A Student-Driven, Technology-Intensive Lab Design for Teaching Neuroscience, Molecular Genetics, and Developmental Biology

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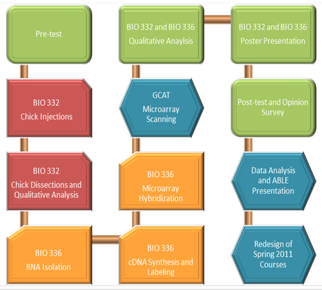
Project Design

Our Original Approach (2010 ABLE Poster)

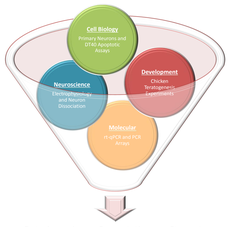
The flowchart to the right illustrates the overall plan for the initial collaboration, beginning and ending with a written test that assessed student understanding of Developmental Biology and Molecular Biology of Microarrays. Green boxes represent tasks performed by all students involved in the project, while red and orange represent Development and Molecular Biology students, respectively. Blue boxes represent tasks not performed by lab students.

The chicken microarrays were obtained from the Genome Consortium for Active Teaching (GCAT), an ongoing project funded by the Howard Hughes Medical Institute to "bring functional genomic methods into undergraduate curricula." GCAT provided low-cost microarrays, slide scanning, and analysis software(www.bio.davidson.edu/GCAT). We participated in this project from 2010-2014.

This work was presented in a poster at ABLE in 2010 and as a major workshop in 2011. Additional presentations of data derived from this study were made at the Society for Neuroscience and American Society for Cell Biology conferences.



Project Expansion (2010-2016)



Data Analysis and Presentations by Students
Assessment of Student Learning Gains
Assessment of Shifts in Student Attitudes Towards Science

- Courses added: Diseases of the Nervous System (2011, 2013), Neuroscience Methods (2014)
- Application for and award of an NSF TUES grant (2013 – 2016)
  - Equipment purchase (Port-a-Patch and Rotor Gene Q)
  - Acquisition of PCR Arrays for Apoptotic Pathways
  - Establishment of summer internship to investigate the effects of apoptosis-inducing chemicals and neuroprotective agents and to enhance the educational experiences of women in science
  - Inclusion of local collaborators
- Independent students working on original research projects (2012 – 2014)
- Potential future facility/resource for teaching faculty interested in incorporating experiments that explore gene expression in neurons and the electrophysiology of channels (2016 and beyond)

Content Survey (Revised for Spring 2014)

- Content questions:
  - Developmental Biology
  - Molecular Genetics
  - Neuroscience
  - Laboratory Techniques and Instrumentation
- Administered before and after class/internship experience
- Data analyzed by a colleague not involved in the laboratory project

Developmental Biology

A teratogen is defined as
a. a molecule that changes DNA sequences.
b. a class B drug that is prohibited during pregnancy.
c. an environmental agent that causes birth defects.
d. a chemical that causes DNA to form insoluble tetramers.
e. a fast-diffusing chemical

Retinoic acid can cause birth defects because
a. it causes cancer in adults.
b. it upregulates expression of Vitamin A.
c. it is not normally active during development.
d. it has an endogenous role in gene regulation.
e. it is found in the drinking water supply due to fracking

Molecular Genetics

Which of the following features is common to DNA, RNA, and proteins?
a. They contain amino acids.
b. They contain nucleotides
c. They contain uracil
d. They are polymers
e. They are found in vesicles

In order to study differences in gene expression at the level of transcription, one can compare
a. Genomes of organisms of the same species
b. Genomes of organisms of different species
c. The transcriptome of differently treated cells
d. Protein content of cells from treated and untreated cells
e. Unspliced and processed mRNA

Neuroscience

Neurodegenerative diseases are hard to treat because
a. adult neurons do not continue to divide
b. degenerating neurons kill the surrounding glia
c. they are so rare that treatments are not profitable to generate
d. neurons cannot be studied outside of the human body
e. human neuronal function is too different from that of animal models

In a typical neuron at rest, the membrane potential is primarily set by channels permeable to which ion?
a. Sodium
b. Potassium
c. Chloride
d. Calcium
e. Phosphate

Techniques & Instrumentation

Which of the following facts regarding the Rotor-Gene Q is TRUE?
a. Each tube spins in a chamber of fast-moving air, keeping all samples at precisely the same temperature.
b. It is an optics-based system with a fluorescent signal detected from each tube every 150 milliseconds
c. The tight-fitting heated lid of the instrument prevents condensation of reaction mixtures on the lid of the tube.
d. All of the above statements are true.
e. Only a and b are true.
f. Only a and c are true.
g. Only b and c are true.

The Port-a-Patch is useful for measuring electrical activity
a. Of a set of neurons
b. Of a neural network
c. Of an individual cell chosen by chance
d. Of a selected individual neuron
e. All of the above

Attitudes Survey (Revised for Spring 2014)

- Majority of questions based on the biology version of the Colorado Learning Attitudes about Science Survey (CLASS) validated instrument (http://www.colorado.edu/sei/class/)
- CLASS questions revised to address each separate subdiscipline (Developmental Biology, Molecular Genetics, and Neuroscience) to identify differences in student attitudes towards specific areas of biology
- Questions added to assess student confidence in her disciplinary knowledge, lab skills, ability to work as a collaborator, and presentation skills

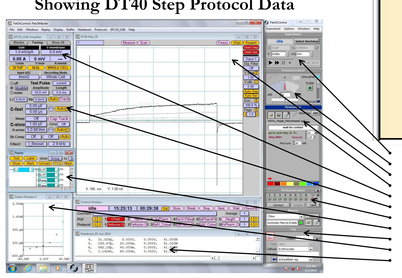
Modified CLASS Questions

The subject of _____ has little relation to what I experience in the real world.
Developmental Biology
Molecular Genetics
Neuroscience
Neuroscientists do not need to understand _____
Developmental Biology
Molecular Genetics
(Strongly Disagree 1 2 3 4 5 Strongly Agree)

Additional Questions

I am confident in my ability to prepare a research poster.
I am confident in my ability to present a research poster
I am confident in my ability to design a research project.
I am comfortable working with other students as collaborators.
I feel that there is a value to student collaborations
I am confident in my knowledge of Developmental Biology.
(Strongly Disagree 1 2 3 4 5 Strongly Agree)

Interface Screen for the Port-a-Patch Instrument Showing DT40 Step Protocol Data



Suction Control and Program Progress
Suction Level Monitor
Data Window (Oscilloscope)
Selection of Suction Control Protocol
Membrane Potential and Resistance Monitor
Transient Current Adjustment
Perfusion Control
Protocol History
Annotation Window
Current/Voltage or Current/Time Plot
Progress of Experiment

Acknowledgements

This project is supported by an award from the National Science Foundation (TUES Grant #1245526).

Technical expertise in the use of the Port-a-Patch was provided by Michael Finley, Ph.D. (Merck, Inc.) and general technical support was provided by Sherry Soost.

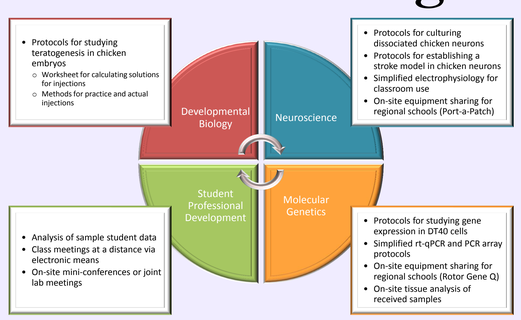
Students who contributed to this work include:
- The 2013 Summer Interns
  - Gabrielle Conant, Bethany Kulp, and Melissa Moyer
- The 2014 Summer Interns
  - Cindy Cabrera, Jinal Patel, Angela Snyder*3, Tayler White*2, and Alicia Zook
- Molecular Genetics II Students:
  - Rebecca Grady*1, Julia Kelly*1, and Melissa Moyer*1
- Independent Research Students:
  - Allison Osborne and Gabrielle Conant

* Provided data specifically shown on this poster
(1 = PCR Array data, 2 = Viability Curves, 3 = Microcopy Images)

Interested in Collaborating?

Areas of Potential Collaboration

- Student-friendly protocols are currently being developed to facilitate instruction and classroom use
- On-site campus visits are possible for shared instrument use
- For distant institutions, tissues could be shipped for PCR array analysis (*material fees will apply)
- Interaction between students in lab classes and/or research labs can be encouraged formally or informally through electronic or on-site conferencing



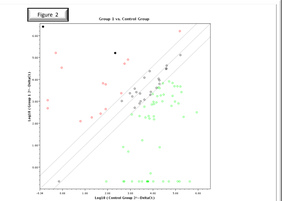
Student Data

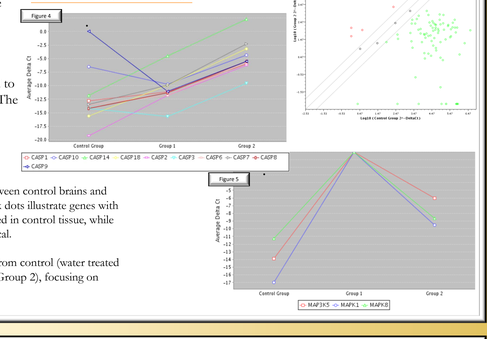
Sample Student PCR Array

- Students performed chicken teratogenesis experiments using alcohol (0.08% ethanol), Red Dye 40 (7ug/g body mass, Allura Red AC), Glutamate (1.47 mg/ g body mass), and control (water) injections into eggs containing 3 day old embryos.
- Eggs were dissected at 10 days, and gross anatomy observations were made and recorded. Deformities ranged from undersized embryos to possible brain hemorrhages to everted organs.
- RNA was isolated from chicken embryonic brain tissue, copied to cDNA, and assayed for apoptotic gene expression using a *Gallus gallus* apoptosis PCR array and the Rotor Gene Q. Each of the three students chose a single brain from a differently treated embryo to perform the analysis, and the data was compared across the student samples.
- Data was analyzed using the SABiosciences web-based analysis software (RT2 Profiler PCR Array Data Analysis version 3.5, http://pcrdataanalysis.sabiosciences.com/pcr/arrayanalysis.php).
- Students used data from the PCR arrays to explore differences in the expression of apoptotic genes from brains of chicken embryos exposed to water (control), Allura Red AC (group 1), and ethanol (group 2) *in ovo*. The figures are excerpts from their lab notebooks showing some of the preliminary data that they generated and analyzed.
  - **Figure 1:** Raw data (Ct values) generated by the Rotor Gene Q
  - **Figures 2 and 3:** Graphical depiction of the changes in gene expression between control brains and brains from chicks exposed to Allura Red AC (Red Dye 40) or ethanol. Black dots illustrate genes with minimal shift in expression. Red dots are genes that are more highly expressed in control tissue, while green are more highly expressed in the tissue exposed to experimental chemical.
  - **Figures 4 and 5:** Student-generated data showing gene expression changes from control (water treated embryos) to experimental treatments (Allura Red AC, Group 1 and ethanol, Group 2), focusing on specific caspase and MAPK genes.

Figure 1

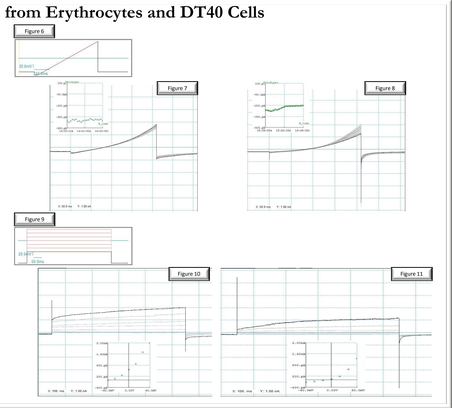
Gene Symbol	Control (water)	Group 1 (red dye)	Group 2 (EtOH)
BAK1	17.49		18.92
BCL10	24.38		20.66
BCL2	21.86		17.75
BDNF	17	21.01	19.54
BID	16.05	18.14	17.7
BIK	24.47	22.03	
BRAF	18.33		18.16
CASP1	20.22	21.84	19.65
CASP3	18.79	17.28	16.08
CASP6	19.49	21.18	19.89
CASP7	19.51	23.08	23.37
CD28	28.19	23.86	21.48
CD40	17.37	23.59	22.86
CD40LG	16.56	18.19	29.86
FADD	20.63	22.86	18.91
FAS	21.91	27.79	21.09
FASLG	18.27	37.02	23.85
IL10	16.58	17.66	24.25
IL2	18.79	20.61	23.45
MAP3K5	19.13		19.62
MAPK1	16.01		16.1
MAPK8	21.73		17.01



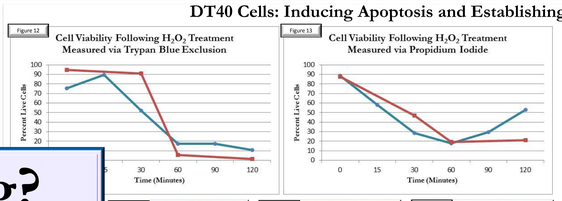


Electrophysiological Recordings from Erythrocytes and DT40 Cells

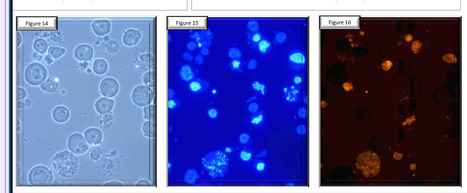
- Currents Measured Using Ramp Protocol (**Figure 6**)
- **Figure 7 (**Erythrocytes) and **Figure 8** (DT40 Cells) Electrophysiology
  - Voltage is raised gradually from -100 mV over 200 ms
  - Current change is measured over time
  - Results: Voltage-gated channels open at higher voltages (main panel)
  - Consistent with the literature on both cell types
  - *Insert panels show current change over time*
- Currents Measured Using Step Protocol (**Figure 9**)
- **Figure 10 (**Erythrocytes) and **Figure 11** (DT40 Cells) Electrophysiology
  - Voltage is changed from -80 to + 60 in 20 mV steps (500 ms each)
  - Current change is measured over time
  - Results: Voltage-gated channels open at higher voltages (main panel)
  - Kinetics suggest the presence of voltage-gated potassium channels
  - Consistent with the literature on both cell types
  - *Insert panels show Current as a function of voltage*
- Faculty also recorded from HEK293 cells and from chicken neurons
- An independent research student also recorded from sea urchin oocytes



DT40 Cells: Inducing Apoptosis and Establishing Viability Assays



- Student Assays: DT40 Cell Viability Following 20 mM H_2O_2 Exposure
  - **Figure 12:** Trypan Blue Exclusion measurement of cell death in two independent experiments (5 hemocytometer fields counted in each); calculation is number of live cells per total cell count
  - **Figure 13:** Assessment of cell death post-treatment in two independent experiments calculated by 100% - (number of dead cells stained with propidium iodide per total cell count via Hoechst 33342)



- Student Microscopy: DT40 Cells Treated with 85.2 mM Caffeine and Visualized via Fluorescence Staining
  - **Figure 14:** Bright Field image of cells at 2 hours post-treatment
  - **Figure 15:** Same field stained with Hoechst 33342 (nuclear stain). Note that pale (live), bright (early apoptosis), and blebbed (late apoptosis) nuclei are visible.
  - **Figure 16:** Same field stained with Propidium Iodide (excluded by live cells)