



Welcome to The Genotyping Protocol System

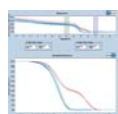
Master Protocol

Strain Name: C57BL/6NJ-Ano8^{em1J}/J
Stock Number: 029360
Allele: Ano8^{em1J}
Protocol Name: Ano8^{em1J}
Method: MELT
Created: 17-June -2016 (JKELMEN) **Updated:** 15-November -2016 (ESCHAAB)

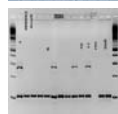
Notes

Expected Results: Mutant = 264 bp
Heterozygote = 104 bp and 264 bp
Wild type = 104 bp

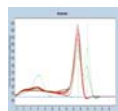
Attachments



View Ano8 HRM.JPG



View Ano8-29360 Gel.jpg



View Ano8 Mplex Melt.jpg

- View Ano8em1jMolDesJun2016.docx

- View Ano8 genomic1.gcs

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
28542	-	GAC CTT CCC AGG TAT CCA CA	-	Common	A
28543	-	GGT CTT GCC TCG AGA GGT G	-	Wild type Reverse	A
28544	-	CTC CAA ATT CGG CCT TCA C	-	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	-
28543	.5 uM	5	-	-	repeat steps 2-4 for 10 cycles (Touchdown)
		6	94	15sec	-

Reaction A (3 primer)		Cycling (TouchDown 65-60_12-12-11)			
28542	.5 uM	7	60	15sec	-
28544	.5 uM	8	72	10sec	-
Glycerol	6.5 %	9	-	-	repeat steps 6-8 for 28 cycles
Dye	1 X	10	72	2 min	-
Kapa 2G HS taq polymerase	.03 U/ul	11	10	-	hold
DNA					

Version 3.2