




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Real time qPCR (Fly Heads)

Mel Feany^{1,2}¹Brigham and Women's Hospital; ²Harvard Medical School1 *Works for me* Sharedx.doi.org/10.17504/protocols.io.q26g7y4r1gwz/v1**Daniel's workspace****Daniel El Kodsí**

ABSTRACT

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

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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.