



Welcome to The Genotyping Protocol System

Master Protocol

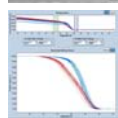
Strain Name: C57BL/6NJ-Med20^{em1J}J
Stock Number: 027272
Allele: Med20^{em1J}
Protocol Name: Med20^{em1J}
Method: High Resolution Melting
Created: 17-July -2015 (JKELMEN) **Updated:** 18-August -2015 (ESCHAAB)

Notes

Expected Results: Mutant = 122 bp
 Heterozygote = 122 bp and 175 bp
 Wild type = 175 bp

Attachments

View Med20-27272 Gel.jpg



View Med20 HRM.JPG

- View Med20 genomic.gcs
 - View Med20_em1j__6-30-15_final.docx

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
24328	-	TGA TGC CTT TGA TTC CAA CA	-	Common	-
24329	-	CAC TCA ATT CCC CAA CAG GT	-	Wild type Reverse	-
24330	-	CCC TTG ACA GAA AAG CAA GC	-	Mutant Reverse	-

Reaction/Components A

Reaction Components	Volume Amt	Final Concentration	Total Volume Amt
ddH2O	4.85	-	-
5 X Kapa 2G HS buffer	2.40	1	-
25 mM MgCl ₂	0.96	2	-
10 mM dNTP KAPA	0.24	.2	-
20 uM 24328	0.30	.5	-
20 uM 24329	0.30	.5	-
20 uM 24330	0.30	.5	-
2.5 U/ul Kapa 2G HS taq polymerase	0.05	.01	-

Cycling

Step #	Temp °C	Time	Note
1	94	2 min	-
2	94	20sec	-
3	65	15sec	-0.5 C per cycle decrease
4	68	10sec	-
5	-	-	repeat steps 2-4 for 10 cycles
6	94	15sec	-
7	60	15sec	-
8	72	10sec	-

Reaction/Components A				Cycling			
20 X EvaGreen	0.60	1	-	9	-	-	repeat steps 6-8 for 28 cycles
DNA	2.00	-	-	10	72	2 min	-
				11	10	-	hold

Number Of Reactions 1

Version 3.2