

RMRC Genotyping Protocol-PCR

No.	RMRC13295	Strain Name	C57BL/6- <i>Gt(ROSA)26Sor^{tm1(CAG-dFLAG-Lmna*)}Cy^h/Narl</i>
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A. Primer

	Primer name	Sequence
1	CHI1228F	caacgctatgtcctgatagcgg
2	CHI1229R	cgaactgttcgccaggctcaagg
3	CHI531F	ctaggccacagaattgaaagatct
4	CHI532R	gtaggtggaaattctagcatcatcc
5	CHI1287F	atggagatgacctgctccatcac
6	CHI1262R	ttacatgatgctgcagttctggg

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	CHI1228F	CHI1229R	166 bp	amplify Neo cassette
2	CHI531F	CHI532R	324 bp	positive PCR control
	Primer set		Product size	Target region
1	CHI1287F	CHI1262R	176 bp	amplify human lamin A/Progerin from nt.1670 (Exon 11)
2	CHI531F	CHI532R	324 bp	positive PCR control

D. Gel Photo

