



Welcome to The Genotyping Protocol System

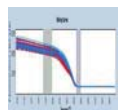
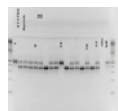
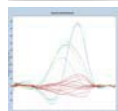
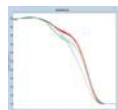
Master Protocol

Strain Name: C57BL/6NJ-Spink12^{em1J/J}
Stock Number: 027573
Allele: Spink12-6847-KOMP CRISPR
Protocol Name: Spink12-6847-KOMP CRISPR
Method: High Resolution Melting
Created: 30-April -2015 (JKELMEN) **Updated:** 04-January -2016 (DTG)

Notes

Expected Results: Mutant = 390 bp
Heterozygote = 390 bp and 500 bp
Wild type = 500 bp

Attachments

[View](#) 300658-norm1.jpg[View](#) 311764-27573.jpg[View](#) 311764-dp1.jpg[View](#) 311764-hrm1.jpg[View](#) - Spink12 genomic.gcs

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
23486	-	TGT CTG CAA GCA TTG TGA AAC	-	Forward	-
23487	-	TGA GGA TCT GTG ACA TCT GTC TG	-	Reverse	-

Reaction/Components A

Reaction Components	Volume Amt	Final Concentration	Total Volume Amt
ddH2O	5.15	-	-
5 X Kapa 2G HS buffer	2.40	1	-
25 mM MgCl2	0.96	2	-
10 mM dNTP KAPA	0.24	.2	-
20 uM 23486	0.30	.5	-

Cycling

Step #	Temp°C	Time	Note
1	94	2 min	-
2	94	20sec	-
3	65	15sec	-0.5 C per cycle decrease
4	68	10sec	-

Reaction/Components A				Cycling			
20 uM 23487	0.30	.5	-	5	-	-	repeat steps 2-4 for 10 cycles
2.5 U/ul Kapa 2G HS taq polymerase	0.05	.01	-	6	94	15sec	-
20 X EvaGreen	0.60	1	-	7	60	15sec	-
DNA	2.00	-	-	8	72	10sec	-
				9	-	-	repeat steps 6-8 for 28 cycles
				10	72	2 min	-
				11	10	-	hold

Number Of Reactions 1

Version 3.2