

RMRC Genotyping Protocol-PCR

No.	RMRC13241	Strain Name	C57BL/6-Gas7 ^{tm1b(GEMMS)} Narl
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A. Primers

No.	Primer name	Sequence
1	Gas7KF	TGGAGATGTGAGTATGTGAGGTGC
2	Gas7KR	GGACTGATAAAGGTTGTGGGTGC
3	Gas7LF	CACTGAGAAGGAAGAAAGGAGCC
4	Gas7LR	GGACTGATAAAGGTTGTGGGTGC

B. Reaction Conditions

Component	Concentration	Volume	Step	Temperature, °C	Time, sec	Number of Cycles
H ₂ O	—	10.6 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 (pmol/µl)	0.3 µl	5	72	180	1
Primer 2	20 µM (pmol/µl)	0.3 µl				
Primer 3	20 µM (pmol/µl)	0.1 µl				
Primer 4	20 µM (pmol/µl)	0.1 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	Gas7KF	Gas7KR	410 bp	Mutant
2	Gas7LF	Gas7LR	390 bp	Wildtype

D. Gel Photo

