



## Welcome to The Genotyping Protocol System

## Master Protocol

**Strain Name:** C57BL/6NJ-Arhhap36<sup>em1J</sup>J  
**Stock Number:** 028321  
**Allele:** Arhhap36<sup>em1J</sup>  
**Protocol Name:** Arhhap36<sup>em1J</sup>  
**Method:** High Resolution Melting  
**Created:** 16-December -2015 (JKELMEN) **Updated:** 21-January -2016 (ESCHAAB)

## Notes

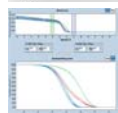
Notes: This allele from project Arhhap36-7011J-LMP1 was generated at The Jackson Laboratory by injecting Cas9 RNA and 3 guide sequences, GATCCAGCCTATGATGGACA, TCTCACGAAAAAACTCCTTG, and TGCCCTCCATTGTTGGAGCA, which resulted in a 180 bp deletion beginning in intron 7 at Chromosome X negative strand position 49,496,527 bp, AAATCCTTGAGGAGCTCAGC, and ending after GAAATGACAGGCTTCAGTAAA at 49,49,6348 bp (GRCm38/mm10) in exon 7. This 180 bp deletion removes the splice acceptor and 87 bp of exon 7 effectively deleting the entire exon and is predicted to result in early truncation 257 amino acids later.

Expected Results: Mutant = 376 bp  
 Heterozygote = 293 bp and 376 bp  
 Wild type = 293 bp  
 X-linked

## Attachments



View Arhhap36-28321 Gel.jpg



View Arhhap HRM.JPG

- View Arhhap36em1jMolDesOct2015.docx

- View Arhhap36 genomic.gcc

## Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
24159	-	CCG GGA GAA GGT TTT ATT GA	-	Mutant Reverse	A
26028	-	AAA GGC TCT CTG ATA CAC ATA GGG	-	Common	A
26029	-	AGC CAC ATC ATG GAC ATT CA	-	Wild type 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
		1	94	2 min	-

Reaction A (3 primer)		Cycling (TouchDown 65-60_12-12-11)			
ddH2O		2	94	20sec	-
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)
26028	.5 uM	6	94	15sec	-
26029	.5 uM	7	60	15sec	-
24159	.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