



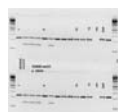
Welcome to The Genotyping Protocol System

Master Protocol

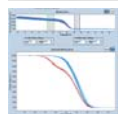
Strain Name: C57BL/6NJ-Med10^{em1}J/J
Stock Number: 029244
Allele: Med10^{em1}J
Protocol Name: Med10^{em1}J
Method: High Resolution Melting
Created: 11-May -2016 (JKELMEN) **Updated:** 01-September-2016 (JKELMEN)

Notes

Expected Results: Mutant = 136 bp
 Heterozygote = 136 bp and 224 bp
 Wild type = 224 bp

Attachments

View 353808-29244 med10.jpg



View med10 HRM.JPG

- View Med10em1jMolDesMay2016.docx
 - View Med10 genomic1.gcs

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
28012	-	TTG TCA TGA GCT GCT GGA AG	-	Common	A
28013	-	TTG GTG TAG AGC TGG GGA TT	-	Wild type Reverse	A
28014	-	TTT GTG TTG TTT TCC ACA GGA	-	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	-
28013	.5 uM	5	-	-	repeat steps 2-4 for 10 cycles (Touchdown)
28012	.5 uM	6	94	15sec	-
28014	.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