

## RMRC Genotyping Protocol-PCR

No.	RMRC13150	Strain Name	C57BL/6- <i>Ngf</i> <sup>tm1a(GEMMS)Narl</sup> /Narl
-----	-----------	-------------	--

### A. Primers

No.	Primer name	Sequence
1	NGF49734F	ACAGAGACAGGCAGACAGATGGAG
2	NGF50174B	ATCAAACACCCCTTACCAAGTCC

### B. Reaction Conditions

Component	Concentration	Volume	Step	Temperature, °C	Time, sec	Number of Cycles
H <sub>2</sub> O	–	10.8 μl	1	94	180	1
PCR buffer	10 x	1.5 μl	2	94	30	35
Formamide	–	0.5 μl	3	60	30	35
dNTP	10 mM	0.3 μl	4	72	30	35
Primer 1	20 μM (μmol/μl)	0.3 μl	5	72	180	1
Primer 2	20 μM (μmol/μl)	0.3 μl				
gDNA	50-250 ng/μl	1 μl				
Taq	5 U/μl	0.3 μl				
Total		15 μl				

### C. PCR Product

No.	Primer set		Product size	Target region
1	NGF49734F	NGF50174B	440 bp	Wildtype
2	NGF49734F	NGF50174B	570 bp	Mutant

### D. Gel Photo

