

RMRC Genotyping Protocol-PCR

No.	RMRC13111	Strain Name	B6.129-Tpt1 ^{tm1(280)Yen} >
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A. Primer

	Primer name	Sequence
1	P1	5-TCTAGAAAAAGTGGAGGCGGAGC-3
2	P4	5-AAAGCAGATCCAGAATAACCCC-3
3	P5	5-GGTGACTACTGTGCTTTCGGTA-3

B. Reaction Conditions

Component	Concentration		Volume		Step	Temperature, °C	Time, sec	Number of Cycles
H ₂ O	--		15.5	μl	1	94	300	1
PCR buffer	10x		2.5	μl	2	94	60	40
dNTP	2	Mm	2.5	μl	3	57	60	40
Primer 1	10	μM(ρmol/μl)	1	μl	4	72	60	40
Primer 2	10	μM(ρmol/μl)	1	μl	5	72	420	1
Primer 3	10	μM(ρmol/μl)	1		6	4	-	-
DNA	--		1	μl	7			
Taq	2	U/μl	0.25	μl	8			
DMSO	100%		1.25	μl	9			
Total			25	μl				

C. PCR Product

	Primer set		Product size	Target region
1	P1	P5	450 bp	Second intron of the TCTP gene (wt)
2	P1	P4	250 bp	Second and forth intron of the TCTP gene (mt)

D. Gel Photo

