Biotechnology lab (Bio-390/590, 2 credits)
Spring 2014

Place and time: Wednesdays 1:50 – 5:50.
Lab discussions: Wednesdays, 1:50 for ~ 30-50 minutes, HS 310
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. 2014. *Experiments in Biotechnology* – under revision. Pending revision, we will use protocols and flow charts for individual experiments, as well as the 2003 manual for reference 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 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.

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 Overview and Learning Objectives

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. During the latter half of the semester, students select a 'mini-project' of choice that they wish to pursue in greater depth. In these 'mini-projects,' students address a question or devise a procedure that may lead to a useful strain, bioproduct, or procedure. Students may chose mini-projects from the listed options or devise one of their own.

Graduate students will be expected to devise a more detailed experimental protocol that could be used to construct a useful bacterial strain, produce a useful bio-product, or answer a specific biological question by means of a 'biotechnology strategy.' All students are expected to submit an outline of major steps to be performed and materials needed for their mini-projects.

Experiments and 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 mini-projects described below, or a project of their own design. Additional mini-project options will be provided separately. Graduate students are expected to take an active role in designing a mini-project of their choice.

Undergraduate 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.

Undergraduate Assignments and Grading

Assignments	**Due date**	**Points**
Lab report 1 -- Transformation of *E. coli* with plasmid pOSH37/GFP and restriction analysis	February 26	100
Genome analysis, gene expression, gene fusion exercise (the same assignment may be used in both the lecture and lab sections)	March 5	50
Lab report 2 -- Expression and affinity purification of a GFP fusion protein from plasmid pOSH37/GFP	April 2	100
Mini-project outline and provisional protocol	March 19	50
Mini-project lab report	May 14	200
Project presentation	May 14	50
Lab notebook and lab maintenance		50
Total points		600

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.

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.***

Additional Information and Requirements for Graduate Students

Graduate students are expected to complete all assignments required of undergraduates as well as additional assignments as described below.

Graduate Student Assignments and Grading

Assignments	Due date	Points
Lab report 1 -- Transformation of *E. coli* with plasmid pOSH37/GFP and restriction analysis	February 26	120
Genome analysis, gene expression, gene fusion exercise (the same assignment may be used in both the lecture and lab sections)	March 5	50
Lab report 2 -- Expression and affinity purification of a GFP fusion protein from plasmid pOSH37/GFP	April 2	120
Graduate students will 1) use mass spectrometry to identify the TRX-GFP-ISF fusion protein or another protein or 2) complete a protein 3D structure analysis exercise	April 30	50
Mini-project outline and provisional protocol	March 19	50
Mini-project lab report	May 14	240
Project presentation	May 14	50
Lab notebook and lab maintenance		50
Total points		730

Graduate Student Grading Policy. 92-100% =A, 90-92% =A-, 88-90% =B+, 82-90% =B, 80-82% =B-, 75-80% =C+. Grades of C or lower are considered failing for graduate students. Grades may be curved if necessary.

Graduate students are expected to submit a detailed experimental protocol, submit an additional analysis assignment, take the lead on an independent 'mini-project,' show a more comprehensive understanding of the material, and answer additional questions on lab reports.

Graduate Student Objectives

1) Data Analysis and Critical Thinking. Special emphasis will be placed on the ability of graduate students to understand and interpret data and think analytically and critically about information necessary to understand and perform lab experiments. Graduate students are further expected to develop the ability to design experiments or develop procedures to address interesting problems in biotechnology or produce useful bio-products. To assess accomplishment of this objective, graduate students **will address additional questions in lab reports** and will **submit a detailed protocol describing the objectives, materials, and procedure they will use to address a problem in biotechnology or produce a useful bio-product.**

2) Clear and Logical Expression in Writing. Graduate students will be expected to write more clearly, logically and correctly than undergraduates. To assess accomplishment of this objective, **graduate students will be held to a higher standard on lab reports.** Their lab reports will be in the form of short, scientific papers that are clearly written and describe objectives or hypotheses tested, results found including necessary data tables and figures, and conclusions drawn. They will also **conduct an additional assignment and submit an additional report on either protein 3D or global gene expression analysis**. These assignments should show a more detailed understanding of the subject than those by undergraduates.

3) Clear and Logical Oral Presentation. Graduate students will be expected to clearly and logically present the objectives, results, and conclusions of their mini-project in an oral presentation. This objective will be **assessed by the quality of the graduate student oral presentation** of their mini-project research at the end of semester. This presentation should be well organized and logically presented. Graduate students are expected to show a deeper understanding of the topic presented, field questions, provide thoughtful answers, and in general will be held to a higher standard in their presentations than will undergraduates.

4) Ability to Synthesize Information and Develop Creative Work. Graduate students will be expected to use information discussed and experiments performed in the lab class, and/or found in the scientific literature, to synthesize and develop new and creative work (e.g. genetic strategies) to tackle important and interesting questions in biology. To assess accomplishment of this objective, **graduate students will develop a detailed experimental protocol** describing the objectives, materials, and procedure to be used to address a problem in biotechnology as described above. The ability to synthesize information and develop creative work **will be further evaluated in their oral presentation of the mini-project.**

5) Leadership. Graduate students will be expected to take a leadership role in the laboratory class. They will be **expected to lead a lab group and mentor undergraduate partners**, participate actively in class discussions, ask questions and show a deeper understanding of the concepts and procedures involved. They will be expected to **help undergraduates understand often difficult concepts and laboratory procedures.** To assess accomplishment of this objective, I will keep track of graduate student leadership and work with undergraduates. Assessment of such leadership activities may elevate or lower the final grade of graduate students, particularly those at the borderline between grades.

Some Mini-Project Options – Undergraduate and Graduate Students

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 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 (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. The first steps in these experiments – the rapid isolation of high-quality RNA – are the same as those for RT-qPCR. Students have successfully done microarray experiments in the past, but their feasibility depends on time commitment and also the availability of funds.

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.)*

Example: Bioproducts - Biofuels connections, RT-qPCR analysis of MEP pathway genes:
Objective: As mentioned, we have introduced *IspS* and *IDI* genes into *Synechococcus* PCC 7002 cyanobacteria to produce isoprene. The precursor for isoprene, dimethylallyl diphosphate (DMAPP) is synthesized via the 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway. Thus conditions that increase MEP pathway activity should 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. We have a photobioreactor that can be programmed for day-night and temperature cycles from which samples could be extracted for RT-qPCR analysis.

5. Quantitative PCR (qPCR) analysis of gene and/or plasmid copy number. Objective: One way to increase bioproduct yields is to increase the copy number of genes and mRNA transcripts in the metabolic pathway leading to the product. We are using this strategy to increase isoprene production in *Synechococcus* PCC 7002 cyanobacteria. We have targeted *IspS-IDI* genes (described above) both to a high copy plasmid (pAQ1) and to a chromosomal site (work of Ola Aremu). Thus an interesting question is the copy number of introduced or over-expressed *IspS-IDI* genes in different strains of these cyanobacteria. This can be addressed by qPCR. A related question is the copy number of a large plasmid, pAQ4, that carries interesting stress response genes but that is lost from some strains of *Synechococcus*. This plasmid is a potentially interesting site for targeting introduced transgenes and its presence and copy number can be addressed by qPCR.

6. Cyanobacterial knockout mutants, electron transport, gene expression, and bioproducts.
Objective: To investigate the impacts of mutations in metabolic pathway or regulatory genes on electron transfer reactions, gene expression, and/or bioproduct production. Students in recent Microbial Genetics or Biotech labs have constructed 'knockout' mutations of cyanobacterial electron transport, metabolic, or regulatory genes. Such mutations provide insights into solar energy conversion and metabolic pathways of cyanobacteria, which will be important for engineering production of bioproducts in these organisms. These projects might involve genetic construction of mutant cyanobacteria, characterizing their impacts on electron transfer reactions (with the BioLogic JTS-10 spectrophotometer), gene expression (by RT-qPCR), or the production of bioproducts such as isoprene (via Gas Chromatography – Mass Spectrometry or a real-time, Fast Isoprene Sensor).

Examples: Several very interesting PCR-based (e.g. Frigaard et al., 2004 *Methods in Molec. Biol.*) and 'Gibson Assembly' (Gibson and Russello, 2012 *NEB Expressions*) methods are available for generating mutations and gene modifications without the need for 'cut and paste' gene cloning. Examples of interesting mutants are SdhB (constructed by Sheriff Otun and Brandon Brummeyer), YrdC (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 very 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.

Many other knockout mutations would be interesting. For example, we now believe that a CpcB mutant, of the major phycocyanin, light-harvesting complex of cyanobacteria, would be more useful

than the ApcF light-harvesting mutant. By knocking out CpcB and 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. Andrea Felton has initiated work on a CpcB knockout. GlgC for ADP-Glucose pyrophosphorylase, and SpsA for sucrose phosphate synthase, would be other interesting targets, the inactivation of which should increase carbon flux to isoprenoid bioproducts.

7. Inexpensive, *ex vivo* method using *E. coli* extracts to assemble DNA fragments.

Objective: To test a potentially very interesting, recently reported method (Fisher et al., 2013 *Frontiers Bioengineering Biotechnology* 1, 12) that used *E. coli* cell extracts to efficiently assemble overlapping DNA fragments. If this method works well, it would accomplish the same thing that we can do with 'Gibson Assembly' reactions, but at a fraction of the cost. The goal would be to make a 'knockout' or other mutation, such as those described above, by this method and see how well this works compared to e.g. the 'Gibson Assembly' method.

8. More biofuels connections – gene expression regulation and isoprenoid 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 could involve 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 be assessed by the intensity of YFP fluorescence. Meghan Raebel has initiated this work.

Another interesting promoter and regulatory gene (that could be amplified by PCR from *Synechocystis* 6803) is a nickel (Ni) ion regulated promoter. It would be interesting to test such a construct in a YFP reporter plasmid and in *Synechococcus* 7002 cyanobacteria.

9. Synthetic gene construct for β-pinene synthesis in cyanobacteria. Objective: To achieve regulated expression of β-pinene, a precursor for jet fuel, which may, however, be toxic to the cyanobacteria. A set of codon-optimized genes (GPPS and mono-TPS) has been designed and synthesized to enable synthesis in *Synechoccoccus* 7002 cyanobacteria of β-pinene (work of Rhiannon Carr). To achieve pinene synthesis in the cyanobacterium, a constitutive or regulatable strong promoter needs to be integrated upstream of these genes and the gene construct needs to be targeted to either a plasmid or chromosomal site in *Synechococcus*. Versions on this theme would be the objectives of this project.

10. A gene to alleviate the vitamin B_{12} requirement of *Synechoccocus* 7002 cyanobacteria.

Objective: To eliminate the need for vitamin B_{12} for growth of *Synechococcus* 7002 and to develop a useful selectable marker. Vitamin B_{12}, needs to be included in the growth medium to enable methionine biosynthesis (the only step for which B_{12} is required in these cyanobacteria). The requirement for vitamin B_{12} can be alleviated by introducing a *metE* gene that encodes an alternative (non-B_{12} requiring) enzyme for methionine biosynthesis (Don Bryant, Penn State, personal communication). A *metE* gene from *E. coli* should work. This project would involve PCR amplification of a *metE* gene, inclusion of a cyanobacterial promoter, and targeting to a plasmid or chromosomal site (currently being pursued by Rhiannon Carr). The *metE* gene also opens interesting possibilities for use as a selectable marker.

11. 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.

Additional mini-project options are possible and encouraged! Further options are described in the lab manual and in a 'Mini-Project Ideas' handout to be discussed later.

Tentative Schedule – Undergraduate and Graduate Students

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	**Experiment 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	**Genome analysis, gene-expression exercise.** DNA sequence analysis and *in silico* plasmid construction via sequence databases and Gene Construction Kit software. Select a gene for a protein of interest. Design PCR primers to construct a plasmid that carries the gene and may be used to express its protein product. **Project options:** Actually order the PCR primers, amplify and clone the gene and express the encoded protein. Construct the recombinant plasmid and analyze by restriction digestion, PCR, and possible DNA sequencing. Possible further analysis of expressed proteins by gel electrophoresis (SDS-PAGE) and MALDI or ESI mass spectrometry.	- 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	**Experiment 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.	- Cell culture - Gene & protein expression - Cell harvest & breakage - Affinity chromatography - Thrombin cleavage - SDS-PAGE (possible immunoblot) - Possible MALDI or ESI mass spectrometry

7	**Project options: Production 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!*
7	**Project options: Oligonucleotide array analysis of cyanobacterial global gene expression:** Described above.	- Cyanobacterial cultures - RNA isolation - cDNA synthesis & labeling - Array hybridization - Data analysis, bioinformatics, & statistics
7	**Project options: Real-Time quantitative PCR analysis of gene expression (RT-qPCR)** **e.g. – Bioproducts connections, RT-qPCR analysis of MEP pathway gene expression for isoprene production.**	- Cyanobacterial cultures - RNA isolation - Design of primers and 'TaqMan' probes for selected genes - RT-qPCR experiments - Data analysis
7	**Project options: Construction and analysis of cyanobacterial mutants.** Described above.	- Megaprimer PCR or 'Gibson Assembly' methods to construct knockout mutations
7	**Project options:** Other mini-project options described above, in the mini-project handout, or possible student-designed mini-projects	
14	**Project presentations – May 14**	
9-14	Complete work in progress ***End of semester celebration at Fratello's! (May 16)***	