



Welcome to The Genotyping Protocol System

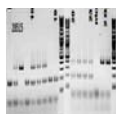
Master Protocol

Strain Name: C57BL/6NJ-Vwa5a^{em1J}/J
Stock Number: 028515
Allele: Vwa5a^{em1J}
Protocol Name: Vwa5a^{em1J}
Method: High Resolution Melting
Created: 20-November -2015 (JKELMEN) **Updated:** 04-May -2016 (LUCASM)

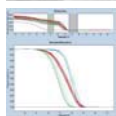
Notes

Expected Results: Mutant = 180 bp
Heterozygote = 180 bp and 358 bp
Wild type = 358 bp

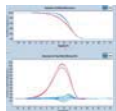
Attachments



View 28515.jpg



View 28515.JPG



View Vw5a Difference Plot.JPG

- View Vwa5aem1jmollescNov2015.docx
- View Vwa5a genomic1.gcs

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
25708	-	TCA CCA TGG AGC ATC ACT GT	-	Common	A
25709		TAT CCT GCA ACT CTG CCA CA		Wild type Reverse	A
25710	-	TGT GAA CTT TAC AAT TCA TCT GGT	-	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	-
25708	.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)			
25710	.5 uM	7	60	15sec	-
25709	.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