Biol-311L Cell Biology Techniques
Proposal Instructions

A written proposal of a *maximum of five pages* (space and a half if needed) that is comprised of three sections: **Background, Specific Aims and References**. See below for a detailed explanation of what is to be included in each of the three sections.

Title Page (not part of the 5 page limit)
Title, Name, Course and Date

Background
Introduce your project by discussing the biological process in which your gene is involved and the importance of this biological process.

From this broad beginning, think about the following list of questions as you formulate an introduction and build down to the biochemical function(s) and cellular role(s) of your specific gene/protein.

Two figures of maximum height of 2" are allowable. Figures should enhance the text, not be redundant with it. All figures require a *legend*.

What gene/protein are you planning to work with?
What are the Big Questions in the Field about this gene/protein?

What is known about this protein (*in all contexts*)?
- In what organisms has it been studied and what is it called in those organisms?
- Does it have any interesting domains and what do they do?
- What cellular process does the protein seem to play a role in?
- In what tissue/organ is this process required?
- How does this cellular process impact the tissue and whole organism?

What are some subQuestions (the Approach/Experiment for each becomes a Specific Aim)?

I. *What might you expect if your gene did not express its protein in C. elegans?*
- Is the cellular process that your gene is involved in essential?
- Is your gene essential in the process?
- Do phenotypes allow deduction of a cellular role?

II. *What molecular experiment would you do to determine **cellular role**?*
- A. Does your protein have a biochemical activity?
- B. Does your protein have any structural elements?
- C. Does your protein have any interactions with other proteins?
- D. Where (in what tissues and at what time) is your protein expressed?
- E. Might a different RNAi approach yield more information?

What are your hypotheses (what are your proposed answers to these questions)?

Specific Aims

I. RNAi

Write a short narrative in which you outline the approach and experimental procedures. Level of detail should include *C. elegans* strains used and a brief description of the overall protocol used. Since we use a non-published protocol for RNAi, these conditions should be described with more detail (see example). This section should be written in *first person* (and in the future tense as if you hadn't done it yet).

II. Molecular Cloning

Write a short narrative in which you propose to clone wild-type or mutant forms, full-length or fragments of your gene for your chosen purpose. Provide a rigorous rationale for why the chosen experiment is the necessary to help understand the cellular role of your protein. These experiments are often protein-specific and I encourage you to talk about your ideas with your peers and instructor. Use *first person*.

References

Proposals must include a minimum of **five** references that are not Internet sources. These can include primary as well as review journal articles or books (excluding the textbook).

Reference format:

1. For journal articles, use (Timmons et al., 2001) in the text in the appropriate location, and use the following Author-Date format for the list of References at the end:
Timmons, L., Court, D. L., and Fire, A. (2001). Ingestion of bacterially expressed dsRNAs can produce specific and potent genetic interference in *Caenorhabditis elegans*. *Gene* **263,** 103-112.
2. For information gathered from the **Internet**, the reference should provide a document title or description, a date (either the date of publication or update or the date of retrieval), and an address (in Internet terms, a uniform resource locator, or URL). Whenever possible, identify the authors of a document as well:
WormBase. (2005) www.wormbase.org/db/gene/gene?name=par-1. Release WS148. Accessed 10/05

Other information

Use the following conventional forms for *C. elegans* nomenclature:

Gene models are as they appear in WormBase:	H39E23.1
Gene common names are italicized all small letters:	*par-1*
Proteins are CAPITALS	PAR-1
Phenotypes are First cap, rest small:	Par or Pvl or Let
RNAi of a gene or gene model	*par-1*(RNAi) or H39E23.1(RNAi)

Where do I find information about my gene/protein?

First, try the Bibliography section of the WormBase page.

The clearinghouse of protein domains (Interpro) will help you gather information about biochemical function:
http://www.ebi.ac.uk/interpro/
http://www.ebi.ac.uk/InterProScan/
paste your sequence into this second URL and have Interpro send it to all the databases at once.

Or try PubMed, the public database for biomedical research:
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=PubMed

Or BioSis, available through the Lavery Library web site when on campus. Judy vanBuskirk in the library can help with either of these two databases.

In most cases, abstracts of papers or meeting presentations should suffice to give you enough information. If you want the full text of a paper, see Dr. Hurd.

One other worm specific database can tell you if and when your gene gets expressed:
http://nematode.lab.nig.ac.jp/dbest/keysrch.html
Dark areas of these pictures are tissues/organs that express the gene. See Dr. Hurd for help with this.

Biol-311 Cell Biology Techniques
Help, Hints and Evaluation of Writing Assignments

In preparing for any scientific writing assignment, you should first think about what major points you want to make, which concepts are relevant, and what sets of experiments and observations you will use to support or illustrate your statements. Then **MAKE AN OUTLINE**. First make a crude outline with only the major points. As you continue to prepare (for example, library research for a grant or a review or study through the course of the semester for a take-home exam) you will be able to make your outline more and more detailed.

Good scientific writing will consist of a **clear** and **concise** presentation of ideas *supported by experimental or observational evidence*. The experimental evidence should be sufficiently detailed to allow one to see that you understand how the experiment relates to the concept, hypothesis, or idea in question. Truly excellent work shows a **synthesis of ideas** drawing relationships between different concepts and experiments and utilizes sources beyond those we actually cover in class.

Discussing your ideas with each other or me is acceptable (and encouraged!), but you should be careful about thoughtlessly using other people's interpretations as your own; they could be wrong. Preparation of outlines or complete works by committee, however, is not acceptable.

Grading Criteria

General Appearance/Nomenclature

5: Contains separate title page with title, author name, course, date; body of paper uses appropriate spacing (one and a half in most cases, references single spaced), with 1" margins all around, 12 point Times Roman (or other normal) font, clear headings and subheadings, all pages numbered and page limits respected. Nomenclature rules (***like italics***) followed.
4: Rare nomenclature mistakes
3: Lacking headings or title page or page numbers, far too long, and some nomenclature mistakes
2: 3 plus sloppy, inconsistent format
1: Not typed, or major problems with printing, etc.

Organization

5: Sections are clearly identified and follow guidelines; objectives/topics laid out in introduction are followed in the same order in the body of the work. Organized by topics.
4: Organized by topics but presentation in body not in the same order given in introduction.
3: No topics given in introduction, but other parts organized by topics
2: Contains some topic-by-topic organization.
1: One or more of the major sections missing OR contains little topic-by-topic organization.

Writing Style

5: Normal sentence structure; each paragraph with topic sentence and logical flow (coherent structure). Neither choppy nor run-on sentences, clear formal, scientific English.
4: Each paragraph with topic sentence and logical flow (coherent structure). Some grammatical (noun-verb agreement, choppy or run-on sentences) and/or spelling problems. Clear English, but a bit conversational.

3: Each paragraph with topic sentence and logical flow (coherent structure). Some grammatical and/or spelling problems, or jargony/dense/conversational.
2: Confusing (multiple threads, lack of topic sentences). Many grammatical and/or spelling problems. Extremely conversational English
1: Paragraphs confusing and many grammatical or spelling problems.

Introduction: Overview
5: Provides focused overview of topic(s), incorporates appropriate amount of literature citations, naturally leads to objectives, of appropriate length (about 20% of total work).
4: Provides focused overview of topic, incorporates appropriate amount of literature citations, naturally leads to objectives, but too long.
3: Provides brief overview of topic, incorporates appropriate amount of literature citations, naturally lead to objectives, but too short (uninformative).
2: Provides brief overview of topic, incorporates too few literature citations, no clear lead to objectives (lack of clear flow, unfocused), too short.
1: No real overview or poor use of background information, too few literature citations.

Introduction: Purpose/Objectives
5: Introduction ends with transition material outlining major topics, goals, and objectives to be covered in body of work. These are often in a separate paragraph.
4: Introduction ends by clearly outlining main purpose along with major topics, goals, and objectives to be reviewed, but not in separate paragraph.
3: Unclear purpose, no separate paragraph, but with specific topics given.
2: Ends with vague statement of purpose of paper, no specific topics given.
1: After overview, introduction just stops.

Formation of Questions and Hypotheses
5: Question(s) is (are) clearly stated, relevant and clearly follows from what is already known. A scientifically rigorous hypothesis and any alternative hypotheses are clearly presented.
4: Relevant questions and hypotheses are vague.
3: As above and alternatives are not mentioned. Varied amount of critical evaluation or scientific rigor.
2: As in number 3, and details sketchy.
1: Questions are present, but not supported by observation or prior knowledge. Hypotheses are not scientifically rigorous.

Body of Work and Content

25: Ideas are sufficiently explained, scientifically rigorous and clearly organized. Information is synthesized and critically evaluated and from a variety of sources. Clear transitions are used between sections and subheadings are used for clarity.
20: As above, but clear transitions lacking or varied amount of scientific rigor.
15: As above, but varied amount of critical evaluation or scientific rigor and lack of transition material.
10: As above, but details sketchy.
5: Facts, observations, concepts and citations are vomited onto the pages to be deciphered for meaning by the instructor.

Use and Citation of Literature

5: Information from sources presented in student's own words, sources adequately documented (referenced); appropriate application of Author-Year citation method
4: Information from sources presented in student's own words, sources adequately documented (referenced); rare errors
3: Information from sources presented in student's own words, sources not always adequately documented (referenced); some errors
2: Information from sources presented in student's own words. Sources poorly documented (referenced) OR inconsistent application of citation method
1: Information from sources NOT presented in student's own words (plagiarism) OR sources undocumented (referenced)

Figures

5: Presented in a timely and appropriate manner; are not redundant with text, but rather add to text, numbered and include a clear legend.
4: As in 5, but redundant with text.
3: Redundant, out of position or not cited in narrative. Inadequate legend.
2: As in 3, but no clear purpose for figure; simply added for flash.
1: Poor/confusing use of figure with inappropriate labels, legend, title OR undecipherable.

Conclusions

5: Scientifically rigorous summaries and appropriate conclusions drawn that reflect overall purpose and each topic given in introduction and through the body of the work.

3: Brief summaries and appropriate conclusion given for overall purpose and each topic where appropriate. Some conclusions missed or 'stretched' beyond supporting data.

1: Inappropriate conclusion(s) given or absent.

Literature Cited Section

5: Correct format for Author-Year method used for each source; all sources cited in text are in literature cited section and all sources in literature cited section are used in paper; entries arranged alphabetically.
4: Rare errors.
3: Some errors.
2: More errors.
1: Complete overhaul needed.

Revised Papers

5: Revision addresses all concerns and annotations made on original.
4: Revision addresses all concerns and annotations made in original, but some revisions do not fix problems
3: Revision adequately addresses most of the major concerns, with some concerns not addressed
2: Revision adequately addresses some of the concerns and annotations made on original.
1: Revision addresses few or none of the concerns and annotations on original

Biol-311L Cell Biology Techniques

Oral Presentation Instructions

Prepare a 10-12 minute presentation using **PowerPoint**. You will present your PowerPoint to a group of colleagues at one of the presentation sessions (date and time TBA, see BlackBoard). This presentation is physically due by email/Digital Drop Box (Bb) or on some sort of disk (CD or thumb drive) three hours before the start of the session. The order of presentations will be determined at the beginning of the session, so be prepared to go first. If you made the presentation on a home/personal computer, then you should try it on a college computer prior to submitting it.

Consider the following:

What **Background** information needs to be understood?

What cellular processes and what are the Big Questions about the cellular process?

What gene/protein are you talking about?

What is known about this protein?

Does it have any interesting domains?

What are their molecular/biochemical functions?

Does it have relatives (paralogues or orthologues) and what do they do?

How are the biochemical functions and cellular role required by the tissue and whole organism?

What is not known?

Questions?

The focus of the presentation is one to three figures worth of ***primary data***. The data that you present may be your own RNAi experiment **or** published data about the worm gene **or** a homologue. *Data that provides insight into some significant aspect of the biology of the gene/protein is **required**.*

What are the **Approaches** being employed to answer the **Questions**?

Is there an hypothesis (what is the proposed answer to the questions)?

What is the **Experiment** being presented? How did you (or someone else) generate the **Result**?

What is the **Result**(s)?

What is the **Literal** interpretation of the **Result**?

What is/are the **Author's or your** interpretations of the **Result**?

What can be concluded?

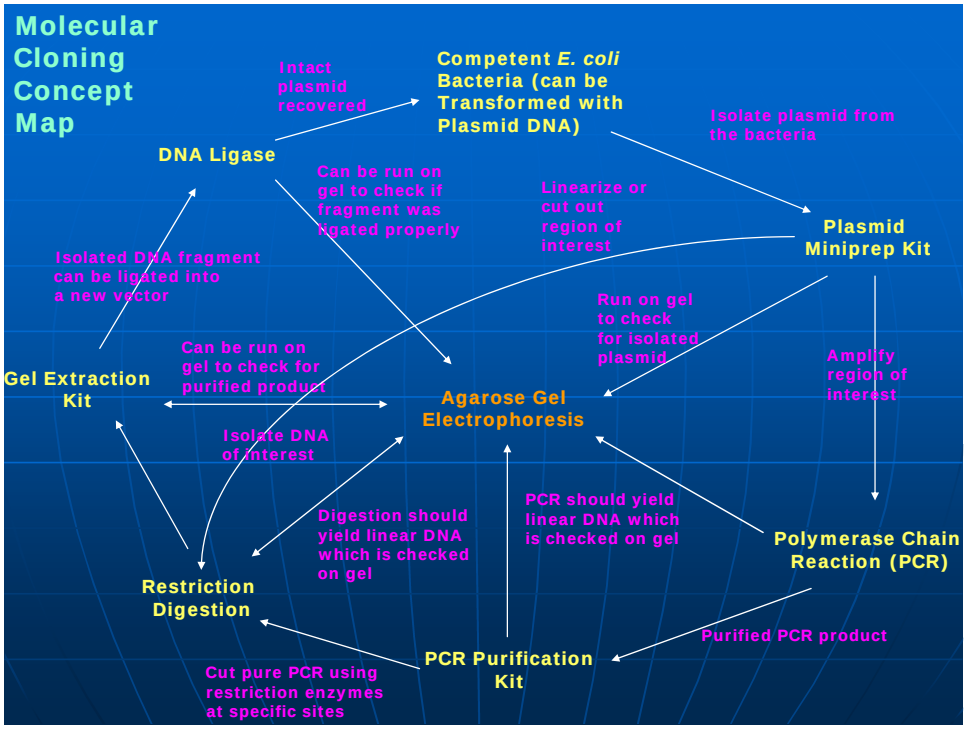
What might be done next?

Evaluation:

Each student will be evaluated on their ability to provide a deeper understanding of the function of the specific molecule that they have chosen to present. Please see criteria in **Oral evalution** document. In addition, each student is required to ask a **minimum** of **two questions** of their colleagues, which count toward the lab participation portion of the final lab grade.

Biol-311 Cell Biology Techniques
Evaluation of Oral Presentations

	< Adequate	Average	Outstanding
Background (10)	not clear or implied	clear, concise description of what is known	'hook' provided with relevant and adequate *detail*
Question (5)	lacking	made obvious and linked to background (what is not known?)	as above with description of the significance and relevance
Approach (5)	as above	made obvious and compared to alternatives	links Background to Question, compared to other possibilities
Experiment (5)	glossing over detail factual errors	completely and precisely explained with relevant technical detail	as above with complete understanding of caveats
Result (5)	glossing over data, factual errors	define/explain what figures depict including scales, axes and all other relevant elements	as above with accurate, relevant detail and mention of shortcomings
Literal Interpretation (5)	lacking or too simplistic	explain and identify the conclusion	separate literal from author interpretation and critically evaluate both
Author's Interpretation (5)	no separation between literal and author interpretation, not/too ambitious	identify how the data move the field forward, evaluate author's bias	as **all** of the above


Molecular Cloning Concept Map
Intact plasmid recovered
Competent E. coli Bacteria (can be Transformed with Plasmid DNA)
Isolate plasmid from the bacteria
DNA Ligase
Can be run on gel to check if fragment was ligated properly
Linearize or cut out region of interest
Plasmid Miniprep Kit
Isolated DNA fragment can be ligated into a new vector
Run on gel to check for isolated plasmid
Amplify region of interest
Can be run on gel to check for purified product
Gel Extraction Kit
Agarose Gel Electrophoresis
Isolate DNA of interest
Digestion should yield linear DNA which is checked on gel
PCR should yield linear DNA which is checked on gel
Polymerase Chain Reaction (PCR)
Restriction Digestion
Purified PCR product
PCR Purification Kit
Cut pure PCR using restriction enzymes at specific sites