Optimization of RNA Extraction and cDNA Synthesis in *C. elegans*

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The normal development of an organism is tightly regulated and highly orchestrated by microRNAs (miRNA), short pieces of regulatory RNA. The timing of the expression of these miRNAs determines stage-specific processes in development. Lin-4 and Lin-14 are two post-transcriptional miRNAs which promote progression through the L1 to the L2 larval stage in *Caenorhabditis elegans* (*C. elegans). C. elegans* is a free-living nonparasitic nematode that makes an excellent model organism for studying gene expression and development due to a completely mapped cell lineage and fully sequenced genome. This study developed our RNA isolation and cDNA synthesis techniques. These techniques will enable future studies designed to delineate the impact of developmental and growth regulating therapeutic drugs on the expression levels of lin-4 and lin-14 miRNA. To isolate high-quality RNA, we optimized the RNeasy Qiagen RNA extraction standard protocol. This protocol utilizes a 2ml sample tube, Qiagen RLT Buffer to collect the specimen, and a QIAshredder to disrupt the *C. elegans* tissue. We discovered that collection of synchronized worms from two Petri dishes with 750µl of water instead of RLT buffer and the utilization of a 1.5ml Eppendorf tube instead of a 2 ml tube produced the best worm pellet. These changes optimized the amount of worms collected and reduced the possibility of stress or lethality (due to the RLT buffer), which could impact miRNA expression. The shape of the 1.5ml Eppendorf tube, which has a pointed bottom versus the round bottom of a 2ml tube, made a critical difference in the production of a compact worm pellet. The pointed bottom resulted in a reduction in the loss of tissue and promoted a high concentration of very pure RNA. Next, to access the RNA, it was necessary to break open the worms and lyse the cells. Instead of using the Qiagen QIAshredder, we broke the worms open by freezing the worm pellet on dry ice and then allowing them to thaw on wet ice. This freeze/thaw cycle was repeated five times and was followed by disruption of the worm pellet with an 18-gauge syringe. The freeze/thaw and syringe steps were indispensable components of the protocol as it enhanced cell lysis, enabled extraction of RNA, and kept the samples cold so as to prevent RNA degradation. Combined, these improvements yielded high-quality RNA at concentrations ranging from 12.9 ng per µl to 1067.4 ng per µl, whereas the standard protocol did not produce any viable RNA. Once RNA was extracted, gene-specific primers were designed that allowed for the synthesis of RNA into cDNA. The next step will be to optimize the cDNA synthesis by selecting the best primer sets and enzymes that will allow the lin-4 and lin-14 miRNA to be detected via real-time RT-PCR. These protocols will enable future research on the developmental impacts of modulation of the expression of specific regulatory miRNAs in *C. elegans* at different larval stages.