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Tetrahymena thermophila Lack a Homologue of the Caenorhabditis Elegans Lin-4 miRNA

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Tetrahymena thermophila lack a homologue of the *Caenorhabditis elegans* lin-4 miRNA

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INTRODUCTION

The netrin family of proteins was first discovered because of their role in axonal guidance during development. Netrin homologues are important developmental signals in organisms ranging from vertebrates to the nematode, *Caenorhabditis elegans*, and netrin-like proteins have even been found in the ciliated protozoan, *Tetrahymena thermophila*. Since the lin-4 miRNA regulates netrin signaling in C. elegans, we hypothesized that a lin-4 homologue might exists in *Tetrahymena thermophila*. In order to test this hypothesis, we purified total miRNA from *T. thermophila*, used this miRNA to make cDNA, then used RT-PCR to quantitate the amount of lin-4 specific cDNA we obtained. Our sample was positive for total cDNA, but not for the lin-4 cDNA specifically, suggesting that this miRNA may not have a homologue in *Tetrahymena*.

MATERIALS AND METHODS

- miRNA was purified using the Qiagen miRNAeasy™ Mini kit, following the manufacturer's instructions
- cDNA was synthesized using the TaqMan Advanced™ cDNA synthesis kit from Advanced Biosciences, following the manufacturer's instructions.
- *cel* lin-4 primer was obtained from Advanced Biosciences.
- RT-PCR was done using the TaqMan Advanced™ Master Mix, following a protocol given to us by Dr. Kaleb Pauley.

RESULTS

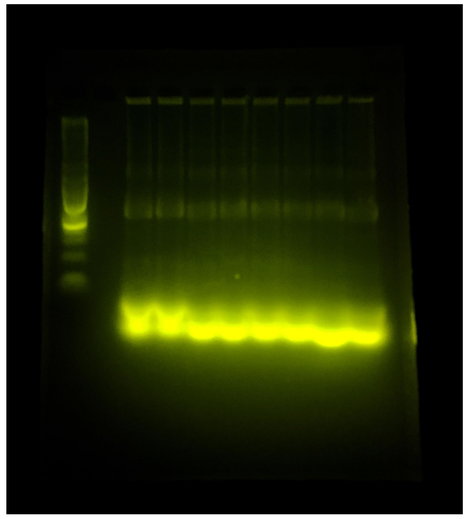

Figure 1. miRNA from *Tetrahymena thermophila.* Low molecular weight fragments represent miRNA. Higher molecular weight fragments likely represent DNA or larger RNA molecules.

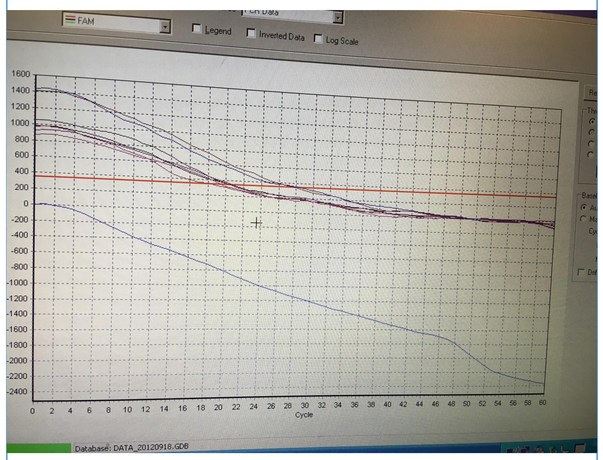

Figure 2. Fluorescence reading from RT-PCR indicates that the primer is not binding to our cDNA. If the primer bound to the cDNA, we should have seen an increase in fluorescence as the complementary cDNA was synthesized.

RESULTS

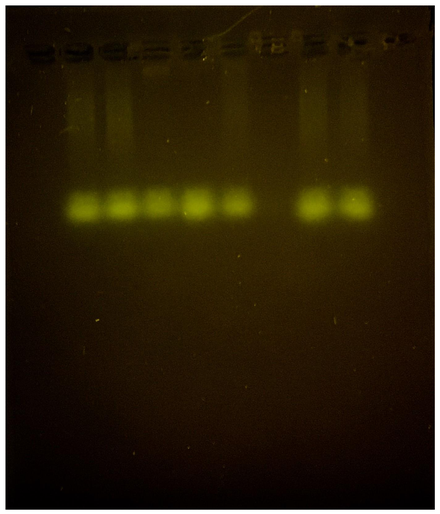

Figure 3. 3% TAE-Agarose gel indicates that cDNA synthesis from miRNA was successful. After our RT-PCR was unsuccessful, we determined that cDNA was present in our samples, indicating that our original miRNA purification was successful. This implies that our primer is unable to bind to our cDNA.

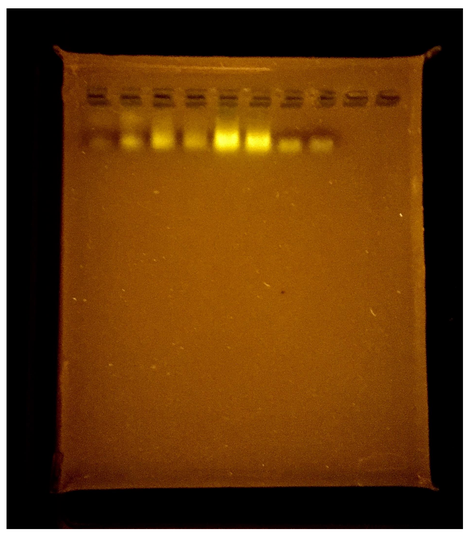

Figure 4. miRNA from *Homo sapiens.* We used the miRNAeasy™ kit to successfully purify miRNA from our own epithelial cells.

CONCLUSIONS

- miRNA purification was successful in *Tetrahymena thermophila* and *Homo sapiens.*
- cDNA synthesis was successful even though RT-PCR was not.
- The lin-4 primer we purchased was unable to hybridize with cDNA created from *Tetrahymena thermophila*.
- Since the *Tetrahymena* genome has been sequenced, we may want to design a custom primer if we wish to search for a homolog of lin-4 in this system.

ACKNOWLEDGEMENTS

We would like to thank Dr. Kaleb Pauley for giving us reagents and recipes, ordering the new, multistep cDNA synthesis kit, helping us run the thermal cycler, and helping us interpret our data.

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