



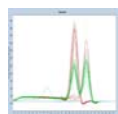
Welcome to The Genotyping Protocol System

Master Protocol

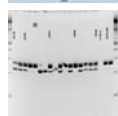
Strain Name: C57BL/6NJ-Lamp5^{em1J}J
Stock Number: 029245
Allele: Lamp5^{em1J}
Protocol Name: Lamp5^{em1J}
Method: MELT
Created: 12-May -2016 (LUCASM) **Updated:** 23-August -2016 (ESCHAAB)

Notes

Expected Results: Mutant = 230 bp
 Heterozygote = 230 bp and 272 bp
 Wild type = 272 bp

Attachments

View 29245.jpg



View 29245.jpg

- View Lamp5 genomic.gcs
- View Lamp5em1jMolDesMay2016.docx

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
28048	-	GGT AGA CTA GAG AAA TGG CTT GC	-	Common	A
28049	-	TGC AAA CTC TGC CAT GAG AC	-	Wild type Reverse	A
28050	-	GAT GCG CGT CCA AAC TCT	-	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	-
28050	.5 uM	5	-	-	repeat steps 2-4 for 10 cycles (Touchdown)
28049	.5 uM	6	94	15sec	-
28048	.5 uM	7	60	15sec	-
Glycerol	6.5 %	8	72	10sec	-
		9	-	-	repeat steps 6-8 for 28 cycles

Reaction A (3 primer)		Cycling (TouchDown 65-60_12-12-11)			
Dye	1 X	10	72	2 min	-
Kapa 2G HS taq polymerase	.03 U/ul	11	10	-	hold
DNA					

Version 3.2