



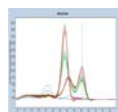
Welcome to The Genotyping Protocol System

**Master Protocol**

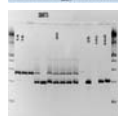
**Strain Name:** C57BL/6NJ-Dgkhem1J/J  
**Stock Number:** 028873  
**Allele:** Dgkhem1J  
**Protocol Name:** Dgkhem1J  
**Method:** MELT  
**Created:** 29-February -2016 (JKELMEN) **Updated:** 26-May -2016 (ESCHAAB)

**Notes**

Expected Results: Mutant = ~280 bp  
 Heterozygote = 205 bp and ~280 bp  
 Wild type = 205 bp

**Attachments**

View Dgkh-28873 Mplex Melt.JPG



View Dgkh-28873 Gel.jpg

- View DgkhMolDesFeb2016.docx  
 - View Dgkh genomic.gcs

**Protocol Primers**

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
23392	-	GCT GTC CTG GAA CTC ATT TTG	-	Mutant Reverse	A
26965	-	GCT AAA GCT AGC CCG TGA AG	-	Common	A
26966	-	CTG GGC CAC CTA CGA AGA C	-	Wild type 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	-
23392	.5 uM	5	-	-	repeat steps 2-4 for 10 cycles (Touchdown)
26966	.5 uM	6	94	15sec	-
26965	.5 uM	7	60	15sec	-
Glycerol	6.5 %	8	72	10sec	-
		9	-	-	repeat steps 6-8 for 28 cycles

<b>Reaction A (3 primer)</b>		<b>Cycling (TouchDown 65-60_12-12-11)</b>			
Dye	1 X	10	72	2 min	-
Kapa 2G HS taq polymerase	.03 U/ul	11	10	-	hold
DNA					

Version 3.2