



Welcome to The Genotyping Protocol System

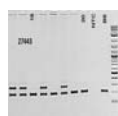
Master Protocol

Strain Name: C57BL/6NJ-Kcnd3^{em1}J/J
Stock Number: 027443
Allele: Kcnd3^{em1}J
Protocol Name: Kcnd3^{em1}J
Method: Standard PCR
Created: 24-July -2015 (JKELMEN) **Updated:** 16-October -2015 (ESCHAAB)

Notes

Expected Results: Mutant = 294 bp
 Heterozygote = 203 bp and 294 bp
 Wild type = 203 bp

Attachments



View 27443.jpg

- View Kcnd3 genomic.gcs

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
24406	-	TTG GGA TTA TAG CCC CGA CT	-	Common	-
24407	-	TCT TAG GCA CCA TGT CTC CA	-	Wild type Reverse	-
24410	-	TGG CTG GGG GTA TAG AAA TG	-	Mutant Reverse	-

Reaction/Components A

Reaction Components	Volume Amt	Final Concentration	Total Volume Amt
ddH2O	4.25	-	-
5 X Kapa 2G HS buffer	2.40	1	-
25 mM MgCl ₂	0.96	2	-
10 mM dNTP KAPA	0.24	.2	-
20 uM 24406	0.30	.5	-
20 uM 24407	0.30	.5	-
20 uM 24410	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