



Welcome to The Genotyping Protocol System

Master Protocol

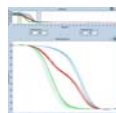
Strain Name: C57BL/6NJ-*Iqj*^{em1J}/*J*
Stock Number: 026272
Allele: *Iqj*^{em1J}-HRM
Protocol Name: *Iqj*^{em1J}-HRM
Method: High Resolution Melting
Created: 11-March -2015 (JKELMEN) **Updated:** 22-April -2015 (JKELMEN)

Notes

Notes: This allele from project *Iqj*-5923J-A (line 103_M_4293) was generated at The Jackson Laboratory by injecting Cas9 RNA and guide sequence CCTTTAGAACAAGTTGACGA, which resulted in an 11 bp deletion **AACAAGTTGAC** in exon2 beginning at Chromosome 3 positive strand position 68041225 bp (GRCm38/mm10) and a 6 bp insertion **TAAATG**. This mutation is predicted to cause amino acid sequence changes after residue 13 and an early truncation 8 amino acid later.

Expected Results: Mutant = 55 bp
Heterozygote = 55 bp and 66 bp
Wild type = 66 bp

Attachments



View IQCJ- 26272 PR.JPG

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
22940	-	GGA AGA ACT AAA GAG ACT ACA GAA TCC	-	Forward	-
22941	-	TTC TCA AGC AAA TAT TTT CCA TC	-	Reverse	-

Reaction/Components A

Reaction Components	Volume Amt	Final Concentration	Total Volume Amt
ddH2O	5.15	-	-
5 X Kapa 2G HS buffer	2.40	1	-
25 mM MgCl ₂	0.96	2	-
10 mM dNTP KAPA	0.24	.2	-
20 uM 22940	0.30	.5	-
20 uM 22941	0.30	.5	-
2.5 U/ul Kapa 2G HS taq polymerase	0.05	.01	-
20 X EvaGreen	0.60	1	-
DNA	2.00	-	-

Cycling

Step #	Temp °C	Time	Note
1	94	2 min	-
2	94	20sec	-
3	65	15sec	-0.5 C per cycle decrease
4	68	10sec	-
5	-	-	repeat steps 2-4 for 10 cycles
6	94	15sec	-
7	60	15sec	-
8	72	10sec	-
9	-	-	repeat steps 6-8 for 28 cycles

Cycling			
10	72	2 min	-
11	10	-	hold

Number Of Reactions 1

Version 3.2