



Welcome to The Genotyping Protocol System

Master Protocol

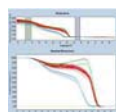
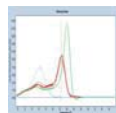
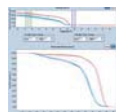
Strain Name: C57BL/6NJ-Trip13^{em1J/J}
Stock Number: 027094
Allele: Trip13^{em1J}
Protocol Name: Trip13^{em1J}alternate1
Method: High Resolution Melting
Created: 15-September-2015 (JKELMEN) **Updated:** 25-October -2015 (ESCHAAB)

Notes

Notes: This allele from project Trip13-6637J F-2781 was generated at The Jackson Laboratory by injecting Cas9 RNA and 3 guide sequences, CACTAAAGTATAGCTAGGTC, TTGTGTTTGGAGATTACGTC and CGGGTTACTACCTATCTGT which resulted in a 556bp deletion beginning in intron 1 at (CTAGCTATACTTTAGTGGG) Chromosome 13 negative strand position 73936559bp (GRCm38) and ending after (TAGCGGGTTACTACCTATC) at position 73936004 bp in intron 2. This mutation deletes exon 2 and is predicted to cause amino acid sequence changes after residue 31 and early truncation 3 amino acids later.

Expected Results: Mutant = 366 bp
Heterozygote = 229 bp and 366 bp
Wild type = 229 bp

Attachments

[View](#) 27094.JPG[View](#) Trip13-27094 Gel.jpg[View](#) Trip13 Mplex Melt.jpg[View](#) Trip13 HRM.JPG

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[View](#) Trip13em1j_6-30-15.docx

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
24316	-	AGG GAA GGT GTG TGT CTG TG	-	Common	-
24317	-	CCT GTT CCG AAA AAC CAA GA	-	Wild type Reverse	-
24992	-	AGC TGC CAA GAT GCT ACA CA	-	Mutant Reverse	-

Reaction/Components A

Cycling

Final

Total

Step

Temp°C

Time

Note

Reaction/Components A				Cycling			
Reaction Components	Volume Amt	Concentration	Volume Amt	#			
ddH2O	4.85	-	-	1	94	2 min	-
5 X Kapa 2G HS buffer	2.40	1	-	2	94	20sec	-
25 mM MgCl2	0.96	2	-	3	65	15sec	-0.5 C per cycle decrease
10 mM dNTP KAPA	0.24	.2	-	4	68	10sec	-
20 uM 24316	0.30	.5	-	5	-	-	repeat steps 2-4 for 10 cycles
20 uM 24317	0.30	.5	-	6	94	15sec	-
20 uM 24992	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