

RMRC Genotyping Protocol-PCR

No.	RMRC13307	Strain Name	C57BL/6- <i>Rictor^{tm1cNarl}</i> /Narl
-----	-----------	-------------	---

A. Primers

No.	Primer name	Sequence
1	RictorLF	AGATGTTGGAACCTGATGCATGTGT
2	RictorLR	CATCTAAATGATCAGTGCCGTGTCT

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 (μmol/μl)	0.3 μl	2	94	15	35
Primer 2	20 μM (μmol/μl)	0.3 μl	3	60	15	35
gDNA	50-250 ng/μl	1 μl	4	68	30	35
LongAmp*	2X Master Mix	7.5 μl	5	68	180	1
Total		15 μl				

*M0533 LongAmp Hot Start Taq 2X Master Mix from New England Biolabs.

C. PCR Product

No.	Primer set	Product size	Target region
1	RictorLF RictorLR	330 bp	Wildtype
2	RictorLF RictorLR	430 bp	Mutant

D. Gel Photo

