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

Spring 2011

Place and Time: Halsey 457: 9:40 – 11:10 TuTh

Instructor: Dr. Toivo Kallas

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Office Hours: Tu 11:20 – 12:20, 3:00 – 5:00, Th 11:20 – 12:20. 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).

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 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. 2007. *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**, 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 databases such as Medline and Web of Science 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 and Overview:

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 see 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." Special emphasis will be placed on concepts and strategies of genetic engineering that have allowed DNA manipulation *in vitro* to yield genetically modified microbes, plants, animals, and novel bioprocesses. We will discuss aspects of the current revolutions in genomics, "metagenomics," proteomics, metabolomics, and bioinformatics that are having tremendous impacts on our understanding of living organisms and applications in biotechnology.

Throughout the semester, we will discuss research and review articles on topics in biotechnology. Our objectives 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 strategies, global gene expression and proteomic studies, transgenic plants and animals, molecular probes, genetic diagnosis, environmental biotechnology, and engineering of metabolic pathways and organisms for development of carbon-neutral biofuels.

Grading and Requirements

Journal article reports	6 reports @ 10 points each *(2 additional reports may be submitted for extra credit)*	60
Genome analysis/gene expression exercise	due February 24	50
MIDTERM EXAM	March 11-18 (due March 18)	150
Protein 3D structure or microarray gene expression exercise	due April 21 (one of these exercises will be required for graduate students, optional for undergraduates)	(50)
MINIREVIEW	due May 5	100
MINIREVIEW presentations	Week of May 5	50
FINAL EXAM	May 5-13 (due May 13)	150
Total (undergraduate/graduate)		560/(610)

Journal Article Reports. To encourage exploration of topics in biotechnolgy, students will read journal articles on topics of interest and write brief reports (***no more than 1 page each***). Six reports are required with up to two additional for extra credit. These reports should describe the **objective** of the study or questions asked, the **methods** used, and the main **conclusions** of the work. We may use some of these articles 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 and discuss them in class.*** Students will not be expected to, and may not, fully understand these papers ahead of class, but grades can be improved by participating actively and asking questions.

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

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 the format for the minireview will be described separately. To share minireview findings, students or pairs of students will give a 15-20 minute presentation near the end of the semester. Graduate students will give individual presentations.

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

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.***

Topics and Tentative Schedule

(Glick, Pasternak, Patten (GP), ASM 2010, is the main reference text. Some sections of Primrose, Twyman, & Old (PTO), Crueger and Crueger (CC), and Glazer and Nikaido (GN) are listed for reference. Relevant materials from these are included in the Powerpoint presentations available on D2L.)

Week	**Topic**	**Text chapters,** suggested but not limited to:
1	**Introduction and course overview**	**1 GP,** 1-4 PTO
1-2	**Review of basic genetic engineering techniques**	**2-4, 6-7 GP**
	• Genetic basis and history of gene cloning	**(review 2 GP)** **1, 3 GP**, 1-2 PTO
	• Restriction & modification of DNA, cutting & joining DNA molecules	**3 GP**, 3 PTO
	• Cloning vectors, host strains, DNA introduction into cells, selection and screening for recombinants	**3 GP**, 4-6 PTO
	• Polymerase chain reaction (PCR) & cloning applications	**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 PTO
	• DNA sequencing & ***introduction to genomic databases & bioinformatics*** • Revolution(s) in DNA sequencing – ***"Next generation" "454-pyrosequencing," "Illumina,"*** & ***"next-next generation," single-molecule Helicos & Pacific Biosciences*** DNA sequencing technologies and their implications	**4-5 GP**, 7 PTO
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 & generation of biodiversity**	**8 GP**

	• Classical microbial & biochemical screening strategies	
	• "Smart screens" for discovery of novel bioproducts	
	• ***Mutagenesis strategies***	**8 GP**, 7 PTO
	• *In vitro* and site-directed mutagenesis	
	• 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	**Genomic Databases, Transcriptomics, Proteomics, & Metabolomics**	
	• ***Genomic databases*** & ***microarray*** gene expression studies for molecular diagnostics, screening, and product discovery	**5 GP**
	• ***"Deep mRNA (cDNA) sequencing"*** – the future of 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), MALDI-TOF/TOF, ESI (electrospray-ionization), and LC-ESI-MS/MS (liquid chromatography, electrospray, tandem) mass spectrometry	
	• Metabolic labeling & isotope-assisted, quantitative proteomics	
	• ***Metabolomics*** for molecular diagnostics	
	• ***The data analysis challenge!***	
	• Two-hybrid & protein array screens for probing molecular interactions	1, 14 PTO

	MIDTERM EXAM March 11-18 (due March 18)	
	SPRING BREAK! March 19-27	
8-9	**Principles of cell culture for bio-product production**	
	• 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 & biofuels: the renewable energy challenge***	
	• ***Solar energy conversion, plant & algal biofuels***	
	• Introduction to microbial biotransformations & bioremediation	
	• Metabolic pathway 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	**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 liquid chromatography (HPLC) strategies	
	• Biotech disasters & controversies, regulatory issues & genetically modified organisms (GMOs)	**22-23 GP**
	• Advances in bioseparation strategies	
	• ***Fusion proteins & affinity purification tags***	
	• Genetic engineering of protein conformation, stability, & export	**6-8 GP**, 5, 9 PTO
10	**Protein folding, degradation, & misfolding** *(importance to biology, biotechnology, & medicine)*	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	
14	**Transgenic animals**	**21 GP**, 11 PTO
	• Vectors & methods of DNA introduction	
	• Embryonic stem cells	
	• Somatic cells & reproductive cloning	
	• Applications of transgenic animals, examples	
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)*	
	MINIREVIEWS due May 5	
13	**STUDENT PRESENTATIONS (week of May 5th)**	
	TAKE-HOME FINAL EXAM May 5 – 13 (due May 13)	
	End of semester celebration at Fratello's! (May 13)	