



## Welcome to The Genotyping Protocol System

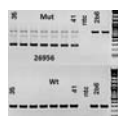
## Master Protocol

**Strain Name:** C57BL/6NJ-*Chn1<sup>em1J</sup>*  
**Stock Number:** 026956  
**Allele:** *Chn1<sup>em1J</sup>*  
**Protocol Name:** *Chn1<sup>em1J</sup>*alternate1  
**Method:** Separated PCR  
**Created:** 31-August -2015 (JKELMEN) **Updated:** 11-September-2015 (ESCHAAB)

## Notes

Expected Results: Mutant = 273 bp  
 Heterozygote = 273 bp and 297 bp  
 Wild type = 297 bp

## Attachments



View 26956.jpg

- View *Chn1em1j\_final\_6-30-15.docx*

- View *Chn1 genomic.gcs*

## Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
24376	-	AAT TAC AGC CCC AAT TTG TTC	-	Common	-
24377	-	GGA GAA CAA AAG CAG AAA TGT CA	-	Wild type Reverse	-
24825	-	ACT ACA ATT CAT TAC AAA ATT ATG CTG	-	Mutant Reverse	-

## Reaction/Components A

Reaction Components	Volume Amt	Final Concentration	Total Volume Amt
ddH <sub>2</sub> O	4.55	-	-
5 X Kapa 2G HS buffer	2.40	1	-
25 mM MgCl <sub>2</sub>	0.96	2	-
10 mM dNTPS-kapa	0.24	.2	-
20 uM 24376	0.30	.5	-
20 uM 24377	0.30	.5	-
5 mM 10x Loading Dye	1.20	.5	-
2.5 U/ul Kapa 2G HS taq polymerase	0.05	.01	-
DNA	2.00	-	-

## Cycling

Step #	Temp °C	Time	Note
1	94	2 min	-
2	94	20sec	-
3	65	15sec	-0.5 C per cycle decrease
4	68	10sec	-
5	-	-	repeat steps 2-4 for 10 cycles
6	94	15sec	-
7	60	15sec	-
8	72	10sec	-
9	-	-	repeat steps 6-8 for 28 cycles
10	72	2 min	-
11	10	-	hold

Number Of Reactions 1

Version 3.2