



Welcome to The Genotyping Protocol System

Master Protocol

Strain Name: C57BL/6NJ-Fxn^{em1J}/J
Stock Number: 025732
Allele: Fxn^{em1J}-SEQ
Protocol Name: Fxn^{em1J}-SEQ
Method: Sanger sequencing
Created: 13-March -2015 (JKELMEN) **Updated:** 29-May -2015 (JKELMEN)

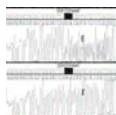
Notes

Mutant =ATGTGGGCG----GAGGTCGCG

Notes: Wild type =ATGTGGGCG**ttcg**GAGGTCGCG

4 bp deletion of TTCG

Attachments



View 25732-controls.JPG

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View Fxnem-DJL-4-15.SPF

Sequence

Sequence GCAGGAGCCCAGCCGCGGCGCTCGGGGGGGGGGGGGGGCGGGGGGGGGCGGGGCG
GCCGCGG
AGCTGGAGTAGCATGTGGGCG(**ttcg**)GAGGTCGCGCAGCCGTGGGCTTGCTGCCCCGGA
CGGCGT
CCCGGGCCTCCGCCTGGGTCGGAACCCGCGCTGGAGGAACCGATCGTAACCTGC

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
20657	-	GCG GAG CTG GAG TAG CAT	-	Forward	-
20658	-	ACG ATC GGT TCC CTC CAG	-	Reverse	-

Reaction/Components A

Reaction Components	Volume Amt	Final Concentration	Total Volume Amt
ddH2O	4.55	-	-
5 X Kapa 2G HS buffer	2.40	1	-
25 mM MgCl2	0.96	2	-
10 mM dNTPS-kapa	0.24	.2	-
100 uM 20657	0.30	.5	-
100 uM 20658	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	-
5	-	-	repeat steps 2-4 for 10 cycles
6	94	15sec	-

Reaction/Components A				Cycling			
50 % Glycerol	1.20	5	-	7	60	15sec	-
2.5 U/ul Kapa 2G HS taq polymerase	0.05	.01	-	8	72	10sec	-
DNA	2.00	-	-	9	-	-	repeat steps 6-8 for 28 cycles
				10	72	2 min	-
				11	10	-	hold

Number Of Reactions 1

Version 3.2