



Welcome to The Genotyping Protocol System

Master Protocol

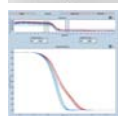
Strain Name: C57BL/6NJ-Arhgap29^{em1J}J
Stock Number: 028869
Allele: Arhgap29^{em1J}
Protocol Name: Arhgap29^{em1J}
Method: High Resolution Melting
Created: 29-February -2016 (JKELMEN) **Updated:** 20-December -2016 (LUCASM)

Notes

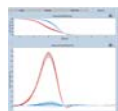
Expected Results: Mutant = 294 bp
 Heterozygote = 226 bp and 294 bp
 Wild type = 226 bp

Attachments

View 334111-28869 Arhgap gel.jpg



View 334111-28869 Arhgap hrm.JPG



View 334111-28869 Arhgap hrm B6.JPG

- View Arhgap29MolDesFeb2016.docx

- View Arhgap29 genomic1.gcs

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
26984	-	TGT GTT GGA TTG TGT CAT GC	-	Common	A
26985	-	TTG CAG TAA GTG GAG GAA AAC A	-	Wild type Reverse	A
26986	-	AGA AAC ACG TAA GAA TCC CAT TAC	-	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	-
26984	.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)			
26985	.5 uM	7	60	15sec	-
26986	.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