



Welcome to The Genotyping Protocol System

Master Protocol

Strain Name: C57BL/6NJ-Smarcd3^{em1J}/_J
Stock Number: 029338
Allele: Smarcd3^{em1J}
Protocol Name: Smarcd3^{em1J}
Method: MELT
Created: 17-June -2016 (JKELMEN) **Updated:** 08-September-2016 (ESCHAAB)

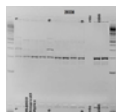
Notes

Mutant = 428 bp
 Heterozygote = 199 bp and 428 bp
 Wild type = 199 bp

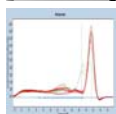
 Expected Results: Actual:

 Mutant = ~300 bp
 Heterozygote = 199 bp and ~300 bp
 Wild type = 199 bp

Attachments



View Smarcd3-29338 Gel.jpg



View Smarcd3 Mplex Melt.jpg

- View Smarcd3em1jMolDesJun2016.docx
- View Smarcd3 genomic.gcs

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
28550	-	CAT GCA AGG AGA CGC TGA G	-	Common	A
28551	-	GGA GGT GAA GCA GAA ACA GG	-	Wild type Reverse	A
28552	-	CTC CCC AAA GAC TGA AGC A	-	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	-
		5	-	-	repeat steps 2-4 for 10 cycles (Touchdown)

Reaction A (3 primer)		Cycling (TouchDown 65-60_12-12-11)			
28552	.5 uM	6	94	15sec	-
28550	.5 uM	7	60	15sec	-
28551	.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