



Welcome to The Genotyping Protocol System

Master Protocol

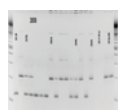
Strain Name: C57BL/6NJ-Zfp641^{em1J}J
Stock Number: 028358
Allele: Zfp641^{em1J}
Protocol Name: Zfp641^{em1J}alternate1
Method: MELT
Created: 21-January -2016 (JKELMEN) **Updated:** 25-February -2016 (ESCHAAB)

Notes

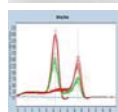
Notes: This allele from project Zfp641-7067J-M4958 was generated at The Jackson Laboratory by injecting Cas9 RNA and 3 guide sequences, ACGGGAACATAACAAGCCAGT, GCAGCCGCACCTTCTGCAGC, and AAGTGCATCCTGGGAAAGAT, which resulted in a 214 bp deletion beginning in intron 3 at Chromosome 15 negative strand position 98,293,024 bp, GAAAGATGGGTAGTTCATC, and ending after CTTTGGCATCATCCCACTGG at 98,292,811 bp (GRCm38/mm10) in intron 4. The 214 bp mutation deletes all of exon 3 and is predicted to result in a change of amino acid sequence after amino acid 61 and early truncation 64 amino acids later.

Expected Results: Mutant = 121 bp
Heterozygote = 121 bp and 206 bp
Wild type = 206 bp

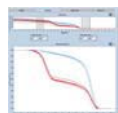
Attachments



View 28358.jpg



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View 321583-28358 Zfp641 alt1 hrn.JPG

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View Zfp641 genomic1.gcs

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
25271	-	AGG GGG AGT AAA GCT CAC CT	-	Wild type Reverse	A
26474	-	TTT ACC AGT GAG CAC AGA TGG A	-	Common	A
26475	-	AAA CAT TGC ACA GCC CTT AAA C	-	Mutant Reverse	A

Number Of Reactions 1

Reaction A (3 primer)		Cycling (TouchDown 65-60_12-12-11)			
Component	Final Concentration	Step #	Temp°C	Time	Note
ddH2O		1	94	2 min	-
		2	94	20sec	-

Reaction A (3 primer)			Cycling (TouchDown 65-60_12-12-11)			
Kapa 2G HS buffer	1.3 X		3	65	15sec	-0.5 C per cycle decrease
MgCl2	2.6 mM		4	68	10sec	-
dNTP KAPA	.26 mM		5	-	-	repeat steps 2-4 for 10 cycles (Touchdown)
25271	.5 uM		6	94	15sec	-
26474	.5 uM		7	60	15sec	-
26475	.5 uM		8	72	10sec	-
Glycerol	6.5 %		9	-	-	repeat steps 6-8 for 28 cycles
Dye	1 X		10	72	2 min	-
Kapa 2G HS taq polymerase	.03 U/ul		11	10	-	hold
DNA						

Version 3.2