A research-based Molecular and Cell Biology Lab Manual

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Abstract

Members of the Pennsylvania Academy of Science collaborated to develop a manual that pulls together several research-based labs that they have previously run in their upper-division college lab courses. The activities include bacteria, yeast, and fruit fly microbiology, zebrafish-based cell biology, invertebrate and plant metagenomics, bioinformatics, and genetic engineering in bacteria and yeast. Advanced laboratory techniques include end-point PCR, qPCR, RT-qPCR, bar coding, in situ hybridization, in silico and in vitro gene cloning and editing, and site-directed mutagenesis and mutation detection. The activities can be combined to create multi-week lab explorations or run individually as standalone experiments.

Idea Development
- Pedagogical discussions at PAS Meetings
- PAS Board Meeting conversations about educational initiatives
- Assembly of interested authors

PAS Book Proposal
- Proposal: book summary, names and affiliations of contributors, proposed list of chapters
- Approved by the PAS Board

Book Development
- Layout developed (modular format)
- Chapters written and submitted
- Peer review
- Student review
- Professional editor review

Printing and Publication
- Pre-print produced (sample available)
- Printed versions commercially available (coming soon)
- Website with pdf version available (coming soon)

Lessons Learned

- Pandemics provide a great opportunity for collaborative projects
- The academic year is a difficult time to move writing projects forward
- Sabbaticals provide the impetus for creative work
- The virtual platform greatly enhances collaborative work through shared files and virtual meetings
- Online platforms design and use advanced over the course of this project
  - Initially: faculty hesitant to use shared files and had access difficulties
  - End: faculty could easily connect via Teams for project discussions
- Including students in pedagogical projects provides valuable insight for faculty authors and leadership moments for the students themselves

The Pennsylvania Academy of Science

The Pennsylvania Academy of Science (PAS) was established in 1924 to educate and support scientists throughout the state of Pennsylvania, promoting science, technology, education through its annual conference, journal editions, and book publications. One of the Academy goals is to "support educational initiatives devoted to the development of scientific skills and knowledge" and the annual meeting provides a great opportunity for students to learn about science communication by attending research talks and presenting their own data. It also affords faculty an opportunity to discuss teaching pedagogies and research approaches best used in undergraduate research labs.

As a non-profit organization, PAS initiatives are supported through several grants and donations. The E. Willard and Ruby S. Miller Book Fund was established to subsidize the publication of books on a variety of subjects. Past publications have primarily promoted scientific research, centered on a specific theme such as the impact of emerging issues on bird conservation (*Avian Ecology and Conservation: A Pennsylvania Focus with National Implications* (2010)), this history and science of influenza (*Pandemic Influenza Viruses: Science, Surveillance and Public Health* (2011)), and the bat conservation crisis (*Ecology and Conservation of Pennsylvania's Bats in a Time of Crisis*(2016)). The fund is also intended to assist in the development and distribution of information relating to science and science education, especially in the Commonwealth of Pennsylvania, but in recent years, there have been no education-based publications.

Broad Questions that Inspired this Project

- Are there other lab activities that I can do to engage my students? If so, how do I find them?
  - Commercially available lab manuals are not always grounded in research-based questions
  - Networking among faculty at similar institutions inspired a protocol exchange of sorts
- After years of perfecting lab protocols, are there others who would be interested in using these in their labs?
  - As faculty near retirement (an author retired during the production of this book), there is an interest in allowing their work to persist
  - There is an interest in using labs that have been tested in undergraduate labs and have the personal connection to the faculty who developed them
- Can we develop a format and procedure for the publication of lab protocols to be disseminated to a broader community?
  - PAS supports science education initiatives that seek to promote the academic pursuit of scientific studies among students; thus, the Academy provides a solution to the dissemination question
  - PAS has published research-based books, but this takes the Academy's publication into a different direction.
  - As a non-profit organization with funding from the Miller Book Fund, PAS was able to support this project
  - Future projects could be developed (e.g., a manual on Ecology or Chemistry labs) based on what we have learned from this project

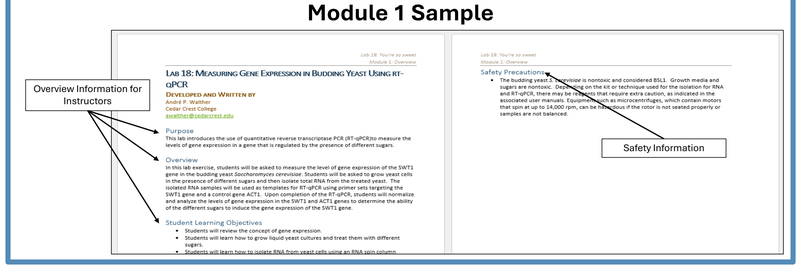
About the Book

- Twenty laboratory exercises
  - Written as individual labs
  - Suggestions for combining them into a series of activities
- Designed for the undergraduate classroom
- Primarily focused on molecular genetics with connections to microbiology and bioinformatics
- Modular in format
  - First module ("Overview"): purpose, overview, student learning objectives, and safety precautions
  - Second module ("Lab Information"): background information to contextualize the activity and review relevant techniques
  - Third module ("Assessment and Extension Activities"): procedural information for the students
  - Fourth module ("Information for Instructors"): lab prep instructions, recommended schedules, connections to other labs

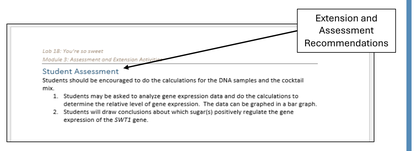
About the Labs

Lab 1: Fruit Flies and the Gut Microbiome
Lab 2: Isolation and Characterization of Plant Growth-Promoting Bacteria
Lab 3: Observing Antibiotic Resistance
Lab 4: Bacterial Growth and Response to Antibiotics
Lab 5: DNA Barcoding I: Sample Collection and Documentation
Lab 6: DNA Barcoding II: DNA Isolation and PCR Amplification
Lab 7: DNA Barcoding III: Agarose Gel Electrophoresis and Sequencing
Lab 8: DNA Barcoding IV: BLAST Analysis
Lab 9: Understanding the Molecular Evolution of SARS-CoV-2 by the Use of Phylogenetic Trees
Lab 10: Structural analysis of the attachment of SARS-CoV-2 to human cells
Lab 11: NCBI and Restriction Mapping for Developing a Cloning Strategy
Lab 12: Recombinant DNA Techniques in silico
Lab 13: DNA Technology I: Generating a Vector through Golden Gate Assembly for CRISPR-Cas9 Gene Editing
Lab 14: DNA Technology II: CRISPR-Cas9 Gene Editing and Genotyping
Lab 15: in vitro Site-Directed Mutagenesis Strategies and Verification
Lab 16: PCR-RFLP Assay for Mutation Detection
Lab 17: HRM Analysis of SNPs to Identify Genetic Disorders: What Will Happen to Izzy and Jimmy Handy?
Lab 18: Measuring Gene Expression in Budding Yeast Using rt-qPCR
Lab 19: Identification of Gene Expression throughout Zebrafish Development Using rt-PCR
Lab 20: Whole-Mount in situ Hybridization Using Zebrafish Embryos

Module 1 Sample

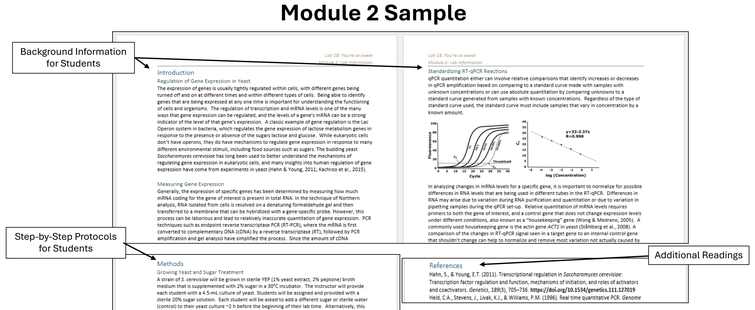


Module 3 Sample

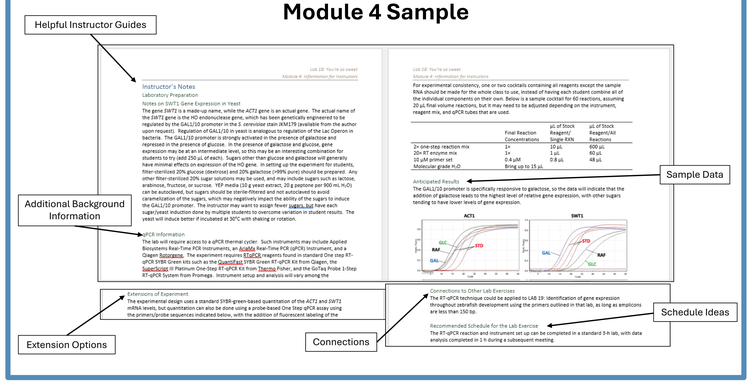


Downloadable Samples

Module 2 Sample



Module 4 Sample



Connecting Different Labs

Lab 3: Observing Antibiotic Resistance → Lab 4: Bacterial Growth and Response to Antibiotics → Lab 16: PCR-RFLP Assay for Mutation Detection

There are several labs that can be easily connected to extend student learning across several weeks and to incorporate a variety of lab techniques. The example shown above begins with Lab 3 where students repeatedly expose a bacterial strain to an antimicrobial (triclosan) so that they may observe derived resistance in that strain over time. Students can further characterize their derived strains by following the Lab 4 procedures, creating growth curves for the initial and final strain in the presence and absence of triclosan to observe growth differences. Finally, Lab 16 explores a specific mutation that has been observed in triclosan resistant strains and uses PCR and restriction digests to determine whether the derived strains have incurred this known mutation. Each of these labs can be conducted separately but provide a much more in-depth research experience when connected.

About the Junior Editors

Goal: Solicit feedback from undergraduates similar to those who would use the manual

- Students from several Pennsylvania colleges and universities were assembled through a Microsoft Team
- Students were assigned to subteams (three students per group), and a group coordinator was identified
- The subteams were assigned a subset of labs
- Each student was asked to review the files independently, make edits and comments, and upload their annotated files to the subteam project folder on the Teams site
  - Review Instructions: Note basic typographical errors, areas where the grammar is awkward and impedes the readability, places where an image would help illustrate the discussion or methods, sections that could use additional information to help the reader better understand the concept
- The group coordinator convened online meetings of the subteam to review the edits and create a master document for each lab
- Feedback was provided to the authors

About the Authors

K. Joy Karnas, Ph.D., Editor: Professor of Biological Sciences at Cedar Crest College where she has taught upper-level undergraduate and graduate courses in molecular genetics since 2001. Research in her laboratory focuses on gene expression, examining genetic alterations that lead to triclosan resistance in bacteria and the use of epigenetics in identifying bodily fluids in crime scene analysis.

Wendy Boehmler, Ph.D.: Associate Professor of Biological Sciences at York College of Pennsylvania where she has taught molecular biology, genetics, and neurobiology courses and labs. Her research focuses on using the model organism, zebrafish, to investigate genes involved in neurodevelopment and neurodegeneration as well as the teratogenic potential of various drugs and chemicals.

Lara K. Goudsouzian, Ph.D.: Associate Professor of Biology at DeSales University. She teaches courses in molecular biology, cell biology, and cancer biology. Her research interests include factors which influence chromosomal stability in the budding yeast Saccharomyces cerevisiae, as well as metagenomic analysis of soil bacteria using massive parallel sequencing.

Stuart J. Haring, Ph.D.: Associate Professor of Biochemistry at North Dakota State University. His research interests involve understanding how cells duplicate their genetic information with high fidelity and preserve the integrity of their genome in the budding yeast *Saccharomyces cerevisiae*, and in human cell cultures.

Cynthia Keler, Ph.D.: Retired Professor in the Biology Department at Delaware Valley University and retired in 2022. She taught microbiology, molecular biology and virology to upper-level students. While at DelVal she implemented and ran the student research program. Always looking for ways to engage students beyond the traditional microbiology labs she developed the Fruit Fly and the Plant Growth Promoting Bacteria lab for her courses.

Jessica Petko, Ph.D.: Associate Professor of Biology at Penn State York teaching courses in cell biology, genetics, and developmental biology. Her doctoral thesis focused on characterizing developmental gene expression within the zebrafish inner ear using in situ hybridization techniques outlined in this manual. Her lab has since transitioned to studying circadian rhythm genes and their regulation in spiders in an NSF funded project.

Nik Tsotakos, Ph.D.: Assistant Professor of Biology at Penn State Harrisburg. He teaches courses in Genetics, Developmental Biology, Molecular Biology, and Bioinformatics. His research lab focuses on regulation of gene expression by non-coding RNAs, with a special interest in diabetic complications as well as lung immunity.

André P. Walther, Ph.D.: Associate Professor of Biological Sciences where he teaches Principles of Cell and Molecular Biology, Biology of Cancer, and labs in Molecular Genetics, Advanced PCR techniques, and Mammalian Cell Culture and Microscopy. His research focuses on the use of yeast as a model system for understanding the underlying causes of human cancer, using yeast as a novel source of biodiesel production, and genetic characterization of yeasts involved in beer fermentation.