

RMRC Genotyping Protocol-PCR

No.	RMRC13396	Strain Name	C57BL/6N- <i>Cenp</i> ^{tm1Tkt} /Narl
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A. Primer

	Primer name	Sequence
1	CPAPKO2-delta-F	TTCATTAGGTTTCAAAGGGGAAGGT
2	CPAPKO2-WT-F	TGAGGAGAATCCATACGAACATGG
	CPAPKO2-WT-R	CTTCTGTCCAGCACATATCCACACA

B. Genomic extraction :

- ☐ Phenol-Chloroform extraction
 ☐ commercial extraction kit _____
- ☒ Lysis buffer alkaline lysis
☐ other _____

C. Reaction Conditions

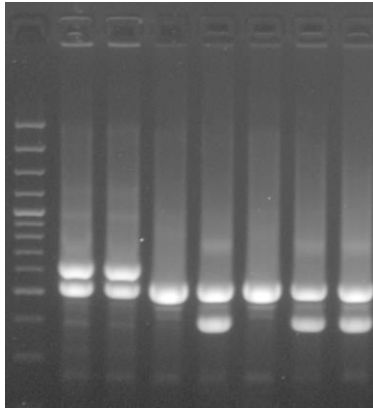
Component	Concentration		Volume		Step	Temperature, °C	Time, sec	Number of Cycles
H ₂ O	--		5.75	μl	1	95	100	
PCR buffer	10x		1	μl	2	95	15	38
dNTP	10	mM	0.2	μl	3	55	20	
Primer 1	5	μM(ρmol/μl)	1	μl	4	72	30	
Primer 2	5	μM(ρmol/μl)	1	μl	5			
DNA	--		1	μl	6			
Taq	5	U/μl	0.05	μl	7			
Mg ²⁺		mM		μl	8			
Total			10	μl	9			

The information of Taq, including company name and catalog number :

D. PCR Product

	Primer set		Product size	Target region
1			539 bp	WT
2			647 bp	flox allele
			406 bp	KO

E. Gel Photo



(+/flox; +/flox; +/+; +/KO; +/+; +/KO; +/KO)