



Welcome to The Genotyping Protocol System

**Master Protocol**

**Strain Name:** C57BL/6NJ-Krt77<sup>em1J</sup>J  
**Stock Number:** 025927  
**Allele:** Krt77<sup>em1J</sup>-EP  
**Protocol Name:** Krt77<sup>em1J</sup>-EP  
**Method:** End Point Analysis  
**Created:** 11-March -2015 (JKELMEN) **Updated:** 02-June -2015 (JKELMEN)

**Notes**

Notes: This allele from project Krt77-5764J-A was generated at The Jackson Laboratory by injecting Cas9 RNA and guide sequence CACTGGCACTTCGTCCTCCACT, which resulted in a 10 bp deletion **GGACGAAGTG** in exon1 beginning at Chromosome 15 negative strand position 101869399-101869408 bp (GRCm38/mm10) and is predicted to cause an amino acid change after residue 70 and a frameshift mutation with early truncation 68 residues later.

Expected Results: Mutant =-----/-----  
 Heterozygote = ggacgaagtg /-----  
 Wild type = ggacgaagtg / ggacgaagtg

**Attachments**

View 276877-ep3.jpg

- View Krt77 CRISPR KO.gcs
- View Krt77 genomic.gcs
- View Molecular description of Krt77-ex1 CRISPR KO\_1-27-15.docx

**Sequence**

Sequence  
 GGGCCTATAGCTCCAGGTCTTCATCAGGCTTTGGAGGTGGGAGACAGGCTCTGGTGTCT  
 GT  
 GAGTCAGTCGAGGAGGTATGGCGGTGATTATGGTGGTGGGTTCCAGTCCAGGAGTCTCT  
 A  
 CAGTCTGGGTGGTAGTAAAGCATCTTTGGGAACCTAGTG[**ggacgaagtg**]CCAGTGGTTTC  
 T  
 GCCAGAGTAGAGGGCCAGGAGGAGGATTTGGAGGAGGAATCGGAGGAGGAATTGGAGG  
 AG  
 GAAGAGGCTTTGGTGGAGGTGGCTTTGGTGGTGGCTATGGCGGAGGTGGCAGATTTGG  
 AG  
 GTGGTTTTGGGGG

**Protocol Primers**

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
22946	-	TTA TGG TGG TGG GTT CAG TTC	-	Forward	-
22947	-	CTC CTC CTG GCC CTC TAC TC	-	Reverse	-
22948	Hex	AGT GGG ACG AAG TGC CAG	Black Hole Quencher 1	WT Probe	-
22949	6-FAM	GGA ACC TAG TGC CAG TGG TTT C	Black Hole Quencher 1	MUT Probe	-

Reaction/Components A				Cycling			
Reaction Components	Volume Amt	Final Concentration	Total Volume Amt	Step #	Temp°C	Time	Note
2 X Kapa Probe Fast QPCR	2.50	.42	-	1	95	10min	-
ddH2O	7.44	-	-	2	95	15sec	-
100 uM 22946	0.02	.17	-	3	60	1min	repeat steps 2-3 for 40 cycles
100 uM 22947	0.02	.17	-				
100 uM 22948	0.01	.08	-				
100 uM 22949	0.01	.08	-				
DNA	2.00	-	-				

Number Of Reactions 1

Version 3.2