Part II: Project Proposal

Project title — Electroporation of DNA into *Drosophila melanogaster* Embryos
Faculty — Christopher Jones, Professor of Biology
Student — Leanna Talotta-Altenburg
Project duration — 10 weeks, Tuesday May 30 through Monday August 7

Description of the project —

Introduction of DNA into model organisms has proven itself to be an indispensable technology in molecular genetic research and has become relatively routine. From simply adding additional genetic material not present in the organism to "rescuing" mutations with the corresponding section of wild-type DNA, being able to place foreign DNA into embryos is versatile and valuable. In order to do this in the fruit fly *Drosophila melanogaster*, DNA constructs are assembled *in vitro* and then injected into eggs, where some fraction of them are successfully incorporated into the genome. As you might imagine, injecting picoliters of liquid into an object significantly smaller than a sand grain is not trivial; most laboratories today opt to send their constructs out to specialized centers to do this work for a fee.

Another way to get DNA into cells, one which has been used for decades with bacteria, is electroporation. In this process, a high-voltage pulse renders the cells momentarily "competent" to take up DNA molecules in the surrounding solution. This approach has also been used for eukaryotic cells, and has been shown to work with *Drosophila* eggs as well, but only transiently: the DNA enters the egg and is expressed, but is not incorporated stably into the fly genome and is instead eventually degraded.

Until recently, that transient expression has limited the utility of embryo electroporation: researchers typically want to incorporate their modified DNA into cells permanently in order to be able to assess their effects over the long term. But the emergence of CRISPR/Cas9 gene editing technology promises to change that. CRISPR/Cas9 is a technique much in the news lately, one which allows researchers to make precise changes to any organism's genome with very high efficiency; it promises to literally revolutionize genetic and genomic research as well as human health, drug development, and many other fields. Transient expression of CRISPR/Cas9 reagents inside embryos may well be enough to allow them to permanently alter the host genome in desirable ways, obviating the need for continued presence of the transgenic DNA construct.

The goals of this project will be two-fold: repeating and improving the rarely-used protocol for transient expression of transgenic DNA molecules in *Drosophila* embryos in order to evaluate its utility for CRISPR-based *in vivo* gene modification, and developing a method to introduce transgenic DNA into embryos such that it is stably integrated into the genome.

Roles and responsibilities —

Leanna will be responsible for background research, growing *Drosophila* in quantity, collecting embryos, preparing solutions, and planning, executing, and analyzing experiments. Her background research will include not only reviewing what has been published about electroporation in relevant systems but also the current state-of-the-art in the world of CRISPR/Cas9 gene editing. She will then decide what DNA construct to use so that it will be relatively simple to quickly assess whether and to what extent it has modified the host genome as well as to what extent it has been successfully incorporated into that genome.

My role will be to guide Leanna's background research, coordinate the various aspects of the project (I will have a better idea than Leanna how much time will be required for the different stages and so will need to plan for how to best optimize the limited time we'll have) and to serve as a voice of experience, having much more experience with *Drosophila*, molecular genetics, and electroporation.

Week 1: Literature research. Leanna will immerse herself in the scientific literature, to learn more about electroporation in general as well as the experiences of other researchers in implementing this technology. In addition she will review the nuts and bolts of CRISPR technology so that we will be able to design effective reagents for her project.

Weeks 2–3: Preparing *Drosophila* cultures for large-scale embryo collection. Purifying a control reporter plasmid which expresses GFP (a fluorescent protein) for experiments. Purifying and appropriately modifying the CRISPR plasmid that I have on hand, then transforming it into bacteria to provide a long-term source.

Weeks 4–9: Carry out electroporation experiments. Analyze results as we go, changing experimental parameters as appropriate to optimize plasmid gene expression or integration.

Week 10: Begin to prepare for presentation at Scholars Day, the NCUR conference, and the Drosophila Research Conference (if appropriate).

Student engagement in discipline-appropriate scholarly research —
Leanna is currently a senior who took Genetics with me in Fall 2015 and Advanced Genetics in Spring 2016; she did very well in both courses, so much so that I'm very excited about the prospect of her being able to conduct this project with me. Leanna is very interested in gaining hands-on research experience, and is currently planning to go to graduate school.

Her involvement in all phases of the project, from the many different hands-on manipulations through analyzing and interpreting her results, will strengthen her command of the research process. As a result, Leanna will be much better prepared to succeed in graduate school.

As for the appropriateness of this work, it is entirely in line with the sorts of tasks that researchers in molecular genetics do routinely: molecular design and purification, processing using specific molecular reagents to create new molecules, and purifying these and confirming their correct structure. If we succeed in our goals, examining the effects these changes have on various phenotypes (e.g. lifespan, behavior, morphology) are also an important part of the (behavioral) molecular geneticist’s toolkit.

Student contributions to the discipline —
If the project is successful, it will absolutely lead to additional experiments which will ultimately be published; from a purely technical perspective, electroporation is far cheaper than embryo injection. Thus developing a protocol for CRISPR/Cas9 gene editing that relies on electroporation will make this technique amenable to use in student research projects around the world. Right now, if I wanted to give each of 20 students a single CRISPR project in Advanced Genetics, the injections alone would cost $4500, far more than is reasonable for almost any college or university. Electroporation would slash that over 100-fold.

If Leanna succeeds it will also be key to several other projects in my laboratory. Hopefully as part of her SOAR project we will also create some biologically interesting mutations which will be valuable in their own right, but the primary focus of the project has to be demonstrating that the technique works, which will require making tried-and-true mutations which in and of themselves won’t be particularly interesting.

Regardless, I believe that the results of this SOAR project will by themselves be more than sufficient to merit presentation at regional and national conferences. In years past my SOAR students have presented their work at the regional Beta Beta Beta convention (Tri-Beta is the undergraduate biology honor society), the National Council for Undergraduate Research conference, and at the national Drosophila Research Conference.

Part III: Student Statement of Purpose

Project title — Electroporation of DNA into *Drosophila melanogaster* Embryos
Student Researcher — Leanna Talotta-Altenburg, Biochemistry major, expecting to graduate December 2017
Faculty Mentor — Christopher Jones
Housing requested — none

I am a non-traditional first semester senior pursuing a bachelor's degree in Biochemistry. To date, I have had the privilege of taking several Biology and Chemistry courses and have thoroughly enjoyed them all. I have taken my education and research opportunities, both in and out of the classroom, very seriously. I have maintained a 3.5 GPA and a membership in the Tri-Beta Honor Society, taking on the role of secretary this semester. I've done this all while maintaining a marriage and a household off campus. I am very grateful for the educational experience I have received and for the scholarships that have made it possible.

Initially, it was my intention to further my education in order that I might attend medical school. However, recently I conducted research with my Chemistry mentor, Dr. Stephen U. Dunham, during winter break, and having felt such fulfillment from our efforts I've decided to pursue a doctorate in Biochemistry. I am currently working on an independent study project with Dr. Dunham, which is an extension of my winter research. The project consists of conducting various assays and protocols utilized in the construction of a model for investigating the incorporation of inorganic rhodium into double-stranded DNA oligomers.

I would now like to work more closely with biological systems and genetic investigatory models. True biochemical research can be fully conducted and optimized with a thorough understanding of DNA recombinant technologies such as cloning and electroporation. DNA is more than just a biological macromolecule; it is the Central Dogma of Life in the scientific community. Biochemical function is most often related to the replication, transcription and translation of an organism's genome. Study of protein expression is done with DNA constructs; and nearly all autoimmune diseases and cancers can be traced to genetic anomalies and mutations.

I am excited to work with Dr. Jones. It is important that I continue to hone my laboratory skills and study new technologies in order to best utilize the resources and monies available for biomedical research. This would include continued practice of some strengths I already possess, such as GFP purification and making a tagged plasmid reporter construct. I want to be an indispensible graduate student and researcher, and working with notable technological advances such as CRISPR/Cas 9 and electroporation will provide a challenge and a chance for growth. It is my expectation that caring for and working with living organisms independently, as well as keeping a detailed record of observations and discussions, will better prepare me for a distinguished Biochemistry graduate program, and the field of biomedical and genetic research.

Part IV: Expense Proposal

Project title — Electroporation of DNA into *Drosophila melanogaster* Embryos
Faculty — Christopher Jones, Professor of Biology
Student — Leanna Talotta

The department now has an electroporation system and most of the basic reagents necessary for this project.

As a positive control, it will be valuable to have the CRISPR constructs that we are trying to electroporate injected into embryos using the currently-preferred method. We will need to pay an outside vendor to do this; currently this runs $225 per injection. We are therefore requesting funding to pay for injection of two constructs ($450) and an additional $50 for a portion of miscellaneous consumables (e.g. the necessary *Bbs*I restriction enzyme).

Additional expenses (e.g. routinely-used laboratory supplies, fly food ingredients, additional reagents) will be covered by the Department of Biological Sciences.