Stefan Taubert's Worm RNA isolation & qPCR protocol

Adapted from: Van Gilst, M.R., H. Hadjivassiliou, A. Jolly, and K.R. Yamamoto. 2005a. Nuclear hormone receptor NHR-49 controls fat consumption and fatty acid composition in *C. elegans*. PLoS Biol 3: e53.

Preparation

-Cool microfuge to 4ºC. Chilled centrifuge is necessary for RNA purification
-Decontaminate work space and equipment with RNase ZAP following manufacturer's instructions (printed on the bottle)
-use barrier-tips and use these tips only for RNA purification

1) RNA isolation

- Harvest (1000-10'000, depending on stage) worms, washed 2-5x w/ M9, freeze liquid N2
- Thaw at RT-65 °C
- Add 1ml Trizol (Invitrogen #15596) per pellet, vortex repeatedly (up to 15' incubation time); worms don't dissolve, you'll see "ghosts"
- Add 100 µl BCP (Molecular Research Center #BP-151) phase separation reagent, vortex, incubate 2-15'
- Spin 12'000g/15'/4$_o$C
- Remove top (aqueous layer) into new tube
- Add 1 vol (~600µl) isopropanol, vortex, incubate 5-10'
- Spin 12'000g/8'/4$_o$C
- Aspirate supernatant (should see white pellet)
- Add 200-1000 µl 70% EtOH
- Spin 7'600g/5'/4$_o$C
- Aspirate supernatant, dry pellet (not necessary to dry if next step is cleanup etc)
- Re-suspend in 100µl H2O (less if ready to use, i.e. 30-80µl)

2) RNA cleanup & DNA digestion (w/ QIAGEN RNeasy kit)

Adapted from the excellent QIAGEN RNeasy handbook (I use the mini size columns): (http://www1.qiagen.com/literature/handbooks/PDF/RNAStabilizationAndPurification/FromAnimalAndPlantTi
ssuesBacteriaYeastAndFungi/RNY_Mini/1016272HBRNY_062001WW.pdf).
All steps @ room temperature unless indicated.
- Adjust volume to 100µl w/ RNAse-free H_2O; add 350µl Buffer RLT (ensure that β-ME is added to RLT: 10 µl BME per ml of RLT); add 250µl EtOH 100%; mix thoroughly by pipetting.
- Apply the sample (700µl) to RNeasy column placed in 2 ml collection tube
- Close the tube gently; spin 15s/≥8000g (≥10'000rpm); discard flow-through
- Pipet 350µl Buffer RW1 onto the RNeasy column, and spin 15s/≥8000g to wash; discard flow-through

- DNA digestion: Use kit QIAGEN kit #79254: Per sample: Add 10µl DNase I stock solution **(I have this in my -20ºC and can give to you)** to 70µl Buffer RDD. Mix by gently inverting the tube (mixing
should be carried out by gently inverting the tube; do not vortex).
- Pipet the DNase I incubation mix (80µl) directly onto RNeasy silica-gel membrane; incubate for 15-30'
- Pipet 350µl Buffer RW1 onto the RNeasy column; spin for 15s/≥8000g; discard flow-through
- Pipet 500µl Buffer RPE (ensure EtOH added) onto the RNeasy column. Close tube gently, and spin for 15s/≥8000g to wash the column; discard flow-through.
- Repeat RPE wash; discard flow-through.
- Spin at full speed for 5' to remove leftover EtOH.
- To elute, transfer RNeasy column to Eppi tube 1.5ml; add 30-80µl RNAse-free water directly onto RNeasy silica-gel membrane; spin 1'/8'000g; keep eluted RNA **ON ICE FROM NOW ON; store at -70 oC**

3) Determine RNA concentration

I determine the concentration of RNA (and DNA) in a spec measuring OD260/280; typically I
get something like 200-1500ng/µl (for something like ~5'000 worms)