCC,TGACAGGGAGCCCCACCTCG,
 AGATGAGCTGCCCTGTGACA, which resulted in a 378 bp deletion in intron 2 beginning at Chromosome 15 negative strand position 101,364,511bp(GTGACAGGGAGCCCCACCTCG) and ending after (CTGGTGGGGTGCACCCAAG) at 101,364,134bp (GRCm38/mm10). This mutation deletes exon 2 and is predicted to cause an early stop after amino acid residue 101.

Expected Results: Mutant = 327 bp
 Heterozygote = 251 bp and 327 bp
 Wild type = 251 bp

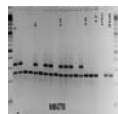
Attachments



View 27793.JPG



View 27793-hrm.JPG



View Krt80-27793 Gel.jpg

- View Krt80em1j_moldesc_9-18-15_in progress.docx

- View Krt80 genomic1.gcs

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
25076	-	TGG TCT CCA CCT TCA TCC TC	-	Common	-
25077	-	GTC TCC AGC AGT TGG TTG C	-	Wild type Reverse	-
25078	-	CAG GCT CAG GAG AGA CTG CT	-	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 25076	0.30	.5	-	6	94	15sec	-
20 uM 25077	0.30	.5	-	7	60	15sec	-
20 uM 25078	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