



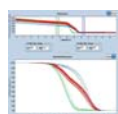
Welcome to The Genotyping Protocol System

Master Protocol

Strain Name: C57BL/6NJ-*Cdrt4^{em1J}*
Stock Number: 028319
Allele: *Cdrt4^{em1J}*
Protocol Name: *Cdrt4^{em1J}*
Method: High Resolution Melting
Created: 20-November -2015 (JKELMEN) **Updated:** 03-December -2015 (ESCHAAB)

Notes

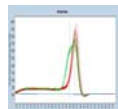
Expected Results: Mutant = 283 bp
 Heterozygote = 203 bp and 283 bp
 Wild type = 203 bp

Attachments

View 28319 Cdrt4 HRM.JPG



View Cdrt4-28319 Gel.jpg



View Cdrt4 Mplex Melt.jpg

- View Cdrt4em1jMolDesOct2015.docx

- View Cdrt4 genomic.gcc

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
25693	-	CTA GGT CAA GGA TGG GTT GC	-	Common	A
25694	-	TGT CCT CTG TCT GTT GCA GTC	-	Wild type Reverse	A
25695	-	AGG GCA CTA GCT GTT CTT TCA	-	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	-
25693	.5 uM	5	-	-	repeat steps 2-4 for 10 cycles (Touchdown)
		6	94	15sec	-

Reaction A (3 primer)		Cycling (TouchDown 65-60_12-12-11)			
25694	.5 uM	7	60	15sec	-
25695	.5 uM	8	72	10sec	-
Glycerol	6.5 %	9	-	-	repeat steps 6-8 for 28 cycles
Dye	1 X	10	72	2 min	-
Kapa 2G HS taq polymerase	.03 U/ul	11	10	-	hold
DNA					

Version 3.2