



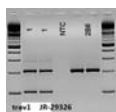
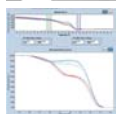
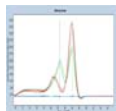
Welcome to The Genotyping Protocol System

Master Protocol

Strain Name: C57BL/6NJ-*Trav1^{em1J}*/*J*
Stock Number: 029326
Allele: *Trav1^{em1J}*
Protocol Name: *Trav1^{em1J}*
Method: MELT
Created: 13-June -2016 (JKELMEN) **Updated:** 10-October -2016 (ESCHAAB)

Notes

Expected Results: Mutant = 101 bp
Heterozygote = 101 bp and 208 bp
Wild type = 208 bp

Attachments[View](#) 29326 trav1.jpg[View](#) trav1 HRM JW.JPG[View](#) trav1 MCA JW.jpg

- [View](#) Trav1 genomic.gcs
- [View](#) Trav1em1j in progress 6-10-16.docx

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
28489	-	ACT CTA AGT TTT CCT GGA CCA CA	-	Common	A
28490	-	AGT TGA CCC GAG CAA AGG T	-	Wild type Reverse	A
28491	-	TCC AAG GAG CCT AGT CTT GA	-	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	-
28491	.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)			
28490	.5 uM	7	60	15sec	-
28489	.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