Biotechnology Applications of Cell and Mol Biology

BIOL 4460 , SPRING Semester 2023

TWTH 8AM-10:30AM; ASCL-126 (aka La Kretz Hall)

**Instructor**

**Dr. Sunjay Jayachandran; Office Hours: Tuesdays 9 AM-10 AM;**

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**Prerequisites: BIOL 4170 and one of these (BIOL 3400 or MICR 3300 or CHEM 4310) or instructor consent.**

**A Laboratory Manual will be provided to all students online. No other textbooks are required.**

**Recommended Textbook: High Throughput Screening in Drug Discovery, by J. Hüser. Wiley-VCH, Weinheim, Germany. ISBN-13: 978-3-527-31283-2.**

**Additional Reference Books: An Introduction to Molecular Biotechnology, M. Wink (ed), Wiley-VCH, Weinheim, Germany. ISBN-13: 978-3-527-31412-6.**

**Student Learning Objectives: After taking this course, the students will be able to:**

** Demonstrate ability to work in a multidisciplinary team environment;**

** Demonstrate ability to become familiar with principles and practices of multiple disciplines;**

** Apply principles of chemical genomics to the drug discovery;**

** Apply laboratory robotics to the drug discovery processes;**

** Self-report a gain in confidence in leading a phrase of the drug discovery process (as a taskforce leader);**

** Evaluate and critique primary research literature relevant in biotechnology;**

** Analyze, synthesize, interpret experimental results and report them to an audience;**

** Evaluate a case and design strategies to fill a technical gap as a team in a case study;**

** Produce and present a case-study presentation as a team to an audience;**

**Attendance**

**Attendance is mandatory. Each student has two allowances for excused or emergent (documentable) absences during the entire quarter but is responsible for making up lost lab work or data or assignments.**

**Grading: 300 points total**

**Quizzes (10 pts per quiz) 60**

**Lab notebooks 50**

**Web-based DNA analysis homework/reports(A,B) 20**

**HTS Assignment 10**

**Cytotoxicity assignment 10**

**Attendance/Participation during group lab exercises 50**

**Team presentation 100**

**Final grades will be based on % of achievable points obtained:**

**Total points earned / 300 points x 100 = % achieved**

**A: 92%** **A-: 90-91% B+: 88-89% B: 82-87%** **B-: 80-81% C+: 78-79 C: 72-77%** **C-: 70-71%**

**D: 62-67% F:  59%**

**In borderline cases (passing/ non-passing or grade levels), course attendance and active participation may be considered for the final outcome.**

**Web-based DNA analysis homework.**

**The students will be assigned a homework involving retrieving, analyzing and comparing a bacterial gene sequence using web-based analysis tools (worth 50 points). Homework report handed-in late will result in deduction of report points (10% deduction per day after due date).**

**Scientific literature search and written report.**

**The writing exercise will be worth 50 points and will consist of a summary report that explains a primary research paper related to HTS/drug discovery. It is the responsibility of the student to find the paper via PubMed or library and print it out after I give you the reference. The student will study the paper in detail and perform a background literature search to obtain at least two additional references (papers) published before and cited by this paper. The report shall cover the following four sections with clear subtitles: The Background of the research (why do it), major Methods used (how), Results obtained (what happened), Conclusions (significance to the field), Your critique (of what the authors did well and where they could improve), and References (naturally including the main reference). The report should follow routine rules relative to the format of species names and gene names. The explanation should be clear enough that a fellow classmate could read it and understand it. The report should be no more than 5 typed pages with double spacing between lines. Fonts of Times New Roman or Arial with 12 point size are to be used. The written reports are due after Thursday class on week 9. Reports handed-in late will result in deduction of final report points (10% deduction per day after due date).**

**Case Study and Team Presentation**

**Case study topics will be assigned to each group. Students of each group will work together to complete its case study and hand in the report. Finally, students of each group will collaboratively prepare and give the team presentation based on research of the assigned case study.**

**General Information**

**There will be no make-up lab sessions. You must provide your own lab coat, safety glasses, permanent markers. The University Academic Honesty Policy (http://www.calstatela.edu/academic/senate/handbook/ ) and the Drop/Incomplete Policy explained in the University General Catalogue will be strictly followed. Students are responsible for the prerequisites for this course and are encouraged to discuss any questions regarding the policies and prerequisites with the instructor. Students with disabilities: please contact the instructor to arrange appropriate accommodations.**

**COURSE SCHEDULE**

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| --- | --- | --- |
| **Week** | **STAGES AND ACTIVITIES** | **EXERCISE PLANS (may be adjusted)** |
| **1**  **(Jan 24-26)** | Laboratory Orientation, Project Overview and Bioinformatics Basics (**Bioinformatics Module**). Students will form teams and learn project/team management skills. They will learn to access GenBank, retrieve sequences, understand ORF definition, and design primers. | **T**: Overview of class; team formation; PPT chemical genomics; review basics of micropipetting video clip; PPT antibiotic drug discovery; HM, |
| **W**: PPT gene cloning & primer design; Ex.2 only four-quadrant streaking; Ex.3 target selection & bioinformatics (in-class demo using computer); Ex.4 primer design (in-class demo using computer); |
| **R**: Ex.4 continue; complete primer design; Observe four-quadrant streaking results, repeat if needed for some teams/persons; each team finalizes a word document containing primer design steps and final primers to be order (IS will order for teams); |
| **2**  **(Jan 31-Feb 2)** | Molecular Cloning (**Molecular Biology Module**). Students will learn to grow bacteria, isolate genomic DNA, perform PCR, and perform agarose gel electrophoresis of PCR products.  During week 3 or 4, student teams will be assigned case studies to work together to write a report and prepare/give a presentation. | **T**: Ex.5 preview TA cloning procedures; Ex.6 bacterial genomics miniprep, Period 2; Ex.6 Period 3, isolation of genomic DNA miniprep scale; If primers arrive, dilute and prep working stocks for PCR Ex.6, nanodrop determination of genomic DNA concentrations; |
| **W**: Ex. 7, PCR using template DNA prepared and primers designed; |
| **R**: Ex.8 agarose gel electrophoresis; |
| **3**  **(Feb 7-9)** | Molecular Cloning (**Molecular Biology Module**). Students will learn to purify PCR products, transform *E. coli* competent cells, streak a colony to assure independent clones, perform plasmid miniprep and perform restriction digest analysis to confirm appropriate size of cloned inserts. | **T**: Ex.9 PCR product purification; |
| **W**: Ex.5, ligation with TA vector; Ex.5 bacterial transformation (note Ex.10 not used) |
| **R**: Ex.5 observe and discuss cloning results; |
| **4**  **(Feb 14-16)** | Molecular Cloning (**Molecular Biology Module**). Students will learn to purify PCR products, transform *E. coli* competent cells, streak a colony to assure independent clones, perform plasmid miniprep and perform restriction digest analysis to confirm appropriate size of cloned inserts (continued) | **T**: Ex.11 plasmid miniprep, inoculation of positive clones |
| **W**: Ex.11 plasmid miniprep; Ex.12 restriction digest set up; |
| **R**: Agarose gel electrophoresis of digested plasmid DNA; |
| **5**  **(Feb 21-23)** | Protein Purification (**Biochemistry Module**). Students will learn to purify His (histidine)-tagged proteins using nickel columns and to determine protein concentrations. | **T**: Ex.13 his-tagged protein over-expression, inoculate starter culture (prepped by IST) into 50 ml medium in flask; add IPTG (inducer) when OD is reached; spin down and freeze pellets; |
| **W**: Ex.13 his-tagged protein extraction; |
| **R**: Ex.13 his-tagged protein purification; |
| **6**  **(Feb 28-Mar 2)** | Western blot analysis (**Biochemistry Module**). Students will learn to perform separation of proteins via electrophoresis and identification of specific protein using antibody-antigen interactions | **T**: Ex.14 polyacrylamide gel electrophoresis of overexpressed proteins; |
| **W**: Ex.14A Transfer proteins from SDS-PAGE to Nitrocellulose |
| **R**: Ex.14A Western Blot analysis with TMB and ECL |
| **7**  **(Mar 7-9)** | Cell-based Assays (**Cellular Profiling Module**). Students will learn to perform cellular profiling using BIOLOG’s OmniLog system and understand physiological differences in bacterial species/strains. | **T**: Ex. 15B Students inoculate plates with environment samples |
| **W**: Ex. 15B Select isolated single colonies and streak on BUG+B plates to obtain cell biomass. |
| **R**: Ex. 15B Set up microplates for BIOLOG profiling experiments. |
| **8**  **(Mar 14-16)** | High Throughput Screening (**HTS Module**). Students will learn to screen a chemical library for cell growth inhibitors and operate HTS robotics. | **T**: Ex. 15B. Discuss results of microbial identification; Ex. 17 Overview of HTS antibacterial growth screening |
| **W**: Ex. 17. HTS – growth inhibitors |
| **R**: Ex. 17. HTS – growth inhibitors |
| **9**  **(Mar 21-23)** | High Throughput Screening (**HTS Module**). Students will learn to screen a chemical library for cell growth inhibitors and operate HTS robotics. | **T**: Ex. 17. HTS – growth inhibitors; data analysis |
| **W**: Ex. 17. HTS – growth inhibitors; hit compounds identification and chemical structure analysis |
| **R**: Ex. 17. HTS – growth inhibitors; hit compounds identification and chemical structure analysis |
| **10**  **(Apr 4-6)** | Tissue culture basics (**Tissue Culture Module**): students will learn basic techniques of mammalian cell culture | **T**: Southern |
| **W**: Southern |
| **R**: Ex. Self- or team-study time due to some students |
| **11**  **(Apr 11-13)** | Impact of Antibiotics on Mammalian Cell-lines (**Tissue Culture Module**): students will perform cytotoxicity assays using antibiotics | **T**: Ex. 16B – Cytotoxicity of Antibiotics |
| **W**: Ex. 16B – Cytotoxicity of Antibiotics |
| **R**: Ex. 16B – Cytotoxicity of Antibiotics |
| **12**  **(Apr 18-20)** | Impact of Antibiotics on Mammalian Cell-lines (**Tissue Culture Module**). students will perform cytotoxicity assays using antibiotics | **T**: Ex. Ex. 16B – Cytotoxicity of Antibiotics – analysis |
| **W**: Ex. Ex. 16B – Cytotoxicity of Antibiotics – analysis |
| **R**: **Elisa** |
| **13**  **(Apr 25-27)** | CRISPR will be performed as well.  If time permits: Team-based case studies (Ideation Module). Students from each project team will work together to “brain storm” ideas for assigned case studies and formulate plans for advance ideas to meet the unmet needs as part of the case study reports and presentations | **CRISPR** |
| **14**  **(May 2-4)** | Team Summary Reports and Team Presentation preparation (**Communication Module**). Students from each project team will work together to produce a summary report of their discovery and prepare PowerPoint slides to present to the whole class (Project/Team reporting skills). Team members will receive points based on evaluation by the instructor and fellow students from other teams. |  |
| **15**  **(May 9-11)** | Team Summary Reports and Team Presentations (**Communication Module**). Students from each project team will work together to produce a summary report of their discovery and prepare PowerPoint slides to present to the whole class (Project/Team reporting skills). Team members will receive points based on evaluation by the instructor and fellow students from other teams. |  |