RNA isolation and cDNA synthesis in the garter snake (Thamnophis elegans)

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Introduction
Obtaining high quality RNA is essential for molecular techniques like the sequencing of different genes and analyzing gene expression (Douglas, 1996; Qiagen, 2010). Sequencing the DNA of an organism is important because it tells us what the cell could do, while analyzing gene expressions tells us what the cell is actually doing. If a gene is being used to make mRNA, or expressed, the gene is considered to be "on". If a gene is not being used to make mRNA it is considered to be "off". Whether a gene is "on" or "off" can depend on many factors such as the type of organism or cell being analyzed, the environment of the organism or cell, the stage of development of the organism or cell, etc.

Genomic DNA, or the entire genetic information of an organism, has many regions of coding DNA (exons) and many regions of noncoding DNA (introns). These introns can act like hundreds to thousands of random letters in the middle of a sentence you want to read, the sentence being gene. It is important to sequence only the coding sections of DNA. To do this, mRNA must first be isolated from the cell and then reverse transcribed into complementary DNA (cDNA). From there, a specific gene can be amplified, as well quantified, or counted.

The objective of this study is to quantify gene expression for garter snakes at different time points in the process of hibernation. This means specific genes from the DNA synthesized will be amplified to see if the amount of gene turned "on" changes with the varying temperatures of hibernation.

Materials and Methods
1. We extracted messenger RNA from 43 baby garter snake livers using the Qiagen RNeasy® Mini Kit. (Fig. 1-3)

2. To ensure the presence and quality of mRNA based on fragment length, we ran the mRNA on an agarose gel using electrophoresis. (Fig. 4)

3. To ensure the presence and quality of mRNA, or expressed, the gene is considered to be "on". If a gene is being used to make mRNA, or expressed, the gene is considered to be "off".

4. To determine the quantity of mRNA extracted, the Nanodrop technology was used. A concentration of at least 150 ng/ul of mRNA is needed to proceed with cDNA synthesis. If the concentration is over 250 ng/ul, the sample must be diluted to the desired concentration of around 200 ng/ul (Table 1).

5. To determine the quality of mRNA extracted, the samples were loaded into a gel electrophoresis chamber and ran at 120 Volts for 30 minutes. The results of the gel electrophoresis match typical results; there were 2 prominent bands that were of equal intensity (Fig. 6).

6. To observe an increase in the expression of certain genes if mitochondria undergo self destruction in response to the hibernation conditions.

Results
Since we successfully extracted mRNA from the 43 baby garter snake livers samples and made cDNA from those samples, the study can continue on to the next step of amplifying specific genes and quantifying their presence. We expect to observe an increase in the expression of certain genes if new mitochondria are being formed in response to the acclimation to cold temperature and caloric restrictions. If the opposite occurs, we expect to observe an increase in the expression of certain genes if mitochondria undergo self-destruction in response to the hibernation conditions.

Conclusions
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Literature cited

Materials and Methods cont.

Figure 3. Photo of spin column. The sample is mixed with different types of buffers and spun in a centrifuge to separate the mRNA onto the membrane, while the rest of the cell material flows through to the collection tube for disposal.

Figure 4. Photo of gel electrophoresis chamber.

Figure 5. Photo of NanoDrop apparatus.

Table 1. Concentration values of mRNA. Sample number corresponds to the lane number in Figure 6.