



Welcome to The Genotyping Protocol System

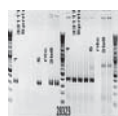
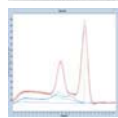
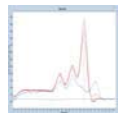
Master Protocol

Strain Name: C57BL/6NJ-Spr1a^{em1J}/J
Stock Number: 028323
Allele: Spr1a^{em1J}
Protocol Name: Spr1a^{em1J} Alternate2
Method: Separated MCA
Created: 08-April -2016 (LUCASM) **Updated:** 28-April -2016 (ESCHAAB)

Notes

Expected Results: Mutant = 395 bp
Heterozygote = 395 bp and 305 bp
Wild type = 305 bp

Attachments

[View](#) 28323.jpg[View](#) 331177-w2.jpg[View](#) 331177-m2.jpg

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[View](#) Spr1a genomic.gcs

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
24181	-	GGA TAG ACA GCA GCC TCA GC	-	Mutant Reverse	A
26137	-	CAG GGC TCT GGC CCC TTG	-	Wild type Reverse	B
27588	-	TGT GGA AGT CAG CAT GGA G	-	Common	A + B

Number Of Reactions 1

Reaction A (2 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
dNTPS-kapa	.26 mM	4	68	10sec	-
24181	.5 uM	5	-	-	repeat steps 2-4 for 10 cycles (Touchdown)
27588	.5 uM	6	94	15sec	-
		7	60	15sec	-

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

Reaction B (2 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
dNTPS-kapa	.26 mM	4	68	10sec	-
26137	.5 uM	5	-	-	repeat steps 2-4 for 10 cycles (Touchdown)
27588	.5 uM	6	94	15sec	-
Glycerol	6.5 %	7	60	15sec	-
Dye	1 X	8	72	10sec	-
Kapa 2G HS taq polymerase	.03 U/ul	9	-	-	repeat steps 6-8 for 28 cycles
DNA		10	72	2 min	-
		11	10	-	hold

Version 3.2