



Welcome to The Genotyping Protocol System

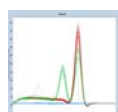
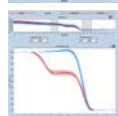
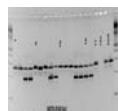
Master Protocol

Strain Name: C57BL/6NJ-Oaz2^{em1J}/J
Stock Number: 029148
Allele: Oaz2^{em1J}
Protocol Name: Oaz2^{em1J}
Method: MELT
Created: 02-May -2016 (LUCASM) **Updated:** 08-August -2016 (ESCHAAB)

Notes

Expected Results: Mutant = 218 bp
Heterozygote = 218 bp and 274 bp
Wild type = 274 bp

Attachments

[View](#) 29148 Oaz2 mca.jpg[View](#) 29148 Oaz2 hrm.JPG[View](#) 29148 Oaz2 gel.jpg[View](#) 29148 Oaz2 Genomic.gcs[View](#) Oaz2em1jMolDesApr2016.docx

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
27838	-	CTT TGA AAT GAC CCT GAT TCA C	-	Common	A
27839	-	CAT CTG GGA TTT CTA CAA ACA GG	-	Wild type Reverse	A
27840	-	AGA AAA ATA GAA ACT GGG GTC AGT 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	-
27840	.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)			
27839	.5 uM	7	60	15sec	-
27838	.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