



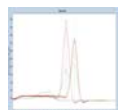
Welcome to The Genotyping Protocol System

Master Protocol

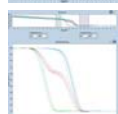
Strain Name: C57BL/6NJ-*Pla2g7^{em1J}*J
Stock Number: 027816
Allele: Pla2g7-7002-KOMP CRISPR
Protocol Name: Pla2g7-7002-KOMP CRISPR alternate1
Method: MELT
Created: 22-March -2016 (LUCASM) **Updated:** 15-April -2016 (ESCHAAB)

Notes

Expected Results: Mutant = 443 bp
 Heterozygote = 263 bp and 443 bp
 Wild type = 263 bp

Attachments

View 327941-2.jpg



View 27816-GS.JPG



View 27816.jpg

- View Pla2g7 genomic1.gcs

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
27235	-	CAG TAA TGT TTC ACA AGC TCC A	-	Wild type Forward	A
27236	-	TGG CAC AGT AGC AAA AGC TG	-	Common	A
27238	-	GTA CGC CTG CCT CCA TTG T	-	Mutant Forward	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	-
27238	.5 uM	5	-	-	repeat steps 2-4 for 10 cycles (Touchdown)
27235	.5 uM	6	94	15sec	-
27236	.5 uM	7	60	15sec	-

Reaction A (3 primer)		Cycling (TouchDown 65-60_12-12-11)			
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

Version 3.2