MICROBIAL GENETICS (BIO-375/575)

Fall 2011

Place and Time: Halsey 237: 9:40 – 11:10 TuTh

Instructor: Dr. Toivo Kallas

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Office hours: TuTh 3:00 – 4:30, W 11:30 – 12:30. Other times by appointment. Anytime by phone or e-mail. If I am not in, please leave a message or check the lab rooms (HS 238, 240, or 163/145 Biosep-Proteomics Labs).

Textbooks and Resources:

Required:

1. Snyder, L. and Champness, W. 2007. *Molecular Genetics of Bacteria*, 3rd edition, American Society for Microbiology, Washington, D. C.
2. Much of the reading material for the course will come from journals such as *Nature, Science, Proceedings National Academy Sciences, J. Bacteriology* and others. These and other reading materials will be posted on the class D2L site. Required readings will be indicated.

Recommended & Other Useful References:

1. McMillan, V. E. 2006. *Writing Papers in the Biological Sciences,* 4th edition, Bedford/St. Martin's.
2. Bushman, F. 2002. *Lateral Gene Transfer*, Cold Spring Harbor Laboratory Press.
3. Kaper, J. B. and Hacker, J. 1999. *Pathogenicity Islands and Other Mobile Virulence Elements*, ASM Press, Washington , D.C.
4. Ptashne, M. 2002. *Genes and Signals*, Cold Spring Harbor Laboratory Press.
5. Miller, J.R. 1992. *A Short Course in Bacterial Genetics: Lab Manual*, Cold Spring Harbor Laboratory Press.

Desire2Learn (D2L) Site: Powerpoint presentations, pdf files of literature discussion and reference articles, and other materials will be available via the class D2L site (**Microbial Genetics Bio-375/575**). To access, go to the UW Oshkosh home page, > click, "D2L, Desire2Learn." On the D2L login page, enter the username and password that you use for UW Oshkosh e-mail.

Some genetics and other resources on the internet:

1. Class D2L site, described above.
2. American Society for Microbiology (ASM) home page: http://www.asmusa.org.
3. BioWeb http://bioweb.uwlax.edu/index.htm. (A collection of data and tools for genetics and biology).
4. **DOE Joint Genomics Institute (JGI)**: http://www.jgi.doe.gov/JGI_microbial/html/index.html (Microbial genome databases and a great resource for genome analysis including BLAST searches.)
5. **ExPASy Molecular Biology Server:** http://www.expasy.ch/. (A very useful site for molecular biology, genomics, and proteomics included predicted peptide mass fingerprints.)
7. **NATIONAL CENTER FOR BIOTECHNOLOGY INFORMATION (NCBI):** http://www.ncbi.nlm.nih.gov/. (This site includes the GenBank and other DNA, protein, and genomic databases and extremely useful search programs such as "BLAST." Includes the PubMed, MEDLINE literature database.)

8. Within **NCBI**, note for example **PubMed** (http://www.ncbi.nlm.nih.gov/pubmed/) for literature database searches and **PubChem** (http://pubchem.ncbi.nlm.nih.gov/) for structures and information about small molecules including metabolites, antibiotics, and inhibitors.
9. TIGR (The Institute for Genomic Research): http://www.tigr.org.
10. *E. coli* Genetics Stock Center: http://cgsc.biology.yale.edu/. (a nice site for gene names, maps, etc.)
11. *E. coli* Genome Center: http://www.genome.wisc.edu
12. **New England Biolabs**, Restriction Enzyme Database (NEB-REB): http://rebase.neb.com.
13. **The RCSB Protein DATA Bank**: http://www.rcsb.org/pdb/. (Site from which to download ".pdb" files of coordinates for viewing and manipulating protein and DNA sequence 3D structures).
14. **PyMOL:** http://pymol.sourceforge.net/ (Site for downloading the PyMOL program for very nice viewing and manipulation of protein and molecular 3D structures on Mac and Windows platforms.)
15. Webcutter (a site for on-line restriction site analysis): http://www.firstmarket.com/cutter/cut2.html
16. Net Primer (a site that allows downloaded or on-line design of PCR primers. They also carry "Plasmid Premier" a program for plasmid design): http://www.premierbiosoft.com/netprimer.html
17. Promega Corporation (Madison, WI): http://www.promega.com/
18. **BioBIKE** (Biological Integrated Knowledge Environment): http://ramsites.net/~biobike/ (Provides integrated databases and access to a 'non-expert' programming language for bioinformatics investigation of biological databases).
19. **CyanoBIKE** (Cyanobacterial Biological Integrated Knowledge Environment): http://cyanobike-community.csbc.vcu.edu/ (graphical interface programming environment for access to integrated cyanobacterial genome databases, manipulation and data mining).
20. **KEGG** (Kyoto Encylopedia of Genes and Genomes): http://www.genome.jp/kegg/ (A very useful bioinformatics resource for linking genomes to biological systems and environments.)
21. **UW-O (Polk) Library:** http://www.uwosh.edu/library/ (Polk Library provides access to a variety of useful literature databases such as Medline and Web of Science and carries on-line, full-text subscriptions of several relevant journals including *Science, the Nature Journals,* Elsevier Journals via Science Direct, and the *American Chemical Society (ACS) Journals*. Follow on-screen instructions or see me.)

Course Objectives:

Understanding microbial genes, genomes, and gene expression is essential for understanding the biology and evolution of microorganisms and their interactions with the environment. Since the discovery of genes in microorganisms, the structure of DNA, and DNA as the molecule of heredity (1940's and 50's), microbes have been used extensively to explore the structure, function, regulation, and evolution of genes. Moreover, microbial genetics is essential for understanding molecular biological studies, the manipulation of eukaryotic organisms, and for practical applications (biotechnology) in diverse areas of life sciences.

Biology and microbial genetics are now in an exciting era of "genomics" and "post-genomics." Complete genome sequences (genetic blueprints) are being solved at astonishing rates and these hold enormous potential for expanding our understanding of life. In this course, we will discuss the structure, function, expression, and evolution of microbial genes and methods for their study and manipulation. Topics include microbial genomes and their evolution; gene discovery, identification, and mapping; mutation; DNA repair; gene transfer among organisms; plasmids; transposable elements; genetic recombination; and gene regulation. We will also discuss molecular genetic strategies or concepts including gene cloning, polymerase chain reaction (PCR) and quantitative PCR, hybridization techniques, microarrays, 'proteomics,' 'metabolomics,' uses of gene expression, directed mutagenesis, gene fusions, 'reporters,' probes, and emerging technologies such as 'Next Generation' DNA sequencing strategies.

Throughout the course, we will discuss research and review articles related to microbial genetics. Our goal is to gain experience in 1) reading and evaluating scientific articles, 2) uses of genetic methods to investigate biological problems, 3) exciting, current topics in microbial genetics, and 4) understanding the role of microbial genetics and molecular biology in the advancement of science and society.

Grading and Requirements

Journal article reports	6 reports @ 10 points each. *(may submit 2 more for extra credit)*	60 points
Genome analysis, gene manipulation, gene expression exercises	2 due Oct 4 and Nov 3. (50 points each). *(Additional exercises may be given for extra credit. Graduate students will complete one additional assignment due Nov 18.)*	100/(150)
MIDTERM EXAM 1	week of October 6	150
MIDTERM EXAM 2	week of November 10	150
Graduate student presentations	week of December 6	(50)
FINAL EXAM	December 8 – 16 (due December 16)	150
Total (undergraduate/graduate)		610/(710) points

**Parts of the exams may be given independently in the form of separate assignments.*

Journal Article Reports: To encourage exploration of current topics, students will be required to read journal articles related to microbial genetics and write brief reports on these **(*no more than 1 page each*)**. Six reports are required with up to two additional for extra credit. These reports should describe the **objective** of the study, the **methods** used, and the main **conclusions** of the work. We may use some of these articles for class discussion. Additional instructions will be given.

Literature Discussion/Analysis: Usually one or more papers per week (from *Nature*, *Science*, *Journal of Bacteriology*, *Molecular Microbiology,* or other sources) will be assigned for class discussion. ***Students are expected to read these papers ahead of class and should be prepared to summarize and discuss them in class.*** Students will not be expected to, and may not, fully understand these papers ahead of class but you can improve your grade by participating actively and asking questions.

Grading Policy: 90-100% =A, 80-90% =B, 70-80% = C, 60-70% = D, less than 60%=F. Grades of A^+, A^-, B^+, B^-, C^+, C^-, D^+, and D^- will be used, at the discretion of the instructor, for borderline scores. For example, scores within 2% of a grade cutoff will be designated minus or plus grades (e.g. 90-92 = A^- and 88-89 = B^+). If the class scores on particular exams or assignments are uniformly low, grades may be adjusted accordingly. Exams will consist of definition, problem, and discussion questions. Exams will typically be 'open-book' and 'take-home.' Undergraduates will be graded separately if graduate student scores are consistently higher.

Graduate Students (depending on their prior experience) will be expected to show a somewhat greater understanding of the material, complete some additional assignments as outlined above, and may be asked to answer additional questions on assignments or take-home exams.

Presentations: Graduate students will give 20 minute presentations on selected current topics. These may be related to journal article reports. Presentations will be optional for undergraduates as time allows.

Late Work: Late work will receive no more than 90% of full credit unless arranged in advance.

Attendance Policy: Students are responsible for obtaining class materials, completing exercises, and meeting requirements. Because this is an advanced course with a small class size, regular attendance is expected to maintain class progress and discussion. Advance notification of absences is expected.

Academic integrity: We operate under the principle of "academic integrity" expected at this university. UW System guidelines state: "*Students are responsible for the honest completion and representation of their work, for the appropriate citation of sources and for respect of others' academic endeavors*." (s. UWS 14.01, Wis. Adm. Code). Cheating or obstruction of the efforts of others will not be tolerated in any form. Students caught cheating will receive an F grade on the exam or assignment and may be subject to further disciplinary action. ***Note in particular that this honor system applies during take-home exams and assignments. Please do not be tempted to***

represent the work of others as your own. This constitutes cheating (plagiarism) and will be treated as described above.

Topics and Schedule:

Week	**Topic**	**Text chapters,** suggested but not limited to:
1-3 Sept 8>	• Introduction & historical perspective. • ***A central theme: How do we identify genes & their function?*** • Genetic nomenclature. • Review of DNA structure • Introduction to genome sequences, genetic & genome databases, & genome analysis. ***The crucial role of bioinformatics!*** • Polymerase chain reaction (PCR) & gene cloning via "5'-add-on" PCR. ***Genome assignment no. 1: Gene identification & cloning via PCR*** • DNA & genome sequencing strategies. • ***The new revolution in DNA sequencing: '454' pyrosequencing & other 'Next Generation' sequencing technologies*** • Review of classical genetic concepts in microbial genetics: complementation, recombination, & mapping.	Introduction (p3-11), Ch 1 Box 1.4, 1.5, Box 2.7 Ch 3 Ch6(320-332)
3-5 Sept 20>	• Structure and replication of DNA. ***How do we know that DNA is the genetic material?*** • The basis for molecular genetics: ***DNA duplexes, melting, reannealing, & the activity of enzymes that bind DNA*** • Review of molecular genetic techniques: restriction analysis, gel electrophoresis, DNA & RNA hybridizations, melting curves, cutting & joining DNA, & gene cloning strategies.	Ch 1
	• (RNA, transcription, translation, protein folding, & membrane proteins. Review mostly on your own)	Ch 2
5-6 Oct 4>	• 'Post-genomic' analyses: ***global gene expression studies via microarrays*** • Spotted vs. oligonucleotide synthesis and 'tiling' arrays • Emerging technologies: Global gene expression studies via ***'deep mRNA sequencing'*** • ***Real-Time, quantitative PCR (qPCR) & reverse transcriptase quantitative PCR (RT-qPCR) for gene expression studies***	Ch13 (p602-607)
	• **MIDTERM EXAM 1 (take-home: Oct 6 – Oct 13)**	
6-7 Oct 11>	• ***Mass spectrometry and 'proteomics'*** as a way to identify gene products and study gene function • *(Possible introduction to 'metabolomics' as a way of assessing the consequences of gene function)*	Ch13 (p602-607)

Week	Topic	Chapter
7-8 Oct 18>	• ***Mutation, DNA repair, and evolution*** • Mutation & DNA repair • Mutagenesis • ***Mechanisms of genome and microbial evolution***	Ch 3 Ch 11 Box 11.1
8-9 Oct 25>	• ***Extra-chromosomal and moveable elements:*** Plasmids: gene cloning and *in vitro* mutagenesis	Ch 4 Box 4.1, 4.4
10 Nov 8>	• ***Gene Transfer:*** Impact on microbial evolution & basis for classical mapping and mutation analysis, • ***Conjugation and conjugative plasmids***	 Ch 5 Box 5.2
	• **MIDTERM EXAM 2 (take-home: Nov 10 – Nov 17)**	
	• ***Midwest-Southeast Photosynthesis Meeting, Nov 11 - 13***	
11 Nov 15>	• ***Gene Transfer:*** • ***Transformation:*** physiological and artificial • ***Transduction and bacteriophages***	 Ch 6 Ch 7, 8 Box 8.3
12 Nov 22>	• ***Moveable genetic elements*** • Transposons, 'illegitimate' recombination, & site-specific recombination • Plasmids and transposons as tools • Microbial introns, retrons, and inteins	 Ch 9 Box 9.2 Box 2.6
	• ***Thanksgiving Break! (no classes Wed, Nov 23 – Sun, Nov 27)***	
Nov 29	• ***Homologous recombination***	Ch 10 Box 10.1-10.3
13-14 Dec 6>	• ***Regulation of gene expression & responses to changing environments*** • Operons, repressors, activators, & paradigms of gene regulation • Global regulatory mechanisms • Regulatory cascades, two component sensors, sensor-kinases & response regulators, enhancers & silencers • ***Regulatory RNAs*** • *Global gene expression studies, further discussion of microarrays, proteomics & new technologies?*	Ch 12 Ch 13, 14 Box 13.1-13.5
13-14 Dec 6> & throughout semester	• Special topics: Genetic analysis of bacteria, strain construction, gene fusions & ***genetic reporters***. Synthetic genes & genomes, *in vitro* genetic manipulations, ***final discussions, & late-breaking news!***	
13 (Dec 6)	• ***Graduate student presentations of selected topics***	
14	• **FINAL EXAM (take-home: Dec 8 – Dec 16)**	
	• *End of semester celebration, Fratello's outing! (Dec 16)*	