UNIVERSITY OF LOUISIANA AT LAFAYETTE

STEP Committee
Technology Fee Application

Contemporary Cell and Molecular Biology Equipment for Seven Lab Courses and Undergraduate and Graduate Research

Title

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Name of Submitter
(Faculty or Staff Only)

Department of Biology

Organization

Title: Modernization of cell and molecular biology lab courses with contemporary equipment Date: 07/15/2018

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ABSTRACT (250 words or less):

We are seeking funds from the Student Technology Enhancement Program (STEP) to purchase an ice machine, CO_2 incubator, and real-time PCR machine (thermocycler) for use in teaching as well as research. **Every year an estimated 180 students, which include approximately 150 Biology majors, 5 Kinesiology majors, and 25 graduate students in Biology will use the purchased equipment.**

The Department of Biology has a proven track record of providing well-rounded education aimed preparing graduates for various career paths such as education, research, healthcare and environmental management and conservation. The working knowledge of modern techniques in biochemistry and cell and molecular biology such as cell culture, quantitative real-time polymerase chain reaction (qPCR), and enzyme assays will provide our students a competitive edge in the job market.

Students will use ice machine to maintain reagents (enzymes) at cold temperature; CO_2 incubator to grow and maintain human/animal cells for experiments; and qPCR (QuantStudio 5) system for real-time monitoring of amplification of target DNA molecules for the quantification of the gene expression.

Learning to effectively perform enzyme assays, cell culture, and qPCR will enhance the technical credentials of our graduates and facilitate their employment in various disciplines such as pharmaceutical and biotechnology industry, academic research and clinical laboratories where these techniques are routinely used as diagnostic and research tools.

3. DESCRIPTION

3a. Purpose of grant and impact to student body as a whole

We are seeking STEP funding to purchase ice machine, CO_2 incubator, and qPCR machine, which will allow our students to gain expertise in cutting edge techniques in biochemistry and cell and molecular biology such as cell culture, qPCR and enzyme assays. These machines will be used to provide hands-on training in biomedically focused laboratory courses (Table 1) which are offered by Biology department and are highly popular amongst undergraduate Biology and Kinesiology Majors. In addition, these machines will also be used by undergraduate and graduate researchers in Biology.

Table 1: Biology courses and number of students impacted by this proposal

Course	Number of Students	When offered?	Faculty
General Microbiology (BIOL 263)	30	Fall and Spring	P. Antley
Microbiology Lab (BIOL 264)	30	Fall and Spring	P. Antley
Neurobiology Lab (BIOL 424G)	20	Fall	K. Smith
Immunobiology Lab (BIOL 443G)	20	Spring	R. Kulkarni
Molecular & Cellular Engineering Lab (BIOL 454)	15	Spring	Y.Wang S. Plouviez
Advanced Cell Biology Lab (BIOL 458)	20	Fall	B. Ding
Mentored Research (BIOL 410)	20	Fall and Spring	Multiple*
Master's and PhD Dissertation Research	25	Fall and Spring	Multiple*
TOTAL NUMBER OF STUDENTS =	180		

* research conducted in the research laboratories of various faculty members

According to the results from Biology department alumni survey, healthcare is the most sought-after career option for our undergraduates. More than half of our BS graduates go on to earn advanced degrees in biology or biomedical or allied sciences and pursue careers as physicians, dentists, veterinarians, researchers, pharmacists, optometrists, physical therapist and biotechnologists. Our undergraduate students have repeatedly expressed a strong desire to gain experience in modern techniques in cell and molecular biology such as cell culture and qPCR, which constitute highly marketable group of skills. Students who graduate with the knowledge of these techniques are better prepared for careers in academic research, biotechnology industry, and clinical laboratories. This is why we are requesting funds to purchase ice machine, CO_2 incubator and QuantStudio 5 qPCR system. Each of the faculty member involved in this proposal, plans to include these techniques in their course syllabi. A brief description of these plans is provided below.

Ice machine (40lb storage capacity) will generate 191 lb./24hr flaked ice of to students enrolled in courses listed in Table 1 as well as those performing research in a Wharton Hall lab. The flaked ice is used for keeping reagents (such as enzymes) cold during experimentation. This is critical because at room temperature, enzymes undergo rapid denaturation. The current ice machine in Wharton Hall is more that 15 years old, has broken down multiple times in the last few years and its replacement parts are expensive and not easily available.

CO_2 incubator will be used for cell culture, which is a process of growing a variety of human and animal cells (primary cells or immortalized cell lines) in a controlled environment. CO_2 incubator maintains an ambient environment (37^0C temperature, high humidity and 5% CO_2 atmosphere) required for the growth of cell cultures. CO_2 incubator will be installed in Wharton Hall lab

VLW416. The maintenance and manipulation of cell cultures must be carried out in an aseptic environment to avoid contamination by air-borne bacteria and fungi. For this, students will use CleaTech 6 ft benchtop laminar flow hood that was purchased through a STEP award (FA17-34) to Dr.s Wang and Plouviez. The laminar flow hood is already installed in VLW416.

QuantStudio 5 real-time PCR System consists of an advanced software and instrumentation for performing a wide array of genomic assays. This will be used by students for a variety of cutting-edge techniques in molecular biology and genomics such as SNP genotyping, gene expression analysis, microRNA expression, gene detection, viral load analysis, etc.

We have obtained a quote from Fisher Scientific which is a preferred vendor with our university and does not charge freight/shipping. Fisher Scientific charges $4.45 for fuel and surcharge for each order. The ice machine, CO_2 incubator and QuantStudio 5 qPCR system will be purchased in Fall 2019. **The department will use lab fees to pay for the service contracts as well as the costs associated with CO_2 cylinder rental and refill.**

The undergraduate students enrolled in mentored research and laboratory courses as well as graduate students (Table 1) would benefit tremendously from the addition of ice machine, CO_2 incubator and QuantStudio 5 qPCR system. This benefit would come through the hands-on training these students receive. For instance, in the laboratory of Dr. Kooyers, both undergraduates and graduate students use qPCR to assess how genetic variants from different populations across the geographic ranges of various widespread plant species contribute to differences in timing and ability to flower. Other students in the Biology Department also incorporate qPCR into their research to examine the contributions of copy-number variation in producing phenotypic variation, create genotyping panels for model species, and detect viruses within hosts. Additional detailed plans for including these machines in laboratory courses are provided below:

Immunobiology Laboratory (BIOL 443G) taught by **Dr. Ritwij Kulkarni** currently relies on ready-to-use kits that use simulated samples and provides detailed recipes to get "flawless" results. Inclusion of Cell Culture and qPCR will shift this course toward asking more open-ended experiments using established protocols to get results that may be "less flawless" but more authentic. Students will cell culture facilities (CO_2 incubator and laminar flow hood) to grow cultures of macrophages, neutrophils, and T-cells isolated from mouse bone marrow. Students will then treat these cells with pathogenic and non-pathogenic bacteria and compare cytokine production using QuantStudio 5 qPCR system. My long-term vision for this course is to have students carry out semester-long, discovery-based projects to answer questions such as: (i) Can the immune system differentiate between pathogenic and non-pathogenic bacteria? (ii) Is the immune system negatively affected by environmental pollutants such as tobacco smoke? (iii) Do pathogenic bacteria fight back against immune responses? How? Students will extensively use cell culture equipment and qPCR machine in these experiments.

Advance Cell Biology Laboratory Course (BIOL 458G) will be taught by **Dr. Baojin Ding** who was recently recruited as Assistant Professor in our department. The ideal model system to study cell biology is cultured mammalian cells which allow students to examine the biological processes at a cellular level. The student will use cell culture equipment to grow and maintain human embryonic kidney (HEK) cells and perform the following experiments: Experiment 1. To examine the membrane integrity of cultured mammalian cells through

nuclear uptake fluorescent dyes, such as DAPI which enters cells and binds dsDNA if the membrane integrity is lost. Students will compare the effects of different conditions on membrane integrity by studying the cells under fluorescent microscope. Experiment 2. To examine the nucleocytoplasmic transport, cultured mammalian cells will be transfected with reporter genes that encode *green fluorescent protein* fused with nuclear export signal (GFP-*NES*) and *red fluorescent protein fused with* nuclear localization signal (RFP-NLS). Under conditions such as disease, ageing or mutations, nucleocytoplasmic transport is impaired and lead to the messy distribution of green and red signals. In addition to cell culture, this experiment will train students in transfection techniques.

Tissue culture capabilities would add a new dimension to the **Neurobiology Lab (BIOL 424G),** taught by **Dr. Karen Müller Smith** by allowing students to culture primary neurons and examine synapse formation, axon outgrowth on plates treated with attachment factors or cytokines, or growth cone collapse on neurons treated with chemorepellents. The class currently lacks a more molecular and cellular approach to neuroscience and incorporating tissue culture experiments would provide the ability to incorporate this important area of Neuroscience to the Neurobiology Laboratory. One potential application in the classroom setting would be to derive cortical neurons from mouse embryos and culture them on coverslips with and without extracellular matrix attachment factors so that the students can appreciate the importance of attachment factors on neurite outgrowth. This can be combined with phase contrast microscopy and treatment with proteins that can induce growth cone collapse and axon repulsion such as commercially available preparations of SLIT proteins. Cultured neurons can also be treated with neurotransmitters, stained for synaptic markers, and number of synapses assessed, to examine the effect of neural activity upon synaptic strengthening.

The qPCR experiments will be used to quantify the expression of AMPA-type glutamate receptors after treatment of cultured neurons with the neurotransmitter glutamate. We will also test the effects of stimulation with growth factors such as FGF upon the proliferation of isolated neural stem cells. For these experiments, students would isolate cells on Week 1 and treat cells with no growth factor, growth factor at different concentrations. In week 2, students would quantify the number of cells in the different treatment conditions. RT-PCR could also be used to assess expression of Cell Cycle proteins induced by FGF treatment.

Molecular and Cellular Engineering Laboratory Course (454G) taught by **Dr. Yi-Hong Wang and Dr. Sophie Plouviez** strives to provide students an understanding of the latest advancement in transcriptomics, genomics, and proteomics in academic research and industry. This challenging class always reaches its maximum capacity (20 students) Techniques such as quantitative real-time PCR (qPCR), Sanger and Next Generation Sequencing, or CRIPSR are explained and discussed during lectures (453G). However, the lack of advanced equipment in the molecular lab creates a gap between the cutting-edge theory learned in the lectures, and the experiment that can be developed with the limited equipment that is currently available. The requested qPCR machine would open up various hands-on opportunities such as comparing gene expression of two experimental conditions, quantifying parasites in a host, or testing the efficiency of a CRISPR experiment.

3b. Projected lifetime of enhancement

Ability to perform enzyme assays, cell culture and qPCR in an undergraduate laboratory using ice machine, CO_2 incubator and qPCR system purchased with STEP funds will constitute a significant enhancement that will last at least for the next 10 years till 2029. The ice machine, CO_2 incubator, real-time PCR machine, software and laptop will be maintained and upgraded through lab fees in the future.

3c. Person(s) responsible for:

3c.i. Implementation: Dr.s Kulkarni, Smith, Wang, and Plouviez will be responsible for the implementation of this proposal by purchasing the requested equipment.

3c.ii. Installation: Dr.s Kulkarni, Smith, Wang and Plouviez will be responsible for the installation of the equipment requested though this proposal.

3c.iii. Maintenance: Dr. Sophie Plouviez, one of the submitters of this proposal, is our instrumentation manager. Dr. Kulkarni will coordinate with Dr. Plouviez for managing the use and maintenance of ice machine, CO_2 incubator and QuantStudio 5 qPCR system. Dr. Plouviez will be responsible for maintaining service contracts through the lab fees.

3c.iv. Operation: Dr.s Kulkarni and Plouviez will provide training and help with troubleshooting when necessary. However, individual faculty members will be responsible for the safe operation of these machines in their teaching labs.

3c.v. Training (with qualifications): All proposers have experience in using ice machine, CO_2 incubator and QuantStudio 5 qPCR machine. It will be the responsibility of each faculty member to train their teaching assistants and undergraduate students enrolled in their sections to operate these machines. However, Dr.s Kulkarni and Plouviez will be available for training and troubleshooting when necessary.

3d. Budget Justification.

Equipment Requested	Price	Purpose	Justification
Flaked ice machine	$4,800	To maintain enzymes and reagents at 4^0C.	The current ice machine in Wharton Hall is >15 years old, breaks down frequently and is difficult to repair
Thermo Scientific™ Forma™ SteriCycle™ CO_2 Incubator	$6,850	To maintain mammalian cell cultures	CO_2 incubator is NOT available for use in undergraduate lab courses to introduce students to cell culture techniques
Quantitative real time PCR (qPCR) system, including software and laptop	$28,000	Real-time monitoring of DNA amplification	Real-time PCR machine is NOT available for use in undergraduate lab courses to introduce students to various modern techniques in molecular biology and genomics.

4. BUDGET PROPOSAL FORM

Budget Proposal

1.	Equipment	
	Flaked ice machine	$04,800.00
	Thermo Scientific™ Forma™ SteriCycle™ CO_2 Incubator	$ 06,850.00
	Quantitative real time PCR system	$ 28,000.00
2.	Software	$ 0
3.	Supplies	$ 0
4.	Maintenance	$ 0
5.	Personnel	$ 0
6.	Other	
	Fuel and Surcharge	$ 4.45
	TOTAL:	**$ 39, 654.45**