

Syllabus for MMBB440: the MMBB Capstone Laboratory

SPRING 2006

Laboratory: Tuesday, Thursday 1:30 – 5:20 pm LSS167
Friday 3:30 - 4:20 pm LSS163

The goals for this class are to:

1. Develop a hypothesis and design an experiment to test the hypothesis.
2. Analyze, manipulate, and annotate raw genome data; become adept at recognizing the multiple factors that are used to determine if an open reading frame is a gene
3. Generate figures and tables to effectively communicate the information within the genome to other scientists
4. Learn specific techniques
 - how to generate a mutation
 - oligonucleotide design
 - PCR amplification
 - Quantify DNA
 - DNA gel analysis
 - how to characterize a phenotype
 - clone a gene for expression in a foreign host
 - induce expression of a gene
 - purify a protein (His-tagged)
 - do a protein assay
 - pour and develop a protein gel
 - perform immunoblot analysis using anti-His antibody
5. Write a manuscript to communicate your findings with other scientists

Your grade in MMBB440

25% of your grade will depend on your lab notes. We will grade your manual twice – once before spring break and again at the end of the semester. It is your responsibility to check with either the TAs of the faculty members to determine if your note area in reasonable order.

50% of your grade will be determined from the **manuscript** you submit at the end of the semester. Part 1 of the manuscript is due on FRIDAY MARCH 3 at 5pm. The first part of your manuscript will contain all of the sequence annotation and figures related to the 25kb of sequence that you were given at the beginning of the semester. **The final manuscript is due on May 5 at 5pm.**

25% will be from attendance at all of the Tues-Thurs lab sessions.

Teaching assistants:

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Participating MMBB, Biological Sciences and IBEST faculty:

Celeste Brown Biological Sciences

Allan Caplan MMBB

Douglas Cole MMBB

Jill Johnson MMBB

Kurt Gustin MMBB

Patricia Hartzell MMBB

Luke Sheneman IBEST

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| Jan 12 | Meet in LSS 167 | Hartzell | Introduction of the TAs and instructors for the wet lab; review the syllabus, requirements for lab notebook |
| Jan 17, 19 | Computer lab check in; | Hartzell/Brown/Sheneman | Learning the ropes: reading the vector NTI manual; Assigning sequence (25,000 kb per student, individual sequence data), review record keeping: naming open reading frames Jan 17 - Molecule display windows, Database explorer Jan 19 – LabShare, Internet connectivity and tools, Bioannotator |
| Jan 20 | Your time | | Please use this time to start developing figures and tables that document your sequence annotation. |
| Jan 24 Jan 26 | Vector NTI | Luke Sheneman | PCR analysis and primer design, AlignX Continue sequence analysis: ID open reading frames first 5,000 kb: look for ORF, blast search |
| Jan 27 | | | Please use this time to work on figures and tables that document your sequence annotation. |
| Jan 31 Feb 2 | Vector NTI | Luke Sheneman | Molecule editing, Formatting molecule graphics, Molecule construction; continue sequence analysis: ID open reading frames next 10,000 kb: look for ORF, blast search |
| Feb 3 | | | Please use this time to work on figures and tables that document your sequence annotation. |
| Feb 7 | Vector NTI | Celeste Brown | Molecule design, Advanced molecule design, Gel display |

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| Feb 9 | | | windows; continue sequence analysis: identify biosynthetic pathways, group putative ORFs by function: motility, secretion, biosynthesis, virulence. |
| Feb 10 | Please use this time to complete the figures and tables that document your sequence annotation. You must present your annotation tables and figures to the instructors on Feb 10. We will compile all of your tables and figures and make a handout for the entire 625 kb for all students, which will be handed out on Feb 14. | | |
| Feb 14 Feb 16 | Discussion of the wet lab portion of MMBB440 | Hartzell/Minnich | Sequence analysis: joining all sequence to form 625kb of continuous sequence. On Thursday, spend time looking at the information obtained by other students in the class. |
| Form teams of <u>three</u> . Using the annotation tables generated by all the students in MMBB440, select a gene you suspect to be involved in motility, symbiosis, or secretion (or a secreted protein). Discussion and communication are critical here – you cannot work on a gene selected by another team. Prepare a restriction map of this gene. | | | |
| Feb 17 | | | Select the gene you propose to study during the wet lab and present it to the TA for faculty/TA approval. We will need to see a restriction map of this gene. Preview of experiments for next week |
| Feb 21 Feb 23 | Begin Part 2, the wet lab portion of MMBB440 | Hartzell | Students carrying out experiments 1 and 2 will purify chromosomal DNA and design oligonucleotides; students carrying out experiment 3 within each group must begin the mutagenesis experiment. |
| Feb 24 | Cloning and mutagenesis | | Transposon mutagenesis |
| Feb 28 Mar 2 | Week 2 wet lab | | PCR amplification |
| Mar 3 | Cloning background, part II | Hartzell | Cloning: types of cloning vectors, use of restriction enzymes, DNA miniprep procedures, agarose gel, ligation, screen and selection, use of genes, such as <i>sacBR</i> , for counterselection |
| Mar 7 Mar 9 | Week 3 wet lab | | |
| Mar 10 | | | Friday before spring break |
| Mar 21 Mar 23 | Week 4 wet lab | | |
| Mar 24 | | | |
| Mar 28 | Week 5 wet lab | | Electroporation of clone (or transposon tagged plasmid) into |

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| Mar 30 | | | bacterial strain. Protein expression: induction week and protein gel electrophoresis |
| | | | Expression of foreign proteins in <i>E. coli</i> |
| Apr 4 Apr 6 | Wet 6 wet lab | | Phenotypic analysis of auxotrophic mutants (after two transfers on selective medium) Protein purification on metal affinity matrix |
| Apr 6 | | | |
| Apr 11,13 | Week 7 wet lab | | Phenotype: does the mutation affect the ability of <i>Xenorhabdus</i> to infect the nematode host? Protein: immunoblot analysis with anti-His |
| Apr 14 | | Johnson | Immunoblot analysis |
| Apr 18, 20 | Week 8 wet lab | | |
| Apr 21 | | Cole | Protein purification:metal affinity |
| Apr 25, 27 | Week 9 wet lab | | |
| Apr 28 | | Caplan | Bacterial:nematode interaction |
| May 2, 4 | Week 10 wet lab | | Writing manuscript |
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