

PCR Genotyping of TTP KO mice

Tails processed (name/method):

Tail numbers genotyped:

0.25µl 20 mM dNTP (20 mM of each dNTP)		
2.5µl 10x (NH ₄) ₂ SO ₄ buffer		
0.25µl 10mg/ml BSA		
1.5µl 25 mM MgCl ₂		
16.05µl water		
0.2µl Taq DNA Polymerase (1U)		
1µl forward primer (10 pmole)		
1µl reverse primer (10 pmole)		
1.25µl 100% DMSO		
24 µl		
+ 1 µl water or DNA		
25µl total volume		

PCR primers to use:

MGTTP/f5832-5856	endog. 305nt	MGTTP/f5832-5856	Neo 1106nt
MGTTP/r6137-6115	Neo 1445nt	pMC1PolA Neo r1328-1307	
MGTTP/f5832-5856	endog. 435nt	MGTTP/f5832-5856	Neo 1106nt
MGTTP/r6267-6247	Neo 1575nt	pMC1PolA Neo r1328-1307	
MGTTP/f5925-5945	endog. 342nt	MGTTP/f5925-5945	Neo 1013nt
MGTTP/r6267-6247	Neo 1482nt	pMC1PolA Neo r1328-1307	

PCR program: 94° C, 2 min After PCR reaction is done, add 2.5µl
 94°, 30 sec of 10x R-stop. Load 12-14µl on a
 62°, 30 sec 1.5% agarose gel (TAE buffer).
 72°, 3 min
 cycle 29 times
 72°, 10 min
 hold at 4°

Forward primers:

5'-CGAATCCCTCGGAGGACTTTGGAAC-3' (MGTTP/f5832-5856)

5'-GGGCCGAAGCTGTGGCTGGGT-3' (MGTTP/f5925-5945)

Reverse primers:

5'-CGAAGTTCACCCAGGCCGTGAGC-3' (MGTTP/r6137-6115)

5'-CTGGCCAGGGAGAGCTAGGTC-3' (MGTTP/r6267-6247)

Neo reverse primer:

5'-GATATTCGGCAAGCAGGCATCG-3' (pMC1PolA Neo r1328-1307) pMC1PolA Neo is from Stratagene

The numbers I have used for the TTP primers are based on our genomic sequence. The GenBank TTP cDNA is accession number M57422. The equivalent bases for the primers in the cDNA sequence are shown below:

genomic TTP	TTP cDNA
f5832-5856	f103-127
f5925-5945	f196-216
r6137-6115	r408-386
r6267-6247	r538-518

I have been using the primer pair that is in bold. If you have trouble seeing the larger Neo containing band you can also set up the adjacent PCR reaction using the TTP forward primer and the Neo reverse primer.

10x ammonium sulfate PCR buffer:

- 160 mM ammonium sulfate
- 670 mM Tris-HCl (pH8.8)
- 0.1 % Tween-20
- 50 mM β -mercaptoethanol