



Welcome to The Genotyping Protocol System

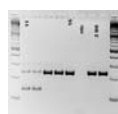
Master Protocol

Strain Name: C57BL/6NJ-*Pcnx2*^{em1J/J}
Stock Number: 028969
Allele: *Pcnx2*^{em1J}
Protocol Name: *Pcnx2*^{em1J}
Method: High Resolution Melting
Created: 25-March -2016 (JKELMEN) **Updated:** 21-July -2016 (ESCHAAB)

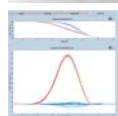
Notes

Expected Results: Mutant = 136 bp
 Heterozygote = 136 bp and 234 bp
 Wild type = 234 bp

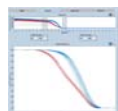
Attachments



View 28969 *Pcnx2* gel.jpg



View 28969 *Pcnx2* B6.JPG



View 28969 *Pcnx2* hrm.JPG

- View *Pcnx2*^{em1J} in progress 3-21-16.docx

- View *Pcnx2* genomic.gcs

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
27319	-	GGT TGA ATC CTG CTA CTT CTC G	-	Common	A
27320	-	TGA GCA CAA GTC AGG GGA GA	-	Wild type Reverse	A
27321	-	TTT CCT TTG GGA GGA GCA GAC	-	Mutant Reverse	A

Number Of Reactions

Reaction A (3 primer)		Cycling (TouchDown 65-60_12-12-11)			
Component	Final Concentration	Step #	Temp°C	Time	Note
ddH ₂ O		1	94	2 min	-
Kapa 2G HS buffer	1.3 X	2	94	20sec	-
MgCl ₂	2.6 mM	3	65	15sec	-0.5 C per cycle decrease
dNTP KAPA	.26 mM	4	68	10sec	-
27320	.5 uM	5	-	-	repeat steps 2-4 for 10 cycles (Touchdown)
		6	94	15sec	-

Reaction A (3 primer)		Cycling (TouchDown 65-60_12-12-11)			
27321	.5 uM	7	60	15sec	-
27319	.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