Biology 431/531 Winter 2006
Recombinant DNA Techniques Laboratory
424 Science Building 1

		E-mail	Room	Office Hours
Instructor	Justin Courcelle Michael Bartlett	justc@pdx.edu	432,SB2	Mon 10–12 PM
Teach Asst Section 001	Adam Clore	clore@pdx.edu	544,SB1	Fri 3--4 PM
Teach Asst Section 002	Michael Micorescu	psu12417@pdx.edu	544,SB1	Fri 3--4 PM

Required text: "Unraveling DNA" by Winfrey, Rott, and Wortman (1997). Prentice Hall, Upper Saddle River, NJ.

Supplies: You will need your own laboratory notebook (a standard lab notebook is recommended) and a supply of permanent, water resistant, extra fine point lab markers.

Website: http://web.pdx.edu/~justc/courses.html (has handouts and lectures)

ATTENDANCE IS MANDATORY: No make up labs will be offered. If you miss a lab period for any reason, you may obtain relevant data from your lab partners in order to complete lab write-ups. Missing more than one day of lab will result in a failing grade for the course.

Time commitment: In addition to normal lab hours, you will need to visit the lab in off hours to maintain experiments.

Grading (Bi431 -- 215 points, Bi531 -- 255 points)

Quizzes (45 points): Preparation for the laboratory exercises is essential. Many of the lab periods will be very busy, and this preparation will help you to focus your efforts and finish in a timely manner. Nine quizzes (5 points each) will be given during the term to check for preparation. The quizzes will use short answer or multiple choice questions to test basic knowledge of the day's laboratory exercises. To prepare for the quizzes you must read the lab exercises before class and understand the basic ideas as well as the relevant calculations.

Lab notebook checks and participation (40 points): Your laboratory notebook is extremely important—you must keep complete records of each day's experiments and data. Adam or Mike will check your notebook occasionally, and will deduct points if your notebook is incomplete or not up to date. Be a good lab citizen—please leave your lab bench cleaner than you found it, and keep equipment and other community materials organized. Messes will result in point deductions.

Laboratory Reports (110 points): Eight typed lab reports will be due during the term (see Schedule for due dates). A 4 page limit applies (including figures and tables). Each lab report will be worth 15 points, with the exception of the first (worth 5).

The format for each exercise (there are several in each report) should follow these guidelines (also see the lab report guidelines from WebCT):

- *Title and brief summary*: This section should be no more than 5-10 sentences, you do not need to copy the entire procedure into the report. Clearly state the purpose of the experiment and how it was pursued.
- *Methods*: Methods may be referenced from Winfrey et al. Any significant changes from the manual (different reagents, incubation times, etc.) must be reported
- *Data*: This section includes all of the data generated in the experiment. Graphs, pictures, and tables are all very useful. Include enough detail to make the data interpretable by the reader. Include all calculations.
- *Observations and relevant experimental details*: This section allows you to support and clarify the data.
- *Discussion*: In a nutshell, what did this experiment tell you? Did the experiment work? If not, why not? What could you do to make the experiment work better? What further experiments would you propose? This section should also include answers to the assigned study questions at the end of each exercise.
- Grading will be based on your understanding of the experiments and the critical thinking that goes into the report. ALL REPORTS MUST BE TYPED. Reports that are not typed will be returned without grading. Reports should be written as concisely as possible while explaining the data thoroughly.
- For the experiments that are not in "Unraveling DNA," Adam or Mike will provide information before the lab, and discussion questions for the report.
- We recommend completing reports soon after the end of each exercise (when the experiments are fresh in your mind), rather than saving them for the day before lab reports are due.

Environmental bioluminescent microbe isolation (20 points): Each student will isolate their own bioluminescent microbe from seawater or from a sea creature by filtration, screening, streaking for purification, and preservation as a freezer stock. This microbe will be preserved in the BI431/531 freezer (under your name) for use by future researchers. 15 points are awarded for creation of the freezer stock, and an additional 5 points will be awarded if this freezer stock is shown to be a pure culture (all bioluminescent colonies). A handout describes details of this project.

Summary (BI531 only, 40 points): A 2-3 page (typed, single-spaced) summary of the experiments you've done this term (excluding exercises 1, 2,4,). A brief recap of all of your results, and how they fit into the larger project. The summary will naturally divide into two parts, cloning of the lux genes, and environmental isolation and characterization of bioluminescent microbes. This report must be handed in by 5PM Monday, June 12.

Schedule

Wk	Date	Lab	Reading	Reports Due
1	10-Jan	Micropipetting, manipulating microbes Concentration and dilution exercises Isolation of bioluminescent microbes from seawater	ex.1, pt I ex.1 pt II A,B ex.3	
1	11-Jan	Plasmid preparations Check for luminescent colonies on plates and cultures	ex.6, plus handout.	
2	17-Jan	Intro to restriction digestion, agarose gel electrophoresis	ex.4	
2	18-Jan	Agarose gel analysis and discussion Media and stock preparation	ex.2	Ex.1, pt I & II
3	24-Jan	Photobacterium leognathi or Vibrio fischeri genomic DNA isolation	ex.5	
3	25-Jan	Finish genomic isolation Spectrophotometric analysis of DNA	ex.5 ex.7	Ex.4 & 6
4	31-Jan	Restriction digestion of genomic DNA	ex.8 plus handout	
4	1-Feb	Agarose plate fluorescence quantitation of DNA Ligation of genomic DNA into plasmid vector	ex.9B ex.10 pt1	
5	7-Feb	Check ligation efficiency	ex.10 pt II	Ex.5, 7, 8
5	8-Feb	Competent cell preparation and test transformation	ex.11 plus handout	
6	14-Feb	Transformation of competent cells with recombinant plasmid; Creation of a genomic library	ex.12 pt I&II plus handout	Ex.9B, 10 part I & II
6	15-Feb	Screening of library transformation		
7	21-Feb	Start plasmid minipreps from positive clones \ PCR screen of transformants for luxA gene	ex.13 ex.14 PCR handout	Ex.11 & Electroporatio n (handout)
7	22-Feb	Plasmid miniprep and restriction analysis	ex.14	
8	28-Feb	Agarose gel analysis of luxA PCRs PCR luxA gene from environmental isolates		
8	1-Mar	Agarose gel analysis of PCRs		Ex12 pt I & II, 13, 14
9	7-Mar	Sequencing of luxA from enviromental isolate		
9	8-Mar	Clean up sequencing reactions from enviromental isolate and send it off for sequencing Computer analysis of sequencing results		
10	14-Mar	Tentative ID of environmental isolates by BLAST and alignments, streak bioluminescent freezer stocks (to determine pure culture), and free lab time.		PCR screen transformants and environmenta l samples
10	15-Mar	Graduate Presentations		