

PBIO 3660L, Fall 2021: Molecular genetics with plants

Time and venue: Mon/Wed/Fri 10:20 AM – 12:15 PM, 1604 Miller Plant Sciences

Instructor: Wolfgang Lukowitz, Plant Biology, 4606 Miller Plant Sciences, phone: 706-542-1121, email: lukowitz@uga.edu. Office hours by appointment – please email me!

Course prerequisites: Introductory biology & associated lab (BIOL 1108 & BIOL 1108L; or BIOL 2108 & BIOL 2108L; or PBIO 1210 & PBIO 1210L; or PBIO 1210 & PBIO 1220L), or permission of the department.

Course objective

This is an intensive lab course aiming to introduce participating students to the variety of research questions and methods that are used by plant biologists in an increasingly integrative discipline. Students will work in small teams on self-contained independent projects to develop an understanding of the biological questions at the base of their research project as well as the experimental approaches adopted to address them. Emphasis will be placed on experimental design and critical evaluation of the resulting data.

Project outlines

After fertilization, plant zygotes typically elongate and then divide into a small apical cell and a large basal cell. These two cells then follow very different developmental trajectories: the apical cell produces a spherical pro-embryo, while the basal cell produces a support structure called suspensor. Root formation is started across the suspensor/pro-embryo boundary, such that the main body axis (apical-basal or top-bottom) can be traced back to the division of the zygote. We will pursue two projects that will hopefully contribute to understanding how this asymmetric first division is organized and how it affects axis formation.

Project 1: Recently, comprehensive lists of the genes that are active in the zygote as well as in the very young embryo have been compiled and published. Can we use these lists to identify potential regulators of zygote elongation and/or the different developmental pathways adopted by the apical and basal daughter cells of the zygote? Let's discuss potential criteria! Once we have settled on a number of candidate regulators, we'll try to test their function by knocking down or eliminating their activity. Towards this aim, we'll build T-DNA constructs for loading the egg cell with artificial micro RNAs targeting our candidate genes; we'll then transform these T-DNAs into plants. We won't have enough time in the semester to generate transgenic lines and assess the effect of the artificial micro RNAs – this will be left for a future class.

Project 2: Mitogen-activated protein (MAP) kinase cascades are molecular switches employed by all eukaryotes for the purpose of cellular signaling. One of the known regulators of zygote elongation and the asymmetric first division is a MAP kinase cascade converging on the two MAP kinases MPK3/MPK6. A previous class has assembled and transformed T-DNAs to characterize the MAP kinase kinase (MKKs) of this cascade. We will grow these transgenic lines and evaluate the phenotype of transgenic embryos to determine which MKK genes can partake in early development.

Please keep in mind that these experiments are open and ongoing – which means that (with any luck) we'll be surprised. There is little doubt that we'll need to adapt this plan and improvise over the course of the semester.

Technical skills you will acquire

- General lab skills: preparation of solutions and buffers; pipetting and operation of lab equipment.
- Basic molecular cloning techniques: design of PCR primers and amplification of DNA; agarose gel electrophoresis and isolation of DNA fragments from the gel; ligation of DNA fragments; transformation of *E. coli* and *Agrobacterium* with plasmids; isolation of plasmid from *E. coli*; analysis of DNA by restriction digest; Sanger sequencing.
- Basic bioinformatics: BLAST searches; finding the cDNA and genomic sequence of a gene in a database.

- Production of transgenic plants: construction of a T-DNA plasmid and transformation into *Agrobacterium*; *Agrobacterium*-mediated transformation of Arabidopsis by floral dip and selection of transgenic progeny.

Items you need to bring to class

- Laptop computer – you will be required to install software for handling DNA sequence files.
- Lab notebook (no loose papers, please!) and pen/pencil for keeping records.

Study material

We will draw on information from the primary literature and publically available data resources, such as GenBank, to develop the research projects. Relevant background material will be shared on eLC, including: published papers; lab protocols; ppt slides, screen recordings, or short movies prepared by the instructor; preliminary results of class experiments; and assignments relevant to the project. There will be no assigned textbook.

Assessment and grading

The grading system is traditional (A-F). There will be no exams. Final grades will be based on your participation and performance in class. Briefly, grades will depend on:

- completion of take-home assignments that will be given out throughout the semester (40%).
- participation and note keeping in the lab (40%).
- a final presentation related to the class project (details to be announced; 20%).

Please note that **attendance is required**. In case of emergencies, please notify the instructor by email ahead of time and provide adequate written documentation. Students missing more than four lab days without documentation will receive a grade of "I" (incomplete).

Lab safety

You are required to follow UGA lab safety guidelines (<https://research.uga.edu/research-announcements/2016/10/13/new-uga-chemical-and-laboratory-safety-manual-available-online/>) when performing research experiments in the lab. Please familiarize yourself with the rules. We will discuss the relevant safety measures for specific experimental procedures when the procedures are introduced in class.

Academic honesty

All issues of academic honesty will be handled according to the procedures outlined in the booklet "A Culture of Honesty: Policies and Procedures on Academic Honesty", available from the Dean's Office or online (www.uga.edu/honesty/ahs/ahs.htm).

UGA student honor code. "I will be academically honest in all of my academic work and will not tolerate academic dishonesty of others." A Culture of Honesty, the University's policy and procedures for handling cases of suspected dishonesty, can be found at www.uga.edu/ovpi.

Mental health and wellness resources

- If you or someone you know needs assistance, you are encouraged to contact Student Care and Outreach in the Division of Student Affairs at 706-542-7774 or visit <https://sco.uga.edu>. They will help you navigate any difficult circumstances you may be facing by connecting you with the appropriate resources or services.
- UGA has several resources for a student seeking mental health services (<https://www.uhs.uga.edu/bewelluga/bewelluga>) or crisis support (<https://www.uhs.uga.edu/info/emergencies>).

- If you need help managing stress anxiety, relationships, etc., please visit BeWellUGA (<https://www.uhs.uga.edu/bewelluga/bewelluga>) for a list of FREE workshops, classes, mentoring, and health coaching led by licensed clinicians and health educators in the University Health Center.
- Additional resources can be accessed through the UGA App.

COVID-19 pandemic

We all certainly hope that the worst of this pandemic is behind us. However, the situation is still volatile, and it remains a vital priority to continue limiting the spread of COVID-19 amongst fellow students, faculty, staff, and –last but not least!– the local community. Below, a summary of current university rules, recommendations, and related information – please note that this guidance is subject to change based on recommendations from the Georgia Department of Public Health, the University System of Georgia, or the Governor’s Office or. For the latest on UGA policy, you can visit coronavirus.uga.edu.

Face coverings. Following guidance from the University System of Georgia, face coverings are recommended for all individuals while inside campus facilities.

COVID-19 vaccine. University Health Center is scheduling appointments for students through the UHC Patient Portal (https://patientportal.uhs.uga.edu/login_dualauthentication.aspx). Learn more here – <https://www.uhs.uga.edu/healthtopics/covid-vaccine>.

The Georgia Department of Health, pharmacy chains and local providers also offer the COVID- 19 vaccine at no cost to you. To find a COVID-19 vaccination location near you, please go to: <https://georgia.gov/covid-vaccine>.

In addition, the University System of Georgia has made COVID-19 vaccines available at 15 campuses statewide and you can locate one here: <https://www.usg.edu/vaccination>

What do I do if I have COVID-19 symptoms? Students showing COVID-19 symptoms should self-isolate and schedule an appointment with the University Health Center by calling 706-542-1162 (Monday-Friday, 8 a.m.-5p.m.). Please DO NOT walk-in. For emergencies and after-hours care, see, <https://www.uhs.uga.edu/info/emergencies>.

What do I do if I test positive for COVID-19? If you test positive for COVID-19 at any time, you are **required to report it** through the [DawgCheck Test Reporting Survey](#). We encourage you to stay at home if you become ill or until you have excluded COVID-19 as the cause of your symptoms. UGA adheres to current Georgia Department of Public Health (DPH) quarantine and isolation [guidance](#) and requires that it be followed. Follow the instructions provided to you when you report your positive test result in DawgCheck.

Guidelines for COVID-19 Quarantine Period (As of 8/1/21; follow DawgCheck or see DPH website for most up-to-date recommendations): Students who are fully vaccinated **do not** need to quarantine upon exposure unless they have symptoms of COVID-19 themselves. All others should follow the Georgia Department of Public Health (DPH) recommendations:

Students who are not fully vaccinated and have been directly exposed to COVID-19 but are not showing symptoms **should self-quarantine for 10 days**. Those quarantining for 10 days must have been symptom-free throughout the monitoring period and continue self-monitoring for COVID-19 symptoms for a total of 14 days. You should report the need to quarantine on [DawgCheck](#) (<https://dawgcheck.uga.edu/>), and communicate directly with your faculty to coordinate your coursework while in quarantine. If you need additional help, reach out to Student Care and Outreach (sco@uga.edu) for assistance.

Students, faculty and staff who have been in close contact with someone who has COVID-19 are no longer required to quarantine if they have been fully vaccinated against the disease and show no symptoms.