

7.16 Proposal Instructions

Team Project Proposals should be approximately 3-5 pages in length (double-spaced, 12 point font, 1" or 1.25" margins, pages numbered, excluding references) and should have 5 sections: 1) Title; 2) Specific Aims (SA); 3) Background & Significance (B&S); 4) Research Design & Methods (RD&M); and 5) References. Include a title at the top of the first page, and list the team members who will be working on the project, with the author's name first. A diagram/figure may be included. The style, content and level of detail expected are illustrated with sample text below. The example given refers to a hypothetical project lab whose goal is to study genes that regulate the shape of human fibroblast cells using over-expression techniques.

The title should describe what the project is about but not be too long, e.g.,

"An Over-expression Screen for Genes that Control Cell Shape in Cultured Human Fibroblasts"

The specific aims should be numbered and should be concise and specific, e.g.,

"SA 1. To identify genes whose products make cells rounder.

SA 2. To identify genes whose products make cells longer and thinner."

The B&S section should provide background that is directly relevant to the question that is being asked and an indication of why it is important. Ideally, it should be intelligible and interesting to any biologist. Here's an example:

"The regulation of cell shape is critical in a number of developmental processes, such as blah, blah and blah, and misregulation of cell shape can lead to diseases such as blah and blah (refs). An increased understanding of the genes involved in cell shape control could lead to development of improved drugs that inhibit or activate these genes and thereby restore proper cell shape in individuals with blah syndrome. Genes of the "rotund" family have been implicated in making cells rounder in a number of systems (refs). This gene family is characterized by a conserved N-terminal domain (the "rotund" domain), which interacts with actin, and a variable C-terminal domain. Rotund family members are thought to regulate cell shape by affecting the polymerization and branching of actin microfilaments. A search of the literature identified 6 different rotund family genes: rotund-1, rotund-2, rotund-3, pudgy, chubby, and beachball. Blah blah blah ...

Genes of the "twiggy" family tend to oppose the activity of rotund family members, either by binding and sequestering rotund family proteins, or by interacting directly with actin microfilaments to inhibit branch formation. Blah blah blah ..."

The RD&M section should contain a complete but concise description of the experiments that will be done and the techniques that will be used. The use of subheadings to organize this section is encouraged. In describing techniques, include only relevant details, e.g., variables that you will alter in your experiments, not those which are present in the protocols in your binders. For example:

Cloning and Transfection Experiments

“We will clone the cDNAs of the 6 rotund family genes identified through literature searches (rotund-1,-2,-3, pudgy, chubby and beachball) into the XYZ vector, downstream of a strong, constitutive cytomegalovirus (CMV) promoter and terminating with the beta-globin polyadenylation signal sequence. These constructs will be transfected into cultured fibroblasts, and cell morphology will be monitored using light microscopy and digital image processing. Briefly, captured images of cells will be analyzed using the CellGeometry™ software package, which calculates the ratio of cell circumference to cell diameter as a measure of “roundness”. The rotund-1 construct provided by the course instructors will be used as a positive control, since over-expression of this gene is known to cause rounding-up of cultured human fibroblasts (ref). A green fluorescent protein (GFP) expression construct will be used as a negative control, since GFP expression has no effect on cell morphology (but makes the cells glow an attractive shade of green). As an additional control, cells will be transfected with an “empty” vector, i.e. a vector containing the CMV promoter and globin polyadenylation site but lacking any inserted cDNA sequence.

We will also do a TBLASTN search with each of the six known rotund family proteins as a query against the human genome using the NCBI BLAST web server [<http://ncbi.nlm.nih.gov/blast>]. BLAST hits will be manually inspected for presence of the conserved rotund domain and for presence of the ‘ACT’ motif, which is known to be critical for interacting with actin microfilaments (ref). Full-length cDNAs will be obtained from the IMAGE Consortium [<http://...>] for each potential novel rotund family member identified by BLAST, and these cDNAs will be cloned into the expression construct and tested as for the known rotund family genes. Blah blah blah ...

References

Cite relevant references for statements of known results, hypotheses, or background information. About 5-10 refs is probably appropriate for this exercise. Cite them in the text like this:

“In response to death stimuli, ubiquitination of DIAP1 is enhanced by interaction with Reaper (Martin, 2002; Palaga and Osborne, 2002).”

For references with one or two others, list all authors (and year), as above. For references with three or more authors, cite as first author *et al.* (Latin for ‘and others’), followed by the year. Include an alphabetical list at the end (ordered by last name of first author), in this format:

Martin, S. J. (2002). Destabilizing influences in apoptosis: sowing the seeds of IAP destruction. *Cell* **109**, 793-796.

Palaga, T. and Osborne, B. (2002). The 3D’s of apoptosis: death, degradation and DIAPs. *Nature Cell Biol.* **4**, E149-E151.

Note that the journal name is italicized and the volume number is in bold. In the reference list, include all authors, even if there are more than two. Make sure that all references cited in the text appear in the reference list at the end and vice versa.

Tips for Writing Team Project Proposals

1. *Begin with the literature.* First, read the relevant papers in your course binder. Ask an instructor if unsure of which papers are relevant to your project. Next, do a PubMed search at [<http://ncbi.nlm.nih.gov/entrez>] using a few relevant keywords (e.g., *Drosophila*, caspase, apoptosis, DIAP1, phosphatidyl inositol, etc.). Try different combinations of keywords. Read the abstracts of any papers that have relevant-sounding titles. If the paper still sounds relevant, download it and read it. Collect a list of gene names related to your project. Search for these genes in FlyBase and on the OpenBiosystems web site. You may want to break up the reading of the papers and the searching between team members and then pool your insights.
2. *Include some bioinformatics if appropriate.* For example, you may want to do some searches of FlyBase [<http://flybase.net/>] (or other servers) or do some BLAST searches against known *Drosophila* proteins or against the *Drosophila* genome. If the bioinformatics seems complex, describe what you plan to do in the proposal rather than doing it beforehand.
3. *Come up with specific aims.* Roughly 1-3 aims is suggested. The aims are very important, so spend some time thinking about them. If you read your aims aloud and they don't seem clear or don't seem interesting, then keep working on them. Aims should be as concise as possible.
4. *Design a set of experiments that address your specific aims.* You should discuss the experiments with your teammate(s), but of course write them up independently. Be sure to include positive and negative controls. Be as specific as possible. List the names and accession numbers of the genes that you will study, perhaps in a table. Describe any time courses or titrations you plan to do, and what range of times or concentrations you will explore. Describe how you will analyze/interpret your data and how you will deal with expected and unexpected results. If one set of experiments is contingent on the outcome of another set of experiments, explain this clearly, and explain what you will do if the first set does not turn out as expected. Convince the reader that you have thought about all the potential obstacles in carrying out your research and have a plan for overcoming each of them.
5. *Follow the advice of the writing instructor in planning, writing and editing your proposals.* In particular, make an outline early on, then write a draft, read it over to yourself, make revisions, and ask for comments from the writing instructor, Marilee Ogren. Be sure to contact her several days before the due date to allow time for her to give you feedback and for you to revise your proposal.
6. *Remember that what you propose is what you will do.* For the rest of the semester. So make sure that your proposal makes scientific sense and is as interesting to you as possible (while still remaining doable in the time available). Of course, some deviation from plans in the face of unexpected results (or technical difficulties) often occurs in scientific research.