



Welcome to The Genotyping Protocol System

Master Protocol

Strain Name: C57BL/6NJ-Tomm40^{em1J}
Stock Number: 029277
Allele: Tomm40^{em1J}
Protocol Name: Tomm40^{em1J}
Method: High Resolution Melting
Created: 03-June -2016 (JKELMEN) **Updated:** 30-August -2016 (ESCHAAB)

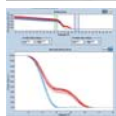
Notes

Expected Results: Mutant = 226 bp
Heterozygote = 156 bp and 226 bp
Wild type = 156 bp

Attachments



View 353811-27277 tomm40 8-30.jpg



View tomm40 HRM.JPG

- View Tomm40em1j in progress 5-31-16.docx
- View Tomm40 genomic.gcs

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
28309	-	AGT TGA GGT GAC TGC ACT GG	-	Common	A
28310	-	CTG AAC TGG AAA CAG CTC TGC	-	Wild type Reverse	A
28311	-	TGG AAT TCT TGA GTC TTC CTG 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	-
28311	.5 uM	5	-	-	repeat steps 2-4 for 10 cycles (Touchdown)
28310	.5 uM	6	94	15sec	-
28309	.5 uM	7	60	15sec	-
Glycerol	6.5 %	8	72	10sec	-
		9	-	-	repeat steps 6-8 for 28 cycles

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

Version 3.2