

## *C. elegans* RNA Isolation and RT-PCR

### Reagents Needed:

M9 (common stock)	RNase-free H <sub>2</sub> O
Trizol (stored at 4°C)	iScript cDNA Synthesis Kit
chloroform	Ambion DNA- <i>free</i> Kit
2-propanol	BioRad iQ SYBR Green Supermix
70% EtOH	

### Procedure:

- \* *Wear gloves at all times.*
- \* *Only use filter tips.*
- \* *Keep everything on ice as much as possible to avoid RNA degradation.*

#### I. RNA Isolation

1. Pick 10 worms into 20µL of M9 in an RNase-free Eppendorf tube.
  2. Pellet worms by spinning briefly at 14,000 rpm.
  3. In the hood, add 250µL of Trizol.
  4. Vortex by hand for about 30 seconds and then vortex at 4°C until the worms dissolve (about 20 minutes)
- Optional (for large samples):
- a. Let sit at room temperature for 10 minutes.
    - \* At this point the worms can be frozen. Remove excess supernatant and flash freeze in liquid nitrogen or dry ice/EtOH. Store samples at -70°C.
  - b. Centrifuge at 14,000 rpm for 10 minutes at 4°C.
  - c. Transfer the supernatant into an RNase-free Eppendorf tube.
5. In the hood, add 50µL of chloroform.
  6. Vortex for 30 seconds.
  7. Let sit at room temperature for 3 minutes.
  8. Centrifuge at 12,000 rpm for 15 minutes at 4°C.
  9. Transfer the clear top layer (~125µL) into a new RNase-free Eppendorf tube.
  10. Repeat steps 5-9.
  11. Add 125µL of 2-propanol and invert to mix.
  12. Let sit at room temperature for several minutes.
  13. Spin down at 12,000 rpm for 10 minutes at 4°C.
  14. Carefully decant the supernatant. Leave a few µLs at the bottom so as not to disturb the pellet.
  15. Wash pellet with 250-500µL of 70% EtOH (use RNase-free H<sub>2</sub>O).
  16. Spin down at 14,000rpm for 5 minutes at 4°C.
  17. Remove as much supernatant as possible and air dry pellet or use the air vacuum.
  18. Dissolve the pellet in 10µL of RNase-free H<sub>2</sub>O.
  19. Optional: Heat for 10 minutes at 60°C to help dissolve the RNA.

### Optional: RNA/DNA Contamination Quantification

\* You will not get an accurate reading with small samples.

$OD_{260}/OD_{280}$ : 1.8 for pure DNA

2.0 for pure RNA

Sample should be somewhere in between.

Make a 1:1,000 dilution

$[RNA] = (OD_{260}) \times (40 \mu\text{g/mL}) \times 1000$

$[Total RNA] = [RNA] \times 19$

### II. DNA-Free Protocol (Ambion Kit)

1. For a 20 $\mu\text{L}$  reaction, add to an RNase-free Eppendorf tube:

10 $\mu\text{L}$  RNA

2 $\mu\text{L}$  10x DNase I buffer

7 $\mu\text{L}$  RNase-free H<sub>2</sub>O

1 $\mu\text{L}$  DNase I

2. Incubate at 37°C. for 20-30 minutes.

3. Add 2.5 $\mu\text{L}$  of DNase Inactivation Reagent.

4. Incubate for 2 minutes at room temperature with occasional mixing.

5. Spin down at 14,000 rpm for 1.5 minutes at 4°C.

6. Transfer supernatant, without disturbing the white pellet, to a new RNase-free Eppendorf tube.

### Optional: RNA Quantification

\* You will not get an accurate reading with small samples.

Make a 1:1,000 dilution

$[RNA] = (OD_{260}) \times (40 \mu\text{g/mL}) \times 1000$

$[Total RNA] = [RNA] \times 49$

### III. cDNA Synthesis

1. For one 20 $\mu\text{L}$  sample, add:

4 $\mu\text{L}$  5x iScript Mix

1 $\mu\text{L}$  Reverse Transcriptase

10 $\mu\text{L}$  RNA

5 $\mu\text{L}$  RNase-free H<sub>2</sub>O

2. Program:

① 25°C for 5 minutes

② 42°C for 30 minutes

③ 85°C for 5 minutes

④ 4°C  $\infty$

3. Store at 4°C or -20°C until needed.

### IV. RT-PCR

1. For one 25 $\mu$ L sample, add to one well of a 96-well plate:
  - 1.5 $\mu$ L cDNA
  - 0.5 $\mu$ L Forward primer (1:10)
  - 0.5 $\mu$ L Reverse primer (1:10)
  - 12.5 $\mu$ L CybrGreen buffer
  - 10.0 $\mu$ L sterile dH<sub>2</sub>O
2. Pipet each sample in triplicate.
3. Use the Biorad iCycler in the Keck Biophysics Facility on the 4<sup>th</sup> floor of Cook.  
NOTE: You must sign up to use the iCycler and either be trained to use the machine or go with someone who knows how.

### **Recipes:**

#### M9 (1L)

\* Common lab stock in worm room.

5.8g Na<sub>2</sub>HPO<sub>4</sub>•7H<sub>2</sub>O

3.0g KH<sub>2</sub>PO<sub>4</sub>

5.0g NaCl

0.25g MgSO<sub>4</sub>•7H<sub>2</sub>O

ddH<sub>2</sub>O to 1L

• Filter (0.22 $\mu$ m) and bottle.

### **Reference:**

This protocol has been modified by many Morimoto lab members over the years.