



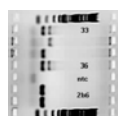
Welcome to The Genotyping Protocol System

**Master Protocol**

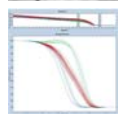
**Strain Name:** C57BL/6NJ-Dalrd3<sup>em1</sup>J/J  
**Stock Number:** 028996  
**Allele:** Dalrd3<sup>em1</sup>J  
**Protocol Name:** Dalrd3<sup>em1</sup>J  
**Method:** High Resolution Melting  
**Created:** 04-April -2016 (JKELMEN) **Updated:** 27-October -2016 (SCASTOR)

**Notes**

Expected Results: Mutant = 234 bp  
 Heterozygote = 152 bp and 234 bp  
 Wild type = 152 bp

**Attachments**

View 28996\_Dalrd3\_GEL.JPG



View 28996\_Dalrd3\_HRM.JPG

- View Dalrd3em1jin progress 3-25-16.docx  
 - View Dalrd3 genomic1.gcs

**Protocol Primers**

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
27496	-	TCG TGT TCG AGA CTT CTT GG	-	Common	A
27497	-	AAC ACG CAC TCT GGC ACC T	-	Wild type Reverse	A
27498	-	AGG TGC TCT TCG AGG CAG T	-	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	-
27496	.5 uM	5	-	-	repeat steps 2-4 for 10 cycles (Touchdown)
27498	.5 uM	6	94	15sec	-
27497	.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