



Welcome to The Genotyping Protocol System

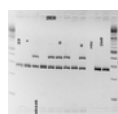
Master Protocol

Strain Name: C57BL/6NJ-Rbpms2^{em1J}/J
Stock Number: 029030
Allele: Rbpms2^{em1J}
Protocol Name: Rbpms2^{em1J} Alternate1
Method: SEPARATED MELT
Created: 25-July -2016 (LUCASM) **Updated:** 04-August -2016 (ESCHAAB)

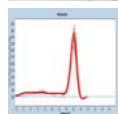
Notes

Expected Results: Mutant = 395 bp
Heterozygote = 268 bp and 395 bp
Wild type = 268 bp

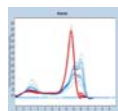
Attachments



View Rbpms2-29030 Gel.jpg



View Rbpms2 WT Melt.jpg



View Rbpms2 MUT Melt.jpg

- View Rbpms2 genomic.gcs
- View Rbpms2em1jMolDesApril2016.docx

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
27992	-	ACA CTG GTG GGA TTT GGT GT	-	Wild type Reverse	A
27993	-	TCC TCA AGA CCA GGG AAA GA	-	Mutant Reverse	B
28960	-	TGA AAG TCT CAT CAC TCC ACA TCT	-	Common	A + B

Number Of Reactions 1

Reaction A (2 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
dNTPS-kapa	.26 mM	4	68	10sec	-
28960	.5 uM	5	-	-	repeat steps 2-4 for 10 cycles (Touchdown)
		6	94	15sec	-

Reaction A (2 Primer)		Cycling (TouchDown 65-60_12-12-11)			
27992	.5 uM	7	60	15sec	-
Glycerol	6.5 %	8	72	10sec	-
Dye	1 X	9	-	-	repeat steps 6-8 for 28 cycles
Kapa 2G HS taq polymerase	.03 U/ul	10	72	2 min	-
DNA		11	10	-	hold

Reaction B (2 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
dNTPS-kapa	.26 mM	4	68	10sec	-
28960	.5 uM	5	-	-	repeat steps 2-4 for 10 cycles (Touchdown)
27993	.5 uM	6	94	15sec	-
Glycerol	6.5 %	7	60	15sec	-
Dye	1 X	8	72	10sec	-
Kapa 2G HS taq polymerase	.03 U/ul	9	-	-	repeat steps 6-8 for 28 cycles
DNA		10	72	2 min	-
		11	10	-	hold

Version 3.2