

# **BCHB 526 CORE METHODS IN BIOTECHNOLOGY**

Room LA4 3 credits

Lecture 1 and 1A Tuesday 3:30-5:00 PM

All Other Lectures Tuesday & Thursday 10:30 AM - 12:00 noon

**FALL 2007; Course Directors: Dr. Dean Rosenthal (Biochem) & Dr. Mary Beth Martin (Biochem)**

This course is designed to introduce the student to the field of biotechnology and the techniques used. The major goal of the course is to allow the student to read articles in current journals (e.g. Cell, J. Biol. Chem., Science, Nature) and understand the terminology and methodology utilized in the study.

Each lecture session will consist of didactic lecture material and review sessions will attempt to clarify the didactic material by working through homework problems and having a question/answer session. Each lecture session will have a set of take-home problems, which will be discussed in the review sessions. There may, or may not, be new didactic material presented in the review sessions, as indicated by each lecturer.

## **1. Introduction to terminology of molecular biology**

DNA structure - chromatin - 5',3' - base pairs; transcription, translation, post-translational modification, sense & antisense

introns & exons - coding region - start sites for transcription & translation; promoters, enhancers - cis & trans - transcriptional regulatory factors. Vectors - plasmid, virus - transformation, infection, transfection; bacteria - antibiotics. oligonucleotides

### **1A. Review questions from Lecture 1**

## **2. Enzymes**

Restriction enzymes - 8,6,5,4 cutters - blunt vs sticky ends - 3',5' overhang; DNA ligase - T4 DNA kinase - Alkaline phosphatase; Terminal deoxytransferase - S1 nuclease - DNase I; RNase - Reverse transcriptase - DNA Polymerase (Klenow, T7); TAQ Polymerase (Intro to PCR)

### **2A - Review questions from Lecture 2**

## **3. PCR**

PCR - Technique - theory; Issues in PCR primer selection; Uses of PCR; Quantitative PCR

## **4. Gel Electrophoresis; Analysis of RNA & DNA**

Agarose gel electrophoresis, Polyacrylamide gel electrophoresis

DNA/RNA purification; How to make probes; Dot/Slot blots; Run on/off

DNA/RNA identification

Southern Blotting

Measurements of mRNA

Northern Blotting; *In situ* hybridization; S1 nuclease protection; RNase protection; Primer extension

### **4A - Review questions from Lecture 4**

### **3A - Review questions from Lecture 3**

## **5. DNA Sequencing**

DNA Sequencing

**First exam Covers lectures 1-5.**

## **6. Microarrays**

cDNA; oligonucleotides, tissues, antibodies

## **7. Transcriptional regulation**

Footprint analysis; Gel retardation; Template commitment; Methylase protection/interference; *In vitro* transcription;

Transcription run-on; DNase I; Chips

### **6A-7A. Review questions from lecture 6 and 7**

## **8. Cloning and utility of plasmid vectors**

Introduction to plasmids

Subcloning DNA fragments

Bacterial expression vectors

Mammalian expression vectors

## **9. Plasmids and Yeast two-hybrid Screening**

Mammalian expression vectors (cont)

yeast plasmids

Components of yeast two-hybrid

Library screening using yeast two-hybrid

## **10. Synthesis and Bacteriophage-based vectors**

Synthesis and cloning of cDNA

introduction to bacteriophage vectors

Genomic library construction

BACs

### **8A-10A. Review questions from Lectures 8, 9, and 10**

**Second Exam Covers lectures 6-10.**

## **11. Protein Purification and Characterization**

Detection of proteins; Gel electrophoresis; Purification - Gel filtration, Ion exchange, Isoelectric focusing, Reverse phase chromatography, Affinity chromatography  
Protein sequencing -N-terminal; Internal

### **11A. Review questions for Lectures 11**

## **12. Antibodies**

Properties of antibodies and antigens - Production of polyclonal and monoclonal ABs: Antigens used; proteins used, peptides, others; Antibody purification - Detection of ABs: Directly: labeling of ABs (iodine, biotin, enzymes, fluorochrome); Indirectly: anti-ABs, Protein A, G - Immunoassays Immuno-blot (Western-blot), -precipitation, -staining; Immunoaffinity chromatography; Others: ELISA, RIA

### **12A. Review questions for Lectures 12**

## **13. Microscopic Imaging**

Studying cells and tissues at the light and **electron** microscopic levels. Preparation procedures for and the kinds of information that can be obtained by using light microscopy and transmission and scanning electron microscopy to study biological specimens.

## **14. Stem Cells**

### **Thanksgiving 11/22**

## **15. MR application to neurodegeneration models I**

Lecture 1- This lecture will touch on basic theories of NMR spectroscopy and MR imaging (MRI/MRS), instrumentation, and design of NMR experiments and data analysis

## **16. MR application to neurodegeneration models II**

Lecture 2- This lecture will focus on the application of MR spectroscopy to study biochemical and bio-energetic pathways both *in vivo* and *in vitro* models of neurodegenerative diseases.

## **17. MR application to neurodegeneration models III**

Lecture 3- This lecture will discuss various strategies of *in vivo* MR imaging (MRI) in models of neurodegeneration to detect lesions and anatomical or morphometric changes in the brain.

### **17A Review questions for Lectures 15-17**

**Third Exam Covers lectures 11-17.**