

RMRC Genotyping Protocol-PCR

No.	RMRC13406	Strain Name	B6.129- <i>Tpt1</i> ^{tm2Yen} /Narl
-----	-----------	-------------	---

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 QuickExtract DNA Extraction Solution other _____

C. Reaction Conditions: for Tpt1 wt and Tpt1-floxed allele

Component	Concentration		Volume		Step	Temperature, °C	Time, sec	Number of Cycles
H ₂ O			8.9	μl	1	95°C	90 sec	1
2x Master Mix	2X		10	μl	2	95°C	20 sec	10
Primer 1	TCTP-P3	20 μM	0.3	μl	3	63°C -.5°C/cycle	20 sec	10
Primer 2	TCTP-P4	20 μM	0.3	μl	4	72°C	30 sec	10
DNA	--		0.5	μl	Go to 2 for 10 cycles			
Total			20	μl	5	95°C	20 sec	28
					6	58°C	20 sec	28
					7	72°C	30 sec	28
					Go to 5 for 28 cycles			
					8	72°C	60 sec	1

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

PB10.33-05 PCRBIO Ultra Mix Red

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

