



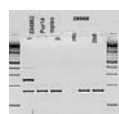
Welcome to The Genotyping Protocol System

Master Protocol

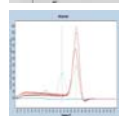
Strain Name: C57BL/6NJ-*Nectin4*^{em1J/J}
Stock Number: 028968
Allele: *Nectin4*^{em1J}
Protocol Name: *Nectin4*^{em1J}
Method: MELT
Created: 01-June -2016 (ESCHAAB) **Updated:** 01-June -2016 (ESCHAAB)

Notes

Expected Results: Mutant = 247 bp
 Heterozygote = 163 bp and 247 bp
 Wild type = 163 bp

Attachments

View Pvr14-28968 Gel.jpg



View Pvr14 Mplex Melt.jpg



View Pvr14 HRM.JPG

- View Pvr14 genomic.gcs
- View Pvr14em1j in progress 3-21-16.docx

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
25593	-	CAT GCA CTT TTA GCC CTC AC	-	Reverse	A
27322	-	ACT GTG ATG CCA CAG GAG GT	-	Common	A
27323	-	CTC TTC TCC CTC TGC TGG AC	-	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
ddH2O		1	94	2 min	-
Kapa 2G HS buffer	1.3 X	2	94	20sec	-
MgCl2	2.6 mM	3	65	15sec	-0.5 C per cycle decrease
dNTP KAPA	.26 mM	4	68	10sec	-
27323	.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)			
27322	.5 uM	7	60	15sec	-
25593	.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