MCDB 3500 LEARNING GOALS

Molecular Biology MCDB 3500 – Course and Topic Learning Goals

Students enrolling in this course should be able to demonstrate achievement of the learning goals for Introductory Biology MCDB 1150 and 1151 or MCDB 1111 Biofundamentals, as well as Genetics MCDB 2150 and 2151.

Teaching efforts toward achieving the following learning goals is expected to occupy 70%-80% of class time. The remaining course content is at the discretion of the instructors. The relative emphasis placed on the goals below and the order in which they are dealt with may also vary according to the tastes and interests of individual instructors (this is not intended to be a syllabus). Some of the learning goals may overlap with those in previous courses because of their importance to molecular biology. However, all students who receive a passing grade in the course should be able to demonstrate achievement of the goals below.

After completing this course, students should be able to:

1. Compare the structures of DNA and RNA

a) Compare the A, B, and Z structural forms of DNA.
b) Describe procedures for "melting" and "re-annealing" (denaturing and renaturing) double-stranded nucleic acids, the methods used to measure degree of double-strandedness, and the practical applications of these techniques (for example in PCR).
c) Describe the secondary and tertiary structure of RNAs in solution and cite examples where RNA folding is functionally significant.

2. Describe techniques for manipulating and characterizing cloned DNA.

a) Describe the role of restriction endonucleases in gene cloning.
b) Rationalize the procedures by which DNA fragments can be ligated into plasmid vectors, introduced into recipient cells, and their presence selected for in the cell population.
c) Compare the procedures for making clone libraries from genomic DNA and cDNA as starting materials, and cite examples where each would be useful.
d) Describe how PCR works and how it can used to generate enough starting material for cloning.
e) Describe the key elements required for a plasmid to be useful as a cloning vector.
f) Compare different types of cloning vectors in terms of their applications for cDNA, genomic, and expression cloning, as well as the production of proteins.
g) Explain how to screen clones and/or entire libraries for a DNA segment of interest.

3. Describe the key features of commonly used molecular biology techniques.

a) Choose the most appropriate techniques for separating nucleic acids of different sizes, proteins of different sizes, and native proteins of similar sizes.
b) Compare the methods that can be used to detect the presence of a particular nucleotide sequence in a genomic or cDNA library, a preparation of DNA or RNA, or a fixed cell preparation.
c) Compare the methods that can be used to detect the presence of a specific protein in a

cell extract or a fixed cell preparation.

d) Distinguish between Southern, Northern, and Western blots and their applications.
e) Explain the rationale of the Sanger procedure for sequencing DNA and describe its use in automatic DNA sequencers.
f) Compare the common methods for detecting and quantifying transcription, in cell extracts and whole cells.
g) Compare the common methods for demonstrating interactions between proteins.

4. Explain the process of DNA replication.

a) Interpret results of the experiments that distinguished proposed models of DNA replication
b) Interpret results from ex.periments that distinguish between bidirectional, unidirectional, and rolling circle replication.
c) Compare the functions of the important enzymes and proteins in replicating each of the two strands of a double-stranded DNA molecule (DNA helicases, SSBs,topoisomerases, primase, DNA polymerase, and DNA ligase).
d) Compare the events of replication on the leading and lagging strands.

5. Describe the key steps and molecular components in bacterial transcription

a) Explain the functions of important recognition elements of the promoter region (-10 and –35 elements) and their relationship to the function of sigma factor in transcription initiation.
b) Interpret experimental evidence for the roles of individual subunits of RNA polymerase in transcription, including sigma factors.
c) Compare the mechanisms of Rho independent and dependent transcription termination.
d) Describe how external stimuli can regulate transcription both globally and at the specific gene level.
e) Compare the logic of transcription regulation in negatively and positively regulated bacterial operons, using the *lac* operon and the *trp* operon as examples.

6. Describe the key steps and molecular components in eukaryotic transcription

a) Diagram the structure of a typical eukaryotic gene, showing locations of the transcriptional start site, 5'-untranslated region (5'UTR), exons, introns, donor and acceptor splice site sequences, translational start site sequence, translational stop sequence, 3'UTR, and poly-A addition sequence.
b) Distinguish the types of transcripts made by RNA polymerase I, II, and III, and compare the promoters they recognize.
c) Interpret experiments that identify important cis regulatory elements of the eukaryotic promoter region and compare eukaryotic and bacterial promoter structure.
d) Explain the role of enhancers and silencers in regulating transcription.
e) Explain the roles of the trans factors required for transcription initiation, including general transcription factors and moderator complexes.
f) Describe in general how specific transcription factors function to activate or repress transcription initiation and explain how the domain structure of these proteins relates to their function.
g) Describe several mechanisms by which transcription factors are converted between

active and inactive forms.

i) Explain how combinatorial control helps eukaryotes modulate gene expression.

7. Describe the mechanisms by which epigenetic changes in chromatin structure modulate transcription.

a) Describe the structure of nucleosomes, how their packing is regulated, and how this packing affects chromatin structure and transcriptional activity.
b) Interpret the results of DNAse sensitivity assays and chromatin-immunoprecipitation (ChIP) experiments to determine local chromatin structure.
c) Compare the structure and functions of heterochromatin and euchromatin.
d) Explain how histone deacetylases (HDAC) acetyltransferases (HAT), methylases, and demethylases alter chromatin structure and transcriptional activity.
e) Describe the role of DNA methylation in maintaining chromatin structure and the mechanism by which epigenetic DNA methylation patterns are maintained during chromosome replication.

8. Describe and diagram the steps involved in post-transcriptional RNA processing

a) Describe the three major steps in pre-mRNA transcript processing, including their timing relative to transcription, their location in the cell, and why they are limited to Pol II transcripts.
b) Differentiate between self-splicing of group I and II introns and nuclear pre-mRNA splicing.
c) Describe the nature of splice donor and acceptor sites, the snRNPs that bind to them, and their roles in the processes of simple and alternative pre-mRNA splicing.
d) Interpret experiments that identify interactions between pre-mRNA and the spliceosome.
e) Describe how alternative splicing adds to combinatorial control of gene expression in eukaryotes.
f) Interpret the experimental evidence for RNA editing, and describe mechanisms by which it can occur.
g) Compare processing of rRNA and tRNA to pre-mRNA processing.

9. Describe and diagram the steps involved in mRNA translation.

a) Explain how the genetic code specifies the sequence of amino acids in a polypeptide, and interpret the experiments by which the code was originally cracked.
b) Draw a diagram showing sites important for translation on the two ribosomal subunits, and explain how these sites function during translation of an mRNA.
c) Compare the structural features of an mRNA that are important for translation initiation in bacteria and eukaryotes.
d) Compare the general mechanisms and the factors involved in bacterial and eukaryotic translation initiation, elongation, and termination.
e) Interpret experiments showing that translation initiation rate, stability, or cellular location of an mRNA can be controlled by binding of specific proteins or miRNAs to the 5' and 3'UTRs.
f) Interpret experiments to elucidate the mechanism of RNA interference (RNAi), and compare the mechanism of RNAi to the mechanism of translational control by miRNAs.