



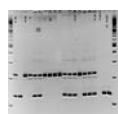
Welcome to The Genotyping Protocol System

**Master Protocol**

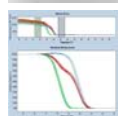
**Strain Name:** C57BL/6NJ-Myo1d<sup>em1J/J</sup>  
**Stock Number:** 029337  
**Allele:** Myo1d<sup>em1J</sup>  
**Protocol Name:** Myo1d<sup>em1J</sup>  
**Method:** High Resolution Melting  
**Created:** 17-June -2016 (JKELMEN) **Updated:** 24-August -2016 (ESCHAAB)

**Notes**

Expected Results: Mutant = 227 bp  
 Heterozygote = 132 bp and 227 bp  
 Wild type = 132 bp

**Attachments**

View 29337.jpg



View 29337 gs.JPG

- View Myo1dem1j in progress 6-13-16.docx  
 - View Myo1d genomic1.gcs

**Protocol Primers**

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
28553	-	TTT AAA AGT AAA TTG AGG GCT GA	-	Common	A
28554	-	GCA AGA ACA AAA GGT TGC TG	-	Wild type Reverse	A
28555	-	GCC TTC AGA ACA AAG ACT AAA GG	-	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	-
28553	.5 uM	5	-	-	repeat steps 2-4 for 10 cycles (Touchdown)
28554	.5 uM	6	94	15sec	-
28555	.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