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Real time qPCR (Fly Heads)

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Protocol status: Working

We use this protocol and it's working

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Abstract

This Protocol describes how to perform RT qPCR with RNA extracted from fly heads.



RNA Extraction

- 1 Homogenize 6 heads in  50 μL Qiazol, then add  450 μL Qiazol.
- 2 Spin  00:10:00 at  4 °C at 12,000 x g. 10m
- 3 Incubate supernatant for  00:05:00 at  Room temperature . 5m
- 4 Add  100 μL chloroform. Vortex, incubate for  00:03:00 at  Room temperature , then spin for  00:15:00 at  4 °C at 12,000 x g. 18m
- 5 Transfer the upper phase to fresh tube, add  250 μL isopropanol, incubate  00:10:00 at  Room temperature , spin for  00:10:00 at  4 °C at 12,000 x g. 20m
- 6 Wash the pellet in  500 μL 75% ethanol (it will be very loose) three times. Do not vortex.
- 7 Air dry
- 8 Resuspend in  15 μL RNase/DNase-free water.
- 9 Measure 260/280 and 260/230 on nanodrop.

RT-PCR

- 10 Use  1 μg RNA to perform RT-PCR reaction.
- 11 Thermocycler settings 2h 15m



- a. 25 °C 00:10:00
- b. 37 °C 02:00:00
- c. 85 °C 00:05:00
- d. 4 °C Hold

qPCR

- 12 Make master mix with primers, SYBR Green and water.
- 13 Dilute DNA 1:50 making a 50 µL stock.
- 14 Add 2 µL DNA to wells with special tips.
- 15 Add 14 µL master mix to wells with regular tips. Pipette up and down once but do not expel a bubble. Keep master mix on ice the entire time to avoid bubbles.
- 16 Check for bubbles and run on machine.