I. Structure and expression of eukaryotic genomes

1. What are the model eukaryotic organisms and why are they model organisms?
2. What is gene expression and why is it important to understand?
3. Before cloning: Different populations of genes are expressed in different cell types at different times.

II. Before sequencing: Recombinant DNA techniques: how to clone your favorite gene (Weaver, Chapters 4&5)

1. Construction and use of recombinant DNA molecules: cDNA and genomic DNA clones

III. After sequencing: Genomics and Proteomics and other omics – High throughput analysis once your favorite genome is sequenced (Weaver, Ch. 24).

1. Genomics: What have we learned from sequencing genomes of model organisms?
2. The transcriptome: assaying amounts of all transcripts under different conditions using gene chips and microarrays
3. Proteomics: study of all proteins present under different conditions
4. Functional genomics and proteomics: investigating gene function by constructing gene knockouts, knockdowns (RNAi) and knock-ins; constructing conditionally expressed genes by PCR, transformation and homologous recombination, epitope-tagging, purification of multimolecular complexes, and identification of constituents by mass spectrometry, protein chips.
5. What does the future hold?

• FIRST HOUR EXAM (~Wednesday, October 1)

IV. Transcription of eukaryotic genes (Weaver, Chapters 10-13)

1. Overview of transcription
2. Temporal and spatial-specific gene expression
3. Cis-acting elements (Weaver, Chapter 10)
   a. Promoters, enhancers and silencers
b. Defining the eukaryotic promoter by mutating it \textit{in vitro}, introducing it into living cells, and assaying expression \textit{in vivo}

4. \textit{Trans}-acting factors (Weaver, Chs. 10-12)
   a. Basal transcriptional machinery: identification by genetic and biochemical methods
   b. Regulatory proteins: enhancer binding proteins and adaptor proteins
   c. DNA-protein as well as protein-protein interactions

5. Role of chromatin/chromosome structure in transcription: histones; nucleosomes; structural and posttranslational modification of nucleosomes, the histone code (Weaver, Ch. 13)

6. Regulation of transcription - \textbf{Example}: the galactose regulon in yeast

\textbf{SECOND HOUR EXAM (~Friday, October 31)}

V. Pre-mRNA processing (Weaver, Chs. 14 and 15)

1. Comparing and contrasting the structure of RNA vs. DNA
2. There are several different classes of processed RNAs and RNA processing reactions.
3. Mechanism of pre-messenger RNA splicing \textit{in vivo} and \textit{in vitro}: essential \textit{cis}-elements
4. \textit{Trans}-acting factors: Splicing factors and the splicing complex (spliceosome)
5. Regulation of splicing and alternative splicing -- another means to generate diversity in gene expression \textbf{Example}: Regulation of sex determination in Drosophila
6. Coupling of transcription and splicing - one large machine?

VI. Export of mRNA from the nucleus to the cytoplasm

1. Is export coupled to transcription or splicing?

VII. Translation of mRNA (Weaver, Chs. 17-19)

1. Role of 5' caps and 3' poly(A): revisiting an old hypothesis
2. \textit{Trans}-acting factors
3. Regulation of translation

VIII. Turnover of nuclear and cytoplasmic RNA

1. Nobodies and P-bodies

IX. Micro-RNAs: Regulators of mRNA turnover, translation, and maybe more

\textbf{THIRD HOUR EXAM (~Friday, December 5)}

\textbf{RESEARCH PROPOSAL (TERM PAPER)}

(Outline due Monday, November 24) (Paper due Friday, December 12)
Your grade in this course will be determined by the following:

Oral and written summaries of journal articles: 20 points each  200 points

First hour exam  ~Wednesday, October 1  100 points
Second hour exam  ~Friday, October 31  100 points
Third hour exam  ~Friday, December 5  100 points
Research proposal outline due  ~ Monday, November 24  50 points
Research proposal  ~ Friday, December 12  200 points  750 points

Research articles from scientific journals are the focus of this course. These are required reading, will be handed out in class, and each will be discussed in subsequent lectures. The exams will be based on the lectures, including information discussed from the articles. The textbook is Molecular Biology, Fourth Edition, (2008…the ink is still wet!) by Robert Weaver, available in the bookstore.
MOLECULAR BIOLOGY OF EUKARYOTES

SUMMARIES OF JOURNAL ARTICLES

Approximately once per week during the course, you will be asked to write a half page summary of one of the journal articles handed out in class. Ordinarily, the summary will be due in class at least one week after each assignment is announced. Generally, I will discuss this article in class soon after the summary is due. Thus you will have read, thought, and written about the paper before we discuss it in class. However, during the first few weeks the course, I will try to discuss the article before your summary is due to help you become acquainted with reading and understanding journal articles.

The summary should be approximately one-half page (some may need to be longer), single-spaced, typed or handwritten legibly. In your own words (not those from the article) briefly describe what previously unanswered questions or issues were addressed by the authors’ research described in the paper, what experiments were done, and what was concluded by the authors. Later in the course I will also ask you to describe what additional questions should be addressed next and by what means (i.e. what experiments should be done). Describe any sections that were confusing or unclear to you and why. This latter point is important in that it will alert me to clarify in class issues that are confusing to you.

If you cannot bring your homework to class, please do not email it to me. Bring me a hard copy as soon thereafter as possible.
MOLECULAR BIOLOGY OF EUKARYOTES

RESEARCH PROPOSAL

Outline due Monday November 24, 2008
7-typed pages, double spaced -- Research Proposal due Friday, December 12, 2008.

The term paper is the most critical "test" in this course. It is designed to assess your ability by the end of this course to critically evaluate the literature, to provide original suggestions for appropriate questions to be asked in eukaryotic molecular biology at this time, and to design appropriate experiments to try to answer these questions.

1. **Specific Aims** (approximately 1 page)

   State concisely and realistically what the research described in your proposal is intended to accomplish and/or what hypotheses are to be tested. Write one or two general paragraphs **introducing the subject and its relevance to biology**, then simply list three or four specific questions to be addressed. This section is critical because it provides a framework for the reader to appreciate your proposal and the "connections" between sections of the proposal.

2. **Significance** (approximately 2-3 pages)

   Briefly sketch the background to your proposal, critically evaluate existing knowledge, and **specifically identify the gaps which the project is intended to fill**. i.e., summarize the general knowledge of the field, and identify where your questions "fit in". This is an important section in that you display your knowledge and understanding of the field and its shortcomings at present. **What are the unanswered questions?**

3. **Experimental Design and Methods** (approximately 3-4 pages)

   Discuss **in detail** the experimental design and the procedures to be used to accomplish the specific aims of the project. Include potential difficulties and limitations of the proposed procedures and alternative approaches to achieve the aims. This section is where you will display the extent to which you have benefited from this course to learn contemporary methods to experimentally test hypotheses in molecular biology. Spend most of your intellectual energy here!

Note that this proposal is analogous to the research proposal at the end of the first year of our graduate program. I will provide you with examples of such term papers from previous years.
MOLECULAR BIOLOGY OF EUKARYOTES

READING A SCIENTIFIC PAPER

We will rely almost entirely on papers from the literature to provide us with both classical and newly developing approaches and ideas in molecular biology. It is essential to your development as a professional scientist that you learn how to read research papers critically and efficiently. We will go over the first few papers in greater detail in class to help you learn how to read journal articles.

A typical outline of a research paper is summarized below:

A. **Title** and **Abstract**: (200 words) This is meant to attract your attention and summarize what the authors consider the most important points of the paper. Little if any background information is provided here, mostly new results.

B. **Introduction**: This is where the authors (try to) provide you with sufficient background information to understand where their work fits into the "big picture". This should not be a comprehensive review -- only salient points that allow you to get directly to the issue at hand. However, some writers often use (abuse) this space to write a comprehensive review article.

C. **Results**: This contains a description of experiments done - how and what was found, usually with some, but minimal interpretations of the significance of the results. It is here that Tables and Figures of the data are presented and explained. Look at the figures. An experienced reader of a well-written paper can determine and assess the results merely from the Figures. A key element to your success in reading papers is the ability to evaluate the credibility of the results including the possible significance of aberrant or unexpected results and sources of revolutionary changes in the field.

D. **Discussion**: Contains interpretations of what the authors find. Are strong conclusions justified? Are alternative interpretations considered? Are shortcomings or limitations of methods and approaches explained? Usually the authors' results are related to others' results -- do they agree or disagree and why?

E. **Methods**: Contains recipes and protocols for what they did. Are they appropriate and adequate? Could you repeat the work with the information provided? Methods may follow Introduction or Discussion, depending on the journal.