

RMRC Genotyping Protocol-PCR

No.	RMRC13286	Strain Name	C57BL/6-Rictor ^{em1aSet}
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A. Primer

	Primer name	Sequence
1	RictorI35F	TGCTAGACTCTGAGCAACCAAG
2	RictorI33B	AGACTGACCTCTTCCCTCGAC
3	PGK-Neo1551F	TGTGTGTTGGTTTTTGTGTGCG

B. Reaction Conditions

Component	Concentration		Volume		Step	Temperature, °C	Time, sec	Number of Cycles
H ₂ O	--		39	μl	1	95	120	1
PCR buffer	10x		5	μl	2	95	20	35
dNTP	10	mM	1	μl	3	60	20	35
Primer 1	20	μM(μmol/μl)	1	μl	4	72	45	35
Primer 2	20	μM(μmol/μl)	1	μl	5	72	600	1
Primer 3	20	μM(μmol/μl)	1	μl	6			
DNA	0.1 μg/μl		1	μl	7			
Taq	5	U/μl	1	μl	8			
Mg ²⁺		mM		μl	9			
Total			50	μl	10			

C. PCR Product

	Primer set		Product size	Target region
1	RictorI35F	RictorI33B	521 bp	Wild type allele
2	PGK-Neo1551F	RictorI33B	433 bp	Rictor 1a allele

D. Gel Photo

