

RMRC Genotyping Protocol-PCR

No.	RMRC13393	Strain Name	B6.129- <i>Prap1</i> ^{tm1Yen} /Narl
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A. Primer

	Primer name	Sequence
1	PRAP1-F	5'-CCTGCGTACCAGCAGAAGAGCTC -3'
2	5p2	5'-AGGGTCCTCAAGGGCAAGGGAGT -3'
3	SPRP2	5'-TCGATAGCTTGGCTGCAGGTCG -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			2	μl	1	94	5	1	
Ampliqon Taq DNA Polymerase 2x Master Mix RED	2X		10	μl	2	94 68 72	1 1 1	30	
Primer 1	PRAP1-F	10 μM	1	μl	3	72	7		1
Primer 2	5p2	10 μM	2	μl	4	4	pause		
DNA	--		5	μl	5				
Total			20	μl	6				

C. Reaction Conditions: for PRAP1 KO allele

Component	Concentration		Volume		Step	Temperature, °C	Time, min	Number of Cycles
H ₂ O			1.6	μl	1	94	5	1
Ampliqon Taq DNA Polymerase 2x Master Mix RED	2X		10	μl	2	94 58 72	1 1 1	30
DMSO			2	μl	3	72	7	1
Primer 1	PRAP1-F	10 μM	1	μl	4	4	pause	
Primer 2	SPRP2	10 μM	0.4	μl	5			
DNA	--		5	μl	6			
Total			20	μl	7			

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-F	5p2	532 bp	
2	PRAP1-F	SPRP2	280 bp	

E. Gel Photo

