RT-PCR

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. SuperScript™ III One-Step RT-PCR System with Platinum™ Taq DNA Polymerase

Protocol

1. Add 29 µL of Master Mixture recipe below to each PCR tube.
   RT-PCR: MASTER MIX.

Reagent	µL per Sample
2x Reaction Mix	25 µl
Forward Primer	1 µL
Reverse Primer	1 µL
Superscript TAQ	2 µL

2. Add 1 µg or 2 µg (depending on project) RNA template + Sterile DEPC H_20 to make up to 21 µL and add to tubes containing master mix.
3. Mix and centrifuge briefly to remove bubbles.
4. Place in thermocycler using program below (50 µL reaction volume).

Step	Temperature	Time	Cycles
cDNA Synthesis	55°C	30 minutes	1
Denaturation	94°C	2 minutes	1
Denaturation	94°C	15 seconds	40
Annealing	55°C	30 seconds	
Extension	68°C	45 seconds	
Final Extension	68°C	5 minutes	1
Hold	4°C	Indefinitely	

5. Analyze on 1% agarose gel using 10 µL PCR product with 2.5 µL 6X Loading Dye and 7.5µL sterile H_2O. Run at 100 volts until front reaches center of the gel. Keep PCR product tubes at -20°C.