BI425/525: Advanced Molecular Biology Research Lab, Winter, 2015

Alice Barkan, Instructor. abarkan@uoregon.edu GTF-Nick DePatie, ndepatie@uoregon.edu

This is an intensive advanced laboratory course that will involve students in research that combines genetic, molecular biology and genomic approaches to dissect a complex cellular process: the biogenesis of the chloroplast. Students will gain hands-on experience with common molecular biology techniques, bioinformatic tools and "next generation" DNA sequencing methods. Students will use their knowledge of genetics, gene expression, and photosynthesis acquired in introductory coursework, and they will build on that foundation by learning about transposable elements, nuclear-organellar interactions, gene families, genome evolution, and more. Lectures and assigned readings will describe the biological context of this research and the theoretical basis of the techniques employed. Student-directed discussion of research articles and current topics related to the course content will be incorporated throughout the term.

The broad goals of the course are to:
• Provide research training as a stepping stone to research in a UO lab and/or post-graduation employment in a research setting.
• Synergize with upper level courses that focus on primary research literature by providing hands-on experience with common experimental methodologies.
• Develop the critical thinking and creative skills associated with data evaluation, hypothesis formulation, and experimental design.

Student Learning Outcomes.
Students in this course will:
• Gain hands-on experience with the theory and practice of common methods in molecular biology (e.g. western blotting, DNA/RNA purification, PCR, molecular cloning, recombinant protein expression, next-generation DNA sequencing).
• Be trained in the use of genome databases and tools for making inferences about gene structure and function.
• Learn basic laboratory skills such as how to keep a proper laboratory notebook, make solutions, etc.
• Learn about a swathe of biology that is only minimally covered in other UO courses (e.g. transposable elements, the biogenesis of chloroplasts and mitochondria, plant biotechnology).
• Develop the critical thinking and creative skills associated with data evaluation, hypothesis formulation, and experimental design.

Grades will be based on the following components:
• **Lab notebook checks** (three graded checks; 15% of grade: *Is the goal of the experiment clearly stated? Are the procedures outlined in sufficient detail? Is the data properly labeled? Are the conclusions summarized? Are implications and suggestions for future experiments noted?*
• **Quizzes** (Four quizzes, 28% of grade)
• **"Journal club" presentation** (15%)**.** *Students will work in small groups to present a research article or overview of current topic related to the course content. See syllabus for topic choices.*
• **Final Symposium Talk** (15%): *Grades will be based on clarity of presentation, understanding of the material, ability to answer questions clearly and correctly.*
• **Final Paper** (20%)- *This project summary should include the question posed, experimental approach, and conclusions at each step, the final conclusions and the reasoning that led to them. Describe new questions that arise from your results and where you would go from here if you were to continue the project.*
• **Discretionary points** (7%)- *Are equipment and reagents used properly? Is the student cooperative? Does the student participate actively in discussions and display independent thought?*

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Overview of Experimental Goals

Two independent projects will be interwoven during the term:

Goal #1: Identify the gene whose disruption causes a specific non-photosynthetic mutant phenotype in maize

- analyze mutant phenotypes by Western blot analysis of photosynthetic complexes
- identify candidate causal mutations: Mu-Illumina analysis to find *Mu* insertions that cosegregate with this phenotype
- prioritize candidates for followup: Bioinformatic analyses of genes disrupted by cosegregating *Mu* insertions
- test best candidates by PCR to evaluate genetic linkage to the mutant phenotype
- test best candidates by RT-PCR to determine the degree to which the insertion disrupts gene expression.

Techniques: SDS-PAGE, Western blotting, PCR, principles of genetic analysis, Illumina sequencing, use of genome database resources, RT-PCR

Goal #2: Express a recombinant protein in E. coli for production of antiserum

- choose a conserved region of the assigned protein for expression
- clone the DNA segment encoding these region into an E. coli expression vector
- express the protein and purify it by nickel affinity chromatography

Techniques: Multiple sequence alignments, molecular cloning by Gibson Assembly, E. coli transformation, expression and purification of his-tagged proteins from E. coli

Tentative Class Schedule

The scheduled course hours are provided only as a guideline. On some days we will not need the full scheduled time, but on others it may be necessary to stay late or come in outside of class hours. Thus, the course schedule shows the order of topics and assignments, but the precise dates are likely to change as the term progresses.

Date	Lecture	Lab	Assignments (Readings available on Blackboard)
Week 1			
Tues Jan 6	Course overview: biological context and goals of experiments. Lab basics: Safety, Lab Notebooks, Pipetting, Making Solutions	Pipetting 101. Making solutions Introduction to your mutants (mark mutants for harvest at next class).	Reading: Dorrell and Howe (2012) What makes a chloroplast? Reconstructing the establishment of photosynthetic symbioses. J. Cell Sci. 125: 1865-
Thurs Jan 8	Principles of Electrophoresis & Western blotting	Protein extraction and quantification. Make detailed plan for protein gels: how much of what will be loaded where? Harvest tissue for future protein, RNA, DNA extraction.	Ten things every molecular biologist should know. pp 2-11
Fri Jan 9	Review of photosynthesis.	Quantify photosynthetic enzymes by SDS-PAGE/Western blotting: run gels and make blots Finish harvesting tissue	Nelson & Ben-Shem. (2004) The complex architecture of oxygenic photosynthesis. Nat Rev Molec Cell Bio **5,** 971–982
Week 2			
Tues Jan 13	Where your mutants came from: The "PML" mutant collection	Probe Western blots with first set of antibodies. Method reinforcement: Prep and quantify replicate protein samples.	Reading: Stern, Hanson, Barkan (2004) Genetics and genomics of chloroplast biogenesis: maize as a model system. Trends in Plant Sci 9: 293-301
Thurs Jan 15	Working with Protein, DNA, and RNA	Run gels of new protein samples and blot Reprobe first Western blots with 2nd antibody cocktail	Work on lab notebooks
Fri Jan 16	Molecular cloning of gene segments into plasmid vectors for protein expression in E. coli.	Probe new western blots with first set of antibodies.	Ungraded lab notebook check. **Chapter 8.1-8.4 Recomb DNA Technology and Molec Cloning** Optional: Chapter 9.2 Molecular Cell Biology 5th https://books.google.com/books?id=sLSdqxA7wScC&printsec=frontcover&dq=molecular+biology+of+the+cell+4th+edition&hl=en&sa=X&ei=ykSjVKDkF4W4oQSQ4YGoAg&ved=0CCIQ6AEwAQ#v=twopage&q=molecular%20biology%20of%20the%20cell%204th%20edition&f=true

Week 3	Lecture	Lab	Assignments
Tues Jan 20	(Alice at conference) Selecting protein segments for antibody production: multiple sequence alignments to identify conserved regions.	Sequence alignments; design inserts for expression constructs Probe new western blots with 2nd set of antibodies	**QUIZ #1**
Thurs Jan 22	Chloroplast gene expression	New SDS-PAGE/blots to fill in missing protein data. Finalize antigen expression constructs and o*rder "gene blocks" encoding antigens*	Barkan (2011) Expression of Plastid Genes: Organelle-specific elaborations on a prokaryotic scaffold. Plant Phys 2011 Yagi and Shiina (2014) Recent advances in the study of chloroplast gene expression and its evolution. Front Plant Sci 5:61 ***LAB NOTEBOOK CHECK #1.***
Fri Jan 23	Maize genome, Transpsoson tagging, Mu transposons	Group meeting: Discuss data and antigen designs Linearize plasmid for antigen expression clones	Reading: Lisch (2002) Mutator Transposons. Trends Plant Sci 7:498-504
Week 4			
Tues Jan 27	Illumina Sequencing Technology Mu-Illumina Method for Mapping Mu insertions.	Extract DNA from mutants and wt cousins for cosegregation tests Check plasmid digestions on gel. Discuss Mu-Illumina method paper.	http://bitesizebio.com/articles/sequencing-by-synthesis-explaining-the-illumina-sequencing-technology/ Williams-Carrier et al, (2010) Use of Illumina sequencing to identify transposon insertions underlying mutant phenotypes in high-copy Mutator lines of maize. Plant J 63:167-177
Thurs Jan 29	Cosegregation analysis/pedigrees	Quantify extracted DNAs by nanodrop and on gels. Discuss pedigrees	
Fri Jan 30	PCR overview Organize presentations: 1. Plastids and Human Disease- the Apicoplast (2) 2. Chloroplast-Based Biotechnology: BioPharming, BioPlastics (4) 3. Translational Research in Plants and the GMO debate (5)	Gibson Assembly of Inserts/Vectors for protein expression, and E. coli transformation	**QUIZ #2**

Week 5	Lecture	Lab	Assignments
Tue Feb 3	Predicting protein function: conserved domains, orthologous groups, and associated databases (TAIR, POGs, InterPro). Working with Mu-Illumina insertion database/ search interface.	Practice PCR on DNA preps Inoculate cultures for plasmid minipreps	EM Schwarz. (2005) Genomic classification of protein-coding gene families. http://www.wormbook.org Earnshaw (2013) Deducing Protein Function by Forensic Integrative Cell Biology, PLOS Biology e1001742
Thursday Feb 5	Predicting protein function cont'd: protein localization, coexpression (TargetP, Predotar, Atted II)	Practice PCRs: analyze products on gels Plasmid mini-preps Analyze Mu-Illumina data: identify insertions that cosegregate with phenotypes	***LAB NOTEBOOK CHECK #2.***
Fri Feb 6	Strategies for prioritizing insertions for followup. Complications in cosegregation analysis: epigenetic suppression of mutant phenotypes	Viewing Illumina reads with the IGV and identifying insertion sites. Evaluating gene models via POGS2 Plasmid miniprep followup: restriction digestions and/or PCR to check for plasmids with inserts	Barkan and Martienssen (1991) Inactivation of maize transposon Mu suppresses a mutant phenotype by activating an outward-reading promoter near the end of Mu1. PNAS 88:3502-
Week 6			
Tue Feb 10	Primer on Giving Scientific Presentations	Identify cosegregating insertions and evaluate candidates for PCR followup, day 1 Continue evaluation of expression constructs	
Thursday Feb 12		Identify cosegregating insertions and evaluate candidates, day 2 Continue evaluation of expression constructs	
Fri Feb 13	***Group Meeting: Present priorities for validation by PCR***	Identify cosegregating insertions and evaluate candidates, day 3	**QUIZ #3: Take Home and Turn in on MONDAY** (PCR, Functional inferences from ortholog prediction, domain architecture, coexpression data).

Week 7	Lecture	Lab	Assignments
Tues Feb 17		Design PCR primers for evaluating candidates. Order PCR primers. Inoculate cultures for protein expression	**Take-home QUIZ #3 due** **Lab Notebook Check #3**
Thurs Feb 19	Journal Club: Plastids and Human Disease: The Apicoplast *(2 students)*	Wait for primers to arrive. Nickel-affinity purification of his-tagged proteins.	Dooren and Striepen (2013) The Algal Past and Parasite Present of the Apicoplast. Ann Rev Microbiol 67:271-89 Nair&Striepen (2011) What do human parasites do with a chloroplast anyway? PLOS Bio 9: e1001137 Yeh & DeRisi (2011) Chemical Rescue of Malaria Parasites Lacking an Apicoplast Defines Organelle Function in Blood-Stage Plasmodium falciparum. PLOS Biol 9: e1001138
Fri Feb 20	Journal Club: Chloroplast-based Biotechnology: Molecular Pharming, bioplastics *(4 students)*	SDS-PAGE of purified recombinant proteins	Maliga and Bock (2011) Plastid Biotechnology: Food, Fuel, and Medicine for the 21st Century. Plant Physiol 155:1501- Bohmert-Tatarev et al (2011) High levels of bioplastic are produced in fertile transplastomic tobacco plants engineered with a synthetic operon for the production of polydyroxybutyrate. Plant Phys 155:1690- Kohil et al (2014) Oral delivery of bioencapsulated proteins across blood-brain and blood-retinal barriers. Mol Ther. 22:535-46. *and commentary* Shenoy et al (2014) Oral delivery of Angiotensin-converting enzyme 2 and Angiotensin-(1-7) bioencapsulated in plant cells attenuates pulmonary hypertension. Hypertension 64:1248-. *and comment on p. 1159)*

Week 8	Lecture	Lab	Assignments
Tues Feb 24		PCR validations: day 1 Test Gene-Specific Primers	
Thurs Feb 26		PCR validations: day 2 Genotype mutants and WT cousins with the winning primers	
Fri Feb 27	Journal Club: Plant Translational Research and the GMO Debate Part 1: The Big 2 GMOs: Round-up Ready and BT crops Do we need GMOs? The promise and the hazards *3 students*	PCR validations: day 3	http://www.pbs.org/wnet/dna/pop_genetic_gallery/page3.html http://12.000.scripts.mit.edu/mission2014/genetically-modified-crops Nature (2013) v497: Fields of Gold (p5-6), A Hard Look at GM Crops (24-25); Africa and Asia need a rational debate on GM Crops p 31-32 Tabashnik et al (2013) Insect resistance to Bt crops: lessons from the first billion acres. Nat Biotech 31:510- GMs in Willamette Valley : Scientist in the Middle of the GM-Organic Wars. Science (2011) 332: 168 Ronald (2014) Lab to farm: Applying research on plant genetics and genomics to crop improvement. PLOS Biol 12: e1001878 Lau et al (2014) Key applications of plant metabolic engineering. PLOS Biol 12: e1001879 Gurian-Sherman (2014) Are GMOs worth the trouble? Technology Review

Week 9			
Tuesday March 3		PCR validations: day 5	**QUIZ #4: Take Home and Turn in on MONDAY** (identifying candidate insertions; molecular cloning, recomb protein expression)
Thurs March 5		PCR validations, continued. RT-PCR to assay expression of the disrupted gene suspected to underlie the mutant phenotype	*Seek/read literature relevant to your gene identifications and (for graduate students) research proposal*
Fri March 6	Journal Club- Plant Translational Research, Part 2: Applying Genome Sequencing Technologies to Crop Breeding Genome Engineering Technologies in Crops *(2 students)*	PCR and RT-PCR continued.	Varshney et al (2014) Harvesting the promising fruits of genomics: Applying Genome Sequencing Technologies to Crop Breeding. PLOS Biol 12: e1001883 Voytas and Gao (2014) Precision genome engineering and agriculture: Opportunities and regulatory challenges. PLOS Biol 12: e1001877
Week 10			
Tue March 10		Bringing it all together: comparison of mutant phenotypes with inferred functions of genes disrupted by genetically-linked insertions; conclusions about likely causal insertions. Prepare for Symposium	*Seek/read literature relevant to your gene identifications and (for graduate students) research proposal*
Thursday March 12		As above.	"
Friday March 13	**Symposium: Presentation of Results (30 min/group)**		
Wed March 18	**Final Paper Due at noon**.		