MCB 502A (Novosibirsk) - 2017 Syllabus

Not applicable this year

MCB 502A (CRN 39764) is a graduate level general Molecular Biology course that focuses on key experiments that serve as a basis for our understanding of the reproduction of the cell. The emphasis is on evaluation of conflicting ideas, comprehension of the logic behind experiments, familiarization with diverse experimental techniques and interpretation of experimental data. The course objective is to encourage students to derive their own interpretations and conclusions from raw experimental data, rather than memorizing "facts" from textbooks.

See the Schedule for the information about the times, dates and locations of the lectures and discussions.

Lectures: Tuesdays and Thursdays, 8:30 - 10:00 AM, in B102 CLSL
Exams: 4:00 - 5:30 PM in B102 CLSL. Exam #1 will be on Monday, November 07. Exam #2 will be on Friday, December 09.
Discussions: Fridays, either 9:00 - 10:30 AM or 10:30 AM - noon, in B124 (class will be split in two equal-sized groups)

Instructor: Andrei Kuzminov <kuzminov@life.illinois.edu>
C326 Chemical and Life Sciences Laboratory
Office hours: Tuesdays 3 - 4 PM and immediately after class

Teaching Assistant: none this year

Prerequisites: It is assumed that students enrolled in this course have the basic knowledge of the following concepts (all of them will be further elaborated in the course):
— Differences between prokaryotic and eukaryotic cells
— DNA, RNA and protein structure, including nucleotides and aminoacids.
— Genes, ORFs, promoters, terminators, operators.
— Enzymes and in vitro assays.
— DNA replication, transcription, translation.
— Chromosomes, bulk DNA segregation, DNA packing and condensation, histones, chromatin.
— Mutants, mutagenesis, complementation, suppression.
— Transformation, plasmids, mobile genetic elements, viruses.

Textbooks:
This class does NOT have a required textbook, and most students will do fine with lecture notes alone. No single textbook covers the course material sufficiently to serve as a replacement for lectures. The textbook that matches the course best (~30%) is **"Molecular Biology"** by Robert F. Weaver (McGraw-Hill). It is an updated and excellent textbook that I highly recommend in general. It provides in-depth coverage for many topics discussed in MCB 501/502 and nicely complements (but does not replace) the lecture material.

Discussions (For 2017-Novosibirsk):
Saturday discussions will be in the following format: every student has to bring three questions on (or relevant to) the material of the last four lectures. I will pick one of them, and we will discuss them together, in English. I will lead the discussion or will moderate it, if the students take the initiative.

Дискуссия (на английском) в формате: каждый студент приносит по крайней мере три разных нетривиальных вопроса по поводу материала прошедшей недели; я выбираю из этих вопросов один, и мы все вместе их обсуждаем (автор вопроса или я выступают лидером дискуссии и модератором, студенты имеют шанс попрактиковать разговорный английский и проявить инициативу).

The final assignment (For 2017-Novosibirsk):
Every student has to create a mini-presentation (5 slides minimum, excluding the title slide), with a short original text in English (about half-a-page total), with appropriate illustrations, on any topic that was mentioned but not discussed in detail in lectures. The topics that were not even mentioned are also welcome, as long as they cover either DNA, or genomes, or chromosomes. You cannot use text and pictures from my slides in this presentation — it should be entirely yours. The deadline of submitting this presentation (by email) — Monday, April 24, 2017.

Каждый студент должен создать мини-презентацию из минимум пяти слайдов (можно больше, слайд с одним названием не считается) с небольшим оригинальным текстом на английском (общее количество текста — около полстраницы) и с подходящими картинками по любой теме, которую мы проходили вскользь и которая показалась интересной. Тема должна быть либо упомянута, но не раскрыта в моих лекциях, либо даже не упомянута, но относиться к ДНК, хромосомам и геномам. В этой минипрезентации запрещается использовать текст и картинки лекций, — всё должно быть своё. Срок сдачи минипрезентации — понедельник, 24 апреля.

Home works :
There will be homework assignments after every lecture, a few problems each, with variable points per question. Only the top couple of problems, typically 6-8 points, will be scored. The rest of the problems are for your interest — they will not be scored, and answers for them will not be provided. Some of the unscored problems will reappear on the exam. The total points from the scored homework problems amount to ≥100 by the end of the course. Since total points on an exam in this course is also 100, homeworks altogether effectively comprise "exam #3". Missing a single homework subtracts (on average) 3-4 points from one's total score. Therefore, if you missed a lecture or for whatever reason could not obtain the text of the homework, request it from me by email at once so that you could turn it in at the next lecture.

Completed homework assignments can be turned in any time before the next lecture directly to the instructor. The homework is due by the beginning of the next class. Once the answer key is distributed at the end of the next class, one cannot turn in the corresponding homework. No make-ups for late or missed homework will be given. However, if you missed a homework for a reason beyond your control (such cases are extremely rare, but one in the past was, for example, if you joined the class late and missed the very first homework), make sure I know about it.

It is not only OK, but is in fact encouraged, to discuss homework with other students, as long as the individual answers are unique. **If I find that different students give essentially the same answers to a particular homework problem, the scores for these answers will be automatic "0" for all students involved: "share your thoughts, not your texts".** Paraphrasing will not help, by the way. With this in mind, I cannot emphasize this simple rule that you should have learned from your undergraduate education: DO NOT SHOW YOUR EXACT ANSWERS TO YOUR CLASSMATES UNDER ANY CIRCUMSTANCES. For additional inspiration on this important matter, check out "MCB Statement on Academic Integrity".

Finally, specific for this course, **if I find an answer which is reminiscent of the lecture text, the grade will be also "0". The answer does not have to be a precise copy of the lecture text to raise my suspicion and to trigger this punitive action.** In other words, "not thinking" while answering will not be tolerated either.

Although homework and exam problems are drawn from the same pool of problems, home works are mostly meant to keep you intellectually engaged with the course material, rather than to faithfully represent the problems you will encounter during exams. Occasionally, a homework question may appear either beyond the scope of the lecture material or not covered in the actual lecture, — I expect you to answer it anyway using the lecture notes and, if needed, textbooks and the Internet (although I would not go there myself for the answer).

Exam: There will be two exams, one in the middle and the other at the end of my half of the course, ~100 points each and covering the material of seven lectures each. The exams are 90 minutes long (the duration of a regular class). If you miss an exam, you need an officially documented justification to request a make-up exam. Missing an exam without good and official justification is an automatic "F".

Re-grading: if, after checking the key, you clearly see that you did not get due credit for your answer (either in a homework or exam), you may be entitled for re-grading. This situation is rare in this class, though, as most of the time students cannot convince me that they deserve additional points.

To be eligible for re-grading, 1) the re-grading request should be made within one week of the date when the graded HW/exam was returned to you; 2) your original writing, including the answer, must be in pen; 3) your answer should be a separate line, away from the body of your work leading to this answer; 4) all the corrections in the work or in the answer must be by crossing the incorrect information. No erasing or whitening out!

Accordingly, I will not be able to re-grade in the following circumstances: 1) the regrading request is made later than one week after the return; 2) the original answer is in pencil; 3) part of the original work is whited out, defaced or otherwise erased in any manner; 4) the original answer is embedded in the body of work or is not separated from it by an extra space.

A separate note about inconsistent grading: occasionally another student may get a higher grade for essentially the same answer as yours. It is OK to bring this inconsistency to my attention, and I will be happy to award you the missed points, but only if you tell me the name of the "reference" student and show both works side-by-side. Keep in mind that, if instead I find that too many points were given to your reference student, you will not be awarded additional points, but the reference student will lose the points that were given by mistake.

Type of HW/exam problems. Multiple choice problems are rare in this class. Problems requiring simple regurgitation of the lecture information are a conspicuous minority. Most problems in this class belong to one of the several basic types: 1) outline a concept; 2) compare one phenomenon, or an approach, or a mechanism, to an analogous but a distinct phenomenon/approach/mechanism; 3) complete an experiment; 4) interpret experimental data; 5) predict something unknown (was not given in lecture) from the known (given in lecture).

These types of common problems are meant to represent various steps of experimental science.

Answering homework and exam problems for best scores. Write legibly and succinctly. A lot of time students lose points because they use too many words or because their handwriting is hard to read. Do not use more space for the answer than is provided, unless you found that your answer was incorrect and you are providing a completely different answer instead. At the same time, try to give a complete answer, making a complete argument. If an apparently trivial observation or conclusion is an important part of the logic of the answer, make sure you include it. To earn a complete credit, the answer must be not only essentially correct, but logically complete and self-sufficient.

Grading multiple-choice problems: Unless specifically indicated otherwise in the formulation of the problem, I expect a single answer. Therefore, multiple answers (even if one of them is correct) receive an automatic zero.

Course Grades: The total scores earned in 502A in the ~300-scale (200 from exams + ~100 from homework) will be transferred to the instructor of 502B (Brian Freeman), who will bring the scores earned in 502A and 502B to the common denominator to combine them into the overall scores and then will assign grades. The total scores earned in 502A will be normalized to the scale of 502 B (Dr. Freeman's part of the course), and the two scores will be then combined. The grid of grade cut-offs will be determined by the distribution of the scores.

Attending lectures: A good half of the factual material of this course can be found in any molecular biology textbook. However, this course is not about facts, — rather, it is about the original questions, the ideas, the experimental logic and observations that form the foundation of these facts, — and *this* material rarely receives adequate coverage in modern textbooks. Basically, your grades will depend more on your ability to think logically with a limited amount of data than on your ability to uncritically memorize tons of data. Therefore, attending lectures and taking good notes is in your best interests. Exam problems are based solely on the lecture material.

Taking notes: The goal of this course is to help you to build your own concepts in molecular biology. Concept-building is an active process dependent, among other things, on your active thinking and re-telling the story, laid out in a lecture, to yourself. Therefore, taking good notes and working on your notes after the lecture to fill gaps and white spots will critically enhance your performance in this class. If you cannot complete your lecture notes by using your classmates' notes, or something does not make sense in the text of the lecture (distributed at the end of each class), visit me during the office hours, and I will be happy to help you.

Studying: Keeping operational concepts in your head (positive knowledge) is an active process, rather than a trait, acquired once and for all. Go over your notes after each lecture and try to catch inconsistencies, insufficient evidence, illogical conclusions. Treat your lecture notes as if this is a case your opponent is trying to build without enough evidence, whereas your task is to dismantle it with a limited number of well-placed blows. Read the original papers and alternative texts. Discuss your findings with your classmates. Come and question the instructor. I cannot build your knowledge for you, but I can help you in this process by providing guidance and challenges. Remember: I am committed to giving you my best, as long as you are committed to a serious effort in this course.

Exam preparation: students who received top grades in this course in the past, when asked about their study and exam preparation patterns, told me that their total preparation time for an exam would be 15-25 hours, usually spread over 3-4 days. Preparation included reading the lecture notes and home work material and writing "improved" notes using the provided lecture texts for clarification of vague spots. They uniformly pointed out that regular work on the lecture material in preparation for the homework was the rule for them.

The delivered MCB 502A-2016 Content

L1

Introduction and General Concepts

Course introduction — the questions — the nature of the "scientific fact"
Introduction into Molecular Biology
The difference between "models" and "mechanisms"
The differences between an idea, a hypothesis, a concept and a theory
Molecular Biology and Physiology vs Genetics and Biochemistry
Biopolymers (BPs)
Ways to transfer information in and between biopolymers
The Cellular Organization of Life
The structure and dimensions of a bacterial cell

DNA as the main information carrier of life

Chromosomes: the bio-bridge between the formality of genome and the chemistry of DNA
The biochemistry of chromosomes. The tetranucleotide structure of DNA.
Gene as a unit of information multiplication and transfer
The experiment of Griffith: the "transforming principle"
The experiment of Avery, MacLeod and McCarty on pneumococcal transformation
The experiment of Hershey and Chase
The observations of Chargaff

L2

DNA Structure

The quest for the structure of DNA
Discussion of the DNA structure
DNA bases: why these four?
The structure and chemistry of the DNA backbone

DNA denaturation, renaturation and hybridization

Denaturation of nucleic acids, by temperature
Denaturation of nucleic acids — low osmolarity, pH, polar solvents
Renaturation
Instantly-reassociating DNA
Hybridization reveals evolutionary relationship
Dot-hybridization

L3

Genome evolution

Genomes and their sizes
The two major types of genome evolution

DNA replication

The problem of helix unwinding
Various explanations
Following the fate of parental DNA in bacteriophages
Equilibrium centrifugation
The experiment of Meselson and Stahl
The experiment of Taylor
Break-induced replication outside the S-phase in eukaryotes

L4

Differential DNA strand labeling as a detection approach. SCE.
The picture of the replicating chromosome (Cairns)

Chromosomal organization of genomic DNA

Genome functions
The chromosomal organization of the genome
The differences between prokaryotic and eukaryotic chromosomes

DNA degradation

The basic configurations of the natural DNA molecules
Gel electrophoresis
Southern blotting (detection of DNA degradation in vivo)
TCA precipitation
Major nucleases of *E. coli*

L5

The rate of DNA degradation
Polarity of ss-specific exo
Polarity of ds-specific exo
Processivity of exonucleases
Measuring the processivity
Endonucleases. The cleavage specificity (position): 5'-phosphate VS 3'-phosphate
Substrate-specificity of endonucleases
The biological roles of exo- and endonucleases

DNA synthesis

The oops of Ochoa and Kornberg's DNA polymerase
DNA depolymerization versus DNA degradation
The need for a primer
The ghost of the non-templated synthesis
Nick-translation

L6

DNA polymerization directionality
Why 5'—>3'?
The primer correction activity of Kornberg polymerase
The two nucleotide binding sites of the enzyme
The DNA polymerization rate and the processivity of Kornberg enzyme
Is Kornberg DNA polymerase a replicative enzyme?

Chromosome Replication

Autoradiography of replication forks suggests 3'—>5' DNA synthesis and additional DNA polymerases
Screening for *dna*-minus mutants
Enrichment for *dna*-minus mutants
Isolation of a *polA* mutant

L7

Isolation and properties of DNA pol II and pol III
The "fork and knife" idea
DNA ligase
The Okazaki experiment
Semidiscontinuous DNA replication (uracil incorporation)?
RNA as a primer for DNA synthesis
Primase

EXAM #1

L8

Unwinding of DNA and keeping it single-stranded

DNA unwinding: helix-destabilizing proteins
Chromatography
Alberts protein
The mechanism of helix destabilization
DNA unwinding: DNA helicases
Discovery of helicases
Helicase polarity
Helicases are enzymes in the presence of SSB
Principles and models of active DNA unwinding

Superhelicity and Topoisomerases

The linking number of a DNA helix
Positive and negative supercoiling.
Detection of DNA supercoiling
Topoisomerase I

L9

DNA gyrase
Type I and type II topoisomerases
Chromosomal scaffold

The replication factory

The composition of DNA polymerase III
The structure and function of the replication fork in *E. coli*.
The replisomes that move in opposite directions at the same time
The replication factory

L10

Initiation of the chromosomal replication in *E. coli*

E. coli origin of replication, *oriC*
DnaA and DnaC
Replication origins
Replication initiation in eukaryotes
oriC-dnaA-independent initiation in bacteria

Termination of the chromosomal replication

Discovery of a termination site
Tus protein and the replication fork trap
Topo IV: decatenation of the sister chromatids

The Cell Cycle

The prokaryotic versus eukaryotic cell cycle
Helmstetter's “baby machine”

L11

Replication period as a function of growth rate
The regulation of chromosomal initiation: titration of DnaA
The initiation cascade reflects the eclipse period
Flow cytometry reveals initiation synchrony
Dam and the origin sequestration
SeqA: the eclipse and beyond

Chromosome Organization

Nucleoid administration
Chromosomal replication complexity

Genome logistics

Definition
Compaction makes DNA functional
Eukaryotes and prokaryotes have distinct basic DNA organization strategies

L12

DNA domainization

Condensation versus Packing for DNA transfer
DNA condensation (rosetting)
Chromosome condensation
DNA packing
Principles of the bulk DNA transport
The chromosome cycle

Mutagenesis

The chemical basis of misincorporation
Methyl-directed mismatch removal

L13

One-strand DNA Damage and its Repair

UV irradiation damages DNA
Photoreactivation
The uncertainty principle of DNA repair and how to get around it
Nucleotide excision repair of UV-lesions
The mechanism of nucleotide excision repair
Base excision repair of damaged, modified or inappropriate bases
Repair avoidance

Chromosomal Lesions

DNA modifications do not change behavior of chromosomes
Lesions affecting the behavior of chromosomes
Chromosome cycle-dependent chromosomal lesions

L14

Classification of chromosomal lesions: types of "failures-by-design"
Chromosomal lesions are repaired by Rec-dependent processes

Recombinational Repair

Homologous recombination: exchange by resolution of Holliday junctions
Genetics of Homologous Recombination
The principles of repair of chromosomal lesions
Biochemistry of recombinational repair
Major types of chromosomal lesions and corresponding recombinational repair pathways
Daughter-strand gap repair
When recombinational repair of daughter-strand gaps is impossible
Double-strand end repair
Replication restart

Chromosomal dimer resolution
The alternative double-strand break repair (NHEJ)
Recombinational misrepair: the importance of sister-chromatid cohesion

EXAM #2