



Welcome to The Genotyping Protocol System

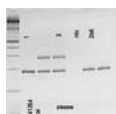
Master Protocol

Strain Name: C57BL/6NJ-Rai14^{em1J}/J
Stock Number: 029006
Allele: Rai14^{em1J}
Protocol Name: Rai14^{em1J}
Method: High Resolution Melting
Created: 04-April -2016 (JKELMEN) **Updated:** 23-June -2016 (ESCHAAB)

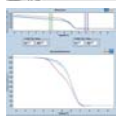
Notes

Expected Results: Mutant = 272 bp
 Heterozygote = 182 bp and 272 bp
 Wild type = 182 bp

Attachments



View Rai14-29006 Gel.jpg



View Rai14 HRM.JPG

- View Rai14em1j in progress 3-28-16.docx
- View Rail4 genomic.gcs

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
27450	-	TGC TGA TGG CTG GTA TTT CA	-	Common	A
27451	-	GTC TGT GGG GCA AAA GGT AG	-	Wild type Reverse	A
27452	-	GCA ACC CTG GTG CTA TTT GT	-	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	-
27450	.5 uM	5	-	-	repeat steps 2-4 for 10 cycles (Touchdown)
27451	.5 uM	6	94	15sec	-
27452	.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