**Appendix 1**

1. **General Information**

For those new to working with *C. elegans* and/or wanting additional background for how to grow, perform RNAi inductions using reverse genetics, and handle worms to examine DNA/RNA, the following links serve as excellent resources and protocol manuals:

1. <http://www.wormbook.org/chapters/www_strainmaintain/strainmaintain.html>
2. <http://www.wormbook.org/chapters/www_introreversegenetics/introreversegenetics.html#d0e691>

The Examining the RNAi Mechanism Kit from Carolina Biological provides a convenient way to purchase all the necessary worm and bacterial feeding strains along with the basic items required for students to learn how to handle worms and perform a RNAi induction experiment. However, the items in the kit can be purchased separately from various vendors if budget or availability is a concern. For example, the control worm strain (N2) and worm strain expressing the *dpy-13* phenotype can be purchased from the Caenorhabditis Genetics Center (CGC). The *dpy-13* RNAi strain (supplied with Carolina Kit) can also be purchased directly from Horizon Discovery. Proper handling and expression of dsRNA in worm strains can be found in the Wormbook protocol manuals listed above. The table below in part B provides additional information on where to purchase items and equipment not included in the kit that are necessary to culture worms and perform gel electrophoresis.

1. **Materials and Protocols**

|  |  |
| --- | --- |
| **Materials and Databases** | **Links** |
| Examining the RNAi Mechanism Kit | <https://www.carolina.com/c-elegans-nematodes/examining-the-rnai-mechanism/FAM_211392.pr> |
| *C. elegans* *N2* and *rrf-3* wild type strains and *dpy-13* expressing worm strain can be obtained from the Caenorhabditis Genetics Center (CGC) | <https://cgc.umn.edu/> |
| Empty pL4400 vector for RNAi expression studies in *C. elegans* can be purchased from the plasmid repository, Addgene | <https://www.addgene.org/1654/> |
| *C. elegans* RNAi Collection of dsRNA feeding strains (Horizon) | <https://horizondiscovery.com/en/non-mammalian-research-tools/products/c-elegans-rnai-collection> |
| Monarch Total RNA Miniprep Kit | **Protocol link:**  <https://www.neb.com/protocols/2017/11/28/quick-protocol-for-monarch-total-rna-miniprep-kit-neb-t2010> |
| First Strand cDNA Synthesis and Reverse Transcriptase | **Protocol link:** <https://www.neb.com/protocols/2016/04/26/first-strand-cdna-synthesis-standard-protocol-neb-m0368> |
| Nematode Growth Media (NGM) | Recipe can be found using the following resource:  <http://www.wormbook.org/chapters/www_strainmaintain/strainmaintain.html#d0e214> |
| *Escherichia coli* HT115 strain - OP50 (wild type feeding strain) | <https://cgc.umn.edu/> |
| Ampicillin and IPTG | Purchase from vendor of choice   * Final concentration is 100 µg/ml Ampicillin and 400 µM IPTG for experimental setup |
| Petri Dishes (60 x 15 mm) | Purchase from vendor of choice |
| Equipment: Thermocycler, Agarose Gel Imaging Station, Stereo Microscopes, 37˚C incubator, Bunsen burners, Metal Spatulas, and Metal Worm Picks and/or Toothpicks |  |
| Integrated DNA Technologies (IDT) – Purchase of Primers | <https://www.idtdna.com/pages/products/custom-dna-rna/dna-oligos>  **NOTE:** The cost of forward and reverse primers for four groups ~ $90.00 |

1. **Websites and Databases**

|  |  |
| --- | --- |
| Wormbook – additional background information on *C. elegans* | <http://www.wormbook.org/index.html> |
| Wormatlas - additional background information on *C. elegans* | <https://www.wormatlas.org/> |
| National Center for Biotechnology Information (NCBI) | <https://www.ncbi.nlm.nih.gov/> |
| Primer Design Tips | <https://www.thermofisher.com/us/en/home/life-science/oligonucleotides-primers-probes-genes/custom-dna-oligos/oligo-design-tools.html> |
| Integrated DNA Technologies (IDT) – PrimerQuest Tool | <https://www.idtdna.com/pages> |

1. **Budget**

Estimated cost for running the lab activities for a total of 16 students (see links above for products):

* $1500 – includes the Examining RNAi Mechanism kit
* $1200 – without kit

**NOTE:** The estimated costs above do not include the equipment listed in the chart.

1. **Possible Genes of Interest**

The following are a list of genes that have given moderate phenotypes for students to observe and score. All genes can be purchased from the *C. elegans* RNAi Collection of dsRNA feeding strains (Horizon). Additional information on the range of possible phenotypes can be found on the Horizon gene database - <https://horizondiscovery.com/en/non-mammalian-research-tools/products/c-elegans-rnai-collection#description>.

* *bli-1*
* *unc-61*
* *egl-18*
* *cdh-12*
* *asg-1*
* *act-4*
* *wsp-1*
* *evl-20*
* *clc-1*
* *vab-9*