

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

Stock No: 002561

Protocol 2120: Restriction Enzyme Digest Assay - Nek8<jck>

Version 1.1

Notes

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 = 308 bp

Heterozygote = 108 bp and 200 bp and 308 bp

Wild type = 108 bp and 200 bp

Separated by gel electrophoresis on a 1.5% agarose gel.

JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
oIMR4004		CTT CCC ACC TGT TGC TGT TT				
oIMR4005		CAG TGG GCT TAC CAC CAT CT				

Reaction A

COMPONENT	FINAL CONCENTRATION
ddH ₂ O	
AB PCR BufferII	1.00 X
MgCl ₂	2.00 mM
dNTP	0.20 mM
oIMR4004	1.00 uM
oIMR4005	1.00 uM
DNA Loading Dye	0.69 mM
Taq DNA Polymerase	0.03 U/ul
DNA	

Cycling

STEP	TEMP °C	TIME	NOTE
1	94.0	--	
2	94.0	--	
3	67.0	--	
4	72.0	--	repeat steps 2-4 for 35 cycles
5	72.0	--	
6	10.0	--	hold

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

Reaction B

COMPONENT	FINAL CONCENTRATION
AB PCR BufferII	X
ddH ₂ O	
BseYI	

