EUKARYOTIC MOLECULAR GENETICS, **MOCB 630**, FALL 2002
http://www.life.umd.edu/classroom/MOCB630/

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Meetings MWF 9:00 PLS 1117. Sept. 4 through December 13.

We will **not** meet Sept. 16 or Nov. 29. There is no final exam.

Prerequisites:
Genetics and at least one advanced course in either Genetics, Biochemistry or Molecular Genetics at the undergraduate level. Those without these prerequisites are encouraged to take BSCI 410 first.

Readings
11 papers from the primary literature in molecular genetics are required reading. Papers and supplemental readings will be on reserve in the White (Chemistry) library and most will be available online; I will provide links through the course web page at **http://www.life.umd.edu/classroom/mocb630**

Texts and background literature

In the book store:

- **Hartwell et al.** Genetics: From Genes to Genomes. 1st edition.2000
This text is **required**, and I will refer you to it for background reading.

- **Gibson and Muse** A Primer of Genome Science. 2002.
This text is **recommended**; you may find it extremely useful as a source of information and explanations for techniques and concepts not in Hartwell.

On reserve in White (Chemistry) library:

- **Weaver**, Molecular Biology**.** WCB McGraw Hill. "2002" (2nd edition).
- Watson et al., **Recombinant DNA** Freeman. 1992 (2nd edition).
- **Alberts** et al., Molecular Biology of the Cell. Garland. 1994 (3rd edition).
This book is online (see **http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=Books**)
- Strachan and Read. **Human Molecular Genetics 2 (HMG2)** Wiley. 1999.
This excellent book is strongly recommended as a supplement. It is a good genetics text in general in addition to being exhaustive on the subject of human genetics.

Permission is required for this course. Anyone who has been accepted into the MOCB or CBMG graduate program will automatically be granted permission by the department or program. Students in other Ph.D. programs will be granted permission from the instructor on a individual basis. This year's class is now full, so only these students may attend. The policy is described in even more detail at http://www.wam.umd.edu/~smount/permissions.html. Keeping the class small is important for the paper presentations.

Coverage.
Methods for genetic analysis in various species commonly used for molecular genetics (model organisms) will be discussed. The primary species are the yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, the worm *Caenorhabditis elegans*, the plant *Arabidopsis thaliana*, the fruit fly *Drosophila melanogaster*, the mouse, and humans. For each species we will consider methods for the identification of informative mutations revealing new genes or new gene functions. We will also discuss methods for testing the expression of modified genes and methods for genetic dissection of biological aspects such as developmental or signaling pathways. We will bear in mind throughout the application of information from model organisms to organisms which are not in this select group.

The course will also involve a great deal of information about gene expression, cell biology and development. These perspectives, particularly gene expression, will be interwoven, but the subject matter is methods of genetic analysis, not the biochemistry of gene expression.

Grading

Your grade will be determined as described below. +/- grading will be used (i.e. grades of A+, A-, B+, C-, etc. are possible). In general, you will not be directly tested on the lecture material except as it applies to the papers, or is reflected in the homework. You are encouraged to write the Honor Pledge on your assignments (see http://www.inform.umd.edu/honorpledge/). I encourage you to work together to prepare your presentations and to discuss the papers and homework, but you are expected to prepare your own homework and you are responsible for your own answers.

Papers The goal is to learn how to extract from a paper:

- what claims are being made
- how the results were obtained (i.e. which experiments were done)
- why the experiments were done the way they were
- whether the data justify the conclusions and what caveats remain
- why these experiments were done at all; and why we should care

On the date that a paper is to be discussed, come to class familiar with the paper, and with a copy of the paper in hand. Your familiarity with the papers will be judged by your presentations, your contributions to the overall discussion, and by your grade on quizzes.

Presentations (20%)
When papers are covered in class, I may call on you to present a defined section of the paper (usually one figure, or a part of a figure). Your presentation will be graded based on the clarity with which you cover the following four things:

1) the overall point of the figure or section
2) the techniques used
3) the results
4) the interpretation.

Many of the presentations will be given a specific time limit (e.g. 5 minutes), and you will know this in advance.

I will give you a grade on a your presentation and a summary by email after class (within a few days). Everyone is subject to being called on at every paper presentation, except that you will be called on only once for a single paper, and you are exempt from presentation on the very next paper. For example, if you are selected to present a figure in paper 2, you will be exempt from presentation for paper 3. This will exempt you from **presentations** on that day, **but not from quizzes**. Those scheduled to present on a particular day will be informed at the beginning of class. The selection of students for presentation will be essentially random except that I will insure that everyone presents at least two times during the course of the semester, and you can be removed from the list (if you want to be), when and if you have presented four times. Figures assigned to specific presenters chosen for the first day of a paper scheduled for presentation over two days will be reassigned if they are not presented. Those who present on the first day will be removed from the pool for the second day **and** for the next paper (but will not be exempt from quizzes).

Quizzes (30%)

A quiz may be given on a paper on presentation day. In some cases, there may be a brief quiz before our discussion of the paper. In other cases, the discussion may be replaced by a full one-hour quiz, or the quiz will come at the end. Some quizzes will just test basic preparation (e.g. can you recognize the authors' words? Did you pay attention during the presentations?) while others will test your understanding of the finer or deeper points of the paper. Accordingly, some quizzes will count more than others. This weighting will be reflected in the total number of points in the quiz.

Discussion (10%)

Contributions to the discussion that aid everyone's understanding of the paper will help your grade. Good questions are especially encouraged. I will never grade anyone down for asking honest questions about the paper.

Homework (40%).

Homework will be assigned at least four days (two class meetings) before the due date. Homework is due at the beginning of class. In many cases, homework questions will direct your reading to essential points of the paper. However, not all papers will have homework, and not all homework will be specifically related to a paper. There will be about 14 homework assignments, each of which will count 20 points. Your lowest four grades (including any not turned in) will be dropped before averaging. Whether or not I accept late homework, and how much it is discounted, will vary depending on many factors, including whether the homework was discussed in class before the late homework was turned in, and why it was not turned in on time.

This year, I am trying something new. Each of you will be asked to select a specific gene that interests you, and each of you will have a different gene. Subsequent homework assignments will have questions regarding your gene.

At the end of the year, the overall scores for homework and quizzes will be normalized before summing. Presentation grades will be averaged as is. I generally give roughly equal numbers of A's and B's and only a few C's, but I do have a sense of what it takes to get an A that is independent of your performance relative to the rest of the class.

Because evaluation during class when papers are presented will count for 50% of your grade, you should be sure to attend class on those days! If you must be absent from class on days when papers are presented (dates in **bold** on the syllabus) you should let me know (by email or telephone), **in advance if possible**, and explain your absence. If there is a legitimate reason why you cannot present on a particular day, you will be taken out of the pool. If you are assigned a presentation and are absent from class, or decline to present, you will be given a presentation grade of C. In the case of conflicts that you know about in advance, try to let me know as soon as you know that you will be missing the class. Unanticipated absences will be dealt with on a case-by-case basis.

If you simply want to be excused from a particular paper, you can be taken out of the pool for one paper in exchange for a grade penalty equivalent to one-third of a grade (e.g. A- instead of A, or B+ instead of an A-) on another presentation. You must ask for this before the assignments are made (by email or voice mail [x5-6934] prior to 8:30 a.m., or when I arrive at the room). This will exempt you from presentations on that day, but it will not exempt you from quizzes.

Office hours: I will normally be available immediately after class on Mondays and Wednesdays, and I am happy to set up an appointment to meet with you in my office at other times. You can do this by talking to me in person, or by making an arrangement by email.

I like to address questions via email so that I can disseminate my answers to the entire class. If you send questions to me at sm193@umail.umd.edu, I will generally remove your name and send my answer to the class mail reflector, MOCB630@umail.umd.edu, so that everyone can see the question and its answer. Of course, not all questions are of a public nature; you should tell me if you don't want the question posted on the class mail reflector. I will also exercise judgment about what matters call for a public answer. Finally, you can send questions to the whole class directly via the mail reflector if you prefer.

Groups and homework: I strongly encourage you to form groups to prepare your presentations and to discuss the homework. In the past, students have always done this on their own, but if you want me to help you find partners, let me know. While discussion of homework is encouraged, you are expected to prepare your own answers. I do not accept homework by email (except under unusual circumstances, typically involving a need to be absent from class on the day that it is due).

Sept. 4 Lecture 1 - **General Introduction**
Outline of the course.
Principal species ; Major techniques of molecular biology;
The flow of genetic information from DNA to RNA to protein.

Sept. 6 Lecture 2 - **Techniques in molecular biology I: cloning & hybridization.**

Sept. 9 Lecture 3 - **Techniques in molecular biology II: PCR**

Sept. 11 **Paper 1 --** Bartel, D. and J. Szostak. 1993. Isolation of new ribozymes from a large pool of random sequences. Science **261**: 1411-1418.
Homework 1 is due.

Sept. 13 **Paper 1,** continued.

Sept. 18 Lecture 4 - **Techniques in molecular biology III:**
Sequencing genomes.

-- Fleischmann et al. (>30 authors). 1995. Whole-genome random sequencing and assembly of *Haemophilus influenzae* Rd. Science **269**: 496-512.

-- Venter et al. 1998. Shotgun sequencing of the human genome. Science **280:** 1540-1542.

-- Myers et al. 2000. A whole-genome assembly of *Drosophila*. Science 287:2196-2204.

Sept. 20 Lecture 5 - **Techniques in molecular biology IV:**
Genome analysis, protein domains, types of homology.

- http://www.ncbi.nlm.nih.gov

- The Arabidopsis Genome Initiative. 2000. Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana.*

- International Human Genome Sequencing Consortium. 2001. Initial sequencing and analysis of the human genome. Nature **409**:860-921

- Doolittle, R. 2002. Microbial genomes multiply. Nature **416:** 697.

- Fitch, W.M. 2001. Homology: A personal view of some of the problems. Trends Genet. **16**: 227-231.

Sept. 23 Lecture 6 - **Molecular genetics: Genetic maps and markers.**
Homework 2 is due.

Sept. 25 Lecture 7 - **Molecular genetics: Humans I:**
Genomics, human gene mapping and positional cloning.
Homework 3 is due.

Sept. 27	**Paper 2 --** Wang et al. 1998. Large-scale identification, mapping and genotyping of single-nucleotide polymorphisms in the human genome. Science **280**:1077-1082. **Homework 4 is due.**
Sept. 30	Lecture 8 - **Molecular genetics: humans II: complex traits, advanced mapping** -- Lander E and NJ Schork. 1994. Genetic dissection of complex traits. Science: **265**: 2037-2048.
Oct. 2	**Paper 3 --** Lai et al. 2001. A forkhead-domain gene is mutated in a severe speech and language disorder. Nature **413**:519-523. **and** Enard et al. 2002. Molecular evolution of *FOX2P*, a gene involved in speech and language. Nature **418**: 869-872. **Homework 5 is due.**
Oct. 4	Lecture 9 - **Gene expression I - DNA recombination, repair, transposition**
Oct. 7	Lecture 10 - **Molecular genetics: Mouse I:** Transgenics, knock-outs.
Oct. 9	**Paper 4 --** de Wind, N, D. Dekker, A. Berns, M. Radman and H. te Riele. 1995. Inactivation of the mouse *Msh2* gene results in mismatch repair deficiency, methylation tolerance, hyperrecombination, and predisposition to cancer. Cell **82**: 321-330. **Homework 6 is due.**
Oct. 11	Lecture 11 - **Molecular genetics: Mouse II:** Inbred lines, QTLs, RDA. Tanksley. ARG *27*: 1993. Mapping Polygenes. pg. 205. Lisitsyn. Representational Difference Analysis: finding the difference between genomes. TIGs. 11: 303.
Oct. 14	**Paper 5 --** Lowrey et al. 2000. Positional syntenic cloning and functional characterization of the mammalian circadian mutation *tau*. Science 288:483-491. **Homework 7 is due.**
Oct. 16	**Paper 5,** continued.
Oct. 18	Lecture 12 - **Molecular genetics: Yeast** -- Ch. 13 of Recombinant DNA: Using yeast to study eukaryotic gene function.
Oct. 21	Lecture 12 - **Gene expression II: Transcription.** -- Roeder. 1996. The role of general initiation factors in transcription by RNA polymerase II. Trends Biochem Sci. **21**: 327-335.
Oct. 23	Lecture 14 - **Gene expression III: Regulation of transcription**.

Oct. 25 Lecture 15 - **Gene expression IV: Microarrays and parallel genetic analysis**
-- DeRisi JL, Iyer VR, Brown PO.1997. Exploring the metabolic and genetic control of gene expression on a genomic scale. Science. 278:680-686.
-- Hughes et al. 2000. Functional Discovery via a compendium of expression profiles. Cell 102:109-126.
-- Cormack BP, Ghori N and S. Falkow. 1999. An adhesin of the yeast pathogen Candida glabrata mediating adherence to human epithelial cells. Science **285**:578-582.
-- E.A. Winzeler et al. 1999. Functional Characterization of the S. cerevisiae Genome by Gene Deletion and Parallel Analysis. Science **285**: 901-906.
-- Hensel M et al. 1995. Simultaneous identification of bacterial virulence genes by negative selection. Science 269:400-3

Oct. 28 **Paper 6 -- Giaever et al. 2002. Functional profiling of the *Saccharomyces cerevisiae* genome. Nature 418: 387-391.**
Homework 8 is due.

Oct. 30 Lecture 16 - **Genetic concepts I: Redundancy and synthetic phenotypes.**
- Guarente, L. 1995. Synthetic enhancement in gene interaction: a genetic tool comes of age. Trends Genet. **9**: 362-366.

Nov. 1 Lecture 17 - **Molecular Genetics: *Arabidopsis thaliana***

Nov. 4 **Paper 7 --** Hua J and E.M. Meyerowitz. 1998. Ethylene responses are negatively regulated by a receptor gene family in Arabidopsis thaliana. Cell **94**: 261-271.
Homework 9 is due.

Nov. 6 Lecture 18 - **Genetic concepts II: Epistasis.**
-- L. Avery and Wasserman, S.. 1992. Ordering gene function: the interpretation of epistasis in regulatory hierarchies. Trends in Genetics **8**:312-316

Nov. 8 **Molecular genetcis**: ***C. elegans***

Nov. 11 Lecture 19 - **Gene expression VI: RNA Processing, stability and localization.**
Nuclear pre-mRNA processing (splicing and polyadenylation)
Nonsense-mediated decay, cytoplasmic polyadenylation, RNA editing.

Nov. 13 **Genetic concepts III**:
Suppression, enhancement and gene interactions.
-- Prelich, G. 1999. Suppression mechanisms: themes from variations. Trends in Genetics **15**: 261-266.
-- Mount and Anderson 2000. Expanding the definition of informational suppression. Trends Genet. 16:157.

Nov. 15 **Paper 8 --** Zhang B, Gallegos M, Puoti A., Durkin E, Fields S, Kimble J and MP Wickens. 1997. A conserved RNA-binding protein that regulates sexual fates in the *C. elegans* hermaphrodite germ line. Nature **390**: 477-484.
Homework (10 and 11) is due.

Nov. 18 **Paper 8,** continued.

Nov. 20 Lecture 20 - **Molecular genetics: *Drosophila* I:**
Introduction, nomenclature, basics.
-- Greenspan. **Fly Pushing**. Cold Spring Harbor Press. 1996.
-- Rubin and Lewis. 2000. A brief history of *Drosophila*'s contributions to genome research. Science **287**:2216-2218.

Nov. 22 Lecture 21 - **Genetic concepts IV**: **The nature of alleles and mutations.**
-- Muller, H.J. 1932. Further studies on the nature and causes of gene mutations. Sixth International Congress of Genetics **1**:231-255.
HMG2 - Ch. 16 Molecular pathology.

Nov. 25 Lecture 22 - **Molecular genetics: *Drosophila* II:**
The many uses of P elements

Nov. 27 Lecture 23 **- Molecular genetics:**
Binary systems for control of gene expression in development.
Binary systems for ectopic expression. Binary systems for controlled deletions.
-- Lindsay et al. 2000 Congenital heart disease in mice deficient for the DiGeorge syndrome region. Nature **401**:379-383
-- Scambler 2000 Engineering a broken heart. Nature **401**:335)

Dec. 2 **Paper 9 --** Rørth et al. 1998.Systematic gain-of-function genetics in *Drosophila.* Development **125**: 1049-1057.
Homework 12 is due.

Dec. 4 Lecture 24 - **Gene expression V: Epigenetic phenomena.** Methylation, imprinting, heterochromatin and maintenance of active or inactive states.
Zamore 2002 Science.

Dec. 6 **Paper 10** -- Schotta et al.. 2002. Central role of *DrosophilaSu(var)3-9* in histone H3-K9 methylation and heterochromatic gene silencing. EMBO J. **21**: 1121-1131.
Homework 13 is due.

Dec. 9 **Paper 10** -- continued

Dec. 11 Lecture 25 - **Gene expression VI:** RNA interference and microRNAs

Dec. 13 **Paper 11** --Grishok et al. 2001. Genes and mechanisms related to RNA interference regulate expression of the small temporal RNAs that control *C. elegans* developmental timing. Cell **106**: 23-34.
Homework 14 is due.