BIOSci 1005: Biochemistry Laboratory - Spring Term 2024

General Information

Lab: Section 1, Wednesdays, 1:00 – 4:50 PM 146 Langley Hall
INSTRUCTOR: Dr. Ping An pia1@pitt.edu A170 Langley Hall

Lab: Section 2, Fridays, 1:00 – 4:50 PM 146 Langley Hall
INSTRUCTOR: Dr. Xiaodong Zhu 144A Langley Hall xzhu@pitt.edu

Office Hours By appointment

Course Description and Objectives

The primary objective of this course is to experience the operation of authentic scientific research in the field of biochemistry. The main experimental design is focused on biochemical approaches in the overexpression, isolation and functional characterization of specific target proteins: the OBD (Origin Binding Domain) region of BK polyomavirus large T antigen for the 2024 season. The hypothesis-driven projects, composed of multiple experiments, are parts of scientific investigations for research labs in the department. This course will teach and reinforce classical and fundamental biochemical laboratory techniques, the theory behind the techniques, development of lab protocols, troubleshooting, identifying and closing gaps in protocols, analysis and interpretation of experimental results, and scientific record keeping. At the end of the course, students should be capable of thinking and planning independently, performing biomedical lab tasks, communicating, and testing scientific ideas. As an authentic research laboratory course, it will be challenging and will involve substantial effort. Being scientifically engaged for a best experience. Your effort can be rewarding and fruitful.

Course Requirements

1. Attendance for Lab.
   - Attendance for the lab section is mandatory. Absences must be justified and require documentation. Students should take the responsibility to inform lab partners and faculty if having health or other issues. Any absence must be excused prior to the class by email directly to the instructors since special arrangements need to be made for such situations. Experiments will be carried out by a lab partner if one of the pair must be absent. Two and more missing labs will need graded make-up assignment/experiment.
   - Pre-lab, post-lab assignments and lab reports need to be turned in on time. Delays will cause deductions.

2. Time Expectations:
   - Expect to spend some time outside of the lecture and laboratory meeting times.
   - Be prepared for the lab – you will write your own plans and protocols.

3. Reading Material:
o No textbook
o Primary research publications and third-party research protocols will be assigned and distributed electronically via Canvas.

4. Communication:
o We will communicate in-person during lab section time, and/or remotely via Canvas, LabArchives and email.

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**GRADING:**

<table>
<thead>
<tr>
<th>Component</th>
<th>Percentage</th>
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</thead>
<tbody>
<tr>
<td>Lab Performance</td>
<td>20%</td>
</tr>
<tr>
<td>Lab Notebooks</td>
<td>30%</td>
</tr>
<tr>
<td>Pre-lab and post-lab assignments</td>
<td>30%</td>
</tr>
<tr>
<td>Journal Club Presentation</td>
<td>5%</td>
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<tr>
<td>Project Poster Presentation</td>
<td>15%</td>
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</tbody>
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**See the Rubrics**

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**LAB PERFORMANCE**

Attendance is mandatory. Absences must be justified and may require documentation. Each absence will be recorded in Lab Performance (as an assignment in Canvas). Two and more missing labs will need a graded make-up assignment/experiment. Each missing lab without a proper reason will get 2% deduction of lab performance scores. Be reliable and responsible in the lab. Specifically, perform experiments, label samples and clean benches at the end of each section. Being late will be recorded in “Lab Performance” and will get 0.5 points off each time from the third late appearance. Being late for 15 or more minutes will get 0.5 points off each time.

**NOTEBOOK AND PRE-LAB ASSIGNMENTS**

Notebooks are an extremely important part of scientific research. We will use an electronic notebook system this term (LabArchives) that is time-stamped for all input and changes. Pre-lab and post-lab assignments should be completed on time. Pre-lab assignments will be posted on Canvas>Files>Pre-labs. Completed pre-lab assignments should be submitted to LabArchives before lab starts. Your notebook should be a real-time document containing all of your original data from your experiments. Gel and plate photos should be clearly labeled. The notebook should be self-contained and organized so that other investigators could understand and reproduce your recorded experiments. Your notebook provides the keys to recognize all samples you save in the freezer or refrigerator. Since this lab course does not require separate lab reports, your notebook should include a discussion/conclusion section as part of each experimental record. Timeliness is an important part of entry validity. Your notebook of each lab should be completed before the end of next lab. In the cases where conclusions are waiting for further investigation, this part may be entered in a separate session without changing the original note timestamps. **Late assignments will take a 10% deduction per day.**

**JOURNAL CLUB PRESENTATION**

Each group will present a primary research paper in the field of DNA-protein interactions. Students will choose the paper and submitted to the assignment portal to be reviewed by instructors. The presentations will be held in the middle of the term.

**PROJECT POSTER PRESENTATION**
An oral poster presentation with your lab partners will be held at the end of the term. Your presentations are a significant portion of your grade, representing your achievement in this investigation, as well as your understanding of the mechanism of this biological process.

**Tentative Schedule:**

<table>
<thead>
<tr>
<th>Weeks</th>
<th>Dates</th>
<th>Lab Procedure</th>
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<tbody>
<tr>
<td>1</td>
<td>1/8</td>
<td>Project overview, Syllabus, notebook, lab Safety, lab equipment. Formation of working hypothesis using results from computational analysis</td>
</tr>
<tr>
<td>2</td>
<td>1/15</td>
<td>Plasmid preparation and restriction digestion for verification.</td>
</tr>
<tr>
<td>3</td>
<td>1/22</td>
<td>Preparation of E. coli BL21 competent cells and transformations</td>
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<tr>
<td>4</td>
<td>1/30</td>
<td>IPTG Induction of protein expression in E. coli cells and analysis of over-expressed proteins by SDS-PAGE gels</td>
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<tr>
<td>5</td>
<td>2/6</td>
<td>Protein affinity purification 1 IPTG induction</td>
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<tr>
<td>6</td>
<td>2/13</td>
<td>Protein affinity purification 2 Affinity chromatography</td>
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<tr>
<td>7</td>
<td>2/20</td>
<td>Protein quantifications (SDS-PAGE) Electrophoresis</td>
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<tr>
<td>8</td>
<td>2/27</td>
<td>Protein quantifications-Bradford and purification yield calculations</td>
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<tr>
<td>9</td>
<td>3/6</td>
<td>Spring Break</td>
</tr>
<tr>
<td>10</td>
<td>3/13</td>
<td>Protein concentration / Journal Club</td>
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<tr>
<td>11</td>
<td>3/20</td>
<td>Electrophoretic Mobility Shift Assay (EMSA) 1</td>
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<tr>
<td>12</td>
<td>3/27</td>
<td>Electrophoretic Mobility Shift Assay (EMSA) 2</td>
</tr>
<tr>
<td>13</td>
<td>4/3</td>
<td>Data analysis and repeating experiments</td>
</tr>
<tr>
<td>14</td>
<td>4/10</td>
<td>Poster preparation</td>
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<tr>
<td>15</td>
<td>4/17</td>
<td>Poster presentations – 15-20 minute each group</td>
</tr>
<tr>
<td>16</td>
<td>4/24</td>
<td>Final’s week</td>
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*Note: This schedule may be modified during the semester. You will be notified of any such changes.*

**General Rules of the Laboratory Safety**

- **Know the Locations of Room Exits, Eye Wash Stations, Shower & First Aid Kits:** know their locations and how to use them.
- **Hazardous Materials** (acids, bases, toxins): know where they are & after use, put them away, back where they came from.
- **Hazardous Waste Disposal:** use them appropriately. Special disposal is expensive.
  - Glass Waste Bin: glass only, no gloves, plastic, tissues etc.
  - Biohazard Waste Bin: experimental plates only.
  - Ethidium Waste Bin: Gels only, not gloves or paper towels.
- **Gloves:** always wear gloves in the lab, except when using computer keyboards.
- **Wash Hands Always:** whenever you leave the lab.
- **Wear appropriate clothing:** Lab coats are available.
- **Goggles/Eyeglasses are recommended & available.**
  - Contact lens wearers must wear eye protection when handling hazardous materials.
- **No food or drink in the labs.**
- **In the event of fire, evacuate the lab** – check to make sure your lab mate makes it out safely.
- **Be a conscientious citizen** – inform lab personnel of concerns or potential hazards, such as spills of unknown origin & do your part to keep the lab clean & organized.
- **Never use equipment without proper instruction:** everything but particularly centrifuges.
Centrifuge Safety

There are two general types of centrifuges in the Langley Teaching Labs:

- **Table-top centrifuges:**
  - for quick spins of 2 ml or less which do not exceed 20,000 rpm
  - using another Eppendorf tube for the balance
  - potentially dangerous

- **Sorvall high speed centrifuges:**
  - referred to as “floor model” centrifuges
  - handle large volume samples (greater than 2 ml to 500 ml)
  - rotors have different maximum speeds
  - must use balance tubes to operate
  - potentially dangerous & very expensive - usage requires training

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**Policy on the Export or Import of Lab Materials:**

We do not permit any student to take any teaching lab samples, reagents or other materials to their research lab or back from their research lab to the Langley Teaching Lab. This policy is directed to biological reagents and samples, and it does not include electronic or paper documents. When students take experiments back to another lab space or carry supplies from their lab to the teaching labs, we cannot control the experiment or personal safety, which it is our responsibility to do.

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**Academic Integrity:**

Students in this course will be expected to comply with the [University of Pittsburgh’s Policy on Academic Integrity](https://www.cfo.pitt.edu/policies/policy/02/02-03-02.html). Any student suspected of violating this obligation for any reason during the semester will be required to participate in the procedural process, initiated at the instructor level, as outlined in the University Guidelines on Academic Integrity.

For online quizzes and the final exam, during the time that the assessment is made available to the class, students may not share the content, discuss the content, or discuss the difficulty with anyone. Students must refrain from any activity that would dishonestly or fraudulently improve their results or someone else’s results, including working with another person (whether enrolled in the course or not) or misrepresenting identity.

Cheating/plagiarism will not be tolerated. Students suspected of violating the University of Pittsburgh Policy on Academic Integrity ([www.cfo.pitt.edu/policies/policy/02/02-03-02.html](https://www.cfo.pitt.edu/policies/policy/02/02-03-02.html)) will be required to participate in the outlined procedural process as initiated by the instructor. A minimum sanction of a zero score for the quiz or exam will be imposed, but sanctions can also include failure in the course.

Violation of the Academic Integrity Code requires the instructor to submit an Academic Integrity Violation Report to the Dean’s Office.

**Disability Services:**

If you have a disability for which you are or may be requesting an accommodation, you are encouraged to contact both your instructor and [Disability Resources and Services (DRS)](https://www.pitt.edu/about/disability_resources_and_services) (DRS), 140 William Pitt Union, (412) 648-7890, [drsecep@pitt.edu](mailto:drsecep@pitt.edu), (412) 228-5347 for P3 ASL users, as early as possible in the term. DRS will verify your disability and determine reasonable accommodations for this course.

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