

C57BL/6J-Nek8^{jck}/J

Stock No: 002561

Protocol 17822: Sanger sequencing Assay - Nek8<jck>

Version 2.0

Notes

Mut = TGT

WT= GGG

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

Sequence

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GACATCAGCCAGGTGGGTGGTCCCCGTACCCTGGAGAG
GGGAAGTGAGCGCAACTCC
CAGGCTGCACCTGCGCTGGGTTAGATCGGGGAAGTACTG
GAAGCTTCCTCCCCAGTG
GCTTCCACCTGTTGCTGTTTCTGCCGCACTGTGCCACTG
TGCGTGCGGTGGGTGTGGT
TGGAGTTAAAGAGACAGTTACCTTCTGAGAATTTGCAGCC
TCCAAC TTCGGCTTGCA
GGGAGGGGACTCAGACCATGGTAGGAGGCAAATCCTTC
ACTTTTTTTTTTCTTTCT
CTAGCCACCATTGTAGAAGCCTTGCT(g/t)G(g/t)CTATGAG
ATGGTGCAGGT
GGCCTGTGGGCCTCTCATGTGCTGGCCCTGTCCACAGA
TGGAGAGTTATTCGCCTGG
GGCAGAGGAGATGGTGGTAAGCCCACTGCCAGCTCCA
GTGTTTAGGGTCCCTCTCA
CAACTTCTCCATCATGCTTTGTTTCATTCTATACCTGTCTA
CCTCTCCGTGGCCATTCT
TTCCTTCCCCCCTTTTTTTTTCTCGAATTCTATTCATTCAT
TGAACGAACCATATTTTCAT
ACTTTATTAGTGCTCAGATAAGATAAATGAGGTGTTGGCAC
ATTGCTTATCTATTTTTGCAA
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JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
25278		TTT TCT TTC TCT AGC CCA CCA		Forward	A	
25279		TAA GCA ATG TGC CAA CAC CT		Reverse	A	

Reaction A

COMPONENT	FINAL CONCENTRATION
ddH2O	
Kapa 2G HS buffer	1.30 X
MgCl2	2.60 mM
dNTPS-kapa	0.26 mM
25278	0.50 uM
25279	0.50 uM
Glycerol	6.50 %
Kapa 2G HS taq polym	0.03 U/ul
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 (Touchdown)
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.

JAX uses a 'touchdown' cycling protocol and therefore has not calculated the optimal annealing temperature for each set of primers.

