

## RMRC Genotyping Protocol-PCR

No.	RMRC13356	Strain Name	C57BL/6- <i>Ccn1</i> <sup>em1Fmj</sup> /Narl
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### A. Primer

	Primer name	Sequence
1	Ccn1 5VF1 10374	GAGACGGATCTGCGCATCTTG
2	Ccn1 5VR1 10757	GCGCTTACCTCGAAACTGTG
3	Ccn1 3VR1 21499	TTGCTCTGAGATCGAGACCG

### Reaction Conditions

Component	Concentration		Volume		Step	Temperature, °C	Time, sec	Number of Cycles
H <sub>2</sub> O	--		15.9	μl	1	95	5 min	1
PCR buffer	10x		2	μl	2	95	30	
dNTP	10	mM	0.2	μl	3	60	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 <sup>2+</sup>		mM	0	μl	8			
Total			20	μl	9			

### B. PCR Product

	Primer set		Product size	Target region
1	Ccn1 5VF1 10374	Ccn1 5VR1 10757	384 bp	Ccn1 WT allele
2	Ccn1 5VF1 10374	Ccn1 3VR1 21499	602 bp	Ccn1 KO allele

### C. Gel Photo

1.2% agarose gel,

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

