



Welcome to The Genotyping Protocol System

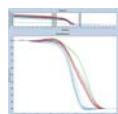
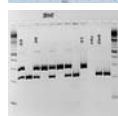
Master Protocol

Strain Name: C57BL/6NJ-*Patj^{em1J}*J
Stock Number: 029147
Allele: *Patj^{em1J}*
Protocol Name: *Patj^{em1J}*
Method: High Resolution Melting
Created: 02-May -2016 (LUCASM) **Updated:** 30-August -2016 (ESCHAAB)

Notes

Expected Results: Mutant = 296 bp
 Heterozygote = 296 bp and 223 bp
 Wild type = 223 bp

Attachments

View [Patj-29147 HRM.JPG](#)View [Patj-29147 Gel.jpg](#)

- View [29147 Inadl Genomic.gcs](#)
 - View [Inadlem1jMolDesApr2016.docx](#)

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
27835	-	CCA AAG CCT GGG ATT AAT TG	-	Mutant Forward	A
27836	-	CAG GTG CAG CGT GGA GAG	-	Common	A
27837	-	CAA GTG TGC GTC TGG CAT AG	-	Wild type 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	-
27837	.5 uM	5	-	-	repeat steps 2-4 for 10 cycles (Touchdown)
27836	.5 uM	6	94	15sec	-
27835	.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