Biotechnology lab (Bio-390/590, 2 credits)
Spring 2013

Place and time: Wednesdays 1:50 – 5:50.
Lab discussions: Wednesdays, 1:50 for ~ 30-50 minutes, HS 237
Lab rooms: Most of the lab work will be done in the *HS 240 Biotechnology Lab & the HS 163-145 Bioseparations-Proteomics Labs*. Students will have access at all times via keypads.

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: M 1:50 – 3:50, Tu 3:30 – 5:30. 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 Bioseparations-Proteomics Labs).
(S.A.F.E. trained – all students are welcome.)

Lab manual. Kallas, T. 2013. *Experiments in Biotechnology* – currently under revision. Pending revision, we will use the 2003 manual and supplementary protocols as needed. These and a Lab Manual appendix of commonly used protocols are posted on D2L.

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.

Some useful Biotechnology lab references

(Note that many useful resources are available via web sites such as those listed below.)

1. Primrose, S. B. and Twyman, R. M. 2006, 2012. *Principles of Gene Manipulation and Genomics*. Blackwell, Oxford.
2. Sambrook, J and Russell, D. 2000. *Molecular Cloning: A Laboratory Manual* 3rd ed., Cold Spring Harbor Laboratory.
3. Veenstra, T. D. and Yates, J. R. 2006. *Proteomics for Biological Discovery.* Wiley.

Desire2Learn (D2L) Site. Materials including the lab manual and supplementary protocols will be posted on the class D2L site (**Biotechnology Lab, Bio-390/590**). The lecture course D2L site (Bio 390/590) will also be accessible to lab students. Go to the UW Oshkosh home page, > "D2L, Desire2Learn." On the **D2L** login page, enter your username and password for UW Oshkosh e-mail.

Some Biotechnology Resources, Websites

1. **Class D2L site**, described above.
2. **American Society for Microbiology (ASM)** home page: http://www.asmusa.org.
3. **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.)
4. **ExPASy Molecular Biology Server:** http://www.expasy.ch/. (A very useful site for molecular biology, genomics, and proteomics included predicted peptide mass fingerprints.)
5. **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.)
6. 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.
7. **TIGR** (The Institute for Genomic Research): http://www.tigr.org.
8. **Kazusa** Genome Research Institute: http://www.kazusa.or.jp. (Includes the database for the genome sequence of the cyanobacterium *Synechocystis* PCC 6803.)
9. ***E. coli* Genome Center**: http://www.genetics.wisc.edu:80/index.html
10. **Human Genome Research Institute**: http://www.genome.gov/
11. **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).
12. **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.)
13. **SWISS-PROT**, University of Geneva, Switzerland: http://expasy.hcuge.ch/sprot/sp-docu.html (Site from which to download the Swiss-PDB viewer program for protein 3D structures).
14. **Frontdoor to PROTEIN EXPLORER**: http://molvis.sdsc.edu/protexpl/frntdoor.htm (Site for online use of the PROTEIN EXPLORER program for protein structure viewing & manipulation).
25. **SINCRIS** information server for crystallography: http://www.lcmp.jussieu.fr/sincris-top/ (A nice site for information and access to programs and databases for viewing and manipulating biomolecules).
16. **Webcutter** (a site for on-line restriction site analysis): http://www.firstmarket.com/cutter/cut2.html
17. **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
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. ***Nature Biotechnology*:** http://www.nature.com/nbt/, (available on-line via Polk Library)
22. ***Trends in Biotechnology***: http://www.trends.com/tibtech/default.htm (via Science Direct, Polk Libr.)
23. **New England Biolabs**, Restriction Enzyme Database (NEB-REB): http://rebase.neb.com.
24. **Promega** Corporation (Madison, WI): http://www.promega.com/.
25. **UW-O (Polk) Library**: http://www.uwosh.edu/library/ (Polk Library provides access to a variety of literature search databases and carries on-line, full-text subscriptions to several relevant journals including *Science, the Nature Journals* (including *Nature* and *Nature Biotechnology, Trends Journals* via Science Direct, and the *American Chemical Society (ACS) Journals*. Follow on-screen instructions or see me.

Course objectives:

1) To gain practical, 'hands-on' experience in techniques of cell culture, product recovery, gene manipulation, and data analysis that are fundamental to many areas of biotechnology. 2) To gain experience in critical thinking and experimental design that may be used to address interesting problems in biology or biotechnology. Topics may include: analysis of DNA sequence databases, DNA amplification by polymerase chain reaction (PCR), gene cloning, gene modification, and expression techniques, cell culture, protein production and purification, DNA and protein gel electrophoresis, protein identification by MALDI-TOF (matrix assisted laser desorption ionization, time of flight) and/or ESI (electrospray ionization) LC-MS/MS (liquid chromatography, tandem) mass spectrometry, and possible DNA sequencing. Students will be able to pursue topics of choice in greater depth as 'mini-projects.' Students will work on one of the mini-project options or on a mini-project of their own design. Students will devise an experimental protocol related to their mini-project.

Experiments & projects. We will begin the semester with an experiment to introduce a plasmid (pOSH37/GFP, which encodes an engineered 'fusion' protein containing parts of the proteins thioredoxin, the jellyfish Green Fluorescent Protein, and an iron-sulfur protein), into a bacterial expression strain (*E. coli* AD494-DE3) so that we can 'overproduce' the fusion protein. We may also introduce the same plasmid into *E. coli* host, ScarabXpress-T7lac (www.scarabgenomics.com) to compare the expression of the TRX-GFP-ISF fusion protein in these two hosts. ScarabXpress-T7lac is supposed to be a leaner and 'sportier' strain for protein expression. We will then purify and analyze the protein, by protein gel electrophoresis (SDS-PAGE) and MALDI or ESI mass spectrometry. The GFP tag provides a nice visual (fluorescent!) marker for tracking the protein. Subsequently, students will work on one of the following mini-projects or a project of their own design. Additional mini-project options will be provided separately.

1. Genome analysis, gene-construction, and protein expression

Objective: To express a gene of interest from a genome sequence database (e.g. cyanobacterial or *E. coli* databases). Students may choose a gene for a protein of their choice, design PCR primers for amplification of these genes, order the primers, use these primers to amplify the gene, clone the PCR product into a plasmid vector (e.g. pET32a, Novagen, Madison, WI), and express the cloned gene to produce the desired protein. If the cloned gene is fused to the thioredoxin (TRX) gene on plasmid pET32a, metal-chelating affinity chromatography may be used to purify the fusion protein (if it is soluble), and the protease thrombin may be used to separate the protein of interest from the thioredoxin affinity tag. Students may further characterize the recombinant plasmids by sequencing and analysis of the protein product. ***All students are expected to complete the computer/database/bioinformatics segment of this project.***

2. Overproduction and purification of recombinant *Thermus aquaticus* or *Pyrococcus furiosus* thermal-stable DNA polymerases

Objective: To grow a fermenter culture of an *E. coli* strain genetically engineered to overproduce one of these DNA polymerases, induce expression of the plasmid-borne polymerase gene, purify by FPLC (Fast Protein Liquid Chromatography), and test the polymerase in a PCR reaction. The purified protein may be characterized in a variety of ways including MALDI mass spectrometry.

3. Oligonucleotide array analysis of cyanobacterial global gene expression

Objective: To discover genes and regulatory elements involved for example in adaptation to specific growth/environmental conditions or other parameters. Through the UW Madison Biotechnology Center and Roche-NimbleGen, we have designed and synthesized 4-plex oligonucleotide arrays for the marine, coastal cyanobacterium *Synechococcus* PCC 7002. Each slide contains four 0.5 x 1.0 cm microarrays each containing ~72,000 probes. In **array design 1**, most of the ~3000 genes in the *Synechococcus* genome are covered with 7 probes repeated three times on each array. In addition

there are ~6000 high-density "tiling" probes covering upstream untranslated (UTR) regions of ~200 genes of interest to map transcription start sites and to define operons. In **array design 2**, most genes in *Synechococcus* are covered with 3 probes repeated three times on each array, but high-density UTR probes are included for all of the predicted genes.

Example 1 (Electron transfer mutants): RNAs might be isolated from wild type *Synechococcus* and electron transfer mutants grown under selected conditions. Mutants PetB-R214H and PetC1-Δ2G both slow electron transfer but only PetB-R214H overproduces oxygen radicals. Another mutant, NdhF, is defective in NAD(P)H dehydrogenase. These RNAs would be converted to cDNAs, labeled, and hybridized against the microarrays. Subsequent work would involve exploring computer programs such as ArrayStar and/or Bioconductor "R" to analyze these data to pick out sets of genes that are differentially regulated in the mutants versus the control. *(Students in previous Microbial Genetics and Biotech labs have initiated such experiments – and obtained beautiful data.)*

Example 2 (Isoprene bioproducts): Matt Nelson has introduced codon-optimized isoprene synthase (*IspS*) and IDI isomerase (*IDI*) genes into *Synechococcus* 7002 and has engineered this strain to produce isoprene, a valuable feedstock chemical for synthetic rubber and liquid biofuels. This strain produces isoprene at a high rate but grows slowly. It would be very interesting to investigate the impact of *IspS-IDI* transgenes (foreign genes) on global gene expression in the *Synechococcus (IspS-IDI)* strain. These data could identify genes that are highly expressed in response to isoprene. The promoter regions of these genes could then be used to obtain inducible, high-level expression of isoprene synthase and other genes to maximize isoprene production.

4. Reverse-transcriptase, quantitative PCR (RT-qPCR) analysis of gene expression.

Objective: To investigate the expression levels of a few selected *Synechococcus* genes as a function of growth/environmental conditions or in mutants. We have a nice instrument (ABI StepOne) for Real-Time quantitative PCR (qPCR), which allows quantitative detection of DNA sequences (e.g. from medically or environmentally important samples) or RNA transcripts. RT-qPCR is often used to confirm selected microarray gene expression data or independently for detailed analysis of selected genes. We would select a few *Synechococcus* genes, perhaps in conjunction with preliminary microarray data, for detailed analysis. 'TaqMan' probes (to be discussed) would be used for these qPCR experiments. *(Several students in previous Biotech labs have done nice RT-qPCR projects to investigate selected gene expression patterns in cyanobacteria.)*

5. Biofuels connections, RT-qPCR analysis of MEP pathway gene expression.

Objective: To investigate the expression levels of selected 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway genes in the cyanobacterium *Synechococcus* sp. PCC 7002. As mentioned, we have introduced *IspS* and *IDI* genes into this cyanobacterium to produce isoprene. The precursor for isoprene, dimethylallyl diphosphate (DMAPP) is synthesized via the MEP pathway. Thus conditions that increase MEP pathway activity may lead to increased isoprene production. This project would involve RNA extractions from *Synechococcus* and 'TaqMan' primers and probes used in RT-qPCR experiments to track the expression levels of selected MEP pathway genes under different growth conditions. For example *Synechococcus* 7002 grows rapidly at high light intensity (full sunlight) and 100% CO_2 as well as under more moderate conditions.

6. Isotope-assisted mass spectrometry and protein expression analysis.

Objective: To discover proteins and regulatory mechanisms involved in adaptation to growth/environmental conditions such as exposures of cyanobacteria to high CO_2 or isoprene. This experiment might complement microarray experiments such as those described above. It would provide information about proteins that are differentially expressed in experimental versus control conditions. This is interesting because proteins do most of the actual work of cells and gene expression levels do not always reflect protein levels in cells. The experiment involves growing control

and mutant cyanobacteria separately in media containing light (N^{14}) and heavy (N^{15}) isotopes of nitrogen, harvesting and mixing these cultures, breaking and fractionating the cells, digesting the mixed proteins with trypsin, and subjecting the protein fragments (peptides) to liquid chromatography and tandem mass spectrometry (LC-MS/MS). For reasons to be explained, this allows both the identification of individual proteins from complex mixtures of proteins, and quantification of relative protein expression levels. *(Previous Biotech students have successfully initiated such experiments.)*

7. Inhibitors and photosynthetic electron transport pathways.

Objective: To investigate the impacts of recently synthesized inhibitors of photosynthesis on electron transfer reactions and gene expression in cyanobacteria. Several students have been involved in synthesis and tests of these inhibitors (collaboration with Drs. Brant Kedrowski and Linfeng Xie, Chemistry). These inhibitors (abbreviated NQNO, 4-1HQ-A, and 4-1HQ-B) act on sites in the cytochrome *bf* electron transfer complex, which is involved in solar energy conversion and signaling. Projects might include investigating the impacts of these inhibitors on electron transport (with our BioLogic JTS-10 kinetics spectrophotometer) and gene expression via RT-qPCR or microarrays.

8. Cyanobacterial mutants, electron transport activities, and gene expression.

Objective: To investigate the impacts of mutations in cyanobacterial electron transport or regulatory genes on electron transfer reactions and gene expression by RT-qPCR or microarrays. Students in recent Microbial Genetics or Biotech labs have constructed 'knockout' mutations of cyanobacterial electron transport or regulatory genes. These mutations may provide insights into energy conversion pathways and adaptation of cyanobacteria, which will be important for solar-energy driven production of bioproducts in these organisms. These projects might involve genetic construction of mutant cyanobacteria, characterizing the impacts on electron transfer reactions with the BioLogic JTS-10 spectrophotometer, and investigating impacts on gene expression by RT-qPCR or microarrays.

Examples of interesting mutants are SdhB (by Sheriff Otun and Brandon Brummeyer), YrdC (by Justin Zangl), GlgA1 (Brandon Thomas and Andrea Felton), GlgA2 (Ola Aremu), and ApcF (Sarah Black). SdhB is defective in succinate dehydrogenase, which may have important roles in electron transfer. YrdC is defective in a 'YrdC-like' regulator of CO_2 uptake and grows extremely poorly at low CO_2. GlgA1 and GlgA2 are defective in synthesizing the major glycogen carbohydrate storage polymer of cyanobacteria. ApcF is defective in a light-harvesting protein. For reasons to be explained, both the GlgA1-GlgA2 and ApcF mutants may increase the yield of bioproducts such as isoprene.

9. More biofuels connections, mutations that may increase isoprene production.

Objective: To investigate the impact of alternative, regulatable promoters, decreased light harvesting, and inactivation of competing carbon pathways on isoprene production. Several students in recent Microbial Genetics or Biotech labs initiated these studies. One project (You Yang and Ehren Snyder) involves modification of a plasmid to allow easy 'swapping out' of promoter sequences to test for increased and regulatable expression of *Isps* and *IDI* genes for isoprene production. A 'reporter' plasmid could also be constructed. We have a plasmid that carries a yellow fluorescent protein (YFP) gene. Promoter regions to be tested could be inserted ahead of the *yfp* gene. Promoter activity can then be assessed by the intensity of the YFP fluorescence.

CpcA phycocyanin light-harvesting protein mutant. A further very interesting mutant would be a *Synechococcus* CpcA 'knockout,' which would lack the major phycocyanin, light-harvesting complex of cyanobacteria. By knocking out most or the light-harvesting complex, cells would not shade each other, and should grow to a higher density and produce more isoprene per culture volume. If the CpcA mutant strain is constructed, it would then be interesting to test its growth, gene expression, and isoprene production.

Additional mini-project options are possible and encouraged! Further options are described in the lab manual and in the "mini-project ideas" 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 serve as team leaders/mentors for less experienced undergraduates.*** Requirements are listed below. Most of the grade will be based on lab reports handed in individually by each student as described below. The criteria for grading will be progress toward successfully meeting objectives, clarity of presentation, and understanding of procedures and concepts. Two small and one major (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. Notebooks may be reviewed midway during the semester. 10% of the grade may be based on lab notebooks.

Lab reports. Guidelines for lab reports are included in the lab manual as are questions specific to the pre-planned (non-project) experiments. 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 take the lead on an independent 'mini-project (such as those listed here), show a more comprehensive understanding of the material, complete the additional requirements listed above, and answer additional questions in lab reports.

Assignments	**Due date**	**Points**
Report: Exp #1, Transformation of *E. coli* with plasmid pOSH37/GFP and restriction analysis	February 22	100
Genome analysis-gene expression exercise (the same report may be used in both the lecture & lab classes)	March 1	50
Report: Exp #2, Expression and affinity purification of a GFP fusion protein from plasmid pOSH37/GFP.	March 22	100
Graduate students: Are expected to 1) work on identifying the TRX-GFP-ISF fusion protein or another protein by mass spectrometry or 2) complete a protein 3D structure analysis exercise	April 26	(50)
Mini-project outline and provisional protocol	March 1	50
Mini-project lab report	May 6	200
Project presentation	week of May 6th	50
Lab notebook and lab maintenance (to be determined)		
Total (undergraduate/graduate)		550/(600)

Attendance. All students are required to attend the weekly discussion meetings. Lab rooms will be accessible at all times for ongoing work. Students intending to work evenings or weekends need to obtain an after-hours pass from the Biology Office.

Academic integrity. Interaction and collaboration among students is encouraged but each student is responsible for submitting his/her own work. Students should be aware of the principle of "academic integrity" expected at this and other universities. The 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."* Cheating or obstruction of the efforts of others will not be tolerated in any form. Students caught cheating will receive an F grade and may be subject to further disciplinary action. ***Note in particular that this honor system applies during take-home assignments. Please do not be tempted to represent the work of others as your own.***

Tentative schedule of experiments (Spring 2013)

The starting weeks of group experiments and possible 'mini-projects' are shown. Mini-projects as well as standard 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 or Project	Components
1	**Exp #1: Transformation of *E. coli* with plasmid pOSH37/GFP and restriction analysis** Introduction of plasmid pOSH37/GFP into *E. coli* expression hosts. Selection of transformants by antibiotic resistance and green fluorescence. Confirmation of plasmid identity by plasmid isolation and restriction analysis. The *E. coli* (pOSH37/GFP) strain produced in this experiment will be used to produce the GFP fusion protein to be purified in Exp #2.	- Plasmid electrotrans-formation of *E. coli* - Plasmid isolation and analysis by restriction digestion and gel electrophoresis
2	**PROJECT #1 (option): Genome analysis/gene-construction/expression.** Analysis of sequence databases via the internet. Selection of an open-reading-frame (gene) for a protein of interest. Design of PCR primers, amplification, cloning, and expression of the gene. Analysis of recombinant plasmids by restriction digestion and DNA sequencing. Possible analysis of expressed proteins by gel electrophoresis (SDS-PAGE) and MALDI or ESI mass spectrometry. ***(All students will work on the database analysis portion. The actual lab portion is a mini-project option.)***	- Internet databases - primer design - Gene Construction Kit (GCK) software - PCR amplification - Gene cloning - Restriction analysis - Gene expression - SDS-PAGE analysis of protein production - DNA sequencing
4	**Exp #2: Expression and affinity purification of a GFP fusion protein from plasmid pOSH37/GFP.** Culture of *E. coli* (pOSH37/GFP, from exp #1 above), gene expression, production of the fluorescent GFP fusion protein, affinity purification, thrombin cleavage to separate the iron-sulfur protein segment, analysis by gel electrophoresis (SDS-PAGE), possible immuno-blotting, and possible MALDI or ESI mass spectrometry.	- Culture - gene & protein expression - Cell harvest & breakage - Affinity chromatography - Thrombin cleavage - SDS-PAGE (possible immunoblot) - Possible MALDI or ESI mass spectrometry

Week	Experiment or Project	Components
6	**PROJECT #2 (option): Overproduction and purification of recombinant, thermal-stable DNA polymerases.** Fermenter culture of *E. coli* expressing *Thermus aquaticus* or *Pyrococcus furiousus* DNA polymerases, enzyme purification by FPLC chromatography, and testing of polymerase activity in a polymerase chain reaction (PCR). Possible protein analysis by gel electrophoresis (SDS-PAGE) and MALDI or ESI mass spectrometry.	- Fermenter culture - Enzyme purification by FPLC - Functional (PCR) assay for polymerase activity - *Production of a ton of valuable Taq DNA polymerase!*
6	**PROJECT #3 (option): Oligonucleotide array analysis of cyanobacterial global gene expression:** Described above.	- Cyanobacterial cultures - RNA isolation - cDNA synthesis & labeling - Array hybridization - Data analysis, bioinformatics, & statistics
6	**PROJECT #4 (option): Real-Time quantitative PCR analysis of gene expression (RT-qPCR)** **or** **PROJECT #5 (option): Biofuels connections, RT-qPCR analysis of MEP pathway gene expression.**	- Cyanobacterial cultures - RNA isolation - Design of primers and 'TaqMan' probes for selected genes - RT-qPCR experiments - Data analysis
6	**PROJECT #6 (option): Isotope-assisted mass spectrometry and protein expression analysis:** Described above.	- Cyanobacterial cultures grown in N^{14}- and N^{15}-NO_3 - Cell breakage, separation of membrane & soluble proteins - Trypsin digestion - LC-MS/MS - Data analysis, bioinformatics, & statistics
6	**PROJECT #8 (option): Construction and analysis of cyanobacterial mutants.** Described above.	- Megaprimer PCR or 'Gibson Assembly' methods to construct knockout mutations
6	**PROJECT: Other mini-project options described above, in the mini-project handout, or possible student-designed mini-projects**	
9-14	Completion of work in progress ***End of semester celebration at Fratello's! (May 10)***	