



Welcome to The Genotyping Protocol System

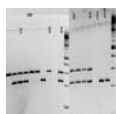
Master Protocol

Strain Name: C57BL/6NJ-Tmcc3^{em1J}/_J
Stock Number: 029140
Allele: Tmcc3^{em1J}
Protocol Name: Tmcc3^{em1J}
Method: MELT
Created: 09-May -2016 (LUCASM) **Updated:** 21-December -2016 (LUCASM)

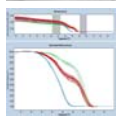
Notes

Expected Results: Mutant = 294 bp
 Heterozygote = 229 bp and 294 bp
 Wild type = 229 bp

Attachments



View 29140.jpg



View 29140.JPG

- View Tmcc3 Genomic.gcs
 - View Tmcc3em1jMolDesApr2016.docx

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
27963	-	GTA AAG CCT GAA AGT TGA GGA CA	-	Common	A
27964	-	GAC AGG AGC CTT AGT GAG CA	-	Wild type Reverse	A
27965	-	CAG GAA ATG TCC GAG TGC 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	-
27965	.5 uM	5	-	-	repeat steps 2-4 for 10 cycles (Touchdown)
27964	.5 uM	6	94	15sec	-
27963	.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