

*Note: this proposal was prepared before the AYURE application was updated . Please see the current application for details.
The cover sheet was removed for web publication*

Pages 2-3: Brief Description of Proposed Research, prepared by faculty mentor (no less than 11pt font, .rtf format)

I accept responsibility for the conduct of this project and assure that the information in this application is correct. Indicate your agreement with the statement with an 'X'.

Question 1: Please provide a title for the proposed project (less than 70 characters with spaces).

Isolation and characterization of an estrogen receptor gene in *Dugesia tigrina*.

Question 2: Provide a BRIEF review of the literature (citations not required), appropriate for the diverse members of the RSCA advisory board, to provide the background information that led you to the proposed project.

Planarians are flat worms that can regenerate themselves when injured. They can regrow their entire body from a tiny fragment. They actually use this as a reproductive strategy (they fragment and regrow). Previous work in our lab has shown that BPA (a component in many types of plastic) has toxic effects on flatworms and interferes with regeneration at low doses. At high doses, it is lethal. Recent work in our lab has shown that a breast cancer drug called Tamoxifen can significantly lessen the toxic effects of BPA and in some cases, reduce lethality. This is of interest because BPA has been shown to work with estrogen receptors and Tamoxifen is known to bind to estrogen receptors. This is very suggestive of a role for vertebrate hormone pathways in invertebrate worms. A bioinformatics study done by Ms. Schappert last Spring of sequenced planarian genes found several candidates for a homologue of the human estrogen receptor in *S. mediterranea* (a different species of planaria).

Question 3: State the purpose of the overall research or creative project. Include the major hypotheses, research question(s) and/or the aims of your project.

We hypothesize that there are members of the estrogen receptor family of genes present in the *D. tigrina* genome. This project will test that hypothesis and examine the genomic DNA to find sequences that are homologous to the well characterized sequences in vertebrates using a candidate sequence discovered in the genome of a closely related flatworm.

Question 4: State the methodology to be employed for the project. Explain the role of the student in the overall project as will apply to the semester's work and the student's qualifications. Please indicate if the project is likely to continue past the award period.

The student will employ standard molecular biological techniques for isolating and amplifying DNA using off-the-shelf reagent kits, PCR and gel electrophoresis. We will utilize routine vendor services to obtain PCR primers and for sequencing the PCR products of interest. Data analysis will employ freely available bioinformatics software and databases maintained by the National Institutes of Health. Mary is very well qualified to perform all of this work. She has experience using all of the molecular techniques, she performed the original bioinformatics search to find the genes in *S. mediterranea*, and she has worked on several independent study projects involving xenoestrogens and flatworms in my laboratory. This particular project should be completed during the Spring of 2012, but there will be ample opportunity for follow-on work based on this project.

Question 5: Provide a brief description of the role of the requested budget items in the project.

The reagents are in kits designed for isolating DNA from animal tissue and running standard PCR reactions in a thermocycler (PCR machine). The primers are used for amplifying the DNA of interest in the PCR reaction. The plastic tubes are specified for use by the sequencing service at Columbia University.

Question 6: State the expected outcomes of the project with regard to the advancement of knowledge in the field or the progress toward new creative works. The outcomes should be described in the context of the current state of the field of study.

We have very strong circumstantial evidence that estrogen pathways are active in flatworms from work done in our own lab with several different species. The *D. tigrina* genome is not likely to be sequenced anytime soon. Finding a homologous sequence in *D. tigrina* would demonstrate the presence of the gene of interest in this species, would provide further evidence that these vertebrate hormone pathways are evolutionarily conserved across phyla in invertebrates, and suggest that the mechanism of action for

estrogen and estrogenic compounds in *D. tigrina* is actually based on the stereotypical estrogen receptor pathway. If we are successful in isolating the estrogen receptor gene, the outcome of this experiment will also provide us with the sequence information required to do gene expression studies and RNAi “knockdown” (gene expression inhibition) studies in the future.

PAGE 3. Faculty Statement, continued

Question 7: Explain how this project provides a learning experience not available in the classroom/ regular programmatic activities/ major requirements.

This is a wonderful opportunity for Mary to tie together her skills gained in lab courses such as molecular biology and genetics and use them in the service of a real scientific investigation. Also, since we are in a very real sense gene-hunting and looking for something that no one has yet found in a place that nobody has yet looked – it is an opportunity for novel and significant work to be done.

Question 8: Describe how the faculty mentor will guide the student. Inclusion of the arrangements for regular, in-person meetings is necessary but not sufficient.

Mary and I have already collaborated extensively on the design of her experiments and have a projected timeline with milestones for achievements. Mary will be working alongside me for the primer design (using the free NCBI tools for primer design). She will work relatively autonomously for the PCR and gel electrophoresis (these are both techniques she is experienced with). I will work closely with her on troubleshooting if the need arises. The analysis of the sequences is fairly straightforward and I expect her to be able to work semi-autonomously on that given her past work in my lab. Mary will be meeting with me in one-on-one meetings at least once per week and has open-door access to me at any time since she is working in my lab. She will also attend and present at our regular lab meetings.

Question 9: (if applicable) Provide a summary of the work accomplished with previous (past 3 years) SURE or AYURE funding. Information on the post-graduation placement of former AYURE and SURE students will be appreciated.

Details removed for web publication

Page 4: Student's statement (no more than one page, no supplementary information)

X I accept responsibility for the conduct of this project and assure that the information in this application is correct. Indicate your agreement with the statement with an 'X'.

Question 1: State the goal of your role in the proposed project.

Candidate estrogen receptor genes were identified in *Schmidtea mediterranea*, a species of planaria, using a species specific EST database (independent study with Dr. Mass in the Spring of 2011). The goal of this project is to attempt to isolate and sequence a homologous gene in *Dugesia tigrina*, another species of planaria.

Question 2: State the specific methodology you will employ in carrying out the proposed project.

Genomic DNA from *Dugesia tigrina* will be extracted by grinding up the worms and using a kit to isolate and purify the genomic DNA. The DNA will be amplified in a PCR reaction using custom primers designed for the target sequence. An agarose gel will be run with a ladder to determine if the amplified sequence is the predicted size. This will determine if the target sequence was, in fact, amplified. The amplified DNA will then be sent out for sequencing in order to run comparisons between *D. tigrina*'s predicted estrogen receptor gene and those of other organisms in which the gene has already been identified.

Question 3: Provide a description of your qualifications to participate in the proposed project.

After almost two years of research in the organic chemistry lab with Dr. Andersen, a Summer internship at Cornell University working with a grape vine pathogen, previous work with Dr. Mass, and molecular class lab with Dr. Morrow and Professor Valens, I feel very prepared to work on this project. I have an understanding of the specific gene that I will be isolating from the planaria because of the work I have already done with Dr. Mass on his xenoestrogen project and because of the BLAST research I did last semester to determine candidate estrogen receptor genes in *Schmidtea mediterranea*. With my experience in a lab setting, along with the skills gained during molecular class lab, I am confident that I will succeed in isolating and sequencing the desired gene from *Dugesia tigrina*.

Question 4: Describe your plans to meet with your mentor in person for guidance.

There will be weekly meetings in which we discuss the project as well as more day-to-day guidance as needed.

Question 5: Describe the expected outcome/ product of your work.

Since *Dugesia tigrina* is a mixoploid species (there is not a stable number of chromosomes in every cell – rather the number of chromosomes is frequently different between cells in the same organism), its genome has never been, and probably never will be, sequenced. By identifying candidate genes using other organisms, such as humans, rodents, flies, and (more importantly) *Schmidtea mediterranea*, the outcome of the isolation and sequencing of an estrogen receptor gene in *D. tigrina* will help to further clarify the conservation of this sequences across phyla. In addition this will provide a valuable tool for future work understanding the mechanism behind the effects of xenoestrogens on these creatures.

Question 6: Describe your plans after graduation and how your involvement in the proposed work relates to these goals.

After graduation, I plan on applying to microbiology/molecular biology programs. Not only will this project give me invaluable experience in a molecular laboratory, it will also help me narrow down professors with whom I would want to work once in grad school.

Question 7: Describe your plans for presenting the results of your research (e.g. the Student Research Symposium).

I am planning on creating a poster explaining my results to present during the Student Research Symposium as well as at the Northeast Society for Developmental Biology Regional Meeting conference in April 2012.