

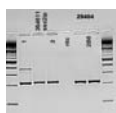
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Welcome to The Genotyping Protocol System

Master Protocol**Strain Name:** C57BL/6NJ-Ssx2ip^{em1J}/J**Stock Number:** 029404**Allele:** Ssx2ip^{em1J}**Protocol Name:** Ssx2ip^{em1J}**Method:** Standard PCR**Created:** 21-July -2016 (LUCASM) **Updated:** 09-September-2016 (ESCHAAB)**Notes**

Expected Results: Mutant = 356 bp
Heterozygote = 219 bp and 356 bp
Wild type = 219 bp

Attachments[View](#) Ssx2ip-29404 Gel.jpg[-](#) [View](#) Ssx2ip genomic.gcs[-](#) [View](#) Ssx2ipem1jMolDesJuly2016.docx**Protocol Primers**

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
28926	-	TCT CCG TCC AGT TTG TAC TCC	-	Wild type Forward	A
28927	-	CAC ACA GGC TTG GTA AAT GC	-	Common	A
28928	-	CCA CCA AAG AGG AAT TCT TGA	-	Mutant Forward	A

Number Of Reactions

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	-
28927	.5 uM	5	-	-	repeat steps 2-4 for 10 cycles (Touchdown)
28926	.5 uM	6	94	15sec	-
28928	.5 uM	7	60	15sec	-
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

Version 3.2