Inducing RNAi of dpy11 and bli-1 genes in C. elegans
(in progress)

Jinming Guan, Yunru Jiang, Siyuan Yang and Lynda McMaster-Schuyler
Biotechnology Program, Department of Natural Sciences, College of Agriculture and Technology, State University of New York, Cobleskill, NY 12043.
Communication: Dr. Lynda McMaster-Schuyler, Ph.D.  Email: McMastL@Cobleskill.edu

Abstract
RNA interference (RNAi) is a biological process in which RNA molecules block gene expression, typically by causing the destruction of specific mRNA molecules. C. elegans is an excellent model system to study because many single gene mutations have been identified and the worms' transparency assist morphological observations. We are studying RNAi techniques by feeding E coli to interrupt the dqy-11 and bli-1 genes in C. elegans, resulting in morphological changes. RNAi constructs were first transformed in E. coli and C. elegans are fed E. coli bacteria containing relevant dsRNA for genes to be silenced. Phenotypic changes in offspring will be observed and documented to verify RNAi had been achieved. Further studies will examine the feasibility of fluorescent dye to enhance offspring traits. These studies have implications for novel medical treatments and gene therapy.

Background
C. elegans (Caenorhabditis elegans) is a small roundworm that lives in temperate soil. Due to its small size, short life span, transparent body, and effective reproduction rate, it is used as an model for studying RNA interference (RNAi). RNAi is a cellular mechanism used to protect cells from RNA viruses, it acts by destroys dsRNA which results in silencing gene function. RNAi can also be used as an important tool to study gene functions.

Mechanism of RNA interference (RNAi)
In C. elegans, dsRNA is recognized by a protein called Dicer which cleaves dsRNA into small interfering RNAs (siRNA). The siRNA binds to RISC (RNA – inducing silencing complex) with an endonuclease called Argonaute (Ago). After one strand of the siRNA is degraded, the other strand is used to bind to the complementary mRNA. The mRNA is then cleaved by Argonaute and thereby the gene function is blocked.

Results to date 4/2/2015

<table>
<thead>
<tr>
<th></th>
<th>Trial</th>
<th>I</th>
<th>II</th>
<th>III</th>
</tr>
</thead>
<tbody>
<tr>
<td>dpy-11 (nos. of worms)</td>
<td></td>
<td>19</td>
<td>105</td>
<td>98</td>
</tr>
<tr>
<td>Total number of worms</td>
<td>378</td>
<td>1532</td>
<td>1013</td>
<td></td>
</tr>
<tr>
<td>Concentration</td>
<td>5.02%</td>
<td>6.85%</td>
<td>9.67%</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Trial</th>
<th>I</th>
<th>II</th>
<th>III</th>
</tr>
</thead>
<tbody>
<tr>
<td>bli-1 (nos. of worms)</td>
<td></td>
<td>62</td>
<td>94</td>
<td>219</td>
</tr>
<tr>
<td>Total number of worms</td>
<td>285</td>
<td>493</td>
<td>1598</td>
<td></td>
</tr>
<tr>
<td>Concentration</td>
<td>21.8%</td>
<td>19.07%</td>
<td>13.7%</td>
<td></td>
</tr>
</tbody>
</table>

Conclusion:
RNA interference of C. elegans requires diligent and consistent attention to growth media, worm life-stage and feeding stock conditions. Our low induction rates were most likely a result of inconsistent worm staging. Future experiments will pay closer attention to staging, concentration and incubation conditions. In addition, we will be investigating the use of fluorescent dyes for phenotypic identification and analysis.

Reference