B6(Cg)-*Ncf1*^{m1J}/J

Stock No: 004742
Protocol 13713: Pyrosequencing Assay - Ncf1<m1J>
Version 4.0

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 = C/C
Heterozygote = A/C
Wild type = A/A

Sequence

GCCTTTGGTCCTATCCTTACAGTATATAAAATGTTATCTTA
AAAAAAAAAACGTAGGGG
CCCTCTTCTGGTGTGTCTGAAGACAGTTGCTGTGTACTCA
TATGAAATAAATAAAATAA
AAAACTTAGAAAGGGAAAGCCAGAAAGAATAGCTCTGAG
GGGACTGAGGCTGTGTG
CTTGTGGTAGTGGCTAGACAGTTGGAAGAAGCTGAGAGTT
GAGGAGACTCTCGCCTC
ACTGGCTGCTCCCTCCGCGGACC(A/C)GGTGAACCGTAT
GTAACCATCAAAGCGTACG
CTGCTGTTGAAGAGGACGAGATGTCCCTGTCTGAGGGTG
AAGCCATTGAGGTCATTC
ATAAGCTCCTGGATGGCTGGTGGGTGGTCAGGTAGGAGA
GCCCCTCCCTCTGCACAC
ACCTCTCTGAGTGGGTTTGGGGAACCTCTCTTGGGCTCTG
AGCTGACTTGGCTGTGCG
ATTCTGGAGTCTTCTGTCCTCATTAGCCGCAGAGTTTCATC
TCCAGGACCTTCTGCAAT
CAAACAAACAAAATAAAAACAAGAGCAGACGAGAAGGAA
AATG

JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
polMR0020		TGT GCT TGT GGT AGT GGC TAG AC				
polMR0021	Fluorophore	GTA CGC TTT GAT GGT TAC ATA CGG				
polMR0022		GCT CCC TCC GCG GAC				

Reaction A

COMPONENT	FINAL CONCENTRATION
ddH2O	
Kapa 2G HS buffer	1.00
MgCl2	2.00
dNTPS-kapa	0.20
polMR0020	0.50
polMR0021	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.