A Cross-Curricular Molecular Genetics Lab in Embryology

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In response to the need for additional interdisciplinary, research-based labs that facilitate student learning across the biology curriculum, a student research project was developed as collaboration between Developmental Biology and Molecular Genetics courses to examine the effect teratogens have on a developing embryo, using both qualitative macroscopic observations and quantitative study of differential gene expression using microarrays. While the entire lab works as a multi-week unit to illustrate experimental design, technical methodology, and data analysis, component activities could be used as independent lab exercises in a variety of lab courses including molecular genetics, developmental biology, neuroscience, and introductory biology.

Keywords: interdisciplinary, functional genomics, cross-curricular, developmental biology, molecular genetics

Introduction

Project Goals and Approval

A research-based, cross-course laboratory experience for undergraduate students at a liberal arts college for women was developed with the primary goal of breaking down barriers between biology sub-disciplines; this goal for improving biology education was proposed as part of a recent National Research Council Report on undergraduate science education (NRC, 2003). Two existing courses were modified to include a multi-week project utilizing microarrays to measure changes in gene expression. Chicken microarrays were obtained from the Genome Consortium for Active Teaching (GCAT), an ongoing project sponsored by the Howard Hughes Medical Institute to "bring functional genomic methods into undergraduate curricula," thus expanding further the interdisciplinary nature of the lab. GCAT provided low-cost microarrays, slide scanning, and analysis software (**www.bio.davidson.edu/GCAT**).

Students participated in conducting the scientific work of this project as part of their course enrollment. The assessment of student learning was approved by Cedar Crest College's Institutional Review Board (# 2006-09) for work with human subjects. Students consented to have their test scores and comments used for research purposes, and could elect not to participate in the assessment process without penalty.

Target Audience and Timeline

This project could be easily divided into four distinct modules. The first module, a chick teratogenesis experiment conducted as part of the Developmental Biology course (BIO 332), is appropriate for a standard upper-level developmental biology course using basic laboratory equipment and required a minimum of two weeks of laboratory time. The second module, used in both courses, consists of a set of commercially available wet-lab and online simulations that could be incorporated into a variety of courses including molecular genetics, developmental biology, bioinformatics, and introductory biology. The simulations could be conducted in a single two to three hour laboratory session. These activities can be scaled up or down, as needed for the time allotted and level of student. In addition, for a greater challenge the activities could easily be expanded to include readings of original research papers. The third module was conducted in the Molecular Genetics II course (BIO 336), and requires advanced molecular genetics students who already have experience with basic molecular cloning techniques, nucleic acid manipulation, and PCR technology. This portion of the project requires access to microarray technology; however, it could be altered to use reverse transcription PCR as the endpoint for analysis on selected candidate genes. If conducting the microarray experiment, three laboratory periods are required, as well as a few hybridization steps carried out between laboratory periods. The final module brought students from both courses together to conduct final data analysis. Students spent two class periods and some out of class time analyzing data, developing research posters, and presenting them at a college-wide research conference.

The Approach

The flowchart (Fig. 1) illustrates the overall plan for the project, beginning and ending with a written test that assessed student conceptual understanding of developmental biology and the molecular Genetics of microarrays. The post-test also allowed students to comment on their percep-

tions of the laboratory experience. Black boxes represent tasks performed by all students involved in the project, while blue and red represent Development and Molecular Genetics students respectively. Grey boxes represent tasks not performed by lab students.
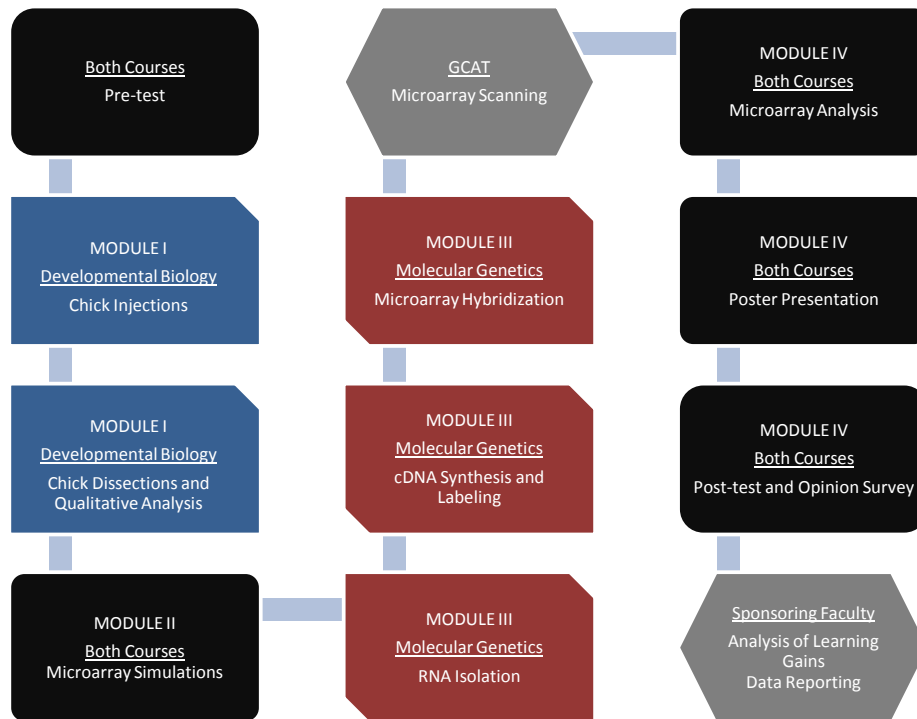
Figure 1. Project Plan. The flowchart illustrates the overall plan for the four modules, beginning in the top left with a student pre-test, and ending in the bottom right with the analysis and reporting of the student learning gains.

Student Outline

Module I: Developmental Biology (BIO 332)

Background Information

Developmental Biology has been a field of inquiry since Aristotle compared the developmental processes of various organisms. An organism undergoes processes of embryonic development from the point of conception to the time of birth; we can also think of the life cycle as extending from gamete formation to time of death.

Many model systems have been studied to understand the conserved and unique mechanisms used during organismal development. The domestic chicken, *Gallus gallus*, has been used extensively for developmental studies because it is easy to obtain, easy to maintain in a lab, and has a short generation time (21 days from fertilization to hatching). Since most of the chick's development takes place outside of its mother, it is also relatively easy to manipulate experimentally. A traditional student laboratory uses teratogens, chemicals known to cause abnormalities when human embryos are exposed to them, to disrupt normal developmental processes in chickens. Teratology, the study of teratogens, is a field which continues to be of interest to both basic scientists and clinicians as we continue to encounter novel chemicals in our environment.

Week 1 (Optional): Injection and Dissection Practice

Description: At the beginning of lab, we will discuss the plan for the teratogenesis experiments and our collaboration with the Molecular Genetics lab sections. By the end of lab TODAY, you should have chosen your teratogen and had it approved by me; if I need to order the chemical, I need the full week to guarantee that it will be available. IF YOU ARE PREGNANT OR THINK YOU MAY BE PREGNANT, PLEASE talk to me privately, and we will determine a teratogen that will be safe for you to use. This week, you will be examining normal chick embryos at various stages of development so that you can recognize the structures present at these stages. Since you will not be preserving the tissue from today's experiment, you do not need to use sterile techniques.

Procedure: With your partner, obtain one 24-hour post fertilization egg and one 48-hour post fertilization egg from the instructor. Note that the eggs are labeled in pencil only; ink can permeate the eggshell and be toxic to the embryo. Weigh the eggs (to the nearest 0.1 g), and record the weights in your notebook.

First, practice injecting the egg with teratogen, in preparation for next week's lab. Using a dissecting needle from your kit, make a small hole in the round/blunt end of the egg. Then inject 1 mL of saline into the egg with a 26-gauge needle attached to a sterile syringe. Do not penetrate far into the egg, as you do not want to cause mechanical damage to the embryo. If a bubble of liquid appears at the surface of the egg, your needle is not in far enough. Withdraw the needle, collect the fluid, and reinject. (during your real experiment, you will have to decide whether to collect and reinject fluid, or simply record in your notes that a smaller-than-intended dose of teratogen was delivered). If you think that you are having trouble injecting the fluid, you can use neutral red dye (0.1% solution in 0.9% saline, non-sterile) for your practice injections to better visualize any fluid that leaks onto the egg surface.

Crack the egg (just like in the kitchen at home!) into a 10 cm petri dish. Use your dissecting scissors to kill the embryo by snipping across its neck; if the embryo is too small to have a visible neck, you can proceed without this step.

Examine the morphology of the embryo, and compare it to the photographs in your atlas. Is your embryo at the developmental stage specified? (Hint: what structures will best help you answer this question?)

To better visualize the somites and other structures, you may inject neutral red dye between the embryo and the yolk, allow it to permeate the embryo for a few minutes, and then rinse with saline.

We will be maintaining a few eggs until next week's lab so that you can observe normal structures at a later point in development. **Before you leave lab today, please confirm with the instructor that you have established a schedule for "turning" these eggs in the incubator and for keeping the water pans full.

Remember that you will need to determine the concentrations of teratogen that you will be injecting next week, and what you will use as a solvent. Think carefully about the typical weight of an egg and a reasonable injection volume.

Week 2 (or 1, if omitting the optional week): Teratogenesis

NOTE #1: IF YOU ARE PREGNANT OR THINK YOU MAY BE PREGNANT, PLEASE talk to me privately, and we will determine a teratogen that will be safe for you to use.
NOTE #2: This week, ALL techniques must be performed under sterile conditions. If any contaminants enter the egg, the embryo will not develop for us to examine… and they will smell horrible!

- For these experimental eggs, we want to minimize the time they spend outside the incubator. You should remove a few eggs at a time, weigh and label them, and then return them to the incubator. Only remove one egg at a time for the injection process.

- After weighing all the eggs, calculate the injection volume for each egg. Assume that 1 g of egg = 1 mL of volume. Prepare your teratogen solution, and control solution, of solvent only, for use in control injections; filter sterilize if necessary.
- ALL necessary materials should now be available on your bench. Wear gloves from this stage forward.
- Remove 1 egg from the incubator. Wipe the egg with 70% ethanol to sterilize the surface. Flame-sterilize and cool a dissecting needle. Make a small hole in the blunt end of the egg. Inject either the teratogen or the solvent, and visually confirm that no fluid leaked onto the egg surface.
- Cover the hole with a round sticker, and return the egg to the incubator. Set the egg on its pointy end, and direct all points to 9:00; this will make turning the eggs easier.
- Continue with the remaining injections, using a new sterile needle for each egg. Make sure that you record any discrepancies in the injection process.

POST-NOTE: Before you leave lab today, please confirm with the instructor that you have established a schedule for "turning" these eggs in the incubator and refilling the water tray. You should turn the eggs 90 degrees clockwise at each timepoint.

Week 3: Analysis of experimental results

NOTE #1: Today you will isolate your embryos, examine their morphologies, and preserve them for RNA isolation. You should maintain an RNase-free environment whenever possible by treating work surfaces with an RNase decontaminant solution.
- Before you begin dissecting, pour RNALater solution (Ambion) into sterile, RNase-free conical tubes. Your embryos will be stored in this solution until the Molecular Genetics students meet to isolate the RNA.
- Isolate the embryos as you did in Week 1. These embryos will be older, and will likely require a neck-snip to terminate them.
- Examine the embryos by eye and under a dissecting microscope. Make careful comparisons between control and treated embryos to help you determine the differences.
- Measure the crown-to-rump distance (you will need to align the removed head for this step), and the distance between the eyes, if possible. You may also see differences in eye or beak size, presence or absence of organs, everted (external) organs, failure of the neural tube to close, etc. Consult your atlas and your instructor when needed.
- Store the embryos in RNALater in the refrigerator. Make sure that your labeled tubes are clearly correlated with your written notes!!!
- Give your instructor a CLEAR table listing the sample number, treatment conditions, and major abnormalities observed for each embryo. This information is needed for the preparation of the microarrays.

Module II: Microarray Simulations

Today you will follow the protocol for the simulations that is included in the Carolina Biological Supply Kit DNA Chips: Genes to Disease (catalog #211520). Because we are conducting advanced analysis, you will also read and discuss a primary research paper that uses these techniques, Wang *et al*. (2006). The exercise below asks you to correlate target genes with spots created on the paper arrays.

Practice Problem: Use the Wang *et al*. (2006) paper to identify the following genes in the paper array simulation:
- Apolipoprotein A1 (Apo-AI)
- Lipoprotein lipase gene (LPL)
- SkTmod mRNA for skeletal muscle type tropomodulin
- Actin-related protein 8 (ARP8)
- GAPDH: enzyme that catalyzes the 6th step of glycolysis
- pax6: control gene responsible for the development of eyes

Module III: Molecular Genetics II (BIO 336)

Introduction to the Project

An exciting aspect of the lab for this year will be a cross-over lab with the Developmental Biology course. They will begin the lab by exposing chicken embryos to various mutagens and comparing them to non-exposed controls. After observing and photographing the phenotypes of the treated embryos, they will dissect out the tissue and store it for your use. Your mission will be to isolate RNA from the control and experimental tissue, create Cy3– and Cy5- fluorescently labeled cDNA, and hybridize it to cDNA microarray slides. After the slides are scanned, it will be your job to analyze the data to determine which genes are differentially expressed in the tissue. The project will culminate with a joint poster presentation with the Developmental Biology students.

RNA Isolation

Today you will begin with RNA isolation from the chicken embryo tissue provided by the Developmental Biology students. The protocol provided is from the usb PrepEase RNA Spin Kit (usb catalog #78766), and will guide you through RNA isolation.

Avoiding RNase Contamination

Why should we care about RNases?
- They degrade RNA…and that's what we are trying to isolate!
- They are everywhere!
- They are very stable and difficult to inactivate.
- The most common source of RNase contamination is you (your skin)! Always wear gloves when working with RNA.
- Another common source of RNase contamination is bacteria or mold present on airborne dust particles. Sterile technique is very important when handling reagents used for RNA isolation and analysis.

Hints for Working with RNA…
- Establish an RNase-free environment in your work area. Spray down all bench surfaces with an RNase Decontaminant or 0.5 M NaOH, and wipe clean. Also moisten a KimWipe and clean pipet handles and shafts.
- Wear gloves, wear gloves, wear gloves…and try not to touch potentially contaminated areas with your gloves.
- Keep all reagent bottles and pipet tip boxes closed when not in use…remember your good sterile technique!
- Pulse spin all tubes prior to opening the caps to reduce aerosols.
- Use sterile plasticware and machine-packaged pipet tips that are labeled as RNase-free. Try not to mix it up with the non-RNase-free stuff in the lab. Pour tubes from bag onto RNase-free area. Do not reach into RNase-free bags!
- When using non-disposable glassware and plasticware that is not pre-packaged and RNase-free, spray with RNase Away or fill with 0.5 M NaOH or H_2O_2 and soak for 15 minutes. Rinse with DEPC-treated water. Glassware could also be baked overnight at 250°C.
- Solutions (water and other solutions) not provided as RNase free should be treated with 0.1% DEPC (diethyl pyrocarbonate). While wearing gloves and working in a fume hood, add 0.1 ml DEPC for each 100 ml of the solution to be treated and shake vigorously to mix. Autoclave for 15 minutes on liquid cycle.
- Keep RNA samples on ice to prevent degradation by RNases (keep them asleep as much as possible).

RNA Analysis

You will analyze the purity of the RNA in two ways: 1) by UV spectrophotometric analysis, measuring absorbance at both 260 and 280 nm and 2) by visual inspection on an RNA gel, using a Lonza brand RNA FlashGel (Lonza catalog #57024). You will be using the Lonza protocols for running your gels, along with the following information on UV spectrophotometry, adapted from Ausubel *et al*. (1999).

UV Spectrophotometric Analysis of Nucleic Acids

Procedure to Follow…
1. Turn on the UV Spectrophotometer, and allow the UV lamp 15-20 minutes to warm up.

2. If measuring RNA, rinse quartz cuvette with RNase Away, and follow with a rinse with DEPC-treated water.

3. Pipet 297 μl of water (use DEPC-treated water if measuring RNA) into the cuvette.

4. Blank the spectrophotometer at 260, 280, and 230 nm.

5. Add 3 μl of sample to the 297 μl of water in the cuvette (1:100 dilution). Place a piece of parafilm on the top of the cuvette, and shake to mix.

6. Take a reading at 260, 280, and 230 nm.

- Concentration of ssRNA (ng/μl) = 40 x A260 x dilution factor
- Concentration of ssDNA (ng/μl) = 37 x A260 x dilution factor
- Concentration of dsDNA (ng/μl) = 50 x A260 x dilution factor
- Note: Highly purified preparations of RNA have A260/A280 ratios of 1.9 to 2.0
- Note: Readings at 230 nm indicates contamination by phenol or urea.
- Note: Dirty cuvettes or contamination by particulates are sometimes indicated by readings at 325 nm.

Microarray Hybridization

Now that you have confirmed the presence of pure RNA, you will be moving on to the cDNA labeling step using protocols from the Genisphere 3DNA Array Detection, Array 350 Cy3/Cy5 Kit for Microarrays (Genisphere catalog #W300180) based on the GCAT Microarray 3DNA Method protocol written by Rosenwald and Eckdahl (2008). This protocol will also guide you through the hybridization of the labeled cDNA to the chicken microarrays provided by GCAT. We will then ship your slides to Davidson College (part of the GCAT consortium) for scanning, and we will access the images via the web next week.

Module IV: Joint Data Analysis, Interpretation, and Presentation

This week you will analyze the scanned slides and correspond with the students in the other course regarding your data. You will use MAGIC Tool, a computer program that was written by GCAT faculty and students as a simple and straight-forward way to compare the gene expression levels. It is available to download for free from the GCAT website (**http://www.bio.davidson.edu/projects/MAGIC/MAGIC.html**). The program and data have been downloaded for you, and we will follow the protocols as outlined on the GCAT website. Remember that you need to prepare your final poster in conjunction with your partners in the other course and present this both in your class and at our college-wide conference on health and wellness.

Materials

Module I: Developmental Biology Lab

This portion of the lab requires chicken eggs, usually purchased from a local farm, an egg incubator, teratogens of interest, ethanol and flames for sterilization, sterile syringes, and sterile 26 gauge needles for injecting the eggs with teratogens. Egg incubators are available from GQF Manufacturing Co. (**www.gqfmfg.com**, Model #1602N Thermal Air Hova-Bator) or from Moyer's Chicks (**www.moyerschicks.com**). Fertilized chicken eggs are available from Moyer's Chicks and Carolina Biological Supply (**Carolina.com**), and fertilized quail eggs are available from GQF Manufacturing . Purchased fertilized eggs often lag 12-24 hours behind their expected developmental stages. Plan to order at least three eggs per experimental or control condition, as some low percentage of eggs is unfertilized. You may also want to include untreated controls for the whole class, along with solvent-treated controls for each student group.

Module II: Microarray Simulations

Two types of microarray simulations were conducted, both relying on the Carolina Biological Supply Kit DNA Chips: Genes to Disease (catalog #211520). The first simulation followed the kit protocol exactly, using microscope slides and a gel-based material to simulate the color change that occurs following DNA hybridization on microarrays. Answers for the microarray are included in the kit, as well as candidate gene descriptions. Note that due to the nature of the kit, the simulated array colors are red and blue instead of the actual red and green colors of microarrays. The second simulation was an adapted version of the paper arrays using a publication (Wang *et al*. 2006) to enhance the lesson. The paper array components are a convenient place to return to the actual colors of microarrays and should therefore be printed on red and green paper, contrary to the instructions included in the kit. After hybridizing paper probes to a paper microarray through Watson-Crick base pairs, students were presented with six candidate genes (apolipoprotein A1, Lipoprotein lipase gene, SkTmod, Actin-related protein 8, and pax6) and asked to match each gene to the most logical corresponding spot on the paper array using the results of the Wang *et al*. (2006) paper as a guide. Suggested answers for the paper array using Wang *et al*. (2006) are as follows:

- Spot #1: Green, highly expressed in layers, ApoAI (based on Figure 1)
- Spot #2: Black, not expressed in either tissue, pax6 (development gene not included in paper)
- Spot #3: Yellow, expressed equally in both, GAPDH (based on equal expression in figure 2)
- Spot #4: Green, expressed in layers at low level, SkTmod (based on Table 2)
- Spot #5: Yellow, down-regulated in broilers, ARP8 (based on Figure 2 and text on page 572)
- Spot #6: Red, expressed in broilers, LDL (based on text on page 571)

Module III: Molecular Genetics II Lab

Kits used in this portion of the lab included usb PrepEase RNA Spin Kit (usb catalog #78766), Lonza RNA FlashGel (Lonza catalog #57024), and the Genisphere 3DNA Array Detection, Array 350 Cy3/Cy5 Kit for Microarrays (Genisphere catalog #W300180). Other supplies are those standard in molecular genetics laboratories, including a UV spectrophotometer for assessing nucleic acid purity.

Module IV: Data Analysis, Interpretation, and Presentation

Data analysis requires MagicTool Software (downloaded free from GCAT website), and materials for creating the final presentation, generally PowerPoint software for poster creation.

Notes for the Instructor

Notes for Developmental Biology Instructors:

The lecture for Developmental Biology has traditionally included both an historical approach to the field and modern, molecular based approaches. Past offerings of the laboratory have been inquiry-based, beginning with several simple model organisms (*Dictyostelium*, planaria, sea urchins) to allow students to design and execute their own experiments. The final weeks of the semester involved a close examination of several stages of chicken development, followed by an experiment exploring the effects of presumed teratogens on developing chick embryos. Students previously selected their chemical of interest (e.g. thalidomide, retinoic acid, ethanol, caffeine, etc.), determined a meaningful concentration(s) using the primary literature, and injected it into fertilized chicken eggs, with appropriate controls. Following a period of incubation (1-2 weeks), students dissected the embryos and macroscopically examined the chick anatomy. Students were able to do a thorough qualitative analysis of the embryos and apply information that they had learned in the lecture portion of the class to this project.

In its first year of implementation, the chicken experiment was carried out early in the semester to facilitate the collaboration across courses, and thus occurred before chicken development was covered in lecture. One pair of students was asked to use retinoic acid as a teratogen based on the extensive literature showing effects of retinoic acid on developing embryos through its action on Hox genes. The second pair of students chose ethanol from a short list of options of less-studied teratogens. Chemical concentrations for injections were based on literature searches by the students; control eggs were injected with solvent only. Embryos were injected at day 0 or day 7, and allowed to develop for one week. The earlier injection date results in higher numbers of resorbed embryos, and is therefore less useful; the later date, however, results in later embryos that the students may be less comfortable handling. Other starting points are equally

valid, but do not let the embryos live longer than embryonic day 14. After qualitatively examining, measuring, and photographing the embryos, tissue samples from experimental and control embryos were collected and stored in RNAlater, an RNA preservative.

To enhance learning, students participated in both an online computer simulation and hands-on lab simulation to demonstrate how microarrays are created and interpreted. In addition, the lecture component of the course included the reading and discussion of one primary literature paper (of four papers total) that used microarrays to address questions in developmental biology. At the close of the project, students spent several lab sessions analyzing the data generated from the arrays and worked in collaboration with the Molecular Genetics students to produce research posters for the College's Health and Wellness Conference. Since each pair of Development students had produced samples for three sets of microarray analysis, these students contributed to each of the three resulting posters.

In its first year of implementation, the microarray project took more time than anticipated, with additional lab time needed for an additional round of egg injections, tissue isolation, and data analysis. Therefore, the inquiry-based aspects of the course were reduced compared to previous years, and students spent less time on self-designed experiments using simple model organisms. This timing issue can be addressed in future years by shifting the inquiry-based projects later in the course to allow for both types of lab experiences. In addition, because the Development lab was so much smaller than the Molecular Genetics lab, each Development student worked on several final poster presentations; working on a single poster might help to increase student satisfaction with collaborating across the courses.

Implementation Timeline

- Optional: One 3-hour lab period for practice injections with dye and dissection of normal embryos
- One 3-hour lab period dedicated to injections
- Twice daily egg rotation ("turning") for 1 week – on a schedule established by the students
- One 3-hour lab period (1 week after initial lab) dedicated to dissections and harvesting of tissue
- One 1-hour portion of lab dedicated to microarray simulation
- Two 3-hour lab periods dedicated to data analysis
- Out-of-class time dedicated to poster development and presentation

Notes for Molecular Genetics Instructors:

Note that the descriptions included in the "Student Instructions" above specify the use of protocols directly from molecular biology kits. This approach can be used to help emphasize how actual scientists use these tools. Alternatively, you may wish to modify these instructions based on your institution's available equipment and facilities and distribute a simplified version to your students.

In past years, one of the projects annually conducted by BIO 336 students involved the selection of an organism and a search of the NCBI database for a gene previously cloned and sequenced from that organism. Students isolated RNA, created cDNA, and then attempted to clone their gene of interest using primers that they designed specifically for that gene. After successfully amplifying the gene by PCR, students ligated their product into an expression plasmid, and then had their choice of various endpoints: restriction map it using enzymes they've identified as useful, sequence it, express the gene and analyze it using SDS-PAGE, etc. Not all students completed all of the possible activities in this lab, but those who had difficulties early on usually learned valuable lessons about how to trouble-shoot experiments and how to design alternative plans for a project (e.g. using genomic DNA as an alternative to RNA and how this changes the final product with the inclusion of introns).

In the first year of implementation, students began their gene cloning as in previous years to gain experience in working with RNA prior to the start of the microarray experiment. After tissue samples were made available by the Development students, the Molecular Genetics students isolated RNA from these samples, copied this RNA to cDNA, differentially labeled control and experimental pools, and hybridized the labeled cDNA to slides that had previously been spotted with more than 21,000 chicken genes. After these slides had been scanned by GCAT (**www.bio.davidson.edu/ GCAT**), students had the opportunity to analyze the data and work in collaboration with the Development students to produce research posters for the College's Health and Wellness Conference. In addition, students participated in the same online computer simulation and hands-on lab simulation as the Development students to learn about how microarrays are created and read.

As this was the first implementation of the microarray project, some challenges arose when the standard microarray protocol was carried out for six slides at a time by twelve individuals, and these issues will be straightforward to address in future experiments. In addition, this project took over the entire semester, and little time was focused on other exercises typically carried out in this course. Streamlining of the protocols and requiring each student to prepare a set of RNA samples would help eliminate the need for having to repeat procedures when not enough RNA was obtained initially. The bioinformatics software recommended by GCAT (Magic Tool) had more of a learning curve than initially anticipated and also had a few quirks that frustrated the students, so instructors may wish to consider other microarray analysis software options. Finally, students voiced difficulty in developing posters with students from the other class; communication seemed to be a challenge for them.

Implementation Timeline

- One 1-hour portion of lab period dedicated to microarray simulations
- One 3-hour lab period dedicated to RNA isolation and RNA gel
- One 3-hour lab period dedicated to cDNA labeling
- One 3-hour lab period and two 1-hour class lecture periods (consecutive days: M morning lecture, T afternoon lab, W morning lecture) dedicated to slide preparation and microarray hybridization
- Two 3-hour lab periods dedicated to data analysis
- Out-of-class time dedicated to poster development and presentation

Notes for Alternative Combinations of Modules:

The overall purpose of this laboratory was to connect two seemingly unrelated courses to illustrate the connectedness of the biological sciences and to extend the chicken embryology study into the realm of molecular genetics. However, microarray analysis is not a readily available technology, and requires a high level of expertise in the area of molecular genetics. Therefore, it is completely reasonable to use only a portion of these modules or have alternate endpoints for the project. Some examples are described below.

- The Developmental Biology Module could be used alone, and quite often is included as part of a classic development lab.
- The Microarray Simulation Module could be a free-standing addition to a variety of courses from introductory biology to a more advanced course in bioinformatics. For the more advanced students, inclusion of a research paper would enhance the educational experience.
- A combination of Developmental Biology Module with the Microarray Simulation Module would reinforce the idea that the gross morphological changes observed in the teratogenesis experiment are resulting from underlying alterations in gene expression at the molecular level. The addition of the microarray simulation to the classic chicken embryology project would allow students to make this connection without requiring the technically complex microarray hybridization and analysis. Students might search the primary literature to identify candidate genes for up- or down-regulation by the teratogen in question.
- The Molecular Genetics Module could be performed with a different starting material. In years when Developmental Biology is not offered, the authors have connected the microarray project to a Neuroscience course, using treated chicken neurons as the tissue for RNA isolation.
- An alternate end point for the entire project could be the use of PCR or qPCR to assess gene expression of target genes. This removes the dependence of the project on microarrays, but would require students to identify candidate genes and design PCR primers to amplify them from isolated tissue.

Acknowledgements

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Audrey J. Ettinger, Ph.D. is an Associate Professor of Biological Sciences and Director of the Neuroscience Program at Cedar Crest College. She completed her undergraduate degree at Bryn Mawr College, a Ph.D. in Neuroscience at Washington University in St. Louis, and postdoctoral train-

ing at Stanford University. This training experience reinforced her belief in the benefits of learning science in a liberal-arts context. Her courses usually include students from multiple majors, and she emphasizes reading the primary literature as a key skill. Her interests in cross-disciplinary work are particularly evident in her First Year Seminar entitled *Visions and Voices: A Novel Approach to Scienc*e, which examines the portrayal of science and scientists in fiction. Her research laboratory, including students from many different majors, uses cichlid fish as a model organism to study questions of neural development and behavioral neuroendocrinology.

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