

Biotechnology Laboratory I, BIO 5503
Syllabus
Fall Semester 2015

Instructors and Contact Information:

Alla Arzumanyan, Ph.D.
Room 446B, BioLife Sciences Bldg.
Phone: 215-204-8359
E-mail: areg@temple.edu
Office hours: by appointment

Darius Balciunas, Ph.D.
Room 435, BioLife Sciences Bldg.
Phone: 215-204-1611
E-mail: darius@temple.edu
Office hours: by appointment

Labs will be held on Wednesdays, 5:30 – 9:30 PM, in Room 247, BioLife Sciences Building, unless otherwise noted by the instructors.

Course Objectives: This course is designed to provide practical, hands-on experience with human cell culture techniques, *in vitro* manipulation of genes and proteins, and data analysis that are fundamental to many areas of biotechnology. The course will encourage teamwork among the students, and will also develop critical thinking skills, the ability to analyze and present data, and proficiency in troubleshooting when experiments fail. This course requires some background knowledge in biology (including genetics), biochemistry and chemistry.

In addition to laboratory safety rules and regulations, you will have Bloodborne Pathogen training (in person) and Initial Safety and Biohazard training (online), since some experiments will be performed in a Biosafety Level 2 (BSL2) cell culture facility.

There will be a field trip to the Moulder Center for Drug Discovery, Temple University, during which students will meet scientists and discuss novel concepts in drug discovery.

If there are questions about material and concepts are being presented, you are welcome to discuss matters during office hours. Please do not wait until the end of the semester to express your concerns.

We will make every effort to help you learn the course material, but you must also study and efficiently utilize available resources. The goal is to acquire both understanding and professional skills at a high academic standard that will be important for the class and your future success.

Laboratory:

- It is important do not miss labs, arrive on time and stay for the entire lab period.
- You must strictly follow laboratory safety rules to avoid the risk of injury. You will be given a Lab Safety Guide which you should sign and return.

- You should be careful with equipment and reagents, keep your lab area organized, and clean up your station at the end of the lab. When using a reagent or material that is to be shared by your lab partners, make sure to return it to the common area.
- You should come to the lab prepared by reading the assigned material beforehand.
- Classroom discussion may be recorded for the personal, educational use and shared with the students enrolled in the class only, and may not be further copied, distributed, or otherwise used for other purposes.
- You should have a bound notebook (which will be provided) during each lab and flash drive to record data analysis when needed. Guidelines and requested format for a notebook should be maintained.

Blackboard: Check Blackboard frequently. There is no formal Lab Manual for this course. On Blackboard, you will find announcements and supplemental material covering the course such as background information, description of the experiments and assignments.

All grades will be posted on Blackboard.

Grading: Grades will be based on the following criteria:

1. **Quizzes** (given at the beginning of the lab, each of duration 20-25 min.) will consist of questions covering background information and concepts discussed in a previous lab material. Problem set questions will be included in some quizzes. There are not quizzes on CRISPR/Cas9 part. There are no make-up quizzes except in the case of a documented emergency.
2. **The notebook** will be submitted for grading at mid-semester and on the last day of the course.
3. **Assignments** will include analysis and presentation of the obtained data (figures, tables, statistics, etc.).
4. **Presentation** will cover CRISPR/Cas9 data analysis.

Extra credits will be given for each successfully done experiment.

Point breakdown (maybe subject to change):

1.	Four (4) quizzes, 40 pts./quiz	160 pts.
2.	Laboratory notebook	60 pts.
3.	Assignments	150 pts. (70 pts. + 85 pts. [CRISPR/Cas9 part])
4.	Presentation [CRISPR/Cas9]	25 pts.

Total: 400 pts.

Final grades will be assigned on a straight scale (there is no curve) using the following weights:
 100 - 90% points: (A and A-); 89 - 78% points: (B+, B and B-); 77 - 67% points: (C+, C and C-).

Disability disclosure: Any student who has a need for accommodation based on the impact of a disability should contact me privately as soon as possible. The Office of Disability Resources and Services (DSR) will coordinate accommodations for students with disabilities. Students who are eligible for extra time on quizzes should talk with the instructors in advance to make arrangements for extended time.

Contact information for DSR:

1301 Cecil B. Moore Avenue

Philadelphia, PA 19122

215-204-1280 (Voice); 215-204-1786 (TTY); 215-204-6794 (Fax)

drs@temple.edu

Student and Faculty Academic Rights and Responsibilities Policy: Temple University has a policy on Student and Faculty Academic Rights and Responsibilities (Policy #03.70.02) that can be accessed through the following link:

http://policies.temple.edu/getdoc.asp?policy_no=03.70.02

Lab Schedule (maybe subject to change)

<p>Week 1 (8/26) Introduction, Lab safety, Pipetting technique, Brightfield microscopy and cell counting, Solution concentration and dilution</p>	<ul style="list-style-type: none"> - Lab safety, Bloodborne Pathogen training (mandatory) will be given by Kisha Grady, EHRS Training Program Coordinator; - Proper pipette usage and accurate pipetting of liquids; - Brightfield microscopy: observation of the slides of liver samples from hepatitis B virus (HBV) infected patients with hepatocellular carcinoma; - Mammalian cell counting; - Solutions, serial dilutions and concentration calculations (molarity, moles, percent composition).
<p>Weeks 2-5 (9/02, 9/09, 9/16, and 9/23) Cell culture, Cell proliferation and cytotoxicity assay, Western blotting</p>	<p>QUIZ 1 (9/02) and QUIZ 2 (9/23)</p> <ul style="list-style-type: none"> - Cell culture technique (cell thawing, plating, passaging and freezing). - Human hepatoblastoma HepG2 cells will be treated with nutlin (a small molecule antagonist of MDM2). Protein extracts will be collected, protein concentrations will be measured, and Western blotting will be performed to evaluate nutlin-induced expression of tumor suppressor protein p53. Protein bands will be visualized using a fluorescent secondary antibody, and protein expression levels will be quantified with the <i>OdisseyFc</i> imaging system (Li-COR). - Drug effect on the cells will be monitored using a cell viability colorimetric assay.

<p>Week 6 (9/30)</p>	<p>Visit the Moulder Center for Drug Discovery, School of Pharmacy, Temple University (http://moulder.temple.edu/). The guided tour will include the Proteomics facility with state-of-the-art equipment (http://moulder.temple.edu/Proteomics%20Facility), the High Throughput Screening facility (http://moulder.temple.edu/research-capabilities/screening-capabilities). Presentation “Lead Optimization for Drug Discovery” will be provided by Dr. Ken Korzekwa, Associate Professor of Pharmaceutical Sciences, School of Pharmacy, Temple University.</p>
<p>Week 7 (10/07)</p> <p>HPLC and mass spectrometry</p>	<p>Identification and mass spectrometry analysis of a bis-peptide using reversed phase HPLC combined with quadrupole mass spectrometer equipped with electrospray ionization (ESI) ion source (Agilent Technologies). Experiment will be conducted in Dr. Chris Schafmeister’s lab, Department of Chemistry, Temple University, under the supervision of JD Northrup, a Ph.D. candidate.</p>
<p>Weeks 8 and 9 (10/14 and 10/21)</p> <p>Primer design and PCR</p>	<p>QUIZ 3 and lab notebook submission (10/14) DNA isolation from fresh beef liver blood. Some blood DNA samples will be contaminated with a viral DNA (HBV). Endpoint PCR will be performed (using primers for the viral surface antigen, HBsAg) to determine which samples are from “HBV-positive patients”. Detailed guide how to design primers and check their specificity (starting from the transcripts of a given gene) will be provided.</p>
<p>Weeks 10-13 (10/28, 11/04, 11/11, 11/18)</p> <p>CRISPR/Cas9 genome editing</p>	<p>QUIZ 4 (10/28) Editing the zebrafish genome using CRISPR/Cas9. Experiments will be focused on the transcription factors with potential role in heart development and regeneration. Based on a published literature and provided guidelines, a gene (that should be targeted) will be selected, and a short guide RNA (sgRNA) will be designed and produced. Cas9 RNA and sgRNA will be co-injected into zebrafish embryos. Genomic DNA will be isolated, and the rate of insertions and deletions at the target locus will be determined by either loss of a restriction enzyme site or by carrying out the Surveyor assay.</p>
<p>Week 14 (11/28)</p>	<p style="text-align: center;">Thanksgiving break, no classes</p>
<p>Week 15 (12/02)</p> <p>CRISPR/Cas9 genome editing</p>	<p>Presentations of the results Lab notebook submission</p>