



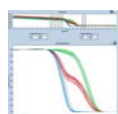
Welcome to The Genotyping Protocol System

Master Protocol

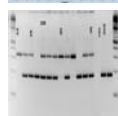
Strain Name: C57BL/6NJ-Rasal1^{em1J}J
Stock Number: 029280
Allele: Rasal1^{em1J}
Protocol Name: Rasal1^{em1J}
Method: MELT
Created: 03-June -2016 (JKELMEN) **Updated:** 20-December -2016 (LUCASM)

Notes

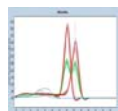
Expected Results: Mutant = 280 bp
 Heterozygote = 182 bp and 280 bp
 Wild type = 182 bp

Attachments

View 29280 gs.JPG



View 29280.jpg



View 29280.JPG

- View Rasal1em1jMolDesMay2016.docx

- View Rasal1 genomic.gcs

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
28303	-	GGA CTG GGT GTG CTT CTC TG	-	Common	A
28304	-	GGG GCT AGG CTA GAG TCT CA	-	Wild type Reverse	A
28305	-	AAA AGG CCA AAC CGA AGT TG	-	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	-
28303	.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)			
28305	.5 uM	7	60	15sec	-
28304	.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