

## RMRC Genotyping Protocol-PCR

No.		Strain Name	C57BL/6- <i>Car10<sup>tm1aNarl</sup>/Narl</i>
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### A. Primers

No.	Primer name	Sequence
1	Car10LF	CAGGAAGTTGCTTGAGATCTTATCC
2	Car10LR	CAGAAAGATGCCCTAGGAAGATTA

### B. Reaction Conditions

Component	Concentration	Volume	Step	Temperature, °C	Time, sec	Number of Cycles
H <sub>2</sub> O	–	5.9 μl	1	94	80	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	60	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	Car10LF   Car10LR	260 bp	Wildtype
2	Car10LF   Car10LR	363 bp	Mutant

### D. Gel Photo

