



Welcome to The Genotyping Protocol System

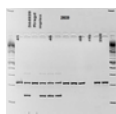
Master Protocol

Strain Name: C57BL/6NJ-Rragd^{em1J/J}
Stock Number: 029039
Allele: Rragd^{em1J}
Protocol Name: Rragd^{em1J}
Method: High Resolution Melting
Created: 11-April -2016 (LUCASM) **Updated:** 14-July -2016 (JKELMEN)

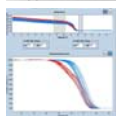
Notes

Expected Results: Mutant = 135 bp
Heterozygote = 135 bp and 221 bp
Wild type = 221 bp

Attachments



View Rragd-29039 Gel.jpg



View Rragd HRM.JPG

- View Rragdem1j_in progress 4-4-16-3.docx
- View Rragd Genomic.gcs

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
27599	-	TGG GTT AGG GCA GTT TTC AG	-	Common	A
27600	-	TCT GGG CTT CAC CTC AGT G	-	Wild type Reverse	A
27601	-	CGG CCA GCA GAA TGA CTC	-	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	-
27599	.5 uM	5	-	-	repeat steps 2-4 for 10 cycles (Touchdown)
27600	.5 uM	6	94	15sec	-
27601	.5 uM	7	60	15sec	-
Glycerol	6.5 %	8	72	10sec	-
		9	-	-	repeat steps 6-8 for 28 cycles

Reaction A (3 primer)		Cycling (TouchDown 65-60_12-12-11)			
Dye	1 X	10	72	2 min	-
Kapa 2G HS taq polymerase	.03 U/ul	11	10	-	hold
DNA					

Version 3.2