



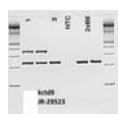
Welcome to The Genotyping Protocol System

Master Protocol

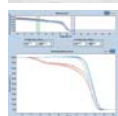
Strain Name: C57BL/6NJ-*Kctd9*^{em1J/J}
Stock Number: 029523
Allele: *Kctd9*^{em1J}
Protocol Name: *Kctd9*^{em1J}
Method: High Resolution Melting
Created: 09-August -2016 (LUCASM) **Updated:** 25-September-2016 (ESCHAAB)

Notes

Expected Results: Mutant = 351 bp
 Heterozygote = 227 bp and 351 bp
 Wild type = 227 bp

Attachments

View 29523-kctd9.jpg



View kctd9 HRM JW.JPG

- View Kctd9 genomic.gcs

- View Kctd9em1jMolDesJuly2016.docx

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
29093	-	GGC ACA GAG TAA TTG CCT ATC AG	-	Common	A
29094	-	CAT CAA TCA GTC CGC CTT TC	-	Wild type Reverse	A
29095	-	ACC TGG GAC ACT GAA AAA CC	-	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	-
29094	.5 uM	5	-	-	repeat steps 2-4 for 10 cycles (Touchdown)
29095	.5 uM	6	94	15sec	-
29093	.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