



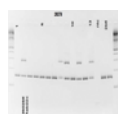
Welcome to The Genotyping Protocol System

Master Protocol

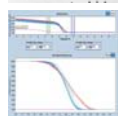
Strain Name: C57BL/6NJ-Rab22a^{em1J}/_J
Stock Number: 029278
Allele: Rab22a^{em1J}
Protocol Name: Rab22a^{em1J}
Method: High Resolution Melting
Created: 03-June -2016 (JKELMEN) **Updated:** 17-August -2016 (ESCHAAB)

Notes

Expected Results: Mutant = 279 bp
 Heterozygote = 149 bp and 279 bp
 Wild type = 149 bp

Attachments

View Rab22a-29278 Gel.jpg



View Rab22a 3 Primer Mplex HRM.JPG

- View Rab22aem1jMolDesMay2016.docx

- View Rab22a genomic.gcs

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
28312	-	GAA GCC TGT TGT GTC TCT CCA	-	Common	A
28313	-	TAC CCA CAC CCG TAT CCT GA	-	Wild type Reverse	A
28314	-	CAT CTG CTC TCT TTC AAG GTG	-	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	-
28314	.5 uM	5	-	-	repeat steps 2-4 for 10 cycles (Touchdown)
28313	.5 uM	6	94	15sec	-
28312	.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