



Welcome to The Genotyping Protocol System

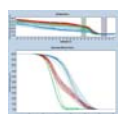
Master Protocol

Strain Name: C57BL/6NJ-*Ier2*^{em1J}/*J*
Stock Number: 029240
Allele: *Ier2*^{em1J}
Protocol Name: *Ier2*^{em1J}
Method: High Resolution Melting
Created: 10-May -2016 (LUCASM) **Updated:** 20-December -2016 (LUCASM)

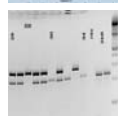
Notes

Expected Results: Mutant = 296 bp
 Heterozygote = 233 bp and 296 bp
 Wild type = 233 bp

Attachments



View 29240.JPG



View 29240.jpg

- View *Ier2* Genomic.gcs
 - View *Ier2*^{em1J}MolDesMay2016.docx

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
27975	-	CCC GTC TAG AAG AGG TGG A	-	Wild type Forward	A
27976	-	CAC AGC TCG CAC CAG CAC	-	Common	A
27977	-	GCC CGT CTT AAC CCA TTC TC	-	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	-
27975	.5 uM	5	-	-	repeat steps 2-4 for 10 cycles (Touchdown)
27977	.5 uM	6	94	15sec	-
27976	.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