Biology 437 Fall 2009 Syllabus
Biology 437: LABORATORY ON DNA MANIPULATION
Instructor: Professor Robert Kranz (kranz@biology.wustl.edu)

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Textbook: none (A "*New England Biolabs*" catalog with technical information will be given to each student).

I. You will be given the following handouts on the first day of class:
1. Student Questionnaire
2. Bio 437 course overview and goals
3. Class Syllabus (subject to change)
4. Guidelines for keeping a Laboratory Notebook (and a sample Notebook Grading Sheet)
5. Guide for the lab write-up
6. Laboratory Safety Rules
7. Handouts for this week's lab (Lab I)
8. *New England Biolabs* catalog
9. A lab notebook and 3-ring binder

II. How is the class graded?
Two Quizzes and two problem sets: 50 points each (there also will be a subjective 50 pts that will be given after the course is over by all instructors --- eg. on missed or late to labs, sloppy, etc). The lowest score of these five items will be dropped, thus **200 points**

Final	**100 points**
Lab Write-up	**100 points**
Lab Notebook	**200 points**
Total	**600 points**

III. Office Hours: (Dr. Kranz and at least one TA will be at all labs and lectures so these 9 hours per week will have ample time for discussion, or alternatively, email Dr. Kranz)

IV. **Bring your lab notebook and binder to every lab. Also, bring your NEB catalog to every lab;** you will refer to it often, including during quizzes and exams!

V. Lectures are in Rebstock 309 on Wednesday from 2:07 to 3:00 p.m.

VI. **Lab attendance is mandatory on both Friday from 1-5pm and Saturday from 9am-1pm.** Labs take place in Rebstock 125 and 126. *Be on time* because a short lecture outlining the day's procedure will take place at the start of most labs.

VII. There is lab on the first Saturday. There is NO LAB on Friday and Saturday of Fall Break. There are no lecture or labs the week of Thanksgiving Break.

AUG 26: LECTURE 1: Introduction to course and the semester projects: cloning & expression of genes in cytochrome c assembly; The basic & applied understanding of nucleic acid (DNA and RNA) chemical structure; DNA purification; vectors (plasmids) and fragments (inserts); agarose gels; fill out student questionnaire.

AUG 28: LAB 1A: **MODULE 1 BEGINS**. Calibrate pipettes; make stock solutions; start overnight (O/N) cultures of *E. coli* for plasmid vector minipreps; start Shewanella etc. cultures to make genomic DNA. In computer lab, start evaluating genes to clone and looking at plasmid maps. Individual meetings with students on their projects

AUG 29: LAB 1B: Purify Shewanella etc genomic DNA for PCR. Minipreps of plasmid DNA from *E.coli*; digests for cloning. Continuing designing oligonucleotides to clone cytochrome c genes using PCR. Individual meetings with students on their projects. (The instructors will order the oligonucleotides to use for next weeks labs.)

SEPT 2: LECTURE 2: Introduction to recombinant DNA and plasmids, restriction enzymes and DNA modifying enzymes; joining pieces of DNA (ligation), quantification of DNA/RNA. PCR introduction.

SEPT 4: LAB 2A: Prepare and quantify oligonucleotides; run PCR reaction for cloning your specific genes; run gel to confirm

SEPT 5: LAB 2B: Set up PCR product and vector for cloning; clean and purify product(s). (Repeat PCR, etc. as needed.)

SEPT 9: LECTURE 3: Polymerase Chain Reaction (PCR): history, principles and reaction components; practical ligation tips.

SEPT 11: LAB 3A: Run PCR fragment to quantify. Upon consultation with instructors, set up ligations at suggested concentrations. (Repeat PCR and ligations if necessary).

SEPT 12: LAB 3B: Transform electro- or chemically-competent *E.coli* cells with ligation mixes and plate on LB with vector drug resistance. Design DNA digestion to confirm cloning for next week.

SEPT 16: LECTURE 4: Transformation of bacteria with plasmids; methods to deliver DNA into cells. Intro to DNA sequencing FREE NOTEBOOK EVALUATION. **PROBLEM SET #1 ASSIGNED**.

SEPT 18: LAB 4A**:** Pick transformed *E.coli* colonies and start for minipreps**.**

SEPT 19: LAB 4B: Miniprep candidate plasmids. Diagnostic digests and gel.

SEPT 23: LECTURE 5: DNA and genome sequencing: vectors, sequencing techniques, ESTs.

SEPT 25: LAB 5A: Sequencing DNA. Finish minipreps. **T**our Washington University Genome Sequencing Center ?? More cloning or subcloning if necessary.

SEPT 26: LAB 5B: More sequencing if needed; subcloning into pBAD or PGEX.

MODULE 2 BEGINS.: start designing *E.coli* chromosomal knockout and/or integration of potential genes involved in cytochrome assembly. Individual meetings with students on their projects. (Have oligonucleotide regions of homology for each gene knockout and/or integration for verification completed so that oligos can be ordered.)

SEPT 30: LECTURE 6: Homologous recombination: why organisms use it and how we can use it to our benefit. Southern blot and PCR analysis to verify success in gene knockout construction.

OCT 2: LAB 6A: DNA sequence from module 1--- Reports on cloning progress and continue subcloning if necessary. Module 2

OCT 3: LAB 6B: Individual meetings with students on their projects. *E.coli* chromosomal knockout and/or integration

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OCT 7: Lecture 7: More stories and background on knockout mutants and genetics in general.

OCT 9: LAB 7A: *E.coli* chromosomal knockout and/or integration. Continue subcloning from Module 1 if warranted.

OCT 10: LAB 7B: Check for transductants (colonies). Repeat Lab 7A, as advised by instructors to optimize successful knock-out generation or integration.

OCT 14: LECTURE 8: The impact of genomic sequences on genetics, metabolic engineering, and more. **Turn in notebooks.**

OCT 16, 17: FALL BREAK, NO LAB!

OCT 21: LECTURE 9: Eukaryotic: analysis of DNA & RNA: Genomic mapping, linkage and genetic maps; microarray, mapping mutations; molecular markers; AFLP anaylsis Notebooks returned.

OCT 23: LAB 8A: Initial PCR verification and other tests of knockout/integration strains.

OCT 24: LAB 8B: **Quiz #1** Finish knockout/integration experiments.

OCT 28: LECTURE 10: Nitrogen stress in plants. .How to measure RNA levels in cells Northern blots, RT-PCR, microarrays.

OCT 30: LAB 9A: Finish knockout/integration studies .

OCT 31 LAB 9B: Finish knockout studies. **MODULE 3 BEGINS:** mRNA studies (microarrays) on the plant, *Arabidopsis thaliana*. Plants that are already growing on the selected media will be observed for reporters and growth properties. Short lecture on the micrroarray project. **Problem set #2 assigned.** Plant tissue preparation for RNA analysis; prepare for microarray study. Computer-based analysis using microarrays to provide sampling of what data is generated.

NOV 4: LECTURE 11: The theory and practice of mRNA studies.

NOV 6: LAB10A: RNA isolation, Microarray data analysis; identification of candidate genes that are controlled by the nitrogen (fertilizer) status.

NOV 7: LAB 10B: " " continued. Design oligonucleotides on candidate genes to validate array results using RT-PCR with oligonucleotides.

NOV 11: LECTURE 12: Transcriptional start, stop and regulation. What comprises a gene? How many genes are there in an organism? Reporter fusions putting DNA into eukaryotes; studying promoters;

NOV 13: LAB 11A: microarray data analysis continued.

NOV 14: LAB 11B: if needed, redesign oligonucleotides on candidate genes to validate array results using RT-PCR with oligonucleotides.

NOV 18: LECTURE 13: DNA/protein interactions; eukaryotic expression systems. Other DNA methods.

NOV 20: LAB 12A: RT-PCR and microarray data.

NOV 21 LAB 12B. **Quiz #2.** Analysis of RT-PCR and microarray data. Discuss individually with each student which module to use in their write-ups.

NOV 25-29: Thanksgiving Break

DEC 2: LECTURE 14: Summarize successes and failures for the semester, what could be done experimentally in the future. A short overview of methods and approaches with DNA and RNA that we did not study this semester. (eg. Identifying interacting proteins via expression tricks; studying gene function *in vivo*; site-directed mutagenesis; RNAi, mutant screens and suppressor analysis.)

DEC 4: LAB 13IA: Wrap up experiments, etc; get help with interpretation of results and notebooks; discuss lab write-up; clean up lab. Student powerpoint presentations.

DEC 5: LAB 13B: Review for final exam. **Have a lab party! Lab starts at 11 am!**

~ DEC ??: **FINAL EXAM TIME: TBD** (Lab write-ups and notebooks are due at this time.)