

RMRC Genotyping Protocol-PCR

No.	RMRC13292	Strain Name	C57BL/6-Lmna ^{tm1(G609G)Cyh}
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A. Primer

	Primer name	Sequence
1	CHI1218F	cctaagaagtcagtcccaaact
2	CHI1219R	aggaggtaggagcgggtgacta
3	CHI1228F	caacgctatgtcctgatagcggt
4	CHI1229R	cgaactgttcgccaggctcaagg

B. Reaction Conditions

Component	Concentration	Volume		Step	Temperature, °C	Time, sec	Number of Cycles
H ₂ O	--	19	μl	1	95	300	
PCR buffer	10x	2.5	μl	2	95	60	30
dNTP	2.5 mM	2	μl	3	60	60	30
Primer 1	100 μM(μmol/μl)	0.15	μl	4	72	30	30
Primer 2	100 μM(μmol/μl)	0.15	μl	5	72	360	
DNA	--	1	μl	6			
Taq	U/μl	0.2	μl	7			
Mg ²⁺	mM		μl	8			
Total		25	μl	9			

C. PCR Product

	Primer set	Product size	Target region
1	CHI1218F CHI1219R	298 bp	G609G C->T mutant site
2	CHI1228F CHI1229R	166 bp	amplify Neo cassette

D. Gel Photo

