



Welcome to The Genotyping Protocol System

Master Protocol

Strain Name: C57BL/6NJ-Pbsn^{em1J}/J
Stock Number: 027794
Allele: Pbsn-6978-KOMP CRISPR
Protocol Name: Pbsn-6978-KOMP CRISPR
Method: MELT
Created: 22-September-2015 (JKELMEN) **Updated:** 11-November -2015 (ESCHAAB)

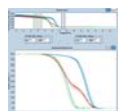
Notes

Notes: This allele from project Pbsn-6978J F#3145 was generated at The Jackson Laboratory by injecting Cas9 RNA and 4 guide sequences:
TTAAGGTCGTGCATTCATTC, ACATTGGAATGTAGATATCA, TCGTATTGTATATTACTCCA, TTTATGTGGAATCAGGACCC, which resulted in a 212 bp deletion in intron 2 beginning at Chromosome X negative strand position 77845123bp (CCCTGGAGTAATATACAATACG) and ending after (TGATATCTACATTCCAATGTAA) at 77844912 bp (GRCm38/mm10). This mutation deletes exon 3 and is predicted to cause amino acid sequence changes after residue 25 and early truncation 3 amino acids later.

Expected Results: Mutant = 203 bp
Heterozygote = 203 bp and 415 bp
Wild type = 415 bp

X-linked

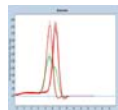
Attachments



View Pbsn HRM.JPG



View Pbsn-27794 Gel.jpg



View Pbsn Mplex Melt.jpg

- View Pbsnem1j_molecular description 9-18-15_in progress.docx
- View Pbsn genomic1.gcs

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
25082	-	TGC AAA CAA CTA ACA GGG TGA	-	Forward	-
25084	-	AAG AAA ATG AGG AGG CTT TCC	-	Reverse	-

Reaction/Components A

Reaction Components	Volume Amt	Final Concentration	Total Volume Amt
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Cycling

Step #	Temp°C	Time	Note
1	94	2 min	-

Reaction/Components A				Cycling			
ddH2O	5.15	-	-	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 25082	0.30	.5	-	6	94	15sec	-
20 uM 25084	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