



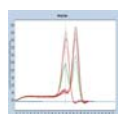
Welcome to The Genotyping Protocol System

Master Protocol

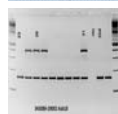
Strain Name: C57BL/6NJ-Asb18^{em1}J/J
Stock Number: 029002
Allele: Asb18^{em1}J
Protocol Name: Asb18^{em1}J
Method: MELT
Created: 05-April -2016 (JKELMEN) **Updated:** 26-June -2016 (ESCHAAB)

Notes

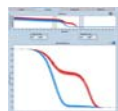
Expected Results: Mutant = 378 bp
 Heterozygote = 154 bp and 378 bp
 Wild type = 154 bp

Attachments

View 29002.JPG



View 343089-29002 Asb18 gel.jpg



View 343089-29002 Asb18 hrm.JPG

- View Asb18em1j in progress 3-28-16.docx

- View Asb18 genomic.gcs

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
27505	-	CTG CTC TCT GTC TGG GGT CT	-	Common	A
27506	-	TAA GGT GAG TGG GGC AAA TG	-	Wild type Reverse	A
27507	-	CTG CAG GGT CTC TGC TGT G	-	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	-
27507	.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)			
27506	.5 uM	7	60	15sec	-
27505	.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