Development of a New Discovery-based Research Course together with a Scientific and Experimental Literacy Assessment Instrument (SELA)

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1. Introduction/project overview:

Discovery-based research courses are one of the five recommendations of the President's Council of Advisors on Science and Technology to improve retention in science, technology, engineering, and mathematics (STEM) disciplines and careers. It has also been suggested that such "authentic scientific experiences" can engage students and increase their understanding of the process of science. The goals of this Chancellor's award project are to impact students' research experiences in MCDB through: i) the development and testing of an upper division discovery-based research course (**MCDB 4100**: Experimental Design and Gene Manipulation in the vertebrate *Xenopus*), ii) to develop and begin the validation process for a scientific and experimental literacy assessment (**SELA**), iii) to gather data on MCDB 4100's effectiveness and impact in terms of students' understanding of the scientific and experimental process; and iv) to begin a discussion with other faculty (in particular Professors Ding Xue and Min Han – MCDB) on the feasibility of developing a freshman version of MCDB 4100, using as a model metazoan the roundworm *Caenorhabditis elegans* and bacterially-delivered siRNAs to manipulate gene expression.

2. Background

In 2012, the President's Council of Advisors on Science and Technology (PCAST) predicted a shortage of ~1 million STEM graduates within the next decade [1]. One major problem with the STEM training system is fewer than ~40% of students entering STEM majors exit with a STEM degree. To close this gap, PCAST made five recommendations, one of which was to replace standard laboratory courses with discovery-based research courses [2], which have recently received significant attention as an instructional strategy [3]. Courses that deliver a discovery-based authentic research experience have been characterized as those that engage students' creativity by enabling them to ask novel questions and generate new (i.e. previously unknown) observations through students' active involvement in the scientific process, including carrying out their own surveys of the existing literature, developing experimental designs, data generation and analysis, and public presentation, something difficult to achieve in more conventional laboratory courses. In such conventional "cook-book / known knowledge" type laboratory courses, faculty frequently encounter difficulties in getting students excited or motivated to follow detailed and laborious protocols just to carry out experiments with already known results. The challenge is getting students to ask questions or think of alternative explanations of observations when they perceive that their only goal is to reproduce already known and correct results. Anecdotally, we (B.B.) have had students' express that they "have taken several laboratory courses but have never heard of a positive control" or they did "not need controls, because all the experiments worked".

Our goal is to circumvent the negative effects of traditional labs by replacing them with what are in fact actual research projects. Only very recently have a number of factors converged to make this possible and practical. We can now use genomic sequence data from both human and the frog *Xenopus laevis* together with CRISPR-Cas9 technology [4, 5] to

carry out novel experiments, with a good chance of success, focused on the manipulation of genes chosen by students. Studies using commercially available purified Cas9 protein and gRNAs targeted to the tyrosinase gene involved in pigment formation, carried out by our collaborators Tyler Square and Dan Medeiros (EBIO, UC Boulder), indicate that CRISPR-based mutagenesis is efficient in *X. laevis.* In this light, we have designed and received departmental approval for a new discovery-based research course, to be offered in the Fall of 2016: MCDB 4100:Experimental Design and Gene Manipulation in *Xenopus.* To help facilitate the delivery, evaluation, and dissemination of this course, we request seed-funding in the form of a Chancellor's award from the Center for STEM learning.

Assessment of scientific literacy and an understanding of experimental design and interpretation: Since the publication of Project 2061 [6-8] and the PCAST report [1], there has been a wide-spread effort to integrate discovery-based laboratory courses in colleges [9, 10]; however, the tools to assess whether these courses are meeting the intended goals of achieving scientific literacy have been lagging. While there are a number of assessment instruments on the nature of science (e.g. VNOS)[11], disciplinary topics [12-15], reasoning [16], experimental design [17], and data analysis [18] there are few, if any, assessment tools that measure all the skills necessary for scientific literacy, that is, the process of science and the establishment of robust experimental observations and conclusions [19]. Also lacking are longitudinal studies and validation across diverse student groups and different majors [20]. To this end we propose to begin work on the development of an assessment instrument in this area. We propose to use the affordances of the beSocratic system [21-24], recently cloned onto CU Boulder servers, to present students with various experimental scenarios, and to develop a rubric that establishes what correct (literate) responses need to contain. By following students through their course sequence we can begin to evaluate the impact of discovery learning courses on scientific literacy and experimental sophistication.

The Scientific and Experimental Literacy Assessment (SELA) will include items addressing question such as:

1. What are positive and negative controls, what is their value in experimental interpretation, and give example of their use in an experimental context.
2. Explain the types of replication studies needed to establish an experimental conclusion.
3. You discover an interesting phenomena, how would you go about making your observation "scientific" and "significant", what does that entail?

While we recognize the realities of assessment development, we expect that once completed the SELA will be useful in a wide range of situations, and can be customized for use in the context of less experimental (more observational) sciences.

MCDB 4100 course design: Experimental Design and Gene Manipulation in the vertebrate *Xenopus* is a three-credit course designed for junior, senior and in some cases sophomore level MCDB and perhaps EBio and iPhy students. The course begins with an introduction into the use of model systems in general, and the clawed frog *Xenopus laevis* in particular, to study aspects of the cell and developmental biology of vertebrates. Students will be called upon to select a gene for study based on their reading of the literature; we expect that students will gravitate to genes known to be involved in human diseases or in biologically interesting process or genes of unknown function. Students will be instructed in the use of on-line reference tools such as PUBMED, OMIM, Unigene, Google Scholar. The students (working in groups of three) will be required to determine whether orthologs of their gene of

interest are present in *Xenopus laevis* (using XenBase). If the gene is not present (an unlikely possibility) or the gene's function has already been characterized via mutagenesis (again, an unlikely prospect) they will be required to choose a new gene. They will then go on to determine (using genomic/RNA sequence analysis) whether the gene contains a >300 base pair exon for use in gene expression / in situ hybridization studies. Once they have chosen their target gene, they will be required to write up a description of their thinking and to make a short (~5-8 minute) in class presentation justifying their choice.

In the next section of the course, the students will be introduced into basic molecular biological techniques involved in the experimental manipulation of *Xenopus* (including appropriate positive and negative control experiments); this includes the process of generating and maintaining embryos, injecting fertilized eggs, polymerase chain reaction (PCR), plasmid construction, genomic DNA isolation, *in vitro* anti-sense RNA and gRNA synthesis for in situ hybridization and gRNA/Cas9 injection, respectively.

Once necessary plasmids have been constructed (by week 4-5) they will continue to design their experiments and learn to inject fertilized *Xenopus* eggs and assay the resulting larvae for the presence of targeted genetic mutations and overt (morphological and behavioral) phenotypes. They will also complete an analysis of the expression of their target gene by in situ hybridization. At this point, they should be in a position to repeat their experimental analysis (to monitor reproducibility using biological replicates). At this point (~week 12 to 15 of the semester) they will extend their original presentation in order to describe the logic of their choice of target gene, their experimental design, their observations, and future objectives. Based on the model established in the course of teaching MCDB/EDUC 4811: Teaching and Learning Biology, we expect that their final presentation will be in the form of a 5 to 8 minute video to be posted on a course YouTube site [25].[1] Gene expression and other data obtained through the project will be posted on the XenBase website [26] with appropriate attribution. It is even possible that students could author manuscripts appropriate for the journal Gene Expression Patterns.[2]

Course Goals: The course aims to provide students with real research experience involving their choice and justification of a target gene, using methods known to work in *Xenopus* and producing results that may well be unexpected and could be publishable. As part of the project, students will consider the importance of control (positive and negative) and follow-on studies, identifying experimental variables and confounding factors. We will emphasize the need to repeat experiments whether they succeed or fail, and we believe that there is time in the semester to repeat experiments (as well as to trouble-shoot earlier PCR and plasmid synthesis aspects of the project. Confirmed data will be submitted to Xenbase for use by the broader research community.

Learning outcomes: This course will involve all four key components of an authentic research experience as established in practice and articulated by HHMI [2]:

1) Students will learn to carry out discovery-based experiments while learning to use cutting edge gene editing technology as opposed to laboratory exercises with pre-determined outcomes.
2) Students will be expected to take ownership of their experiments. Each student group will choose its own gene of interest, mutate that gene and design follow-up studies of the effects of such mutations on phenotype, together with appropriate control studies.

[1] http://klymkowskylab.colorado.edu/Learning-Videos.htm
[2] http://www.journals.elsevier.com/gene-expression-patterns/

3) Students will consider the role experimental replication, alternative interpretations of results, and possible follow-on studies.
4) Students will be introduced to (and become part of) the scientific community: Students will read primary research articles, collaborate with one another other, and present their work to their peers and the broader community.
5) Students will have the opportunity to submit their data to online databases (e.g. Xenbase), produce resources for the scientific community, and contribute to future publications.

If the course is successful, students will develop an ability to read the literature critically, be able to draw useful conclusions, and build working models. They will become familiar with a set of on-line tools used in analyzing genomic data and designing reagents (PCR primers and gRNA-encoding plasmids), as well as the strengths and limitations of specific experimental techniques, including state of the art CRISPR Cas9 system. They should develop a familiarity for the features of a well-controlled and interpretable experiment, and be able to clearly articulate reasonable follow on studies. The ability to meet these goals will be taken as evidence for scientific and experimental literacy.

By having students work together in small research teams they will also be introduced into the important aspects of conveying the logic of their project and the immediate and broader implications of their observations. This will include the scientific, medical, and ethical implications of genetic engineering as applied to humans. They will most likely experience failure with their experiments and learn the necessity for repeating experiments, how to troubleshoot and how to formulate alternative hypotheses. We will insure (working in the background) that failures are not due to poor reagent design. Students will analyze their results and discuss them with their peers and faculty. We will focus explicit discussions on the importance of resilience and the development of realistic attitudes on the process of science, in part to consider issues of stereotype threat and self-discouragement.

Assessing scientific and experimental literacy: To assess student learning as well as engagement, we will analyze pre- and post-class quizzes, written lab reports, lab notebooks, oral presentations and final video projects. We expect to have a working (preliminary) version of the SELA before the semester begins, which we will use to collect pre-/post-course responses. We will also collect one-on-one interviews in order to revise the instrument. We will also collect data on students' self-reported attitudes about science and career choice. Beyond the immediate scope of this project, we intend to extend our studies to include students that take more conventional laboratory courses as well as other discovery research courses to characterize how various types of labs impact student learning outcomes and attitudes.

We anticipate offering the course in future semesters, as well as exploring the possibility of developing a similar course (based on lessons learned in the *Xenopus* course) at the freshman level using the roundworm *Caenorhabditis elegans* (to be developed in collaboration with Professors Ding Xue and Min Han, MCDB and HHMI, UC Boulder). A Chancellor's award will provide the seed-funding needed to offer the first version of MCDB 4100 and to begin the development of a targeted assessment, the SELA.

Bilge Birsoy, Ph.D. has more than 15 years of molecular biology and more than seven years of *Xenopus* research experience, as well as more than five years of undergraduate teaching experience. Dr. Birsoy was a member of the scientific team who got awarded a million dollar HHMI grant to support the two discovery labs developed at University of California, Santa

Barbara. Large Undergraduate Research Experience (LURE), and Research Immersion in Molecular Biosciences (MCDB161L) are now in their fifth year at UCSB. Since Fall 2015 at CU Boulder, she has been teaching MCDB4790 (Experimental Embryology) covering the principles underlying embryonic development with an emphasis on developing critical thinking skills (ORCID: http://orcid.org/0000-0003-4337-4582).

Mike Klymkowsky is a founding fellow of the CSL, a fellow of the AAAS (education section) and a professor in MCDB with extensive experience in course development (CLUE: [27] and Biofundamentals: [28, 29]), the development and interpretation of assessment activities (BCI and beSocratic). He also has a long history of experimental work in *Xenopus* (ORCID: http://orcid.org/0000-0001-5816-9771).

4. Timeline.

In the summer of 2016, the learning goals and the schedule of the experiments will be finalized and pilot experiments will be carried out to ensure that the necessary experimental reagents and resources are ready for the fall semester. The proposed course will be implemented in Fall 2016 with ~9 to 12 (max) students. Assessment of the learning goals will be carried out in Fall 2016 and data analysis will be done in Spring 2017. Input from the DBER community will help us evaluate the success of the course and plan its future trajectory. In 2017, we intend to submit an NSF proposal to seek funding to develop the scientific literacy assessment as well as to expand the course to multiple sections. After two or three semesters of this course offering, we expect to be able to submit these data as part of a research publication.

5. Outcomes/Impacts

The proposed experiments using CRISPR-Cas9 technology are relatively low cost and can be readily expanded to several sections. They are easy to implement in any molecular biology lab setting using various model organisms thus can be replicated or modified to other systems in other departments and institutions.

The use of the vertebrate model organism and the powerful gene editing technology in this course will provide an excellent context to discuss ethical and social implications of animal use in research and the future of editing the human genome.

7. Budget

The bulk of the budget will cover salary for Bilge Birsoy plus the expenses associated with the lab supplies and reagents.

One semester salary	$6365
lab supplies	$3635 (the itemized budget also available in excel sheet)
total	$10000

8. Budget justification:

We are requesting one semester salary for Bilge Birsoy who will be developing the discovery lab exercises and their pre- and post-assessments, grading quizzes, lab books and presentations, collecting and analyzing data for assessments in collaboration with Prof. Michael Klymkowsky. Dr. Birsoy will be the primary person preparing the laboratory reagents and running the labs, and lecturing when Prof. Klymkowsky is away. The course will have 1 hour lecture on Mondays, and 2hour lab sessions on Tuesdays and Thursdays.

The lab supplies will include all the cost of molecular biology reagents necessary for experiments described ($2866.44) plus frogs and hormones for generating fertilized eggs ($628.5). As currently budgeted, each student will be able to either run their own experiment or work with two other students (in a group of three) with the appropriate controls and repeat their experiments at least once.

	cost for 12 students
Oligos+Primers	$216.00
Subcloning enzymes	$299.00
Bacterial plates and liquid LB medium	$168.10
Miniprep kit	$380.00
Sequencing for construct confirmation	$216.00
Transcription kits to synthesize gRNA and in situ probes	$484.80
Cas9 protein	$750.00
FITC-dextran co-injection tracer	$88.10
Frogs (males and females)	$370.00
Hormones	$258.50
Anti-DIG HRP antibody for in situs	$405.00
Supplies total	$3,635.50
SALARY (1 semester)	$6,350.00
TOTAL	$9,985.50

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