Promega

Product Application

Automated Purification of RNA from *C. elegans*

Purify RNA from C. elegans *nematodes using the Maxwell® RSC Instrument and Maxwell® RSC miRNA Tissue Kit.*

Kit: Maxwell® RSC miRNA Tissue Kit (Cat.# AS1460)

Analyses:

- UV absorbance
- Dye-based quantitation
- RT-qPCR
- TapeStation

Sample Type(s): *C. elegans* nematodes

Input: from 1 adult worm to a plate of worms

This protocol was developed by Promega Applications Scientists and is intended for research use only.

Users are responsible for determining suitability of the protocol for their application.

For further information, see Technical Manual TM441, available at: **www.promega.com/protocols**

or contact Technical Services at: **techserv@promega.com**

Materials Required:

- Maxwell® RSC miRNA Tissue Kit (Cat.# AS1460)
- Maxwell® RSC Instrument (Cat.# AS4500)
- Microcentrifuge

Protocol:

1. Grow and collect *C. elegans* worms following standard method[1].
2. Centrifuge at 200rpm for 1 minute and remove supernatant.
3. Prepare 1-Thioglycerol/Homogenization Solution and DNase I Solution as described in Technical Manual TM441 (Section 3.A).
4. Add 200µl of 1-Thioglycerol/Homogenization Solution to worm pellet and vortex.

   Option 1: bead beating can be performed at this step.

   Option 2: homogenized worms can be frozen at -80°C.
5. Add 200µl of Lysis Buffer, 200µl of Lytic Enhancer and 30µl of Proteinase K to the homogenized sample. Mix by vortexing for 20 seconds.
6. Incubate at room temperature for 10 minutes. During this time, prepare the Maxwell® RSC Cartridges as described in TM441 (Section 4.B.). Add 60µl Nuclease-Free Water to elution tubes.
7. Transfer the whole lysate to well #1 of the Maxwell® RSC Cartridge.
8. Add 10µl of DNase I Solution to well #4 of the Maxwell® RSC miRNA Tissue Cartridge.
9. Place the prepared cartridges in the Maxwell® RSC Instrument and run the Maxwell® miRNA Tissue Kit method.

Results:

Total RNA was purified from *C. elegans* worms using the Maxwell® RSC miRNA Tissue Kit (Figure 1). Purified RNA was of high quality and little to no degradation was observed (Figure 1, left and Figure 2). Purified RNA was amplifiable with both mRNA and miRNA-specific primer sets by RT-qPCR (Figure 3). Concentration data (Figure 1) were obtained from the lysis protocol without the optional bead beating step. A bead beating step may help to beak the nematode cuticule and increase purified RNA concentrations.

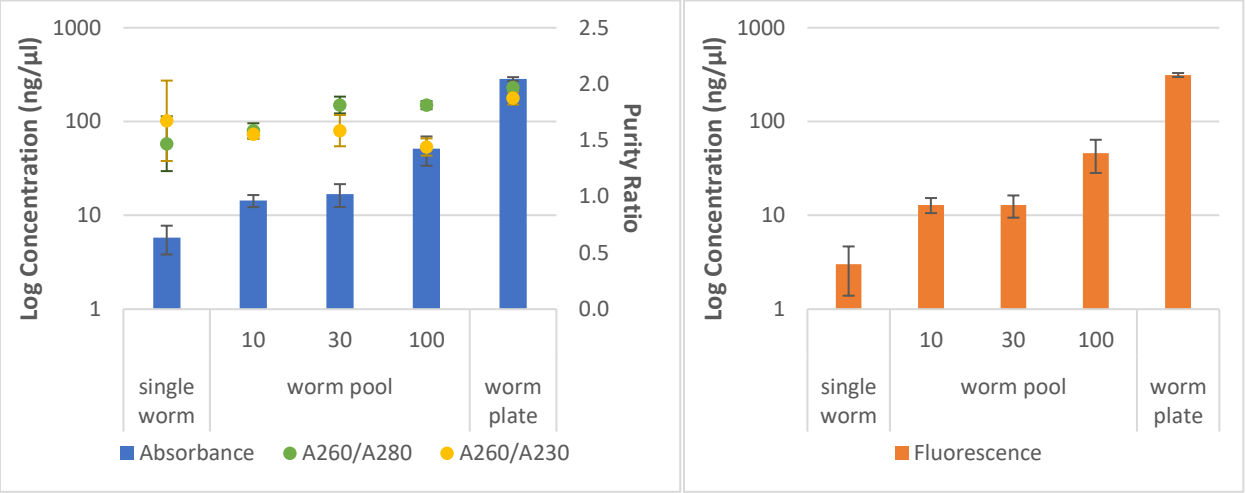

Figure 1: Concentration, yield and absorbance ratios of RNA purified from *C. elegans* worms using the Maxwell® miRNA Tissue Kit. Total RNA was purified from 1, 10, 30, and 100 staged adult wild-type worms (larval stage 4+3 days; N2, Bristol strain)* or from the progeny of 2 wild-type worms grown for 5 days at 20°C (60mm diameter plate) using the Maxwell® RSC Instrument. RNA concentration and purity ratios were measured by absorbance using a NanoDrop™ One Spectrophotometer (Left). RNA concentration was also measured using QuantiFluor® RNA System (Cat.# E3310) on a GloMax® Discover Microplate Reader (Cat.# GM3000). Average values ± standard deviation are shown for n=3 except for single worm extraction for which n=10.

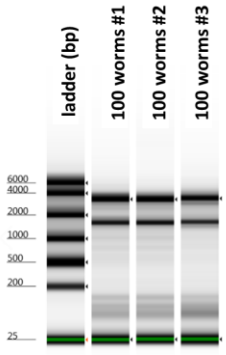

Figure 2: Integrity of RNA purified from 100 *C. elegans* worms. Total RNA was purified from 100 staged adult wild-type worms (N2, Bristol strain)* using the Maxwell® RSC Instrument. Purified RNA was analyzed using 4200 TapeStation System with a RNA ScreenTape according to the manufacturer's instructions (Agilent). The image is a representation of the gel migration of purified RNA.

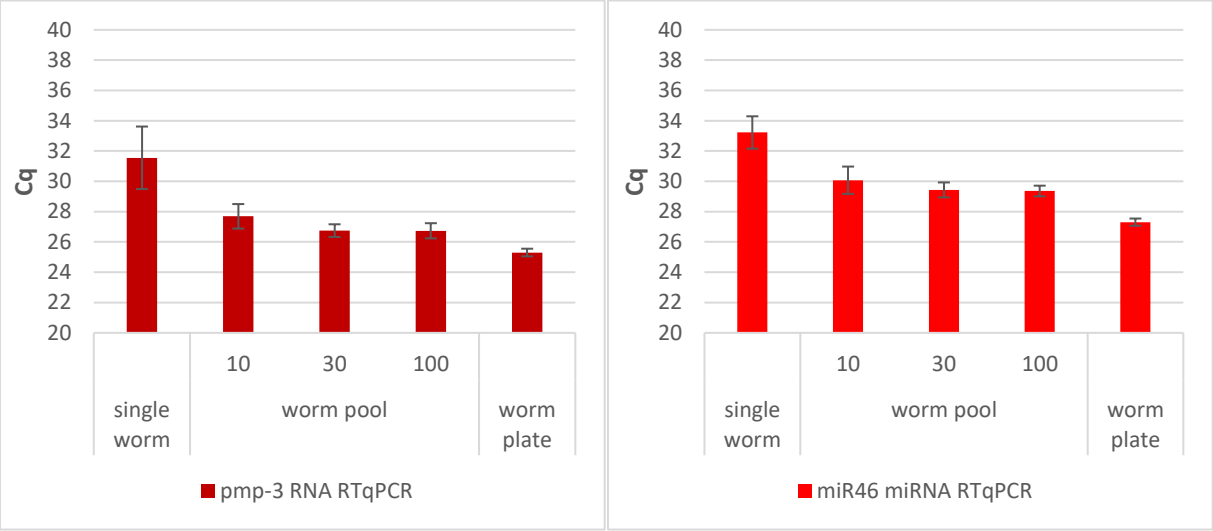

Figure 3. RT-qPCR amplifications of mRNA and miRNA purified from *C. elegans* worms. RNA was purified from *C. elegans* single worm, worm pools or worm plate on the Maxwel® RSC Instrument. RNA purified from 100 worms or worm plate were normalized to 10 ng/µl, and 2 to 20ng of purified RNA was reverse transcribed and amplified with *pmp-3* and *miR-46-3p* gene-specific primers[2,3] using GoTaq® 1-Step RT-qPCR System (Cat.# A6020) following TM355 on a CFX96 Touch™ Real-Time PCR Detection System (BioRad). No-template controls and No RT controls were negative. Shown are the average values for N=6 ± standard deviation. Reaction efficiencies were 93.10% and 103.50% for *pmp-3* and *miR-46-3p* amplifications, respectively, and R^2=0.991 for both reactions.

***C. elegans* worms were kindly provided by Dr Florence Solari from the Genetics and Neurobiology of *C. elegans* research team at Institut NeuroMyoGène, University of Lyon, France.**

References:

1. Stiernagle, T. Maintenance of *C. elegans* (February 11, 2006), WormBook, ed. The *C. elegans* Research Community, WormBook, doi/10.1895/wormbook.1.101.1, http://www.wormbook.org.

2. Mergoud dit Lamarche, A. et al., (2018) UNC-120/SRF independently controls muscle aging and lifespan in *Caenorhabditis elegans*. *Aging Cell.* Apr;17(2):e12713.

3. Kagias K. et al., (2014) Reliable reference miRNAs for quantitative gene expression analysis of stress responses in *Caenorhabditis elegans*. *BMC Genomics*. Mar 21;15:222.