

RMRC Genotyping Protocol-PCR

No.	RMRC13357	Strain Name	C57BL/6- <i>Ccn12^{em1Fmj}</i> /Narl
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A. Primer

	Primer name	Sequence
1	Ccn12 5VF1	GTCGATACGGAGACGGGACT
2	Ccn12 5VR1	TTTGAATGCAGGACTCACGC
3	Ccn12 3VR1-1	GCAAGGACTGCTCTATCAAAGGAC

Reaction Conditions

Component	Concentration		Volume		Step	Temperature, °C	Time, sec	Number of Cycles
H ₂ O	--		15.9	μl	1	95	5 min	1
PCR buffer	10x		2	μl	2	95	30	
dNTP	10	mM	0.2	μl	3	62	30	40
Primer 1	10	μM(ρmol/μl)	0.2	μl	4	72	30	
Primer 2	10	μM(ρmol/μl)	0.2	μl	5	72	3 min	1
DNA	--		1	μl	6	16	∞	
Taq	5	U/μl	0.5	μl	7			
Mg ²⁺		mM	0	μl	8			
Total			20	μl	9			

B. PCR Product

	Primer set		Product size	Target region
1	5VF1	5VR1	410 bp	Ccn12 WT allele
2	5VF1	3VR1-1	514 bp	Ccn12 KO allele

C. Gel Photo

1.2% agarose gel,

Marker: 1 Kb plus DNA Ladder (Thermo Fisher Scientific)

