

RMRC Genotyping Protocol-PCR

No.	RMRC13394	Strain Name	C57BL/6- <i>Prap1</i> ^{em1(E85V)<i>Yen</i>} /Narl
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A. Primer

	Primer name	Sequence
1	PRAP1-I3-F	5'-TACCTACTCCCTTGCCCTTG-3'
2	PRAP1-reverse	5'-CAGTCTCCACCCAGGTCATG -3'
3	E85V-reverse	5'-CAGTCACTACCCATGTCATG -3'

B. Genomic extraction :

- ☐ Phenol-Chloroform extraction
 ☐ commercial extraction kit _____

☒ Lysis buffer _____
 ☐ other _____

C. Reaction Conditions: for PRAP1 wt allele

Component	Concentration		Volume		Step	Tempera- ture, °C	Time, min	Number of Cycles
H ₂ O			3	μl	1	95	3	1
Ampliqon Taq DNA Polymerase 2x Master Mix RED	2X		10	μl	2	95 56 72	0.5 0.5 0.5	30
Primer 1	PRAP1-I3-F	10 μM	1	μl	3	72	3	
Primer 2	PRAP1-reverse	10 μM	1	μl	4	4	pause	
DNA	--		5	μl	5			
Total			20	μl	6			

C. Reaction Conditions: for PRAP1 E85V allele

Component	Concentration		Volume		Step	Tempera- ture, °C	Time, min	Number of Cycles
H ₂ O			3	μl	1	95	3	1
Ampliqon Taq DNA Polymerase 2x Master Mix RED	2X		10	μl	2	95 56 72	0.5 0.5	30
Primer 1	PRAP1-I3-F	10 μM	1	μl	3	72	3	1
Primer 2	E85V-reverse	10 μM	1	μl	4	4	pause	
DNA	--		5	μl	5			
Total			20	μl	6			

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

Taq DNA Polymerase 2x Master Mix RED, 2 mM MgCl₂; Ampliqon #A190303

D. PCR Product

	Primer set		Product size	Target region
1	PRAP1-I3-F	PRAP1-reverse	497 bp	
2	PRAP1-I3-F	E85V-reverse	497 bp	

E. Gel Photo

