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The Jackson Laboratory
Leading the search for tomorrow's cures

Welcome to The Genotyping Protocol System

Master Protocol

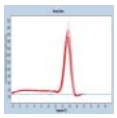
Strain Name: C57BL/6NJ-*Rfc2*^{em1J}/J
Stock Number: 029029
Allele: Rfc2^{em1J}
Protocol Name: Rfc2^{em1J}
Method: SEPARATED MELT
Created: 03-May -2016 (ESCHAAB) **Updated:** 22-July -2016 (ESCHAAB)

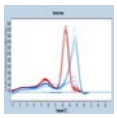
Notes

Expected Results:	Mutant = 299 bp Heterozygote = 190 bp and 299 bp Wild type = 190 bp

Attachments


View Rfc2-29029 Gel.jpg


View Rfc WT Melt.jpg


View Rfc MUT Melt.jpg

\- View 29029 Rfc2em1jMolDesAprl2016.docx
\- View 29029 Rfc2 genomic.gcs

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
27863	-	TCT TGG TTC TGG GGT TTG AG	-	Common	A + B
27864	-	GTG GAG CTG TGA TGC CAA C	-	Wild type Reverse	A
27865	-	GTG TGG TGG TGG TGG ATA TG	-	Mutant Reverse	B

Number Of Reactions 1

Reaction A (2 Primer)

Component	Final Concentration
ddH2O	
Kapa 2G HS buffer	1.3 X
MgCl2	2.6 mM
dNTPS-kapa	.26 mM
27863	.5 uM

Cycling (TouchDown 65-60_12-12-11)

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 (Touchdown)
6	94	15sec	-

Reaction A (2 Primer)

27864	.5 uM
Glycerol	6.5 %
Dye	1 X
Kapa 2G HS taq polymerase	.03 U/ul
DNA	

Cycling (TouchDown 65-60_12-12-11)

7	60	15sec	-
8	72	10sec	-
9	-	-	repeat steps 6-8 for 28 cycles
10	72	2 min	-
11	10	-	hold

Reaction B (2 Primer)

Component	Final Concentration
ddH2O	
Kapa 2G HS buffer	1.3 X
MgCl2	2.6 mM
dNTPS-kapa	.26 mM
27863	.5 uM
27865	.5 uM
Glycerol	6.5 %
Dye	1 X
Kapa 2G HS taq polymerase	.03 U/ul
DNA	

Cycling (TouchDown 65-60_12-12-11)

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 (Touchdown)
6	94	15sec	-
7	60	15sec	-
8	72	10sec	-
9	-	-	repeat steps 6-8 for 28 cycles
10	72	2 min	-
11	10	-	hold

Version 3.2