C. elegans Embryonic RNA Isolation Protocol of 6 Timepoints -Reinke lab

Day 1. Bleach adult worms using standard bleaching protocol and incubate the embryos overnight with shaking.

Day 2. Plate synchronized L1 worms onto 2 peptone enriched 15X15cm plates, 35,000 worms per plate.

Day 5. After 2.5d incubation on enriched plates, bleach adult worms using standard bleaching protocol and incubate the embryos overnight with shaking.

Day 6. Plate synchronized L1 worms onto 4 peptone enriched 15X15cm plates, 35,000 worms per plate.

Day 9. After 2.5d incubation on enriched plates, bleach adult worms using standard bleaching protocol and incubate the embryos overnight with shaking.

Day 10. Plate well-synchronized L1 worms onto 18 peptone enriched 15X15cm plates, 35,000 worms per plate.

Day 12. Start checking the stage of young adult worms under the scope at 48 hours after plating L1 worms, and bleach the worms when most of them have 0 to 2 embryos (it usually takes 49.5-52.5 hours). Bleach worms and collect embryos using 40 µm sterile strainer to remove worm carcasses. Vortex well before using strainer. After bleach, check the stage of embryos. Start timing for embryo development when most of the embryos are at 4-cell stage. According to the gene's expression pattern determined by RNAseq of wild type worm strain VC2010, we chose three time points for RNA isolation: at the beginning of gene expression, at the peak of expression, and after peak expression. We collect embryos again 30min after each of the 3 time-points and consider them as "replicate controls" of each time-point. Culture the embryos with shaking until the time points are reached and immediately spin down and remove the supernatant. Add 500 µL TRIzol (Life Technologies) to each embryo pellet and flash freeze in liquid nitrogen and then place tubes at -80°C.

Day 13 Thaw the pellet in a 37°C waterbath and then flash freeze in liquid nitrogen (repeat this 2 more times). Transfer sample to a 1.5 mL RNase-free tube. Put tubes on ice and add 100 µL of RNase-free chloroform to each sample. Shake 1 minute by hand vigorously and gently vortex for 10 seconds. Put samples on ice for 10 minutes. Spin at 12,000 rpm for 30 minutes at 4°C. Transfer aqueous layer into a new 1.5 mL RNase-free tube and estimate the volume. Add an equal volume of isopropanol and 1 µL of glycogen (Life Technologies) to each sample and mix it well by inverting the tube several times. Put samples at -20°C overnight.

Day 14 Spin samples at 12,000 rpm for 30 minutes at 4°C. Discard supernatant and wash pellet with 1 mL of cold 75% RNase-free ethanol. Spin at 12,000 rpm for 5 minutes at 4°C and remove the supernatant. Repeat ethanol washing again. Dry pellet at RT for 5-10 minutes. Re-suspend pellet with 15 µL RNase-free water and then add 1 µL of SUPERaseIn (Life Technologies).

All the worms are cultured at 20-22°C