

RMRC Genotyping Protocol-PCR

No.	RMRC13304	Strain Name	C57BL/6- <i>Rbfa</i> ^{tm1a^{Narl}} /Narl
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A. Primers

No.	Primer name	Sequence
1	RbfaLF	CAGCACAGGTCTGTATGTTCTGGGT
2	RbfaLR	GGACCCGTGCTACATCTTCCCATCT

B. Reaction Conditions

Component	Concentration	Volume	Step	Temperature, °C	Time, sec	Number of Cycles
H ₂ O	–	5.9 μl	1	94	110	1
Primer 1	20 μM (pmol/μl)	0.3 μl	2	94	15	35
Primer 2	20 μM (pmol/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	RbfaLF	RbfaLR	150 bp	Wildtype
2	RbfaLF	RbfaLR	250 bp	Mutant

D. Gel Photo

