C. elegans RNA prep Eric Greer 11/16/12

If trying to measure the knock-down of a specific gene by feeding RNAi the worms first need to be transferred every day for three days to regular NGM plates with OP50-1 and streptomycin antibiotics to kill any residual RNAi bacteria themselves (which will be detected by the real-time primers). The worms (~200) should then be transferred to NGM plates without any bacteria and then washed off with M9 buffer. If detecting non-knocked down mRNA levels this preparation is unnecessary.

1. Wash worms off plates with M9 (1 NGM strep chunking plate with hundreds of worms is enough); wash 2-3X to get rid of bacteria. Add 10ml Trizol for every ml of packed worms.
2. Vortex tubes for 30 sec, then place in liquid Nitrogen, let thaw at 37 deg C, and repeat 6 times. Then freeze at -80 deg C until ready to prep.
3. Thaw and let stand at room temperature for 5 minutes.
4. Add 2ml chloroform per 1ml of packed worms. Invert 15 seconds, let sit 3 minutes room temperature. Spin 15 minutes at 8250 rpm at 4C. RNA is in aqueous supernatant.
5. Transfer aqueous phase to new tube.
6. Add isopropanol (0.7 volumes of what is already in tube). Gently invert several times. Leave at room temperature for 10 minutes. Spin at 8250 rpm for 10 minutes at 4C. Will see small white RNA pellet at bottom of tube.
7. Pipet out supernatant and wash pellet with ice cold 75% EtOH. Spin 4000 rpm at 4C for 5 minutes. (Make sure to "make" 75% EtOH w/ DEPC treated H2O)
8. Pipet out EtOH. Let sit for ~7 minutes with tube caps open to dissolve all EtOH (but you don't want to dry out the pellet). When almost all the ethanol has evaporated (faint halo around pellet), resuspend in 100ul DEPC-H2O by pipetting and incubate at 60C for 10 minutes.
9. Take 260/280, and 260 (2ul in 98ul H2O). If pure, 2.0 ratio. Expect 1-4 mg/gram of worms.
10. Set up amplification grade DNAse reaction (Invitrogen) or Turbo DNAse (Ambion) using 1-5 ug RNA.
11. Store frozen at -80C.

We use a primer pair that amplifes multiple actins:

pan-actin tcggtatgggacagaaggac catcccagttggtgacgata