



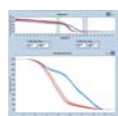
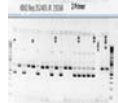
Welcome to The Genotyping Protocol System

**Master Protocol**

**Strain Name:** C57BL/6NJ-*Klh2<sup>em1J</sup>*J  
**Stock Number:** 029268  
**Allele:** *Klh2<sup>em1J</sup>*  
**Protocol Name:** *Klh2<sup>em1J</sup>*  
**Method:** High Resolution Melting  
**Created:** 06-June -2016 (LUCASM) **Updated:** 25-August -2016 (ESCHAAB)

**Notes**

Expected Results: Mutant = 431 bp  
 Heterozygote = 431 bp and ~636 bp  
 Wild type = ~636 bp

**Attachments**View [Klh2primer hrmb 8-24.jpg](#)View [Klh1 352405 JR29268 8-23 Gel 2Primer.jpg](#)- View [Klh2 genomic.gcs](#)- View [Klh2em1jMolDesMay2016.docx](#)**Protocol Primers**

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
26677	-	TCT GCC TCC CTG ACT ACC TG	-	Mutant Forward	A
28336	-	GCA ACA CGT TTA TGG AGG AC	-	Common	A

Number Of Reactions 1

Reaction A (2 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
dNTPS-kapa	.26 mM	4	68	10sec	-
26677	.5 uM	5	-	-	repeat steps 2-4 for 10 cycles (Touchdown)
28336	.5 uM	6	94	15sec	-
Glycerol	6.5 %	7	60	15sec	-
Dye	1 X	8	72	10sec	-
Kapa 2G HS taq polymerase	.03 U/ul	9	-	-	repeat steps 6-8 for 28 cycles
		10	72	2 min	-

**Reaction A (2 Primer)**

**Cycling (TouchDown 65-60\_12-12-11)**

DNA

11 10 - hold

Version 3.2