PCB4917, Principles and applications of cell-free gene expression, 4 credits

#### **CLASS MEETINGS**

Class will meet in Bartram Hall 617 MTWRF - (9:35-12:35 PM). Some meetings will be in Bartram 227 (will be announced)

#### **DATES**

2/12-3/13

#### **INSTRUCTOR:**

Zhanar Abil, Department of Biology

Phone: (352)-846-3781

Office: Wertheim Lab Eng Exc Room 487

Office Hours: By appointment at abilz@ufl.edu. Please request appointments at least one business day

in advance.

#### **COURSE OVERVIEW**

Living cells are extremely complex and difficult to study on a holistic level. Cell-free gene expression systems mimic cellular processes in a minimal, controlled environment and provide a simplified platform for studying complex biological phenomena. Moreover, it enables the rational design and manipulation of biological systems for biotechnological applications. This immersion course will provide hands-on experience on utilizing recombinant cell-free gene expression systems for visualizing the central dogma of molecular biology, cell-free production of recombinant proteins, and design of minimal cell-free biosensors for the detection of environmental contaminants in water. Students will gain experience in cell-free gene expression, PCR, gel electrophoresis, fluorescence plate reading, cell-free biosensor design, building, and testing. The course is ideal for the immersion course format because it allows the students to engage in hour-long experiments spanning several consecutive days and allows leeway for troubleshooting.

### **MAIN OBJECTIVES**

This course will introduce students to major concepts of cell-free systems with a focus on biosensing and give students practical experience with molecular and biochemical methods used to engineer gene circuits for biosensing. Students will gain authentic research experience and prepare a written report and a presentation. Experiments will be conducted with E. coli cell extracts.

**FORMAT** – lectures, discussions, lab activities and data analysis at UF, oral and written projects, no exams.

#### ESTIMATED COURSE FEES PER STUDENT:

\$700

**PREREQUISITES** - Completion of PCB3063 (Genetics) or an equivalent course is recommended before enrolling in the immersion class.

#### TENTATIVE SCHEDULE

# Week 1: 2/12-2/13 (Thursday and Friday):

- Day 1: Introduction to Synthetic Biology
- Day 1: Apply the principles of the Central Dogma of Molecular Biology to understand how proteins are made in the cell.
- Day 2: Introduction to Cell-Free Gene Expression (Riku)
- Day 2: Understand applications of cell-free gene expression systems (Riku)
- Day 1: Understand the rules and reasons of lab notebook keeping
- Day 2: Master pipetting and dilution
- Reading and videos: synthetic biology and cell-free synthetic biology

#### Week 2: 2/16-2/20

- Day 1: Introduction to gene regulation (Carlos)
- Day 2: Introduction to biosensing, cell-free biosensing<sup>1</sup>
- Day 1: Master pipetting and dilution
- Day 2: Master cell-free gene expression. Module 1 Experiment 1: cell-free expression of reporter proteins and Lac repressor (LacI) (Riku)
- Day 3: Master protein expression analysis: SDS-PAGE analysis of Experiment 1 (Carlos)
- Day 4: Master cell-free gene regulation. Module 1 Experiment 2: Dose response of mRFP expression to pre-expressed LacI

- Day 5: Master cell-free gene regulation. Module 1 Experiment 3: Study the effect of LacI, LacO, and inducer (IPTG) on gene expression
- Module 1 Exp 1-3: Answer pre-lab questions and post-lab analysis due next Monday

#### Week 3: 2/23-2/27

- Day 1: Master cell-free biosensing. Module 1 Experiment 4: dose response to IPTG, detect lactose in milk.
- Day 2: Discuss course project: cell-free biosensing to detect fluoride, lead, or copper in a source of choice
- Day 2-4: Troubleshooting, group project design, sample collection
- Module 1 Exp 4: Answer pre-lab questions and post-lab analysis due next Monday
- Work on Module 2 Reports (Introduction, Experimental design) due next Monday

#### Week 4: 3/2-3/6

- Day 1: Module 2 Experiment 1: Pre-express repressor of choice
- Day 2: Module 2 Experiment 2. Tune repressor of choice concentration with reporter of choice
- Day 3: Module 2 Experiment 3. Detect analyte in collected sample
- Day 4: Troubleshooting
- Day 5: Troubleshooting
- Work on Module 2 Reports (Results) due next Monday

#### Week 5: 3/9-3/13

- Work on Module 2 reports (add final data, conclusions, perspectives, respond to feedback)

  due Friday
- Module 2: Work on group presentations
- Friday: Group presentations

#### READINGS

There is no required textbook. Reading material and videos will be selected from available sources or provided.

#### **GRADING**

Participation in class activities	10
Lab Notebook	15
Module 1 Assignments	25
Module 2 Report	25
Research presentation	25
Total	100

Point Range (%)	Letter Grade
≥ 90.00	A
≥ 86.66	A-
≥ 83.33	B+
≥ 80.00	В
≥ 76.66	В–
≥ 73.33	C+
≥ 70.00	С
≥ 66.66	C-
≥ 63.33	D+
≥ 60.00	D
≥ 56.66	D–
< 56.66	Е

Detailed grading policies for the University can be found at:

https://catalog.ufl.edu/ugrad/current/regulations/info/grades.aspx

### **POLICIES**

This course is compliant with the UF academic policies and resources that can be found via this link: https://go.ufl.edu/syllabuspolicies

### Attendance and absences

Attendance is mandatory. If a student needs to miss class for an excused absence, they must notify the instructor ASAP before the scheduled class time to arrange make-ups and alternative assignments.

### Class demeanor

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Students will be expected to spend the majority of the week in class completing experiments and participating in discussions and presentations. Students will need to arrive on time. Cell phones are not to be used during presentations and discussions for personal reasons.

Communication with Dr. Abil

Written communication should be made in Canvas (e.g., mail and announcements) unless there is an emergency. If a student fails to check Canvas, the instructor is not responsible for missed information. Grades will only be made available in person or via Canvas.

#### Teacher Evaluations

Students are expected to provide professional and respectful feedback on the quality of instruction in this course by completing course evaluations online via GatorEvals. Guidance on how to give feedback in a professional and respectful manner is available at https://gatorevals.aa.ufl.edu/students/. Students will be notified when the evaluation period opens, and can complete evaluations through the email they receive from GatorEvals, in their Canvas course menu under GatorEvals, or via https://ufl.bluera.com/ufl/. Summaries of course evaluation results are available to students at <a href="https://gatorevals.aa.ufl.edu/public-results/">https://gatorevals.aa.ufl.edu/public-results/</a>.

Students with Special Needs

Students with disabilities who experience learning barriers and would like to request academic accommodations should connect with the Disability Resource Center at https://disability.ufl.edu/students/get-started/. It is important for students to share their accommodation letter with their instructor and discuss their access needs, as early as possible in the semester.

No accommodations are available to students who lack this documentation. It is the policy of the University of Florida that the student, not the instructor, is responsible for arranging accommodations when needed.

#### *UF counseling Services*

Resources are available on-campus for students having personal problems or lacking clear career and academic goals. The resources include: 1) UF Counseling & Wellness Center, 3190 Radio Rd, 392-1575, psychological and psychiatric services. 2) Career Resource Center, Reitz Union, 392-1601, career and job search services. Many students experience test anxiety and other stress related problems. "A

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Self Help Guide for Students" is available through the Counseling Center (301 Peabody Hall, 392-1575) and at their web site: https://counseling.ufl.edu/.

Your well-being is important to the University of Florida. The U Matter, We Care initiative is committed to creating a culture of care on our campus by encouraging members of our community to look out for one another and to reach out for help if a member of our community is in need. If you or a friend is in distress, please contact umatter@ufl.edu so that the U Matter, We Care Team can reach out to the student in distress. A nighttime and weekend crisis counselor is available by phone at 352-392-1575. The U Matter, We Care Team can help connect students to the many other helping resources available including, but not limited to, Victim Advocates, Housing staff, and the Counseling and Wellness Center. Please remember that asking for help is a sign of strength. In case of emergency, call 9-1-1.