**tTA Genotyping Protocol with MAPT control**

Updated July 2021

These primers amplify the original tTA construct used in CaMKIIα-tTA Line B, ROSA26-LNL-tTA, Pcp2-tTA, GABARα6-tTA, and PITX3-tTA mice. They WILL NOT WORK with the new tTA2 construct used in the Nop-tTA mice.

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| **Reagent** | **Volume/rxn (ul)** |
| ddH2O | 7.6 |
| 50 μM tTA Forward | 0.1 |
| 50 μM tTA Reverse | 0.1 |
| 50 μM MAPT Forward | 0.1 |
| 50 μM MAPT Reverse | 0.1 |
| 2x PCR PreMix (Green Dye) (Syd Labs, MB067-EQ2G-L) | 10 |
| Tail DNA | 2 |

**PCR Program: Touchdown (ABI SimpliAmp)**

1. 94°C for 1 minute
2. A. 94°C for 15 seconds

B. 65 °C for 15 seconds

C. 68 °C for 30 seconds

D. Repeat 2A-C for 10 cycles

1. A. 94°C for 15 seconds

B. 60°C for 15 seconds

C. 72 °C for 30 seconds

D. Repeat 3A-C for 28 cycles

4. 72°C for 7 minutes  
5. Hold at 10°C

**Primer Sequences**

tTA Forward: CGC TGT GGG GCA TTT TAC TTT AG

tTA Reverse: CAT GTC CAG ATC GAA ATC GTC

MAPT Forward (IMR3092): CTC AGC ATC CCA CCT GTA AC

MAPT Reverse (IMR3093): CCA GTT GTG TAT GTC CAC CC

**Gel Percentage:** 2% Agarose Gel with 10 ul of 10 mg/ml ethidium bromide (30min at 175V in 1x SB)

**Ladder:** TrackItTM 100 bp Ladder (Invitrogen, Catalog number: 10488058)

**Expected Products:** Transgenic animals should produce one band from the tTA product at approximately 480 bp.All animals, transgenic and wild-type, should exhibit the 187 bp band indicating successful amplification of the tail DNA.