UNIVERSITY OF LOUISIANA AT LAFAYETTE

STEP Committee
Technology Fee Application

Modernization of Undergraduate Cell and Molecular Biology Laboratories with Contemporary Equipment for Cell Culture and Real-time PCR

Title

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Name of Submitter
(Faculty or Staff Only)

Department of Biology

Organization

Title: Modernization of undergraduate cell and molecular biology laboratories with contemporary equipment for cell culture and real-time PCR Date: 07/13/2018

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ABSTRACT (250 words or less):

The Department of Biology has a proven track record of providing well-rounded education aimed preparing graduates for various career paths such as research, healthcare, environmental management and conservation and education. To maintain their competitive edge, it is important for our undergraduates to be familiar with modern cell and molecular biology techniques such as cell culture and quantitative real-time polymerase chain reaction (qRT-PCR). We are seeking funds from the Student Technology Enhancement Program (STEP) to purchase a CO_2 incubator and a real-time PCR machine for use by undergraduates enrolled in laboratory courses as well as mentored research. We estimate that these machines will be used by 150 undergraduates every year.

A CO_2 incubator is a cell culture incubator that maintains constant (37^0C.) temperature, high humidity and CO_2 atmosphere for the growth of cell culture. Students will use CO_2 incubator (in conjunction with the laminar flow hood purchased through STEP grant awarded in Fall 2017) to grow and maintain human or animal cells for experimental manipulation. The Step One Plus™ system (for qRT-PCR) will be used for continual (or real-time) monitoring of amplification of a target DNA molecule for the quantification of the gene expression. Students will use qRTPCR to study the effects of different stimuli on gene expression.

Learning to effectively use cell culture and qRT-PCR will enhance the technical credentials of Biology graduates and facilitate their entry into various disciplines in academia and industry 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

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 therapists and biotechnologists. The laboratory courses with a strong biomedical focus (Table 1) that are offered by our department are highly popular amongst these students as these courses offer hands-on experience in modern laboratory techniques.

Undergraduate Laboratory Courses with Strong Biomedical Focus			
Course	**Average Number of Students**	**When offered?**	**Faculty**
General Microbiology Lab (BIOL 263)	30	Fall and Spring	P. Antley
Microbiology Lab (BIOL 264)	30	Fall and Spring	P. Antley
Individual Project (BIOL 410)	30	Fall and Spring	Multiple*
Neurobiology Lab (BIOL 424G)	15	Fall	K. Smith
Immunobiology Lab (BIOL 443G)	15	Spring	R. Kulkarni
Molecular & Cellular Engineering Lab (BIOL 454)	10	Spring	Y.Wang S. Plouviez
Advanced Cell Biology Lab (BIOL 458)	20	Fall	B. Ding
TOTAL NUMBER OF STUDENTS =	150		
* Individual Project course is conducted by undergrads in the research laboratories of various faculty members			

Our undergraduate students have expressed a strong desire to gain experience in modern techniques such as cell culture and quantitative real-time PCR (qRT-PCR), which in our opinion are a highly marketable group of skills. The students who graduate with a 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 CO_2 incubator and Step One Plus™ real-time PCR system for exclusive use by undergraduate students. 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.

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°C temperature, high humidity and 5% CO_2 atmosphere) required for the growth of cell cultures. The CO_2 incubator will be installed in Wharton Hall laboratory 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 to Dr.s Wang and Plouviez (Fall 2017). The laminar flow hood is already installed in VLW416.

Step One Plus™ real-time PCR System consists of an advanced software and instrumentation (with Sensitive 3-color optical LED recording system) 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 qRT-PCR, SNP (single nucleotide polymorphism) genotyping, gene expression analysis, microRNA expression, gene detection, viral load analysis, etc.

The CO_2 incubator and Step One Plus system will be available for undergraduate students enrolled in laboratory courses listed in Table 1 as well as for undergrads carrying out mentored research. 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 CO_2 incubator and Step One Plus™ real time PCR system will be purchased in Spring 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.

Following are the plans to include these machines in laboratory courses:

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 qRT-PCR will shift this course toward asking more open-ended questions to get results that may be "less flawless" but more authentic. Students will use 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 Step One Plus™ 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 qRT-PCR 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: <u>Experiment 1.</u> 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. <u>Experiment 2.</u> 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.

qRT-PCR experiments could be incorporated into our experiments with cultured neurons. Such as quantifying the expression of AMPA-type glutamate receptors after treatment with the neurotransmitter glutamate. Another possible application would be to test the effects of growth factor stimulation, such as FGF administration, 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 2 concentrations. On week 2, students would quantify the number of cells in the different treatment conditions. qRT-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 is currently taught every alternate year and reaches its maximum capacity (10 students). The newly hired faculty members joining in the fall 2018 are skilled in these topics. Hence, this course could be offered more often.

Techniques such as quantitative real-time PCR (qRT-PCR), 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 basic practical experiment that can be developed with this limited equipment.

The requested qRT-PCR 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.

The equipment requested through this STEP proposal constitute fundamentally new additions to the departmental resources that directly support undergraduate instruction and research. Access to these machines will significantly impact our ability to introduce undergrads to modern laboratory techniques and to prepare them to become highly productive members of the workforce.

We thank you for consideration.

3b. Projected lifetime of enhancement

Ability to perform cell culture and qRT-PCR in an undergraduate laboratory is a significant enhancement for at least next 10 years till 2029. The CO_2 incubator, the real-time PCR machine, Step One Plus 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, Plouviez and Ding will be responsible for the implementation of this proposal by purchasing the requested equipment.

3c.ii. Installation: Dr.s Kulkarni, Smith, Wang, Plouviez and Ding 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 CO_2 incubator and Step One Plus real-time PCR 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 CO_2 incubator and Step One Plus™ real time PCR 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
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
Step One Plus™ real time PCR system (includes software and laptop)	$23,920	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	
	Thermo Scientific™ Forma™ SteriCycle™ CO_2 Incubator	$ 06,850.00
	Step One Plus™ real time PCR system	$ 23,920.00
2.	Software	$ 0
3.	Supplies	$ 0
4.	Maintenance	$ 0
5.	Personnel	$ 0
6.	Other	
	Fuel and Surcharge	$ 4.45
	TOTAL:	**$ 30, 774.45**