

MICROBIAL GENETICS LAB (BIO-377/577)

FALL 2011

- Place and Time:** Wednesdays 1:50 – 5:50.
Lab discussions: HS 310, Wednesdays, 1:50 to ~2:40.
Lab rooms: HS 240, 163 (Bioseparations-Proteomics lab), and 165. Most of the work will be done in the HS 240 Biotechnology Lab, which will be available for ongoing experiments.
- Instructor:** Dr. Toivo Kallas
- Office:** Halsey 245 (phone 424-7084; e-mail: kallas@uwosh.edu)
webpage: http://www.uwosh.edu/faculty_staff/kallas
- 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).
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Lab manuals:

Kallas, T. 2003 (under revision). *Experiments in Microbial Genetics*, UW-Oshkosh. Pending revision of the lab manual, we will use the 2003 Lab Manual and handouts as needed. The 2003 manual and an appendix of commonly used protocols are posted on the D2L site.

(recommended): Miller, J.R. 1992. *A Short Course in Bacterial Genetics: Lab Manual*, Cold Spring Harbor Laboratory Press. Provides background information and protocols for *in vivo* experiments with *Escherichia coli*.

Other materials:

Important -- Lab notebook for recording ongoing **objectives**, **results**, and **conclusions** of experiments. USB memory stick for saving computer data files.

Locks & pipetors:

A set of 3-4 micro-pipetors will be issued to each lab group together with drawers and locks. Each group is responsible for returning their pipetors in good condition at the end of the semester. Students will be responsible for repair or replacement of damaged or lost pipetors. Replacement costs are \$200 - \$250.

Desire2Learn (D2L) Site:

Materials including the lab manual and supplementary protocols will be posted on the class D2L site (**Microbial Genetics Lab Bio-377/577**). The lecture course D2L site (Microbial Genetics, Bio 375/575) will also be accessible to lab students. To access, go to the UW Oshkosh home page, > then click, "D2L, Desire2Learn." On the **D2L** login page, enter the username and password that you use for UW Oshkosh e-mail.

Other useful microbial genetics laboratory references:

Miller, J. H. 1972 *Experiments in Molecular Genetics*, Cold Spring Harbor Laboratory, NY.

Miller, J. H. 1992 *A Short Course in Bacterial Genetics: Handbook*, Cold Spring Harbor Laboratory.

Sambrook, J and Russell, D. 2000 *Molecular Cloning: A Laboratory Manual* 3rd ed., Cold Spring Harbor Laboratory.

Primrose, S. B. 2007. *Principles of Gene Manipulation and Genomics*. Blackwell, Oxford.

Some genetics and other resources on the internet:

1. Class D2L site, described above.
2. American Society for Microbiology (ASM) home page: <http://www.asmtusa.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 Encyclopedia 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:

1) To provide “hands-on” experience in the investigation and manipulation of bacteria and their genes. 2) To develop the ability to think critically and devise genetic strategies that might be used to address interesting biological problems.

A variety of experimental approaches will be introduced. Most experiments will use *Escherichia coli*, which is used in some stage of virtually every molecular genetic investigation and genetic engineering application of prokaryotic or eukaryotic organisms. Some experiments will use the cyanobacteria *Synechococcus* PCC 7002 or *Synechocystis* PCC 6803. Cyanobacteria perform ~25% of global photosynthesis, they produce oxygen for life on earth, remove CO₂ from the atmosphere, and hold great potential for biofuels applications.

Experiments will include chemical and transposon mutagenesis, analysis of mutants, gene transfer, and strain construction. Molecular genetic procedures such as chromosomal and plasmid DNA isolation, DNA amplification by standard or quantitative polymerase chain reaction (PCR or qPCR), hybridization, gene cloning, and restriction site analysis will also be introduced.

Students will have the latitude to pursue selected topics in greater depth as “mini-projects.” All students will work on one of the mini-project options or on a mini-project of their own design. Mini-project options include construction of “knockout” or site-directed mutations to investigate specific genes, reverse-transcriptase, real-time quantitative PCR (RT-qPCR) for expression analysis of selected genes, and possible microarray hybridizations for global gene expression analysis.

Graduate students will be expected to devise a detailed experimental protocol that could be used to construct a useful bacterial strain, a recombinant DNA molecule, or answer a specific biological question. All students are expected to submit an outline of major steps to be performed and materials needed for their miniprojects.

Experiments & projects: We will begin the semester with a couple of classical, *in vivo* bacterial genetics experiments (chemical mutagenesis with ethylmethane sulfonate, EMS, and transposon mutagenesis with a Tn10 derivative) designed to generate large collections of mutant bacteria. Because “**Mutations Define Genes,**” these classical approaches are still extremely useful, particularly when combined with molecular strategies. Students wishing to gain further experience with *in vivo* gene manipulations will have the option of pursuing further experiments in this area. Otherwise, students will subsequently work on molecular genetic experiments (such as characterization of Tn10 insertions in plasmids from the above transposon mutagenesis experiments) and then for the remainder of the semester on one of the mini-projects listed below, or possibly on a mini-project of their own design.

Some mini-project options:

1. Construction of cyanobacterial “knockout” mutations. We will discuss the construction of knockout mutations to investigate gene/protein function. Several interesting PCR-based methods (e.g. Frigaard et al., 2004 *Methods in Molec. Biol.*) are available for generating knockouts without cloning. It would be interesting to test these methods. This project would involve selection of one or more target genes, design of PCR primers, and construction of PCR products that contain an antibiotic-resistance cassette (e.g. for Km-resistance) inserted into the coding regions of the selected genes. These knockout constructs would be introduced into the cyanobacterium *Synechococcus* PCC 7002 by transformation. If time allows, the potential knockout cyanobacterial strains would be tested for integration their of the inactivated genes and their possible consequences.

2. Site-directed mutagenesis of redox-regulation genes. Directed mutagenesis is a cornerstone of modern genetic analysis designed to probe regulatory networks and protein structure-function in biological processes. We have successfully used the Invitrogen “Quick Change” PCR-based mutagenesis strategy that allows construction of specific mutations in genes carried on any plasmid. In this experiment, students would design primers and create mutations in a cyanobacterial electron transport gene that has been implicated in redox signaling. The modified plasmids would be characterized by DNA sequencing and, if time allows, introduced into cyanobacteria for replacement of the native gene with the mutant allele.

3. Construction of an isiA-YFP or SidA-YFP reporter construct for investigations of iron-starvation in cyanobacteria. We have nice plasmid, pAQ1Ex-PcpB-YFP, that contains a gene (YFP) for a yellow fluorescent protein under the control of a strong, light-harvesting protein (pcpB) promoter. This project would involve amplification by PCR of the promoter region of a gene (*isiA*) that is strongly expressed under iron-starvation conditions and replacement of the pcpB promoter in the plasmid to generate a new plasmid, pAQ1Ex-PisiA-YFP. The pAQ1Ex plasmids (constructed by Yu Xu and Don Bryant, Penn State) are designed to target gene constructs into a high copy number plasmid, pAQ1, of the cyanobacterium *Synechococcus* PCC 7002. The PisiA-YFP region would be introduced into the cyanobacterium by transformation to generate a strain in which iron starvation can be easily detected and quantified by YFP fluorescence.

4. Reverse-transcriptase, quantitative PCR (RT-qPCR) analysis of gene expression. We have a very nice instrument (ABI StepOne) for Real-Time quantitative PCR, which allows the quantitative detection of DNA sequences (e.g. from medical or environmental samples) or RNA transcripts. RT-qPCR is often used to confirm selected microarray gene expression data or independently for the detailed analysis of selected genes. We would select a few *Synechococcus* genes, perhaps in conjunction with data from a microarray experiment, for detailed analysis. This experiment might also involve the design of "TaqMan" primers and probes (to be discussed) for highly specific qPCR or RT-qPCR.

5. RT-qPCR analysis of gene and/or plasmid copy number in a cyanobacterium. We are involved in a project to produce isoprene, a valuable chemical feedstock and biofuel precursor, in the cyanobacterium *Synechococcus* PCC 7002. A question of interest is the copy number of introduced or over-expressed genes designed to promote isoprene synthesis. This can be addressed by qPCR. A related question has to do with the copy number of a large plasmid, pAQ4, that carries interesting stress response genes but that appears to be lost from some strains of *Synechococcus*. The presence of this plasmid and its copy number can be addressed by qPCR.

6. Oligonucleotide expression array analysis of global gene regulation. Oligonucleotide microarrays are made by massive, parallel, "on-chip" synthesis of tens of thousands of "probes" consisting of short (e.g. 60 base) DNA sequences. Through the UW Madison Biotechnology Center and NimbleGen, we have two "generations" of oligonucleotide expression arrays for the cyanobacterium *Synechococcus* 7002. Each array carries 72,000 probes. Each of the ~3000 predicted genes in the genome are represented for most genes by either 3 or 7 probes per gene repeated 3 times per array. Upstream, untranslated regions of selected genes are covered by high-density probes. These allow the identification of transcription start sites and operons and thus regions important in gene regulation. In this experiment we would isolate RNAs from a control cyanobacterium and a mutant cyanobacterium, e.g. one with a defect in photosynthetic electron transport. These RNAs would be converted to cDNAs, labeled, and hybridized against the arrays. As time allows, these data would then be analyzed to pick out sets of genes that are differentially expressed in the mutant relative to the control. Ultimately, this work can provide insight into responses of cyanobacteria to environmental signals, perturbations in electron transport and responses to oxygen radicals.

Additional mini-project options are described in the lab manual and in a handout to be discussed later.

GRADING AND REQUIREMENTS:

Students will work collaboratively in groups of two or three. ***Master's students or advanced undergraduates may be asked to serve as team leaders/mentors for less experienced students.*** Requirements are listed below. Most of the grade will be based on lab reports handed in individually by each student (including 10% for successful results). The main criteria for grading will be clarity of presentation and understanding of procedures and concepts. Two small and one mini-project lab reports are required. The first report may be re-submitted for a higher score. Additional reports may be used to replace low scores.

Lab notebooks: Students are expected to maintain a detailed laboratory notebook where objectives, results, and conclusions (successes and failures) of experiments are recorded. Lab

notebooks may be reviewed midway during the semester. Up to ~10% of the grade may be based on lab notebooks.

Grading:

| | | |
|---|---|-----------|
| Lac and GFP genotype tests & predictions | due September 14 | 10 |
| Lab report #1: Mutagenesis with EMS | due September 28 | 100 |
| Undergraduate students: Mini-project outline | Preliminary, Sept 28 Final, October 19 | 50 |
| Graduate students: Project protocol | Preliminary, Sept 28 Final, October 19 | (100) |
| Lab report #2: Transposon mutagenesis | October 26 | 100 |
| Mini-project lab report | December 14 | 200 |
| Project presentation | December 14 | 50 |
| Lab notebook and lab maintenance | | 50 |
| Total | | 560/(620) |

Lab reports: Guidelines for lab reports are included in the lab manual as are questions specific to each experiment. Target dates for lab reports are shown in the schedule. *All lab reports are due by the last day of the semester. Late assignments will be marked down progressively.*

Grades: 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. 10% of the grade will be based on maintenance of clean and safe conditions in the lab.

Graduate students: are expected to submit a detailed experimental protocol as described above, take the lead on an independent “mini-project,” show a more comprehensive understanding of the material, and answer additional questions in lab reports.

Attendance: Students are required to attend the weekly discussion meetings. Advance notice of unavoidable absences is expected. Lab rooms will be accessible at all times for ongoing work.

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.***

Tentative schedule

The starting dates of experiments or "mini-projects" are shown. "Mini-projects" as well as experiments will typically take several weeks to complete and consist of several sequential components. These experiments do not require constant attention and it may be possible to perform parts of more than one concurrently with the aid of your lab partners. The actual schedule will depend on class and individual interest. I encourage students to seek me out to discuss the progress of experiments and interpretation of results.

| Week | Experiment | Description |
|----------------|---|---|
| 0-1 Sept 7> | 1. (required) (handout & Miller-3, p 135) | Mutagenesis with EMS. Mutagenesis of an <i>Escherichia coli</i> strain expressing a jellyfish green fluorescent protein (GFP) to obtain auxotrophic, fluorescent, and lactose repressor mutants. |
| | 2. (optional) (Miller-6, p157) | Screening for nonsense mutations. Use of nonsense suppressor strains of <i>E. coli</i> to screen for nonsense mutations in the lactose repressor gene. |
| 2 Sept 21> | 3. (required) (handout & Miller-27, p343) | Transposon mutagenesis. Use of transposon Tn10 (carried on a suicide bacteriophage) to obtain "knockout" mutations in <i>E. coli</i> plasmids carrying genes for the lactose operon and the green fluorescent protein (GFP). |
| 3 Sept 28 | | Preliminary mini-project outlines or protocols due Lab report #1 due |
| 3-4 Oct 5> | | Complete transposon mutagenesis experiment |
| 5 Oct 19> | | Mini-project outlines or protocols due Begin work on mini-projects: for example |
| | 3b. (mini-project option-1, see lab manual) TK-1 | Molecular analysis of transposons in plasmid DNA. Isolation of plasmids containing transposon insertions. Restriction and possible sequence analysis to identify location of transposon insertion. |
| | 4. (mini-project option-2) (Miller-28,) | Or, Transposon insertions near a gene of interest. Use of the pool of transposon insertion from experiment 3 and bacteriophage P1 transduction to obtain a transposon tag near a gene of interest. |
| | | Or, one of the mini-projects described above. |
| | | Or, a student designed mini-project of your own. Design a protocol for an experiment that addresses a specific question in microbial genetics, or that would allow the construction of a useful strain or plasmid – then work on this project! |
| Nov 11 | | <i>No class: Fri. Nov. 11 (Midwest Photosynthesis Meeting, Nov. 11-13)</i> |
| 11 | | <i>November 23-27, Thanksgiving break!</i> |
| 8-14 | | Mini-projects and completion of work in progress |
| 14 | | December 14: Mini-project presentations |
| 14 | | Mini-project reports due |
| | | <i>End of semester celebration, Fratello's! (Dec 16)</i> |