

RMRC Genotyping Protocol-PCR

No.	RMRC13301	Strain Name	C57BL/6J-Sln ^{em2Cyt}
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A. Primer

	Primer name	Sequence
1	CHI1265F	GCACAAACCCTCTCCTTTTCCAT
2	CHI1266F	TAGAAAACATGCCAAGGACCTAGT

B. Reaction Conditions

Component	Concentration		Volume		Step	Temperature, °C	Time, sec	Number of Cycles
H ₂ O	--		19.5	μl	1	95	300	1
PCR buffer	10x		2.5	μl	2	95	30	30
dNTP	2.5	mM	2	μl	3	55	30	30
Primer 1	100	μM(μmol/μl)	0.15	μl	4	72	40	30
Primer 2	100	μM(μmol/μl)	0.15	μl	5	72	420	1
DNA	--		0.5	μl	6	4	∞	-
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	CHI1265F	CHI1266R	417 bp	Wild-type allele
2	CHI1265F	CHI1266R	324 bp	Mutant allele

D. Gel Photo

