REDextract Genotyping

Reference

1. Lang, A. E., Riherd Methner, D. N., & Ferreira, A. (2014). Neuronal degeneration, synaptic defects, and behavioral abnormalities in tau_{45-230} transgenic mice. *Neuroscience*, *275*, 322–339. https://doi.org/10.1016/j.neuroscience.2014.06.017

Kit

1. Sigma RedExtract Kit, Cat# XNAT-100

Protocol

DNA Extraction

1. With tails placed cut side down, pipette 100 µL of Extraction Solution (from kit) into the microcentrifuge tube containing the tail clipping.
2. Add 25 µL of Tissue Preparation Solution (from kit) to the tube. Pipette up and down to mix or gently vortex. Double check that the cut-side of the tail is facing down.
3. Incubate samples at room temperature for 10 minutes.
4. Incubate samples at 95°C for 3 minutes (Tissue will not be digested).
5. Let samples cool slightly for ~1 minute.
6. Add 100 µL of Neutralizing Solution B (from kit) to the sample and mix by vortexing.
7. Store neutralized tissue extract at 4°C or use immediately for PCR (store tails in 4°C until results are obtained and accurate).

PCR amplification

1. Dilute 100 µM primer stocks in sterile MilliQ H_2O (1:10 dilution).
2. Make Master Mix Solution: (If using more than two primers, decrease the amount of sterile water added accordingly.)

Reagent	Volume
Sterile H_20	4.4 µL
REDextract-N-Amp PCR Reaction Mix	10 µL
Forward Primer	0.8 µL
Reverse Primer	0.8 µL
Tissue Extract	4 µL
Total Volume	20 µL

3. Add 16 µL Master Mix and 4 µL tissue DNA extract to each PCR tube, centrifuge briefly.
4. Place in Thermocycler using following program and run at 100V.

Step	Temperature	Time	Cycles
Initial Denaturation	94°C	3 minutes	1
Denaturation	94°C	0.5 minutes	30
Annealing	52°C	0.5 minutes	
Extension	72°C	1 minute	
Final Extension	72°C	10 minutes	1
Hold	4°C	Indefinitely	