



## Welcome to The Genotyping Protocol System

## Master Protocol

**Strain Name:** C57BL/6NJ-Prkx<sup>em1J</sup>/<sub>J</sub>  
**Stock Number:** 027813  
**Allele:** Prkx<sup>em1J</sup>  
**Protocol Name:** Prkx<sup>em1J</sup>  
**Method:** High Resolution Melting  
**Created:** 20-October -2015 (JKELMEN) **Updated:** 11-December -2015 (JKELMEN)

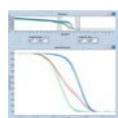
## Notes

Notes: This allele from project Prkx-6999J-M3432 was generated at The Jackson Laboratory by injecting Cas9 RNA and 4 guide sequences, ATAACGTTAGGGAGGTGAGG, CTCTTGGGAGAATAACGTTA, TTATCTTTAAGGCCCCAGAT, and ATGGACATCATGCCAATCTG, which resulted in a 283 bp deletion beginning in intron 2 at Chromosome X negative strand position 77,786,367 bp, TTGGCATGATGTCCATTCTC, and ending after CCACCTCCTCACCTCCCTAA at 77,786,085 bp (GRCm38/mm10) in intron 3. The 283 bp mutation deletes all of exon 2 and is predicted to result in a change of amino acid sequence after amino acid 54 and early truncation 12 amino acids later.

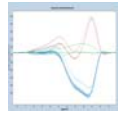
Expected Results: Mutant = 198 bp  
Heterozygote = 198 bp and 481 bp  
Wild type = 481 bp

X-linked

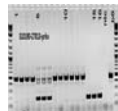
## Attachments



View 27813.JPG



View 310190-dp.jpg



View 310190-27813.jpg

- View Prkxem1jMolDesOct2015.docx

- View Prkx genomic1.gcs

## Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
25302	-	CCC CTT GAT TGA CAG TTG TG	-	Forward	A
25303	-	ACC AAT TGA GCT GCA AAC AC	-	Reverse	A

## Reaction/Components A

## Cycling

Reaction Components	Volume Amt	Final Concentration	Total Volume Amt	Step #	Temp°C	Time	Note

Reaction/Components A				Cycling			
ddH2O	4.55	-	-	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 dNTPS-kapa	0.24	.2	-	4	68	10sec	-
20 U Primer 1	0.30	.5	-	5	-	-	repeat steps 2-4 for 10 cycles
20 U Primer 2	0.30	.5	-	6	94	15sec	-
5 mM 10x Loading Dye	1.20	.5	-	7	60	15sec	-
2.5 U/ul Kapa 2G HS taq polymerase	0.05	.01	-	8	72	10sec	-
DNA	2.00	-	-	9	-	-	repeat steps 6-8 for 28 cycles
				10	72	2 min	-
				11	10	-	hold

Number Of Reactions

Version 3.2