

## B6C3-Npr2<sup>pwe</sup>/SacJ

Stock No: 020812

Protocol 22017: End Point Analysis Assay - Npr2&lt;pwe&gt; End Point

Version 3.0

### 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 = 4 nucleotide deletion

Wild type = 4 nucleotides present

### Sequence

```

TGTGTTTACCCACTTTTCCTATGGTGGTCCTTCCCCTCCTG
CTCACCCCGAGTCCTGTTTCCA
TGGTGCAGACTGTATTGGTGATCACATACCGAGAACCCC
CAAATCCTGAGTATCAGGAGTTT
CAGAATCGC[ctgc]TGATCAGAGCCCGGGAAGACTTTGGTG
TGGAGCTGGCCCCATCCCTGGT
GAGTAGATCTCTGAGGGTCAGAACAGGCTACGAGGAAGG
GACTTCTCACAGAGTCCTTGATC
ATGGAGCAAGCCCCATTTTCAGGCTGTGACTTCCATCCAG
GCTACTACCCTTTGACCCCGGGAC
CGGGGGTGGGGGAGGG
  
```

### JAX Protocol

#### Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
15839		CCC GAG TCC TGT TTC CAT G		Forward	A	
15840		AGC TCC ACA CCA AAG TCT TC		Reverse	A	
15841	Fluorophore-1	AGA ATC GCC TGC TGA TCA GA	Quencher-1	WT Probe		
15842	Fluorophore-2	TCA GAA TCG CTG ATC AGA GC	Quencher-2	MUT Probe		

#### Reaction A

COMPONENT	FINAL CONCENTRATION
Kapa Probe Fast QPCR	1.00 X
ddH2O	
15839	0.40 uM
15840	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

