

C3.B6-*Ift88^{flexo}/J*

Stock No: 019149

Protocol 15044: Pyrosequencing Assay - *Ift88*<flexo>Pyro

Version 2.0

Notes

This genotyping assay uses pyrosequencing technology and is run on the Biotage PSQ 96MA. The Jackson Laboratory is not posting the complete details of our pyrosequencing genotyping assays as the primers for pyrosequencing cannot be used for sequencing using more traditional methods. The wild type and mutant nucleotides and the flanking DNA sequence are provided below.

The genotyping protocol(s) presented here have been optimized for reagents and conditions used by The Jackson Laboratory (JAX). To genotype animals, JAX recommends researchers validate the assay independently upon receipt of animals into their facility. Reaction cycling temperature and times may require additional optimization based on the specific genotyping reagents used.

Expected Results

Mutant = G/G

Heterozygote = A/G

Wild type = A/A

Sequencing primer is on the reverse strand.

Homs are lethal.

Sequence

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AACTTCAGACAATCTGTTTCTAGGCTGTAGACACATTGAA
AATGTTTGAGAAGAAGGACAG
TAGAGTGAAGAGTGCAGCTGCGACCAACCTCTCGTTCT
GTATTATCTGG(tc)AGGTTTTG
GTTTTACAGCTAAGGGTTGTTTCATATGATAGTTTTGCATTA
CTAAATCTGCCTAAGAAAA
GGCCTCCAAAGAAGATTGGTGCAAACCTGTAAGTGGACTG
AGGTGCACCA
```

JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
15557	Fluorophore	CCA ACC TCT CGT TCC TGT ATT ATC				
15558		GAG GCC TTT TCT TAG GCA GAT TTA				
15559		GCT GTA AAA ACC AAA ACC T				

Reaction A

COMPONENT	FINAL CONCENTRATION
ddH ₂ O	
Kapa 2G HS buffer	1.00
MgCl ₂	2.00
dNTPS-kapa	0.20
15557	0.50
15558	0.50
Glycerol	5.00
Kapa 2G HS taq polym	0.01
DNA	

Cycling

STEP	TEMP °C	TIME	NOTE
1	94.0	--	
2	94.0	--	
3	65.0	--	-0.5 C per cycle decrease
4	68.0	--	
5		--	repeat steps 2-4 for 10 cycles
6	94.0	--	
7	60.0	--	
8	72.0	--	
9		--	repeat steps 6-8 for 28 cycles
10	72.0	--	
11	10.0	--	hold

JAX uses a very high speed Taq (~1000 bp/sec), use cycling times recommended for your reagents.

