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Teaching Bioinformatics and Neuroinformatics Using Free Web-based Tools

William Grisham[1], Natalie A. Schottler[1], Joanne Valli-Marill[2],
Lisa Beck[3], Jackson Beatty[1]
[1]Department of Psychology, UCLA; [2] Office of Instructional Development, UCLA;
[3]Department of Psychology, Bryn Mawr College

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Address correspondence to:
William Grisham
Department of Psychology, UCLA
PO Box 951563
Los Angeles, CA 90095-1563
Grisham@lifesci.ucla.edu

ABSTRACT

This completely computer-based module's purpose is to introduce students to bioinformatics resources. We present an easy-to-adopt module that weaves together several important bioinformatic tools so students can grasp how these tools are used in answering research questions. This module integrates information gathered from websites dealing with anatomy (Mouse Brain Library), Quantitative Trait Locus analysis (WebQTL from GeneNetwork), bioinformatics and gene expression analyses (University of California, Santa Cruz Genome Browser, NCBI Entrez Gene, and the Allen Brain Atlas), and information resources (PubMed).

This module provides for teaching genetics from the phenotypic level to the molecular level, some neuroanatomy, some aspects of histology, statistics, Quantitaive Trait Locus analysis, molecular biology including *in situ* hybridization and microarray analysis in addition to introducing bioinformatic resources. Students use these resources to discover 1) the region(s) of chromosome(s) influencing the trait, 2) a list of candidate genes—narrowed by expression data, 3) the *in situ* pattern of a given gene in the region of interest, 4) the nucleotide sequence of the candidate gene, and 5) articles describing the gene. Teaching materials such as a detailed instructor's manual, powerpoints, sample exams, and links to free web resources can be found at http://mdcune.psych.ucla.edu/modules/bioinformatics.

INTRODUCTION

Gregor Mendel's work was nearly lost (Maloney, 1996) and was only re-discovered well after his death in 1884. People with vision have created Bioinformatic tools to prevent such a tragedy in our day. Students need experience with these tools in order to be well-trained scientists and even consumers of data. Yet undergraduate students are afforded little opportunity to learn about these resources that could become important tools in their career. This paper describes an easy-to-adopt module that weaves together several important bioinformatic tools so students can grasp the depth and power that these tools provide in formulating and answering research questions. Moreover, all of the resources used in this module are available for free over the internet.

The core of this module is a Quantitative Trait Loci (QTL) analysis. QTL analysis provides a means of linking variations in a quantitative phenotype to chromosomal loci. QTL has become an exciting topic in biology because the genotypes of so many organisms have been sequenced and published. QTL analyses can suggest candidate genes that could be involved in shaping the phenotype, and QTL analyses are currently being applied to humans as well as animals and even plants to determine the locus of genetically determined/influenced morphological and behavioral traits. Among the growing list of traits are olfactory bulb size (Williams et al., 2001), cerebellum size (Airey, Lu, Williams, 2001), cortex size (Beatty & Laughlin, 2006), alcoholism (Bergen et al., 2003; Grisel, 2000; Radcliffe et al., 2004), anxiety (Dina et al., 2005), Alzheimer's disease proteins (Brich et al.; 2003 Ryman, Gao, & Lamb, 2008), ADHD (Doyle et al., 2008), pain susceptibility (Nissenbaum et al. 2008), IQ (Butcher, Davis, Craig, & Plomin, 2008), obesity (Casellas, Farber, Gularte, Haus, Warden, & Medrano, 2009), and dyslexia (Deffenbacher et al., 2004). In short, almost any morphological, physiological, or behavioral trait that could have at least some genetic basis can be examined by QTL analysis.

Although QTL is analysis is central to this module, this module provides students with an integrated experience that goes from measuring phenotype through identifying candidate genes that are expressed in the tissue and may influence the phenotype. As students journey through this module, they use a succession of bioinformatic tools including the Mouse Brain Library, WebQTL from GeneNetwork, the University of California, Santa Cruz Genome Browser, NCBI Entrez Gene, the Allen Brain Atlas, and PubMed.

METHOD

Below, we provide a synopsis of the steps used in teaching this module. A more complete description is available in the Student/Instructor manual and protocol PDFs that can be downloaded for free from our website http://mdcune.psych.ucla.edu/modules/bioinformatics.

QUANTIFYING THE PHENOTYPE

We use the Mouse Brain Library as a resource for our phenotype. The Mouse Brain Library (see references for URL) provides images of sectioned mouse brains from different recombinant inbred strains as well as pertinent metadata about the given individual animal such as age, body weight, sex, and fresh brain weight. The set of images that we use come from brains have been sectioned in the horizontal plane (see Figure 1) and Nissl-stained, which defines cell bodies. The specific set of images that we employ came from various BXD recombinant inbred strains (RISs) along with F0 C57BL/6 mice (B mice) and DBA/2J mice (D mice). Each BXD RIS has a unique recombination of the DNA from the F0 B and D mice on each chromosome. (Good descriptions of the derivation of recombinant inbred strains can be found in Grisel (2000), in Silver (1995), and at the GeneNetwork website—URL in references). Also, each of these RISs have been genotyped and informative markers mapped denoting if the DNA was from the F0 B or D strain. Thus, RISs can be sorted as to whether they have the B or D marker at a given point on a given chromosome. So differences in the phenotype among RISs can ultimately be correlated with differences in their genotypes. (A good discussion of markers and chromosome mapping can be found in Silver's (1995) free online book on mouse genetics (URL in references).

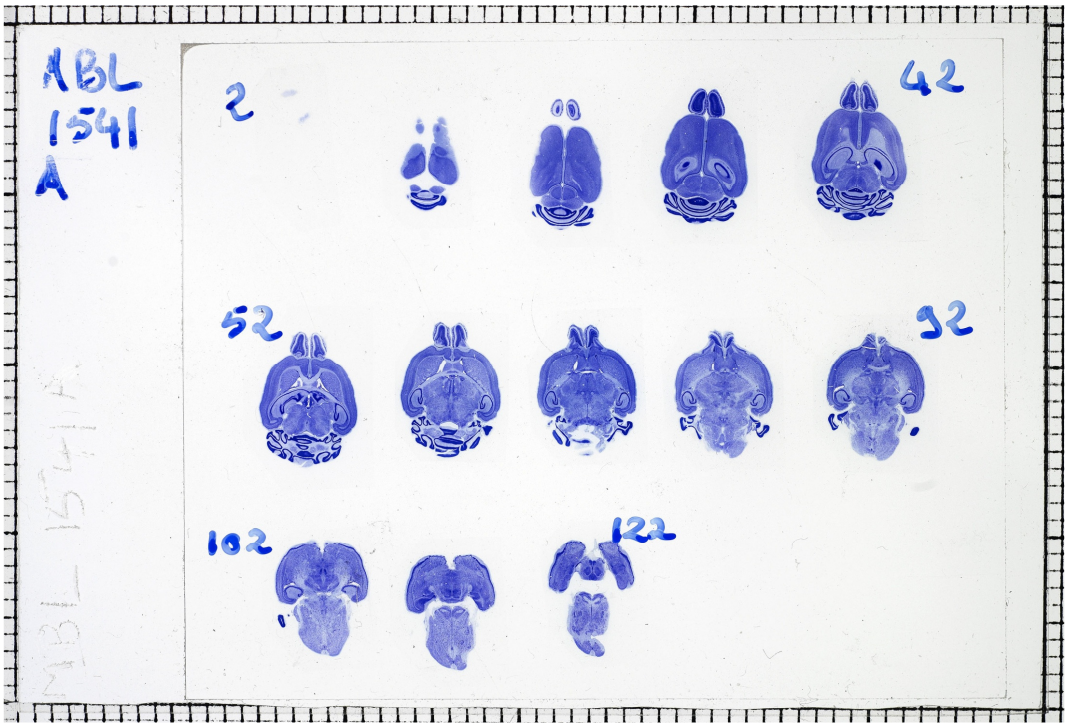

Figure 1. Image of a BXD mouse 1541A from the Mouse Brain Library. This brain was sectioned in the horizontal plane at 30 µm, every 10th section was mounted, and stained with cresyl violet for Nissl. Rostral is up.

Selection and quantification of the phenotype. Although any phenotypic brain trait could be selected, we selected olfactory bulb for ease and reliability of quantification with less inter-observer variability than other brain structures. Also, a published work on

QTL analysis of mouse olfactory bulbs is available for comparison (Williams et al., 2001).

The specific set of images from the Mouse Brain Library that we use can be downloaded from our website http://mdcune.psych.ucla.edu/modules/bioinformatics along with a spreadsheet with the pertinent metadata (by kind permission of Dr. Rob Williams, purveyor of the MBL).

To quantify the olfactory bulb as well as obtain an estimate of the volume of the whole brain, students download NIH Image (see references for URL), which is a software package that allows analyses of digital images. Briefly, the entire olfactory bulb is traced in every section in which it occurs and the volume determined from these data.

Correcting Variability. QTL analyses are sensitive to various sources of variability—not just variability that is due to genotypic variation. Thus, a great deal of emphasis in this module is spent on controlling sources of variability that are not due to genotypic variation. Extraneous non-genotypic variability can come from technical or environmental sources (Williams, 1998) and will result in more Type II statistical errors (false negatives) if it is not differentially distributed across strains. Some sources of variability that this module addresses are technical sources such as differential shrinkage of olfactory bulbs and inter-observer variability, which we seek to minimize and correct (see instructor's manual at http://mdcune.psych.ucla.edu/modules/bioinformatics).

Another important source of variability is subject characteristics that could affect the phenotype apart from the genetic influences acting directly on our region of interest (olfactory bulbs). Brains from the Mouse Brain Library come from animals of diverse ages, body sizes, and brain weights as well as both sexes. To distill the variance uniquely due to genetic influences on olfactory bulbs, these extraneous variables must be controlled for statistically via multiple regression. This step provides an excellent opportunity to teach simple and multiple regression as well as correlation as tools for this purpose.

QTL ANALYSIS

After removing extraneous variance, students then find an average of the residual variance for each recombinant inbred strain and are ready to perform the Quantitative Trait Analysis, using GeneNetwork, a web-based resource provided by the University of Tennessee (URL in references). Again, QTL analysis relates the variation in the phenotype (or residual phenotype) to loci on chromosomes that impact the phenotype. (See resources available at the GeneNetwork and Grisel (2000) for an excellent description of QTL analysis.)

GeneNetwork uses a specific interface in which the data from a given trait can be entered, and a Likelihood Ratio Statistic (LRS) calculated as a function of markers across the genome. (A good discussion of the LRS can be found in Beatty & Laughlin, 2006). The LRS will be high if there is a large discrepancy in the phenotype between mice with the B

vs. D maker at a given chromosomal locus and low when the phenotypes are not discrepant among mice with different forms of the marker. Large LRS values suggest that a gene(s) at or near the markers have a large impact on the phenotype (see Figure 2).

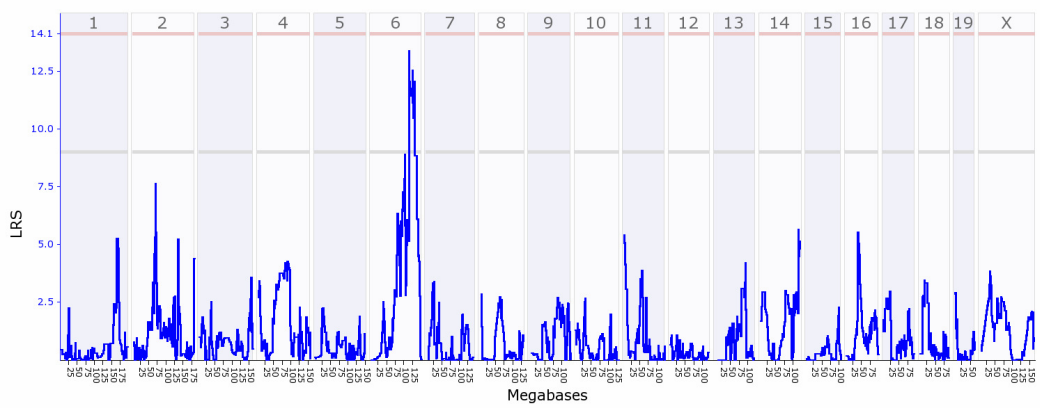

Figure 2. Output from WebQTL in geneNetwork using students' data. These graphs show the Likelihood Ratio Statistic (blue squiggly line) as a function of megabases across all mouse chromosomes except for Y. The criterion for significance is indicated by the pink horizontal line and the criterion for suggested is indicated by the gray horizontal line in both panels. The horizontal blue line represents the genome-wide p level (alpha level) of 0.05. LRS values exceeding this are considered significant, and are indicative of a link between the phenotype and that region of the chromosome. The horizontal green line represents the "suggested" level of linkage (genome-wide p level of 0.67).

In our example (Figure 2), students can see that they obtained a peak LRS scores on the distal end of chromosome 6 that exceeded the "suggested" criterion, and approached the criterion for significance. Figure 3 shows the same graph as Figure 2 but "zoomed-in" on the peak so that only a part of chromosome 6 is displayed. On the top of the graph, there is a track linking directly to the University of California Santa Cruz Genome Browser.

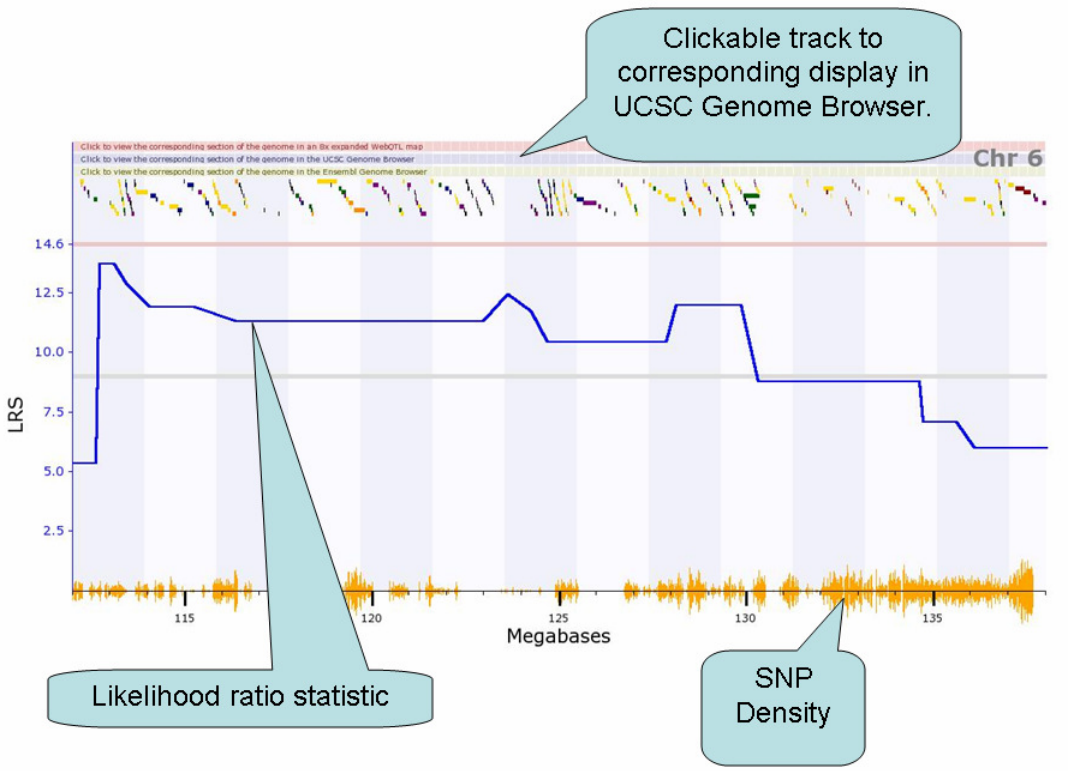

Figure 3. A "zoomed-in" section of chromosome 6 from the same data as displayed in Figure 2. This view shows the SNP track as well as the LRS as a function of marker. Further, there is a clickable track that leads directly to the UCSC Genome Browser information for that particular portion of chromosome. The small colored boxes near the top of the figure represent individual genes and are also hot-linked to further information about individual genes.

USING THE UCSC GENOME BROWSER

The UCSC Genome Browser (Zweig et al., 2008—see references for URL) is a "site [that] contains the reference sequence and working draft assemblies for a large collection of genomes." The UCSC Genome Browser provides a list of the known genes spatially arrayed in the selected region of a given chromosome. In Figure 4, we can see the list of genes on chromosome 6 that is displayed in Figure 3. Students then have a list of candidate genes that may influence the phenotype.

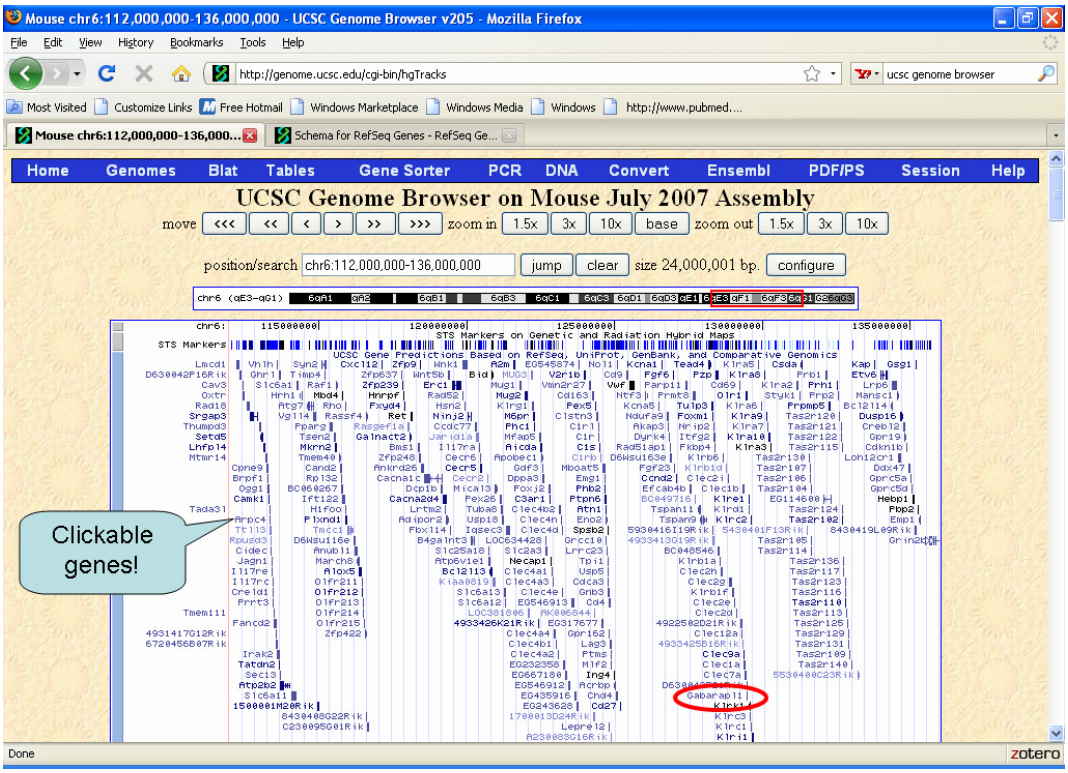

Figure 4. Screenshot of the UCSC Genome Browser for the span of chromosome 6 corresponding to the peak displayed in Figures 2 and 3. (The red box near top displays the portion of Chromosome 6 displayed.) Here we can see list of the known genes in this region of chromosome 6. Names are staggered with relation to where the gene would occur on the chromosome. These names are hot-linked to more information such as the relative degree of expression. As one example, the gene Gabarapl1 (Gamma-aminobutyric acid (GABA(A) receptor-associated protein-like 1) is a gene on the distal end of Chromosome 6 and is known to be expressed in the olfactory bulb.

Students use the microarray data to refine the list of candidate genes. By clicking on the names of genes, students can link to the microarray data, which may include whether the gene is expressed in the olfactory bulb. These microarray data provide an opportunity to discuss this cutting-edge technique (Figure 5). As students identify genes that are expressed in the olfactory bulb, we have them pursue further information about them using other bioinformatic resources. The UCSC genome browser has links to several other bioinformatic resources such as microarray data, the Allen Brain Atlas, NCBI Entrez Gene, and NCBI Pubmed.

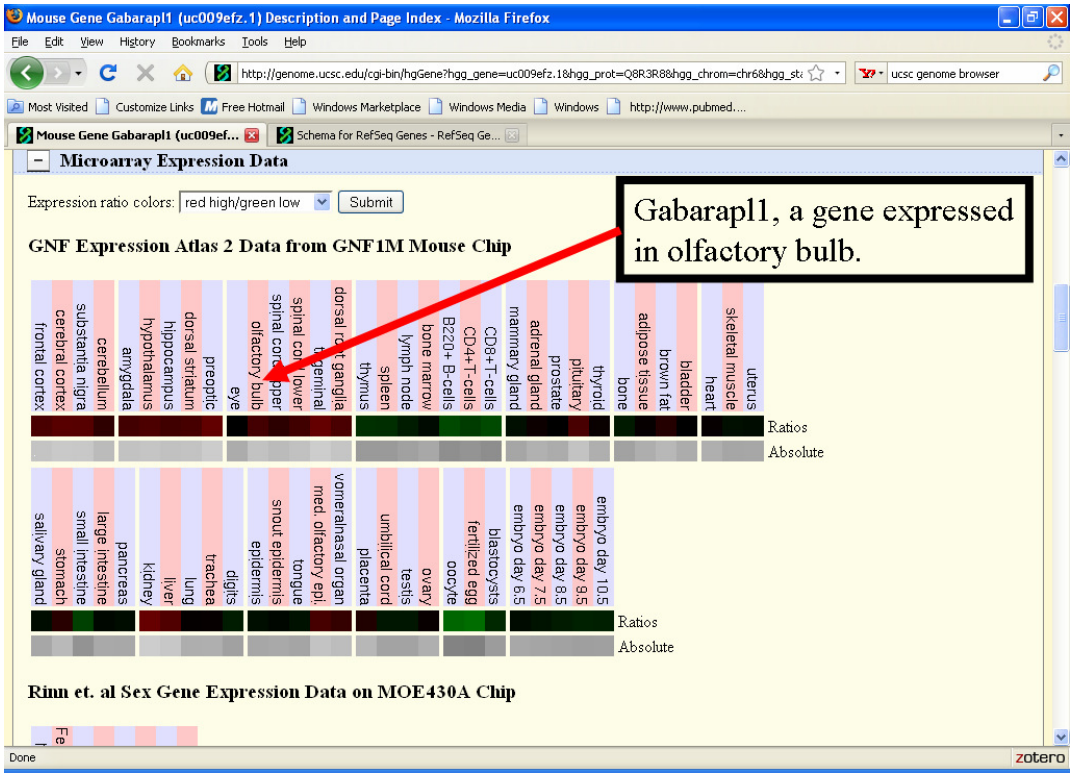

Figure 5. Screenshot from UCSC Genome Browser showing the expression of the Gabarapl1 gene in olfactory bulb relative to other tissues. Although the expression of Gabarapl1 is not extremely high, it might be of interest because it is a gene whose differential expression could affect development.

USING THE ALLEN BRAIN ATLAS

Once students have used the UCSC Genome Browser to identify a gene that is highly expressed in the olfactory bulb, they are then asked to click on the link to the Allen Brain Atlas (Lein, E.S. et al., 2007—URL in the references). The Allen Brain Atlas is an interactive, genome-wide image database of gene expression. In other words, it is database of *in situ* hybridization studies showing the expression pattern of specific genes across brain regions (cf. Ramos et al., 2007). The Allen Brain Atlas gives students the opportunity to learn about *in situ* hybridization as well as some experience with a brain atlas and neuroanatomy.

Specifically, we ask students to describe which olfactory bulb cell layers express their particular gene of interest (refer to Figure 6). Knowing which cell layers express the gene could give clues about the ontogeny of size differences among strains. Using the Allen Brain Atlas brings the students full circle back to the tissue itself, this time armed with the knowledge of a gene that could have affected the development of this structure.

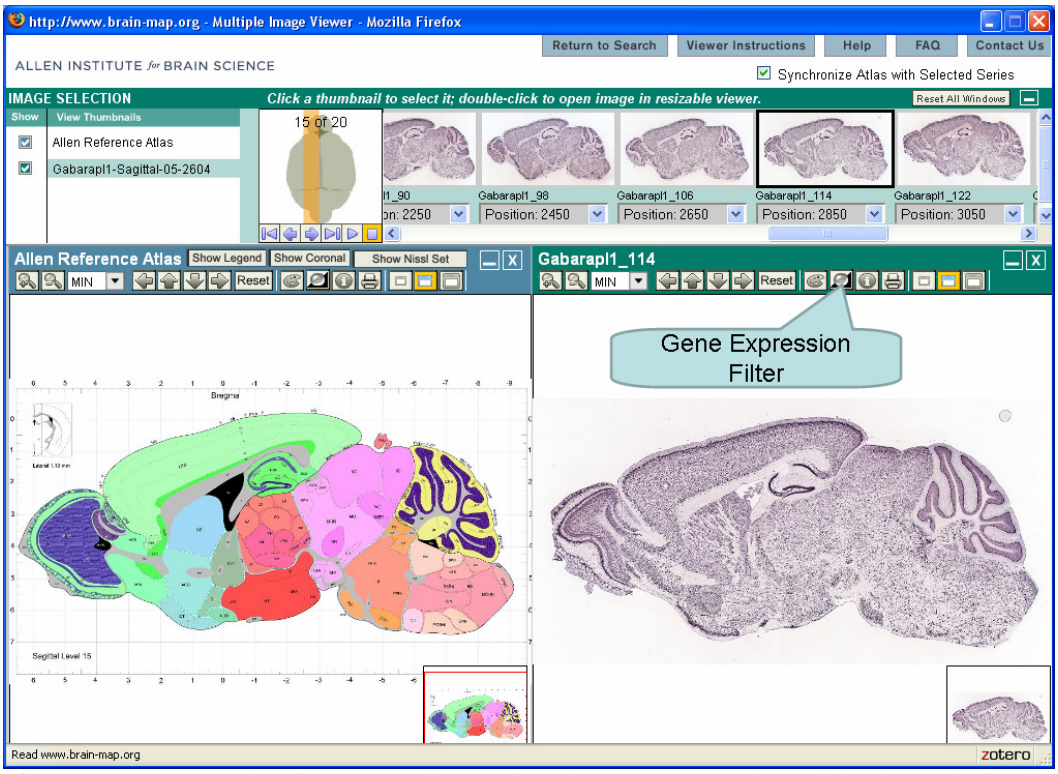

Figure 6. Screenshot from the Allen Brain Atlas. Large panel on right show the in situ expression in a parasagittal section. (Since Gabarapl1 is widely expressed, most of the brain shows some expression of this gene but highly expressed (darker) regions include the olfactory bulb, hippocampus, and one cortical layer. Large panel on the left is a plate from the corresponding atlas so that students can identify the names of the cell layers in which this gene is expressed.

USING ENTREZ GENE

Once genes expressed in the olfactory bulb have been identified, students can use a link from the UCSC Genome Browser to National Center for Biotechnology Information's Entrez Gene resource (URL in references). Using a link out of the UCSC Genome Browser, we have students find the nucleotide sequence of the gene as well as the coding sequence (Figure 7). We use this as an opportunity to talk about introns and exons and why the whole nucleotide sequence does not always coincide to the coding sequence. Students learn that this information is useful for constructing *in situ* probes, quantitative PCR, or antibodies to study the expression of this gene during development.

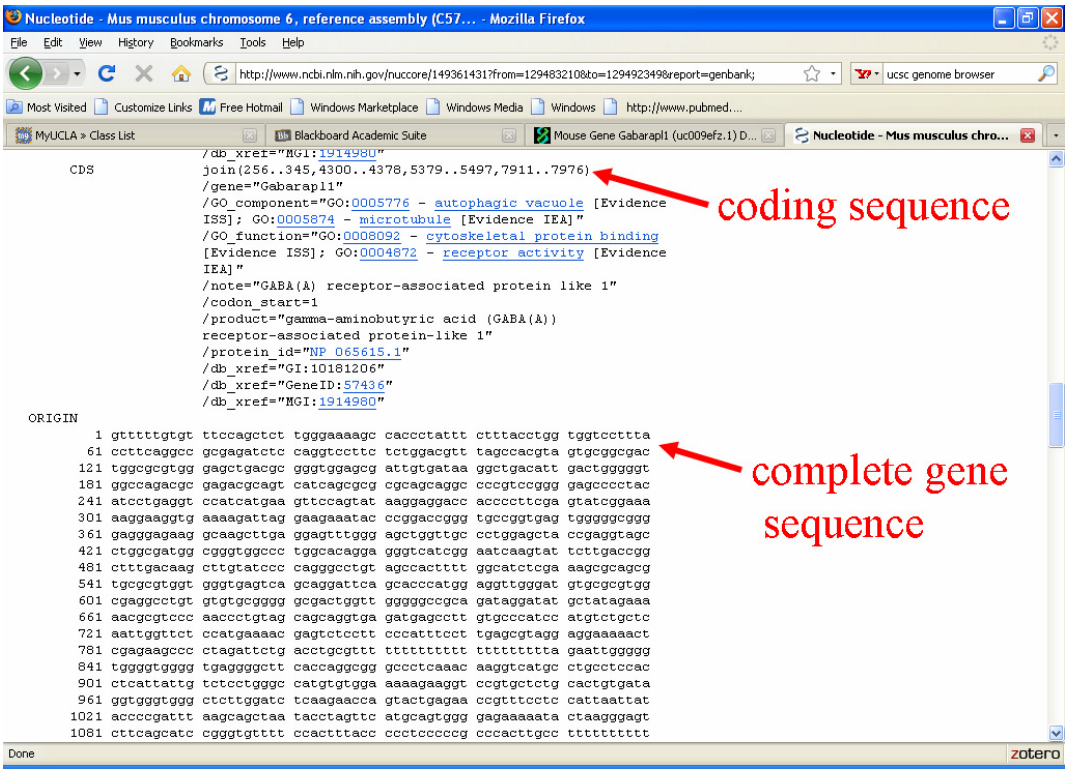

Figure 7. Screenshot from Entrez Gene displaying information about the coding sequence and the nucleotide sequence of the gene.

USING PUBMED

When students locate a gene that is expressed in the olfactory bulb, we ask students to find an article about that particular gene and include a summary of the article in their write-up. The UCSC genome Browser provides a direct link to a listing of the relevant articles in PubMed (URL in references). Although some institutions may have limited library resources, many journals now have content online for free (listing can be found at the Open Directory Project--URL in references), and Pubmed Central provides articles for free. We ask students to find an article that describes something about their gene, preferably relating to function, but not necessarily relating to the olfactory bulb, and write an abstract of the article.

ASSESSING THE EFFECTIVENESS OF THIS MODULE

To assess the effectiveness of this module, we administered a brief quiz to measure gains not only in the content of this unit but also to tap understanding of statistics and logical reasoning before and after exposure to the module (a pre- and posttest design). Because repeated testing can sometimes raise scores by itself (Campbell & Stanley, 1963; Trochim, 1986), we controlled for this possibility by only administering the posttest in a subsequent term after students completed the module.

To assess student perspectives on their learning, we administered a Likert-scale questionnaire. Also, students were asked to respond to the open-ended question, *"Please*

describe the purpose of the QTL (Bioinformatics) module from a learning standpoint in the space below." No further prompts were given and students were not limited on the length of their response. No specific responses were anticipated prior to data collection, so the coding of data was loosely based on a grounded theory model that allowed student perceptions to emerge without a preconceived hypothesis. However, given the nature of the module, there was a strong likelihood that students would comment on content knowledge, the relevance of statistics, and the usefulness of the technology. All assessment measures had IRB approval (UCLA IRB Exemption # 07-211).

RESULTS

We have taught this module for several terms and invariably found that it was an effective learning exercise. When comparing posttest to pretest scores using a paired-t-test, highly significant gains were found (t (91) = 14.58, $p < .001$, see Figure 8). Students who only took the posttest still showed gains relative to the previous term's pretest (t (129) = 10.606, p < .001, independent t-test), and posttest scores did not differ between students who had the pretest and those who didn't (t (129) = 0.06, p > .95, independent t-test—Figure 8). The latter result establishes that the gains that we observed are genuinely due to the instructional module and not due to a confounding factor such as "pretest sensitization" (Campbell & Stanley, 1963; Trochim, 1986). Pretest scores did not significantly correlate with grades on this unit (r (84) = .164, $p > .10$), suggesting that differential student performance was not due to some students being better prepared than others but rather due to genuine gains in learning. Posttest scores did correlate with the grades on the unit r (84) = .537, p < .001 when grades were determined by a multiple choice and short-answer exam but not in the subsequent term when grades were only determined by a short-answer exam, r (34) = .087, p > .60.

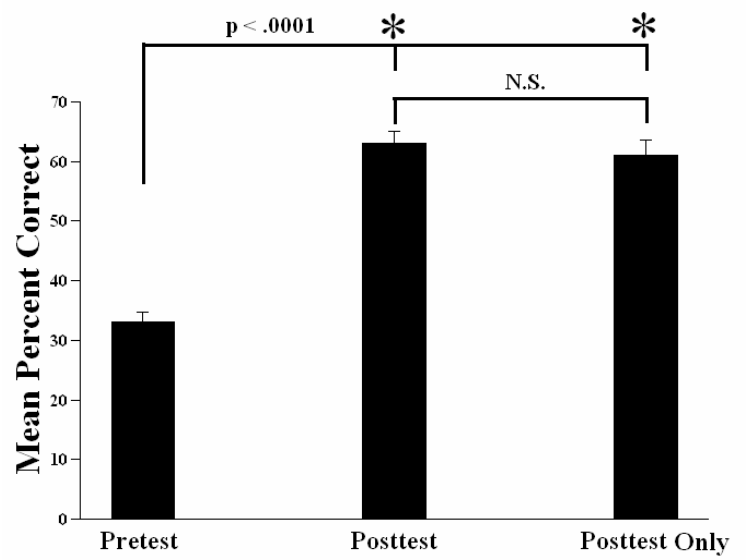

Figure 8. Mean percent correct on pretest and posttest given in one academic term and a posttest alone given in a subsequent term. Asterisks indicate significant differences as determined by a paired t-test (for tests given in same term) and an independent t-test for tests given in different terms. N.S. difference not significance.

On the Likert attitude survey, students indicated that their understanding of bioinformatics databases was enhanced, as was their understanding of statistics, genetics, molecular biology (refer to Figure 9).

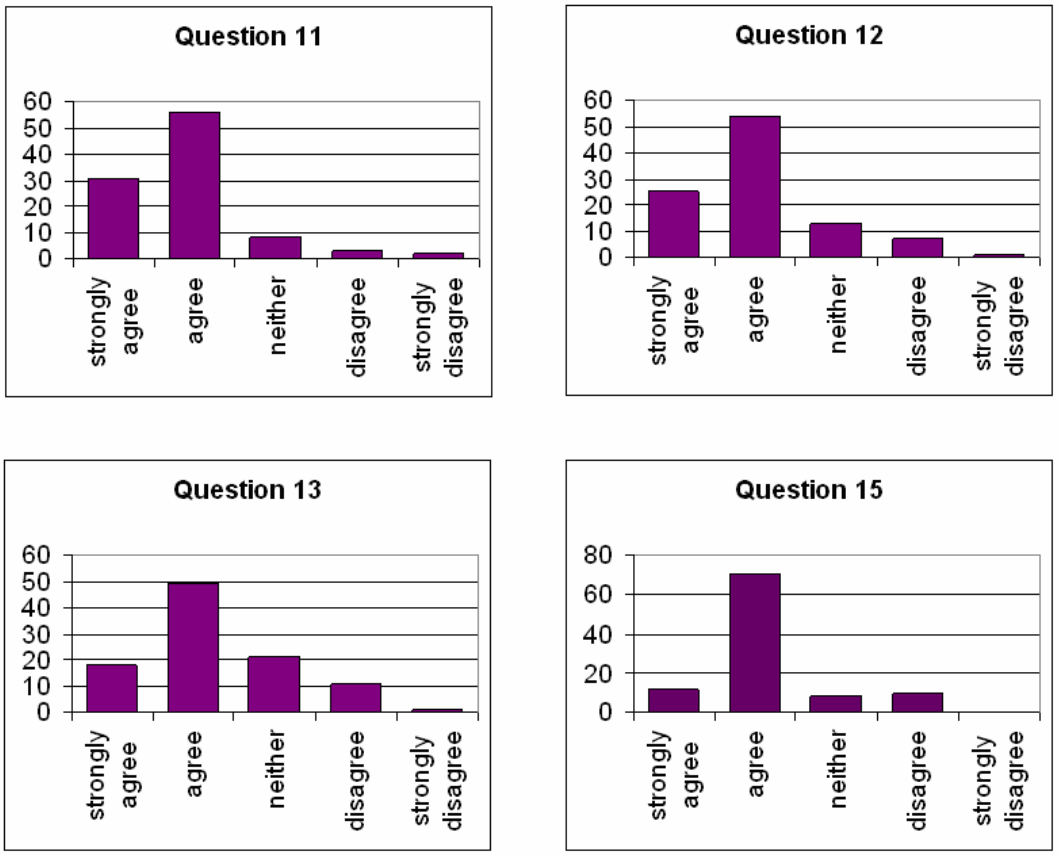

Figure 9. Percent of respondents (n = 95) as a function of scale points. Questions were worded as follows: (*A*) Question 11: My understanding of bioinformatics databases was enhanced by actually doing the computer tasks and examining their data. (*B*) Question 12: My understanding of genetics was enhanced by the QTL (Bioinformatics) module. (*C*) Question 13: My understanding of statistics was enhanced by the QTL (Bioinformatics) module. (*D*) Question 15: I learned something about molecular biology from the QTL (Bioinformatics).

Most responses to the open-ended question, *"Please describe the purpose of the QTL (Bioinformatics) module from a learning standpoint in the space below,"* described a combination of learning objectives that factored down to six main categories: (1) illustrative, (2) content knowledge (3) hands-on learning, (4) technology, (5) statistics, and (6) job related. Responses that were coded as *illustrative* addressed the module's ability to disseminate knowledge without reference to applying skills or analyzing content (terminology such as, "exposed," "showed," "familiarize" or "to see how"). Responses were coded as *content knowledge* if the response acknowledged that learning the material was at least one objective for using the QTL module. Responses that addressed the module's ability to provide an experience to perform a learning-based task were coded as *hands-on learning* (phrases such as "doing the activity," "opportunity to participate," "allows us to locate and analyze," or "hands-on approach"). The *technology*

category incorporated all responses that described learning objectives related to using bioinformatic tools, learning about a computer program or application, or developing computer-related skills. The *statistics* category included any responses that described the QTL module's usefulness for analyzing data. Finally, responses that claimed that learning to use the QTL module helped prepare students for "work in the field," "real world experiments," or "going into research" were categorized as *job-related*. Table 1 shows the frequency of responses by category, along with crosstab data on four categories that had a higher rate of correlation: *hands-on learning* and *technology*, and *technology* and *job related*. Frequency data are also provided on student satisfaction levels, even though the question did not require such commentary. Most student comments were not value based and simply stated the perceived purpose of the QTL module; however, several students offered their opinion of the module's usefulness. Twenty-seven comments included positive adjectives or phrases within the response, such as "reinforced learning," "makes learning easier," "quick and efficient," or "greatly enhanced [learning]." Five comments included negative adjectives or phrases within the response, such as "too fast-paced," "confusing" or "busy work."

Purpose	
Illustrative	**67**
Content Knowledge	**62**
Hands-On Learning	**56**
Technology	**42**
Statistics	**26**
Job Related	**26**

Frequently correlated categories	
Hands-On Learning/Technology	**23**
Technology/Job Related	**15**

Satisfaction	
Positive Comments	**27**
Negative Comments	**5**

Table 1. Student perspectives on the pedagogical objectives of the QTL module—a given student's response may be coded into more than one category.

DISCUSSION

The quantitative, attitude, and qualitative data all indicate that this module is a successful pedagogical unit. The quantitative data showed dramatic differences in pretest vs. posttest clearly showing that students make clear gains in content, quantitative, and reasoning skills used in this unit. The attitude questionnaire reflected students' impression that their understanding of genetics, statistics, and molecular biology was enhanced by this module. In addition to content, responses to the open-ended qualitative item addressed the module's ability to provide a hands-on, learning-based task. Many students made mention of learning technology, statistics, and job/career related skills. No student ever called it a simulation in the open-ended question, and it is not. Rather, students use these digital tools just as professional investigators would. Finally even though we did not specifically ask for value judgments, positive comments outweighed negative by a ratio of 5:1.

With regard to the QTL data produced, our students almost invariably find a peak on the distal end of chromosome 6 that reaches the "suggested" level but not quite the significant level (Figure 2). We have replicated this finding across several terms with different sets of students, and this result is quite robust, so we feel fairly confident about it. Suggested peaks are worthy of further pursuit because: (1) QTL analysis is a tool for generating a list of genes that might influence the phenotype and suggested peaks probably shouldn't be ignored, (2) the alpha level for individual points is extremely stringent, so it is actually difficult to find a significant peak, and (3) student data are likely to underestimate the true relationship between markers and phenotype. When dealing with naïve students making measurements, error variance will probably be large, thus diluting the relationship between markers and the phenotype. Further, suggested relationships are reported in the literature (Beatty & Laughlin, 2006; Doyle et al., 2008; Ryman, Gao, & Lamb, 2008; but also see Williams, 1998). So, instructors should feel gratified when their students can at least find peaks that reach the suggested criterion and use these peaks to generate a list of genes that have a putative impact on the phenotype.

Notably, our students do not faithfully replicate Williams et al. (2001): our students find fewer QTL peaks and although our students do find a peak on chromosome 6, our peak is shifted relative to Williams et al. There are several possible reasons for these differences: (1) we use a slightly different set of strains—Williams et al. use F1 mice whereas we do not, (2) we use volumes rather than weight as did Williams et al., (3) we operationally define the olfactory bulb in a slightly different fashion than do Williams et al. (for greater consistency among students), and (4) the map that we are using probably has more markers than Williams at al. (2001) had, so our peak may be more refined. The difference in the number of QTL peaks is probably due to our students' data having a lot more error variance than Williams et al. (2001). Accordingly, the probability of making a Type II error (false negative) is higher with our students' data, which would mean fewer peaks. We use this as a lesson on what random error variance does to data and why it pays to be painstaking in science.

We use olfactory bulb in this module due to its ease of definition and reliable quantification by naïve students and because there is a published work on this structure to which students can compare their data. Nonetheless, many other brain phenotypes could easily be substituted, such as cerebellum, hippocampus, and cortex size and there are corresponding published papers (Airey, Lu, Williams, 2001; Beatty & Laughlin, 2006; Pierce, Chesler, Williams, & Lu, 2003).

This module not only exposes students to QTL analysis, which is a relatively new tool in molecular biology/genetics but also exposes them to various bioinformatic tools weaving them together into a cohesive, comprehensible unit. Giving students experience with these tools sharpens their understanding of the underlying biology and statistics that were used to construct these bioinformatic tools. Our ultimate goal is to not only expose students to these resources but to also guide them in solving a tractable problem using this module as a vehicle to teach statistical analyses, genetics, neuroanatomy, and molecular biology. Although we do not utilize all of the many features available at GeneNetwork, the UCSC Genome Browser (Zweig et al., 2008), the Allen Brain Atlas

(cf. Ramos et al., 2007), and NCBI databases, we do manage to expose students to these enormously valuable tools that are being used daily in research.

Notably, all of these web-based resources employed in the module are available free to users. So, this module can be replicated by any faculty that have access to computers. We have re-published (with permission) the set of images from the Mouse Brain Library as well as a spreadsheet that contains the metadata about these mice to save instructors time in implementing this module. These and other didactic materials such as a detailed student/instructors manual, PDFs of handouts, powerpoint slides, quizzes, sample exams, etc. are available for free at our website, http://mdcune.psych.ucla.edu/modules/bioinformatics.

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