

## RMRC Genotyping Protocol-PCR

No.	RMRC13327	Strain Name	C57BL/6N- <i>Gnb4<sup>tm1aNarl</sup></i> /Narl
-----	-----------	-------------	--

### A. Primers

No.	Primer name	Sequence
1	Gnb4LF	CTGTTTGGTTCTCAGAACCTGCATC
2	Gnb4LR	GAACACCATGGACACACAGGATGTC

### B. Reaction Conditions

Component	Concentration	Volume	Step	Temperature, °C	Time, sec	Number of Cycles
H <sub>2</sub> O	–	5.9 μl	1	94	110	1
Primer 1	20 μM (μmol/μl)	0.3 μl	2	94	15	35
Primer 2	20 μM (μmol/μl)	0.3 μl	3	60	15	35
gDNA	50-250 ng/μl	1 μl	4	68	30	35
OneTaq*	2X Master Mix	7.5 μl	5	68	180	1
Total		15 μl				

\*M0488 OneTaq Hot Start Quick-Load 2X Master Mix with Standard Buffer from New England Biolabs.

### C. PCR Product

No.	Primer set		Product size	Target region
1	Gnb4LF	Gnb4LR	215 bp	Wildtype
2	Gnb4LF	Gnb4LR	310 bp	Mutant

### D. Gel Photo

