Principles of Biotechnology (Bio-389/589, 3 credits)

Spring 2014

Place and Time: Halsey 367: 9:40 – 11:10 TuTh

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 email. 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.)

Textbooks and Resources

Required:

1. Glick, B. R, Pasternak, J. J., Patten, C. L. 2010 *Molecular Biotechnology.* 4th ed. ASM. Washington D.C. *(This text will serve mostly as a background and reference book.)*
2. Much of the reading/discussion material for the course will come from journals such as *Nature Biotechnology, Trends in Biotechnology, Nature, Science,* and others. These and other readings will be posted on the class D2L site. Required and reference 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. Primrose, S. B. and Twyman, R. M. 2006, 2012. *Principles of Gene Manipulation and Genomics*. Blackwell, Oxford.
3. Glazer, A. N. and Nikaido, H. 2007. *Microbial Biotechnology.* Cambridge University 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 (**Principles of Biotechnology Bio-389/589**). 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 Biotechnology Resources, Websites

1. **Class D2L site**
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.
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 many 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

Biotechnology represents the adaptation and uses of biological processes for practical purposes. The roots of biotechnology date back to the dawn of civilization and agriculture. Modern biotechnology draws on all areas of life sciences, chemistry, engineering, and computer sciences among other fields and has relevance to research and applications in all of these. Our goal is to learn the principles of biotechnology and understand how these have led to numerous exciting applications. A central theme of the course is the discovery and production of useful bioproducts and their improvement through genetic and other means. Topics include methods of screening for novel bioproducts, principles of cell culture ('fermentation'), production and purification of bioproducts, and enzymatic 'bioconversions.' We will emphasize concepts and strategies of genetic engineering that allow DNA manipulations and introduction of modified genes into host organisms to yield genetically modified microbes, plants, animals, and novel bioprocesses. We will discuss ongoing revolutions in genomics, 'metagenomics,' proteomics, metabolomics, and bioinformatics that are having tremendous impacts on society, our understanding of living organisms, and the development of novel applications in biotechnology.

Throughout the semester, we will discuss research and review articles on topics in biotechnology. Our goals in this are 1) to gain experience in reading and evaluating scientific articles, 2) to gain insight into methods and research at the frontiers of biotechnology and 3) to learn about exciting developments in areas such as genomic DNA sequencing, global gene expression and proteomic studies, transgenic plants and animals, molecular probes, genetic diagnosis, environmental biotechnology, and metabolic engineering to produce bioproducts such as high-value chemical feedstocks and carbon-neutral biofuels.

Undergraduate Grading and Requirements

Assignment	Due date	Points
Journal article reports	6 reports @ 10 points each *(2 additional reports may be submitted for extra credit)*	60
Genome analysis - gene expression, gene fusion exercise	due March 4	50
Midterm Exam	March 14-21 (due March 21)	150
Protein 3D structure or microarray gene expression exercise	due May 1 (Optional exercise for undergraduates – may be used as one of the questions on the Final Exam)	(50)
Minireview	due May 6	100
Minireview presentations	Week of May 6th	50
Final Exam	May 9-16 (due May 16)	150
Total points		560

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

Journal Article Reports. To encourage exploration of topics in biotechnology, students will read be required to read six journal articles on topics of interest and write brief reports (***no more than 1 page each***). Two additional reports may be submitted for extra credit. Journal article reports should describe the **objective** of the study or questions asked, the **methods** used, and the main **conclusions** of the work. Some of these articles may be used for class discussion.

Literature Discussion/Analysis. One or more papers per week (from *Nature Biotechnology, Trends in Biotechnology*, *Nature*, *Science* or other sources) will be assigned for class discussion. ***Students are expected to read these papers ahead of class and be prepared to summarize or ask questions about these articles in class.*** Students will not be expected to fully understand journal articles ahead of class. However, to improve grades, students are encouraged to participate actively in discussions and ask questions. Other articles will be posted on D2L primarily for reference.

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.'

Minireviews and Presentations. Each student will write a minireview on a current topic in biotechnology. Minireviews should be 5-10 manuscript pages long (~250 words per page) and contain 20 or more references (no more than 10% may be internet references). Details and format for minireviews will be described separately. To share minireview findings, students (individually or in pairs) will give a 15-20 minute presentation on their minireview topic near the end of the semester.

Late Assignments. Work submitted after deadlines will receive no more than 90% of full credit unless arranged in advance.

Attendance Policy. Students are individually responsible for obtaining class materials, completing exercises, and meeting course 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 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.***

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 Grading and Requirements

Assignment	Due date	Points
Journal article reports	6 reports @ 10 points each *(2 additional reports may be submitted for extra credit)*	60
Genome Analysis - Gene expression, gene fusion exercise	due March 4	50
Midterm Exam	March 14-21 (due March 21)	170
Protein 3D structure or Microarray, global gene expression exercise	due May 1	50
Minireview	due May 6	100
Minireview presentation	Week of May 6th	50
Final Exam	May 9-16 (due May 16)	170
Total points		650

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

Graduate Student Grading Policy and Exams. 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. Exams will consist of definition, problem, and discussion questions. Exams will typically be 'open-book' and 'take-home.' Graduate students will answer two or more additional questions on exams. These will represent an additional ~10 - 20% of exam material relative to undergraduates.

Minireviews and Presentations. Graduate students will write a minireview on a current topic in biotechnology, as described above, and give an individual 15-20 minute presentation on their minireview topic.

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 that has been presented in class. To assess accomplishment of this objective, all **exams will contain 2 or 3 additional questions** for graduate students. These will be data analysis problems or questions that require critical assessment of new information related to topics covered in class.

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 exams and written assignments. They will be expected to **answer additional questions on exams** and **submit an additional written assignment** (on either protein 3D structure analysis or global gene expression analysis). Answers to exam questions and written assignments should be clearly written and show a more detailed understanding of the topic than those by undergraduates. Assignments, such as genome analysis assignments and the minireview on a selected topic, should be logically organized, use vocabulary correctly and show a superior understanding of the topic.

3) Clear and Logical Oral Presentation. Graduate students will be expected to clearly and logically present material orally. To assess accomplishment of this objective, graduate students will be expected to give an **individual oral presentation on a selected topic** in Biotechnology at the end of semester. This presentation should be well organized and logically presented. Graduate students are expected to show a good depth of understanding of the topic presented, field questions, and provide thoughtful answers.

4) Ability to Synthesize Information and Develop Creative Work. Graduate students will be expected to use information presented in class to synthesize and develop new and creative work or genetic strategies to tackle important and interesting questions in biology. To assess accomplishment of this objective, graduate students will complete an **additional analysis assignment** (on either protein 3D structure analysis or global gene expression analysis) and give an **individual oral presentation** on their minireview topic at the end of the semester. In these assignments, they will be expected to synthesize existing information (e.g. from scientific journals) and explore and/or present new or emerging findings or methods that may be used to address interesting biological problems and expand understanding.

5) Leadership. Graduate students will be expected to take a leadership role in the class. They will be expected to **participate actively in class discussions**, ask questions and show a deeper understanding of the material, and **help undergraduates understand fundamental as well as challenging concepts and assignments.** To assess accomplishment of this objective, I will keep track of graduate student participation 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.

Topics and Schedule – Undergraduate and Graduate Students

(Glick, Pasternak, Patten (GP), ASM 2010, is the main reference text. Some sections of Primrose & Twyman (PT), Crueger and Crueger (CC), and Glazer and Nikaido (GN) are listed for reference. Materials from these and other sources will be included in the Powerpoint presentations posted on D2L.

Week	**Topic**	**Text chapters,** suggested but not limited to:
1	**Introduction and course overview**	**1 GP,** 1-4 PT
1-2	**Review – basic and advanced genetic engineering & genome analysis techniques**	**2-4, 6-7 GP**
	• Genetic basis and history of gene cloning	**(review 2 GP)** **1, 3 GP**, 1-2 PT
	• Restriction & modification of DNA, cutting & joining DNA molecules	**3 GP**, 3 PT
	• Cloning vectors, host strains, DNA introduction into cells, selection and screening for recombinants	**3 GP**, 4-6 PT
	• Polymerase chain reaction (PCR) for cloning & gene modifications • 'Recombineering' for cloning & gene assembly • ***Synthetic biology and 'Gibson Assembly'***	**4 GP**
	• ***Introduction: genome analysis – gene expression exercise:*** • NCBI & JGI sites, Gene Construction Kit program, expression plasmids, expression of cloned genes & protein products	**5-7 GP**, 5, 9 PT
	• DNA sequencing & ***introduction to genomic databases & bioinformatics***	**4-5 GP**, 7 PT

	• Revolution(s) in DNA sequencing – ***'Next generation' '454'- pyrosequencing, Illumina, single-molecule Helicos & Pacific Biosciences, 'Ion Torrent,' & Oxford Nanopore*** DNA sequencing technologies and their implications	
3	**Biodiversity and screening for novel bioproducts**	
	• Weird and unusual organisms and their biotechnological potential	**14 GP,** 1-2 GN
	• Molecular methods for exploring microbial diversity	
	• Genome sequences, genes, & bioproducts from 'uncultivated' organisms	
4	**Classical & molecular methods for screening & creating biodiversity**	**8 GP**
	• Classical microbial & biochemical screening strategies	
	• 'Smart screens' for discovery of novel bioproducts	
	• ***Altered genes & organisms -- Mutagenesis strategies***	**8 GP**, 7 PT
	• In vitro and site-directed mutagenesis • In vivo mutagenesis in *E. coli* via 'Recombineering'	
	• Random targeted mutagenesis	
	• *(Possible discussion of RNA-based applications, e.g. interference RNA -- RNAi)*	
	• ***'In vitro' molecular evolution:*** 'gene shuffling' & other methods	
	• Genetic tricks: bacteriophage & microbial surface display of proteins	parts of **6, 10 GP**
5-6	**Gene – protein – bioproduct discovery -- Genomic databases, transcriptomics, proteomics, & metabolomics**	
	• ***Genomic databases*** & ***microarray*** gene expression studies for molecular diagnostics, screening, and product discovery	**5 GP**
	• ***'mRNA (cDNA) sequencing'*** – Next generation sequencing & global gene expression studies	
6-7	• ***Genomic databases*** and ***proteomics*** for molecular diagnostics, screening, and product discovery	
	• Analysis of proteins & protein modifications by: MALDI-TOF (matrix-assisted-laser-desorption-ionization, time-of-flight), ESI (electrospray-ionization), and LC-ESI-MS/MS (liquid chromatography, electrospray, tandem) mass spectrometry	
	• Metabolic labeling & isotope-assisted, quantitative proteomics	
	• ***Metabolomics, biomarkers, & molecular diagnostics***	
	• ***The data analysis challenge!***	
	• Two-hybrid & protein array screens for probing molecular interactions	1, 14 PTO

6-7	**MIDTERM EXAM March 14-21 (due March 21)**	
	SPRING BREAK! March 22-30	
8-9	**Culture strategies -- bioproducts -- biofuels**	

	• Principles of 'Fermenter' or bioreactor operation (batch, fed-batch, and continuous cultures)	**17 GP** 4,5 CC
	• ***Biomass & ethanol**, the potential of crude substrates*	**14 GP**, 10-11 GN
	• ***Biohydrogen, biodiesel, isoprenoids & biofuels -- The carbon-neutral renewable energy challenge***	
	• ***Solar energy conversion, plant & algal biofuels***	
	• Introduction to microbial biotransformations & bioremediation	
	• ***Metabolic engineering***	
	• *(Possible examples of bioproducts and production strategies):*	
	• Pharmaceuticals, enzymes, antibodies, vaccines	**10-12 GP**, 7 GN
	• Antibiotics, biopolymers	**13 GP**, 8 GN
	• Microbial insecticides	**16 GP**, 6 GN
	• Amino acids, vitamins, & small biological molecules	**13 GP**, 13 GN, 6 CC
	• ***Genetically engineered products – genetically engineered organisms (GMOs)***	**13 GP**
9	**Product recovery and purification**	
	• Cell harvest, disruption, & primary separations	**17 GP**, 6 CC
	• ***Chromatography*** for separation of proteins & other biomolecules	
	• Ion exchange, 'normal' phase, 'reverse' phase, gel filtration, & affinity chromatography	
	• High Performance & 'Ultra High Performance' liquid chromatography (HPLC & UHPLC) strategies	
	• Biotech disasters & controversies, regulatory issues & genetically modified organisms (GMOs)	**22-23 GP**
	• ***Bioseparation strategies & engineering***	
	• ***Fusion proteins & affinity purification tags***	
	• Genetic engineering of protein conformation, stability, & export	**6-8 GP**, 5, 9 PTO
10	**Protein folding, degradation, & misfolding** -- *Important for biology, biotechnology, medicine & disease!*	parts of **8 GP**
	• Molecular chaperones, proteasomes, & foldases	
	• Protein folding 'Reporters' & strategies for refolding misfolded proteins	
11	**Enzymes & proteins in biotechnology**	
	• Enzymes as bioproducts *(e.g. in the molecular biology revolution)*	
	• Enzymes as biocatalysts	
	• Enzymes in microbial transformations & bioremediation	**14 GP**, 10-11 GN
	• Immobilized enzymes & enzyme biosensors	parts of **6-7 GP**
	• Protein 3D structures, databases, & structure viewing/manipulation	

	• ***Websites & programs for protein 3D structure analysis***	

12	**Biosensors & molecular probes**	
	• Organisms as biosensors	
	• ***DNA fingerprinting & probe techniques***	**9 GP**
	• Restriction fragment length polymorphisms & DNA fingerprinting	
	• Allele-specific PCR	
	• 'Molecular beacons' & Real-Time or quantitative PCR (qPCR)	
	• Immuno-PCR	
	• Protein interaction probes: Fluorescence resonance energy transfer (FRET)	
	• ***Nanobiotechnology: new approaches to molecular recognition***	
13	**Transgenic plants**	**17-20 GP**, 12PTO
	• DNA introduction by 'Agro-infection'	
	• Universal methods of DNA introduction: electroporation & particle bombardment	
	• Genetically engineered foods & environmental concerns	
	• Applications of transgenic plants, examples	
13 -14	**Transgenic animals**	**21 GP**, 11 PTO
	• Vectors & methods of DNA introduction	
	• Embryonic stem cells	
	• Somatic cells & reproductive cloning	
	• Applications of transgenic animals, examples	
13 -14	**Human gene therapy, diagnosis, & molecular medicine**	**9-12, 22-23 GP**, 14 PTO
	• *ex vivo* & *in vivo* strategies	
	• methods for transgene introduction & detection	
	• role of genomics & proteomics	
	• Embryonic stem cells, therapeutic cloning, & controversies	
	Environmental biotechnology	**14 GP**, 14 PTO
	Ethical And Patent Issues	**22-23 GP**
	Selected Current Topics *(throughout the semester)*	
13	**Minireviews due May 6**	
13	**Minireview presentations (week of May 6th)**	
13 -14	**TAKE-HOME FINAL EXAM May 9 – 16 (due May 16)**	
	End of semester celebration at Fratello's! (May 16)	