

## C57BL/6J-Nek8<sup>jck</sup>/J

Stock No: 002561

Protocol 35038: End Point Analysis Assay - Nek8<jck>-EP

Version 1.0

### 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 = T, T

Wild type = G, G

>[chr11:77982518-77982630](#) 113bp AGGAGGCAAATCCTTCACTT AGGGCCAGCACATGAGAG

### Sequence

```
TGGAGTTAAAGAGACAGTTACCTTCTGAGAATTTGCAGCC
TCCAACCTCCGGCTTGCA
GGGGAGGGGACTCAGACCATGGTAGGAGGCAAATCCTTC
ACTTTTTTTTTTCTTTCT
CTAGCCCACCATTTAGTAAGCCTTGCT(g/t)G(g/t)CTATGAG
ATGGTGCAGGT
GGCCTGTGGGGCCTCTCATGTGCTGGCCCTGTCCACAGA
TGGAGAGTTATTCGCCTGG
GGCAGAGGAGATGGTGGTAAGCCCACTGCCAGCTCCA
GTGTTTAGGGTCCCTCTCA
CAACTTCTCCATCATGCTTTGTTTCATTCCTAT
```

### JAX Protocol

#### Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
45099		AGG AGG CAA ATC CTT CAC TT		Forward	A	
45100		AGG GCC AGC ACA TGA GAG		Reverse	A	
45101	Fluorophore-1	AGC CTT GCT GGG CTA TGA GAT	Quencher-1	WT Probe		
45102	Fluorophore-2	AAG CCT TGC TTG TCT ATG AGA TG	Quencher-2	MUT Probe		

#### Reaction A

COMPONENT	FINAL CONCENTRATION
Kapa Probe Fast QPCR	1.00 X
ddH2O	
45099	0.40 uM
45100	0.40 uM
Wt Probe	0.15 uM
Mutant Probe	0.15 uM
DNA	

#### Cycling

STEP	TEMP °C	TIME	NOTE
1	95.0	--	
2	95.0	--	
3	60.0	--	
4		--	repeat steps 2-3 for 40 cycles
5	4.0	--	Forever

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

Endpoint Fluorescence Scatter Plot

Select Zoom

