

RMRC Genotyping Protocol-PCR

No.	RMRC13175	Strain Name	C57BL/6-Ngf<tm1c(GEMMS)Narl>
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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 ₂ 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

