

RMRC Genotyping Protocol-PCR

No.	RMRC13406	Strain Name	B6.129- <i>Tpt1</i> ^{tm2^{Yen}} /Narl
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A. Primer

	Primer name	Sequence
1	TCTP-P3	5'-GTCTCAGCCCATTTTGTGTAGC-3'
2	TCTP-P4	5'-AAAGCAGATCCAGAATAACCCC -3'

B. Genomic extraction :

- Phenol-Chloroform extraction commercial extraction kit _____
 Lysis buffer _____ other _____

C. Reaction Conditions: for Tpt1 wt allele

Component	Concentration		Volume		Step	Temperature, °C	Time, min	Number of Cycles
H ₂ O			3	μl	1	94	5	1
Ampliqon Taq DNA Polymerase 2x Master Mix RED	2X		10	μl	2	94	1	39
						55	1	
						72	1	
Primer 1	TCTP-P3	10 μM	1	μl	3	72	10	1
Primer 2	TCTP-P4	10 μM	1	μl	4	4	pause	
DNA	--		5	μl				
Total			20	μl				

C. Reaction Conditions: for Tpt1-floxed allele

Component	Concentration		Volume		Step	Temperature, °C	Time, min	Number of Cycles
H ₂ O			3	μl	1	94	5	1
Ampliqon Taq DNA Polymerase 2x Master Mix RED	2X		10	μl	2	94	1	39
						55	1	
						72	1	

Primer 1	TCTP-P3	10 μ M	1	μ l	3	72	10	1
Primer 3	TCTP-P4	10 μ M	1	μ l	4	4	pause	
DNA	--		5	μ l				
Total			20	μ l				

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	TCTP-P3	TCTP-P4	250 bp	Tpt1 WT allele
2	TCTP-P3	TCTP-P4	290 bp	Tpt1-Floxed allele

E. Gel Photo

