

RMRC Genotyping Protocol-PCR

No.	RMRC13405	Strain Name	C57BL/6- <i>Tpt1</i> ^{em2Yen} /Narl
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A. Primer

	Primer name	Sequence
1	Tp1 e5 GT Fw	5'-gccagtgcctctaattgctgg-3'
2	B6 tpt1 E138 Rv	5'-caaccataccatctggattcatgtttc -3'
3	Tpt1 K138 Rv	5'- caaccataccatctggattcatgttctt -3'
4	Internal control Fw	5'-gccacttgctaaagcacctc -3'
5	Internal control Rv	5'-tgcacatggctctcttctcc -3'

B. Genomic extraction :

- ☐ Phenol-Chloroform extraction
 ☐ commercial extraction kit _____

☒ Lysis buffer _____
 ☐ other _____

C. Reaction Conditions: for Tpt1 wt allele

Component	Concentration		Volume		Step	Tempera- ture, °C	Time, min	Number of Cycles
H ₂ O			3.5	μl	1	94	3	1
Ampliqon Taq DNA Polymerase 2x Master Mix RED	2X		10	μl	2	94	45sec	12
						68 (-0.5°C per cycle)	0.5	
						72	35sec	
Primer 1	Tp1 e5 GT Fw	10 μM	0.375	μl	3	94	20sec	23
						62	0.5	
						72	35sec	
Primer 2	B6 tpt1 E138 Rv	10 μM	0.375	μl	4	72	5	1
Primer 4	Internal control Fw	10 μM	0.375	μl	5	4	pause	
Primer 5	Internal control Rv	10 μM	0.375	μl				
DNA	--		5	μl				
Total			20	μl				

C. Reaction Conditions: for Tpt1 E138K allele

Component	Concentration		Volume		Step	Temperature, °C	Time, min	Number of Cycles
H ₂ O			3.5	μl	1	94	3	1
Ampliqon Taq DNA Polymerase 2x Master Mix RED	2X		10	μl	2	94	45sec	12
						68 (-0.5°C per cycle)	0.5	
						72	35sec	
Primer 1	Tp1 e5 GT Fw	10 μM	0.375	μl	3	94	20sec	23
						62	0.5	
						72	35sec	
Primer 3	tpt1 K138 Rv	10 μM	0.375	μl	4	72	5	1
Primer 4	Internal control Fw	10 μM	0.375	μl	5	4	pause	
Primer 5	Internal control Rv	10 μM	0.375	μl				
DNA	--		5	μl				

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	Tp1 e5 GT Fw	B6 tpt1 E138 Rv	233 bp	
2	Tp1 e5 GT Fw	tpt1 K138 Rv	233 bp	
3	Internal control Fw	Internal control Rv	492 bp	

E. Gel Photo

