



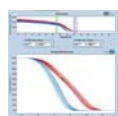
Welcome to The Genotyping Protocol System

Master Protocol

Strain Name: C57BL/6NJ-*Prpf4b*^{em1J/J}
Stock Number: 029395
Allele: *Prpf4b*^{em1J}
Protocol Name: *Prpf4b*^{em1J}
Method: High Resolution Melting
Created: 20-July -2016 (LUCASM) **Updated:** 18-November -2016 (ESCHAAB)

Notes

Expected Results: Mutant = 217 bp
 Heterozygote = 217 bp and 395 bp
 Wild type = 395 bp

AttachmentsView [prpf HRM JW.JPG](#)View [29395 prpf4b.jpg](#)- View [Prpf4b genomic.gcs](#)- View [Prpf4bem1jMolDesJuly2016.docx](#)**Protocol Primers**

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
28917	-	CCT TGA AGA TTT TGA TGT GGA G	-	Wild type Forward	A
28918	-	CAA AAC AAA GAG GAA CAA GTC AG	-	Common	A
28919	-	AGA TTG GGG AGG TCC TTG A	-	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	-
28919	.5 uM	5	-	-	repeat steps 2-4 for 10 cycles (Touchdown)
28917	.5 uM	6	94	15sec	-
28918	.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