UNIVERSITY OF WISCONSIN-MADISON
EUKARYOTIC MOLECULAR BIOLOGY
BIOCHEMISTRY / GENETICS / MEDICAL GENETICS 620
SPRING 2023

TIME AND PLACE
Monday, Wednesday, and Friday 11:00-11:50 AM, 1211 Biochemical Sciences Building

INSTRUCTORS
Dr. David Wassarman, Department of Medical Genetics
Email: dawassarman@wisc.edu
Office hours: Thursdays at 3:00-4:00 PM, Genetics/Biotech Building, Room 4110, or by appointment

Dr. Gaelen Hess, Department of Biomolecular Chemistry and Center for Human Genomics and Precision Medicine
Email: ghess3@wisc.edu
Office hours: Fridays at 12:00-1:00, Biochemical Sciences Building (BSB), Room 4214, or by appointment

COURSE INFORMATION

COURSE DESCRIPTION
Focuses on core principles underlying molecular mechanisms that regulate DNA, RNA, and protein metabolism in eukaryotic organisms, for the purpose of developing hypotheses and designing experiments to solve contemporary problems in eukaryotic molecular biology. Intended for advanced undergraduate students and first year graduate students with a firm knowledge of basic molecular biology.

REQUISITES
Genetics 466, or Genetics 467, or Microbiology 470, or Biochemistry 508, or Microbiology/Biochemistry/Genetics 612, or graduate standing.

CREDITS
3

HOW CREDIT HOURS ARE MET BY THE COURSE
The course meets for three 50-minute class periods each week over the spring semester and carries the expectation that students will work outside of class on course learning activities (pre- and post-review of class notes, watch supplemental videos, meet with study group, work on Discussion assignments, and study for exams) for 6-9 hours per week. Additional information is provided in the sections below on Course Workload and Time Management.

COURSE DETAILS
Classes are on Mondays, Wednesdays, and Fridays at 11:00-11:50 AM. Students are strongly encouraged to attend class in-person, but lectures will be recorded and can be accessed on Zoom (link below). Other course materials (PowerPoint slides, supplemental videos, Discussion assignments, and exams) are provided in Canvas. Time will be reserved in each lecture to answer questions, and the instructors are available to answer questions before and after class, during office hours, and by appointment. The 42 class sessions include 31 lectures on topics central to eukaryotic molecular biology, 7 Discussions, and 4 exams. The goals of the lectures, Discussions, and exams are to help students attain the learning outcomes and to give instructors the opportunity to evaluate and provide feedback on students’ attainment of the learning outcomes.

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STUDY GROUPS
Students are expected to meet in-person or remotely with their study group for at least one hour per week to discuss the lectures, supplemental videos, Discussion assignments, and exams. Students can either make their own study group of at least three people or they can be assigned to a study group by Prof. Wassarman.

CANVAS COURSE URL
https://canvas.wisc.edu/courses/344793

ZOOM URL
Join Zoom Meeting
https://uwmadison.zoom.us/j/8058895992?pwd=YytMQjN1d2tjQ0RmNWRZS3dCNVbudz09

Meeting ID: 805 889 5992
Passcode: 036375
One tap mobile
+13017158592,8058895992#,,,*036375# US (Washington DC)
+13126266799,8058895992#,,,*036375# US (Chicago)

Dial by your location
+1 301 715 8592 US (Washington DC)
+1 312 626 6799 US (Chicago)
+1 929 205 6099 US (New York)
+1 253 215 8782 US (Tacoma)
+1 346 248 7799 US (Houston)
+1 669 900 6833 US (San Jose)

Meeting ID: 805 889 5992
Passcode: 036375
Find your local number: https://uwmadison.zoom.us/u/adh6mRTnxL

Join by SIP
8058895992@zoomcrc.com

Join by H.323
162.255.37.11 (US West)
162.255.36.11 (US East)
Meeting ID: 805 889 5992
Passcode: 036375

TEXTBOOK
There is no required textbook for this course, but it is recommended that students have a textbook on eukaryotic molecular biology as a reference. Any edition of the following textbooks published within the past 10 years will work: “Molecular Biology” by Robert Weaver, “Molecular Biology of the Gene” by James Watson et al., “Molecular Biology of the Cell” by Bruce Alberts et al., “Molecular Cell Biology” by Harvey Lodish et al., “Molecular Biology: Genes to Proteins” by Burton Trop, “Molecular Biology: Principles and Practice” by Michael Cox et al., and “Molecular Biology: Principles of Genome Function” by Nancy Craig et al. Contact Prof. Wassarman if you are considering using a textbook that is not in this list.

SUPPLEMENTAL VIDEOS
Prof. Wassarman has prepared videos of important topics that students should be familiar with through prior courses. The topics include (A) structures and functions of DNA, RNA, and protein, (B) cell processes and signaling pathways, (C) methods to detect DNA, RNA, and protein, (D) methods to detect molecular interactions, (E) experimental systems, and (F) experimental approaches. Details are provided on pages 7-9 of
the syllabus. Videos are located on Canvas under the Kaltura Gallery tab on the left-hand side of the home page, and Powerpoint slides for the videos are located on Canvas under the Lecture 1-Orientation module.

COURSE LEARNING OUTCOMES

• Recall core principles that govern the structure and function of DNA, RNA, and protein.

• Describe techniques for quantifying the expression, interaction, and cellular localization of specific molecules and for determining their necessity and sufficiency in molecular processes.

• Explain how molecular processes that control the synthesis, decay, interactions, localization, folding, and modification of molecules are silenced, initiated, maintained, and terminated.

• Illustrate, using specific examples, how molecular interactions control the specificity of molecular processes.

• Describe how information is transferred between molecules to alter the activity of cells in response to developmental and environmental signals.

• Critique and weigh the credibility of existing molecular data.

• Develop and draw hypotheses that use existing data to account for as yet unexplained molecular processes in eukaryotic organisms.

• Design discovery, loss-of-function, and gain-of-function experiments to test molecular hypotheses.

Graduate student-specific learning outcome
• Implement problem solving strategies in thesis research project.

COURSE WORKLOAD

<table>
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<tr>
<th>Learning Activity</th>
<th>Hours per Week</th>
<th>Weeks</th>
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<tbody>
<tr>
<td>Attend lectures, discussions, and exams</td>
<td>3</td>
<td>14</td>
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<td>Watch supplemental videos</td>
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<td>Review lectures and notes</td>
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<td>Meet with study group</td>
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<tr>
<td>Answer Discussion questions</td>
<td>5</td>
<td>7</td>
<td>35</td>
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<td><strong>Grand Total</strong></td>
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<td><strong>126 hours</strong></td>
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TIME MANAGEMENT: WEEKLY RHYTHM

<table>
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<tr>
<th>Monday</th>
<th>Tuesday</th>
<th>Wednesday</th>
<th>Thursday</th>
<th>Friday</th>
<th>Saturday</th>
<th>Sunday</th>
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<tbody>
<tr>
<td>Lecture, discussion, or exam 11-11:50 AM</td>
<td>Lecture, discussion, or exam 11-11:50 AM</td>
<td>Lecture, discussion, or exam 11-11:50 AM</td>
<td>Watch videos, meet with study group, complete discussion assignments, and study for exams</td>
<td>Meet with Instructor at office hours or by appointment</td>
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DISCUSSION ASSIGNMENTS

Students should upload Discussion assignments in pdf format to Canvas by 11:00 AM on the days indicated in the syllabus. Discussion answers should be less than one 8.5 x 11 inch page (one side). Answers can by typed or hand-written. Instructors will return graded assignments through Canvas with comments to students prior to the Discussion class.
EXAMS
Exams will follow each of the major sections of the course, Exam 1: transcription, Exam 2: chromatin, Exam 3: post-transcriptional events and translation, and Exam 4: DNA replication and repair (see syllabus). Students must work on exams on their own. On the day of the exam (February 17, March 10, April 14, and May 12), the exam can be taken during any 2-hour period from 7:00 AM to 11:59 PM. During exams, students can use notes on one 8.5 x 11-inch page (both sides). The notes page from prior exams can be used on future exams. The format of the exams will be the same as the Discussion assignments. For each exam, students will choose one of two questions to answer. Exams from past years are posted on Canvas. The instructors will hold a review session prior to each exam (5:00-6:00 in BSB 1211 on February 15, March 8, April 12, and May 9).

LATE WORK POLICIES AND MAKE-UP DATES
Every so often a situation may arise that is beyond your control and prevents you from meeting a Discussion assignment or exam due date. If this occurs, contact Prof. Wassarman by email and he will work with you to remedy the situation.

GRADES
Grades will be posted under the Grades navigation tab in Canvas.

Discussion assignments (7): 10 points each (70 total points, 15% of total grade)
Exams (4): 100 points each (400 total points, 85% of total grade)
Total points, 470

<table>
<thead>
<tr>
<th>Letter grade</th>
<th>Percent*</th>
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<tbody>
<tr>
<td>A</td>
<td>88-100</td>
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<tr>
<td>AB</td>
<td>83-87</td>
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<td>D</td>
<td>60-65</td>
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<td>F</td>
<td>below 60</td>
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*Decimals will be rounded to a whole number. For example, 87.5 will be rounded to 88, and 87.49 will be rounded to 87.

Improvement over time will also be considered when determining grades.

UNIVERSITY OF WISCONSIN-MADISON POLICIES

COURSE EVALUATIONS
You will be provided an opportunity to evaluate the course and your learning experience. Your feedback is important for improving the course. The instructors strongly encourage you to participate in the course evaluation.

RULES, RIGHTS & RESPONSIBILITIES
See: https://guide.wisc.edu/undergraduate/#rulesrightsandresponsibilities#text

ACADEMIC CALENDAR & RELIGIOUS OBEYANCES
See: https://secfac.wisc.edu/academic-calendar/#religious-observances

ACADEMIC INTEGRITY
By virtue of enrollment, each student agrees to uphold the high academic standards of the University of Wisconsin-Madison. Academic misconduct is behavior that negatively impacts the integrity of the institution. Cheating, fabrication, plagiarism, unauthorized collaboration, and helping others commit these
previously listed acts are examples of misconduct that may result in disciplinary action. Examples of
disciplinary action include, but are not limited to, failure on the assignment/course, written reprimand,
disciplinary probation, suspension, or expulsion. (Source: https://conduct.students.wisc.edu/syllabus-
statement/)

ACCOMMODATIONS FOR STUDENTS WITH DISABILITIES
If you have formal disability accommodations through the McBurney Center or you need informal
accommodations, please let Prof. Wassarman know at the beginning of the semester.

The University of Wisconsin-Madison supports the right of all enrolled students to a full and equal
educational opportunity. The Americans with Disabilities Act (ADA), Wisconsin State Statute (36.12), and
UW-Madison policy (Faculty Document 1071) require that students with disabilities be reasonably
accommodated in instruction and campus life. Providing reasonable accommodations for students with
disabilities is a shared faculty and student responsibility. Students are expected to inform instructors of
their need for instructional accommodations by the end of the third week of the semester, or as soon as
possible after a disability has been incurred or recognized. Instructors will work either directly with you or
in coordination with the McBurney Center to identify and provide reasonable instructional
accommodations. Disability information, including instructional accommodations as part of a student's
educational record, is confidential and protected under FERPA. (Source:
https://mcburney.wisc.edu/instructor/)

DIVERSITY & INCLUSION
Diversity is a source of strength, creativity, and innovation for UW-Madison. We value the contributions of
each person and respect the profound ways their identity, culture, background, experience, status,
abilities, and opinion enrich the university community. We commit ourselves to the pursuit of excellence
in teaching, research, outreach, and diversity as inextricably linked goals.

The University of Wisconsin-Madison fulfills its public mission by creating a welcoming and inclusive
community for people from every background – people who as students, faculty, and staff serve
Wisconsin and the world. (Source: https://diversity.wisc.edu/)
# Daily Schedule for Eukaryotic Molecular Biology

**Biochemistry/Genetics/Medical Genetics 620**

<table>
<thead>
<tr>
<th>Date</th>
<th>Class #</th>
<th>Topic</th>
<th>Assignments due at 11:00 AM</th>
<th>Instructor</th>
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<td>1</td>
<td>Orientation</td>
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<td>(F) Jan 27</td>
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<td>RNA pol I transcription</td>
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<td>3</td>
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<td>(F) April 28</td>
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<td>CRISPR tools</td>
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<td>CRISPR screens and functional genomics</td>
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<td>(W) May 3</td>
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<td>Single cell genomics</td>
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SUPPLEMENTAL VIDEOS FOR EUKARYOTIC MOLECULAR BIOLOGY
Biochemistry/Genetics/Medical Genetics 620

Videos are located on Canvas under the Kaltura Gallery tab, and Powerpoint slides for the videos are located on Canvas under the Lecture 1-Orientation module.

A. STRUCTURES AND FUNCTIONS OF MOLECULES
A1. DNA structure and function
- Functions of DNA
- DNA is made up of phosphates, sugars, and bases
- Structures and terminology for the parts of DNA
- The three-dimensional structure of DNA has major and minor grooves
- Major and minor grooves of DNA expose different functional groups

A2. RNA structure and function
- Functions of RNA
- RNA is made up of phosphates, sugars, and bases
- Structures and terminology for the parts of RNA
- Differences between RNA and DNA
- Computational approaches predict RNA structures

A3. Protein structure and function
- Functions of proteins
- Amino acids are the basic structural units of proteins
- Structures of the 20 common amino acids
- Proteins are polymers of amino acids
- Secondary structures form by hydrogen bonding between amino acids
- Higher order structures of proteins

B. CELLS
B1. Basic cellular processes
- Cell structure
- Cells grow, divide, and proliferate
- Cell proliferation occurs through four stages of the cell cycle
- Cells undergo fate determination and then differentiation
- Cells move (migrate)
- Cells die through apoptosis (programmed cell death)

B2. Major signaling pathways
- Signal transduction pathways regulate gene expression
- RAS-MAPK signaling pathway
- Target of rapamycin (TOR) signaling pathway
- NF-κB signaling pathway
- Notch signaling pathway

C. METHODS TO DETECT MOLECULES
C1. Gel electrophoresis
- Gel electrophoresis separates molecules based on size
- Agarose versus polyacrylamide gel electrophoresis
- Denaturing versus nondenaturing gel electrophoresis

C2. Detecting specific DNA fragments
- DNA detection methods involve strand unwinding and hybridization
- Southern blot analysis detects specific DNA fragments in vitro
- Generation of radioactive DNA probes by a kinase
- Generation of nonradioactive DNA probes using a modified nucleotide
• PCR (polymerase chain reaction) amplifies specific DNA regions in vitro
• FISH detects specific DNA sequences in vivo

C3. Detecting specific RNAs
• Northern blot analysis detects specific RNAs in vitro
• Generation of radioactive RNA probes
• Reverse transcription-PCR (RT-PCR) detects specific RNAs in vitro
• The amount of DNA doubles with each round of PCR
• Realtime-PCR quantifies the amount of a specific RNA in vitro
• In situ hybridization detects specific RNAs in vivo

C4. Detecting specific proteins
• Western blot analysis detects specific proteins in vitro
• Immunofluorescence detects specific proteins in vivo
• How to produce polyclonal antibodies
• How to produce monoclonal antibodies
• Secondary antibody detection methods
• Epitope tags provide ways to detect overexpressed proteins
• Cellular localization of GFP-tagged NF-κB

C5. Sequencing DNA
• Uses of DNA sequencing
• DNA sequencing-Sanger sequencing/Dideoxy sequencing
• DNA sequencing-Automated sequencing
• DNA sequencing-Illumina sequencing/Next generation sequencing

D. METHODS TO DETECT MOLECULAR INTERACTIONS

D1. Detecting protein-protein interactions
• Coimmunoprecipitation detects protein-protein interactions
• FRET detects protein-protein interactions in vivo
• The yeast 2-hybrid assay detects protein-protein interactions in vivo
• Co-immunoprecipitation-MS detects protein-protein interactions
• Affinity chromatography detects protein-protein interactions
• Size-exclusion chromatography detects protein-protein interactions
• Proximity labeling detects protein-protein interactions in vivo

D2. Detecting protein-DNA interactions
• Electrophoretic mobility shift assay (EMSA) detects protein-DNA interactions
• EMSA of RNA pol II GTFs and promoter DNA
• DNase I footprinting detects DNA binding sites of proteins
• DNase I footprinting of RNA pol II GTFs on promoter DNA
• Chromatin immunoprecipitation (ChIP) detects protein-DNA interactions

D3. Detecting protein-RNA interactions
• EMSA detects protein-RNA interactions in vitro
• Generation of radioactive RNA
• RNA immunoprecipitation (RIP) detects RNAs bound by a given protein
• RNA affinity purification detects proteins bound by a given RNA
• CRISPR/RdCas9 detects proteins bound by a given RNA

D4. Detecting RNA-RNA interactions
• Psoralen crosslinking detects RNA-RNA base pairing interactions
• Compensatory mutations detect RNA-RNA base pairing interactions
• Evolutionary conservation predicts RNA-RNA base pairing interactions

E. EXPERIMENTAL SYSTEMS

E1. Experimental systems
• In vivo and in vitro experimental systems (advantages/disadvantages)
• Major in vivo experimental models
• Major in vitro experimental models
• Procedure for making a nuclear extract

E2. Genetic engineering (cloning)
• Genetic engineering can be used to genetically modify cell and organisms
• Join DNA fragments together using restriction enzymes
• Join DNA fragments together by Gibson assembly
• Plasmid (a type of vector)
• Methods to deliver a transgene to cells and organisms

F. EXPERIMENTAL APPROACHES
F1. Loss-of-function approaches
• There are three general types of experiments
• Gene knockdown by RNA interference (RNAi)
• Conditional RNAi knockdown by the GAL4/UAS system
• Gene knockdown by CRISPR interference (CRISPRi)
• Enzyme inhibition by drugs
• Gene knockout by CRISPR/Cas9
• Gene knockout in yeast by homologous recombination
• Gene knockout in mammals by positive-negative selection
• Conditional gene knockout by the Cre/Lox system

F2. Gain-of-function approaches
• There are three general types of experiments
• Ubiquitous gene overexpression
• Cell type-specific gene overexpression
• Conditional overexpression by the GAL4/UAS system
• Conditional gene expression by an environmental agent
• Gene overexpression by CRISPR activation (CRISPRa)

F3. Discovery approaches
• There are three general types of experiments
• Discovery approaches
• Omics approaches
• Genomics
• Genomics involves predicting the genes in genomes
• Genomics involves predicting other features of genomes
• Comparative genomics compares genomic features of different organisms
• BLAST is used to compare DNA, RNA, and protein sequences
• Transcriptomics
• Proteomics
• Mass spectrometry is commonly used for proteomic analysis
• RNA profiling detects new translation

F4. Experimental controls
• Experimental controls
• Controls test whether samples are equally loaded
• Controls test the size of molecules
• Positive controls test whether the assay worked as expected
• Negative controls test the specificity of an outcome
• Untreated controls test the effect of a treatment
• Wild type controls test the effect of a mutation
• Controls test the timing of molecular events