CAMOSUN COLLEGE - CHEMISTRY & GEOSCIENCE DEPARTMENT

CHEMISTRY 250 MOLECULAR BIOTECHNOLOGY

Course Outline - Winter Semester 2004

This course covers fundamentals of molecular biotechnology and applications to drug, vaccine, and diagnostics development with emphasis on medical biotechnologies, industrial processing, and agrobiotechnology. Topics include gene expression systems, protein engineering, targeted tissue delivery, gene therapy, DNA diagnostics, recombinant DNA vaccines, fermentation, bioremediation, and intellectual property and regulatory issues.

Instructor Jamie Doran

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Office Hours	Monday 14:30 PM to 15:20 PM
	Wednesday 14:30 PM to 15:20 PM
	Friday 13:30 PM to 15:20 PM

Also, whenever my door is open feel free to talk with me.
Appointments can be scheduled at other times.

Course Chem 250

Term offered	Winter
Credits	4 credits
In-class workload	3 h lecture; 3 h laboratory
Out-of-class workload	6 h
Pre-requisites	Biol 202
Pre- or Co-requisite	Chem 255

Lecture times

Monday 10:30 to 11:20 in Room F212
Thursday during the 2:30 - 6:20 Lab/Lecture Slot in Room F360*
Friday 15:30 to 16:20 in Room F360

Laboratory time

Thursday during the 2:30 - 6:20 Lab/Lecture Slot in Room F360*
*Normally about 45 to 50 min of the approximately four hour lecture/laboratory time period will be used for lecture.

Textbook (required)

Molecular Biotechnology: Principles and Applications of Recombinant DNA. Third Edition, 2003. Au. Bernard R. Glick and Jack J. Pasternak. ASM Press, Washington, D.C.

Experimental protocols

These will normally be provided prior to each laboratory session. Biosafety information will be provided in advance of any laboratory work with bacterial cultures.

Information on the proper maintenance of a laboratory notebook will be provided during the first laboratory period.

Safety glasses

Safety glasses are required when handling hazardous chemicals or bacterial cultures. Glasses and shields to protect the eyes and face from UV light are required and will be provided.

Lab coats

Lab coats are <u>required</u> for experiments involving bacteria, recombinant DNA, or hazardous chemicals. These lab coats <u>must remain in the lab</u> after the completion of the experiments, and be stored in a closed plastic bag. These lab coats are not to be used in laboratory work associated with other courses.

Latex gloves

Latex or similar gloves will be available in the lab at all times and are to be used liberally to protect the skin from hazardous chemicals, and to protect labile biochemicals from becoming contaminated with the enzymes on your skin.

Scientific calculator

Calculators are normally required in the lab and occasionally needed in class. You should bring a calculator to the final exam.

Course evaluation:	Projects and Assignments (five)	(total value)	40 %
	Laboratory Performance & Lab Note Book*		20 %
	Final Exam		40 %

* There are no laboratory reports due to be handed in after each experiment but the students will maintain a lab notebook as they would if they were working in a biotechnology research laboratory. This lab book will be graded at the end of the course. There is no separate laboratory exam, however, students are responsible for the principles and practical aspects of the laboratory experiments. These are subject to examination on the final exam.

An outline of the criteria for evaluation of lab performance will be provided in the first laboratory period.

Grade scale

The percentage marks for the course will be converted to grades according to the School of Arts & Science scale:

A+	= 95% to 100%	B-	= 70% to 74%
A	= 90% to 94%	C+	= 65% to 69%
A-	= 85% to 89%	C	= 60% to 64%
B+	= 80% to 84%	D	= 50% to 59%
B	= 75% to 79%	F	= 0% to 49%

Lecture Outline

I. Introduction
- Introduction to molecular biotechnology
- Sectors of the biotechnology industry
- The BC biotechnology industry

II. Intellectual property protection
- Patents, trade secrets, trademarks and copyright

(Where suitable, the material described below in sections III to V will be introduced in the course of conducting the related experiments)

III. Molecular cloning & other recombinant DNA technologies

- review of gene structure, function and regulation
- plasmid cloning vectors
- cosmids, phagemids & bacteriophage-based vectors
- laboratory techniques for molecular cloning
    - DNA purification
    - restriction fragment digestion and ligation
    - transformation, transfection and electroporation
    - DNA amplification technologies

IV. Techniques for the characterization of cloned DNA

- screening and detection of cloned genes
    - Southern, colony, and plaque hybridization
    - autoradiographic, colorimetric & chemiluminescent detection
- physical mapping
    - restriction endonuclease mapping of DNA
- DNA sequencing
    - strategies for enzymatic (dideoxy) nucleotide sequencing
- transcriptional analysis
    - Northern hybridization
    - primer extension
    - reporter genes
- recognition of protein products
    - Western blotting (immunodetection)
    - phage display peptide library screening

V. Gene expression technologies

- prokaryotic expression systems
    - factors affecting gene expression
    - high-level expression systems
- eukaryotic expression systems
    - unicellular yeast systems
    - insect cell systems
    - cultured mammalian cells

VI. Recombinant vaccines

- attenuated vaccines
- subunit vaccines
- DNA vaccines
- heterologous vaccine expression systems
- economic (production and market) considerations
- strategies for the development of HIV vaccines

VII. Fermentation

(this will likely involve a site-visit to a fermentation facility)

- batch, batch-fed, and continuous fermentations
- large-scale fermentation systems
- cell harvesting and disruption, and downstream processing

VIII. Diagnostic molecular biotechnologies

- nucleic acid based diagnostics
  - gene probes
  - nucleic acid amplification-based technologies
  - DNA microarray technologies
  - RFLP analyses
  - forensic applications
  - genetic disease diagnosis
- immunodiagnostics
  - agglutination-based strategies
  - membrane-based immunodetection
  - ELISA
  - biosensor technology

IX. Molecular agrobiotechnology

- applications of transgenic plant technologies
  - genetic engineering of nitrogen-fixing bacteria
  - *A. tumefaciens* & Ti plasmid mediated gene transfer systems
  - genetically engineered crop plants and forest trees
  - plants as bioreactors
- applications of transgenic animal technologies (overview)
  - relevance to agriculture and aquaculture
  - novel protein production systems

X. Molecular biotechnologies for bioremediation
- genetically engineered organisms for biodegradation of environmental pollutants

XI. Biopharmaceuticals
- identification of new therapeutic targets
- genomics, proteomics and bioinformatics
- emerging therapeutics from molecular biotechnology
- combinatorial chemistries & drug design

XII. Technologies for drug delivery
- liposome-based systems
- microencapsulation
- virus-based systems
- targeted delivery

XIII. Strategies for gene therapy
- nucleic acid therapeutic agents including antisense technologies
- human gene therapy

XIV. Regulatory, entrepreneurial, ethical and social issues

Laboratory Outline

The following scheme represents a continuous series of recombinant DNA experiments. Therefore, success in each experiment is required to continue to the next one. Prepare well, work carefully, be observant, ask lots of questions, and think.

1. Preparation of buffers, solutions and media (Week 1)

2. 'Mini-prep' isolation of plasmid DNA (two methods)
(a) Rapid, small-scale isolation of plasmid DNA from bacterial cells.
(b) Restriction enzyme digestion assay.

(c) Agarose gel electrophoresis.
(d) Ethidium bromide staining, UV-transillumination and photography.

3. DNA Subcloning
(a) DNA ligation to form recombinant plasmids.
(b) DNA transformation of competent bacteria.
(c) Use of colorimetric, enzymatic, gene-inactivation assay to visually select recombinant plasmids.
(d) Plating of bacterial cells to obtain isolated colonies.
(e) Use of antibiotic-containing medium to select for transformed cells.

4. Physical Mapping of DNA
(a) Single and double restriction enzyme digestion.
(b) Molecular weight/size determination of linear DNA fragments using agarose gel electrophoresis.
(c) Physical mapping of DNA fragments by data analysis.

5. Southern Blot Analysis
(a) Southern transfer technique.
(b) Southern hybridization technique using nonradioactively-labeled probe.
(c) Detection of DNA hybridization.
(d) Interpretation of hybridization results.

6. DNA Amplification by the Polymerase Chain Reaction
(a) Performance of PCR reactions using a thermocycler.
(b) Analysis of amplified DNA using by agarose gel electrophoresis.

7. DNA Sequencing Techniques
(a) Techniques for pouring a DNA sequencing gels
(b) Dideoxy DNA sequencing reactions.
(c) Preparation and operation of a manual DNA sequencing apparatus.
(d) Handling of a DNA sequencing gel in preparation for autoradiography.
(e) Analysis of autoradiographs and data from automated DNA sequencing.

8. Bioinformatics: Computer Analyses of DNA and Proteins Sequences
(a) Application of BLASTN, BLASTX and BLITZ, and similar software packages to analyze sequences by comparison to worldwide DNA and protein databases.
(b) Analysis of open reading frames (ORF's) for consensus sequences for transcription and translation (dependent on available software), and comparative protein sequence analyses by CLUSTAL W or similar software.