



Welcome to The Genotyping Protocol System

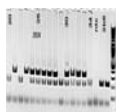
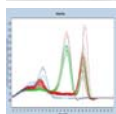
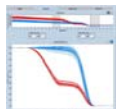
Master Protocol

Strain Name: C57BL/6NJ-Zfyve1^{em1J}/_J
Stock Number: 029134
Allele: Zfyve1^{em1J}
Protocol Name: Zfyve1^{em1J}
Method: MELT
Created: 05-May -2016 (LUCASM) **Updated:** 10-August -2016 (ESCHAAB)

Notes

Expected Results: Mutant = 311 bp
Heterozygote = 205 bp and 311 bp
Wild type = 205 bp

Attachments

[View 29134.jpg](#)[View 29134.JPG](#)[View 29134 Zfyve1 hrm.JPG](#)

- [View Zfyve1em1jMolDesApr2016.docx](#)
- [View Zfyve1 Genomic.gcs](#)

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
27936	-	GAG AAC AAC ACC ACC AGG TC	-	Common	A
27937	-	CCA GAG AGC CTG ACA AGA TG	-	Wild type Reverse	A
27938	-	GTG TGG GGA AGG GTG ATG T	-	Mutant Reverse	A

Number Of Reactions

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	-
27938	.5 uM	5	-	-	repeat steps 2-4 for 10 cycles (Touchdown)
		6	94	15sec	-

Reaction A (3 primer)		Cycling (TouchDown 65-60_12-12-11)			
27936	.5 uM	7	60	15sec	-
27937	.5 uM	8	72	10sec	-
Glycerol	6.5 %	9	-	-	repeat steps 6-8 for 28 cycles
Dye	1 X	10	72	2 min	-
Kapa 2G HS taq polymerase	.03 U/ul	11	10	-	hold
DNA					

Version 3.2