



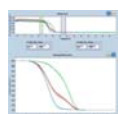
Welcome to The Genotyping Protocol System

Master Protocol

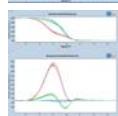
Strain Name: C57BL/6NJ-E130311K13Rik^{em1J/J}
Stock Number: 028322
Allele: E130311K13Rik^{em1J}
Protocol Name: E130311K13Rik^{em1J}alternate1
Method: High Resolution Melting
Created: 13-January -2016 (JKELMEN) **Updated:** 25-February -2016 (ESCHAAB)

Notes

Expected Results: Mutant = 278 bp
 Heterozygote = 209 bp and 278 bp
 Wild type = 209 bp

Attachments

View 28322.JPG



View 28322-2.JPG



View 321599-28322 Rik gel.jpg

- View E130311K13RIKem1jMolDesOct2015(2).docx
- View E13011K13Rik genomic.gcs

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
26030	-	CCA CAT CCA CTG CCC TTA AC	-	Common	A
26031	-	TTT GAG GTC TGT TGG TTG ACA	-	Wild type Reverse	A
26388	-	TAT TAA ATG CCA TAA CAA TCA CTA CA	-	Mutant 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	-
26030	.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)			
26388	.5 uM	7	60	15sec	-
26031	.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