



Welcome to The Genotyping Protocol System

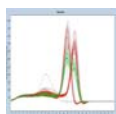
Master Protocol

Strain Name: C57BL/6NJ-Mpv17^{em1J}/J
Stock Number: 028912
Allele: Mpv17^{em1J}
Protocol Name: Mpv17^{em1J}
Method: MELT
Created: 02-March -2016 (JKELMEN) **Updated:** 11-June -2016 (ESCHAAB)

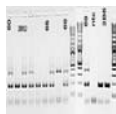
Notes

Expected Results: Mutant = ~390 bp
Heterozygote = 176 bp and ~390 bp
Wild type = 176 bp

Attachments



View 28912.JPG



View 28912.jpg

- View Mpv17MolDesMar2016.docx
- View Mpv17 genomic.gcs

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
27022	-	CCA TCC TTG AGA AGC AGA GG	-	Common	A
27023	-	ATG TCA CCT ACG CCC ATC AG	-	Wild type Reverse	A
27025	-	CCC CAG CCC TTT TCT CTT AG	-	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	-
27022	.5 uM	5	-	-	repeat steps 2-4 for 10 cycles (Touchdown)
27023	.5 uM	6	94	15sec	-
27025	.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