Title
Gel Scramble: An E-Tool for Teaching Molecular Neuroscience

Authors
Grisham, William
Keller, Lani
Schottler, Natalie

Publication Date
2015-05-19

Peer reviewed

ARTICLE
Gel Scramble: An E-Tool for Teaching Molecular Neuroscience

William Grisham[1], Lani Keller[2], & Natalie Schottler[1]
[1]Department of Psychology, University of California at Los Angeles, Los Angeles, CA 90095; [2]Department of Biology, Quinnipiac University, Hamden, CT 06518.

In this completely digital teaching module, students interpret the results of two separate procedures: a restriction endonuclease digestion, and a polymerase chain reaction (PCR). The first consists of matching restriction endonuclease digest protocols with images obtained from stained agarose gels. Students are given the sequence of six plasmid cDNAs, characteristics of the plasmid vector, and the endonuclease digest protocols, which specify the enzyme(s) used. Students calculate the expected lengths of digestion products using this information and free tools available on the web. Students learn how to read gels and then match their predicted fragment lengths to the digital images obtained from the gel electrophoresis of the cDNA digest. In the PCR experiment, students are given six cDNA sequences and six sets of primers. By querying NCBI BLAST, students can match the PCR fragments to the lengths of the predicted *in silico* PCR products. The ruse posed to students is that the gels were inadvertently mislabeled during processing. Although students know the experimental details, they do not know which gel goes with a given restriction endonuclease digest or PCR—they must deduce the answers. Because the gel images are from actual students' experiments, the data sometimes result from mishandling/mislabeling or faulty protocol execution. The most challenging part of the exercise is to explain these errors. This latter aspect requires students to use critical thinking skills to explain aberrant outcomes. This entire exercise is available in a digital format and downloadable for free at http://mdcune.psych.ucla.edu/modules/gel.

Key words: molecular neuroscience; gel electrophoresis; PCR; endonuclease digest; virtual laboratory; distance education; digital learning

Advances in molecular biology have influenced and transformed neuroscience in the past few decades. The advent of recombinant DNA technology along with the completion of the Human Genome Project have spawned new areas of research in cellular and molecular neuroscience which together seek to understand the complexities of how the human brain functions. Recently, large-scale research efforts such as the BRAIN (Brain Research through Advancing Innovative Neuro-technologies) Initiative aim to develop innovative technologies with the long-term goal of uncovering the mysteries of learning and memory and to help shed light on brain disorders. The development and employment of powerful tools for characterizing both the structure and function of neurobiological systems relies on a thorough understanding of molecular biological techniques. To this end, it is essential that all students within neuroscience programs have a strong background in not only the concepts of molecular biology but also in hands-on practical applications, such as PCR, endonuclease digestion, and interpretation of DNA gel electrophoresis results.

Although students pipette DNA into agarose gels every academic term, students are not usually challenged to critically evaluate and interpret the results. Students are often provided with pictures of perfect gels—or at least the ones that "worked." Science, including molecular biology, is actually not so neat and clean. Undergraduate voices claim that students need to "learn how to work with real data; learn to deal with ambiguity, and that science can be messy" (Vision and Change, 2011).

Gel Scramble provides a completely digital module that requires students to engage the mental processes behind doing an endonuclease digest and a PCR experiment. Students are given the pertinent information about the nucleotide sequences and protocols but are not told which gel image matches with a given endonuclease digest (in the first task) or with a given PCR (in the second task). Students must deduce the answers based on the provided protocols. Essentially students are challenged to "reverse engineer" these experiments and determine which result likely came from which protocol.

At first blush, this task may seem relatively simple once students learn the basics of reading gels and how to work through the protocols. The challenge comes because the protocols were not always followed faithfully (Figure 1). Students are not only challenged to figure out which gel goes with which protocol, but also are challenged to provide explanations for unexpected outcomes when they are present. The gel images come from UCLA students' experiments, and improper sample handling and / or protocol execution inevitably confounding some results. This messy reality requires critical thinking to properly interpret the outcomes.

Both tasks are completely digital so they require no equipment other than a computer. The Gel Scramble web page provides agarose gel images, as well as lab protocols and additional information that students will need to interpret the gel pictures, and a complete student tutorial at http://mdcune.psych.ucla.edu/modules/gel. An instructor manual and various keys are also provided to instructors who establish a faculty account through

http://mdcune.psych.ucla.edu/. All materials on the Gel Scramble web page, including those for faculty and other tools utilized in these exercises, are available for free.

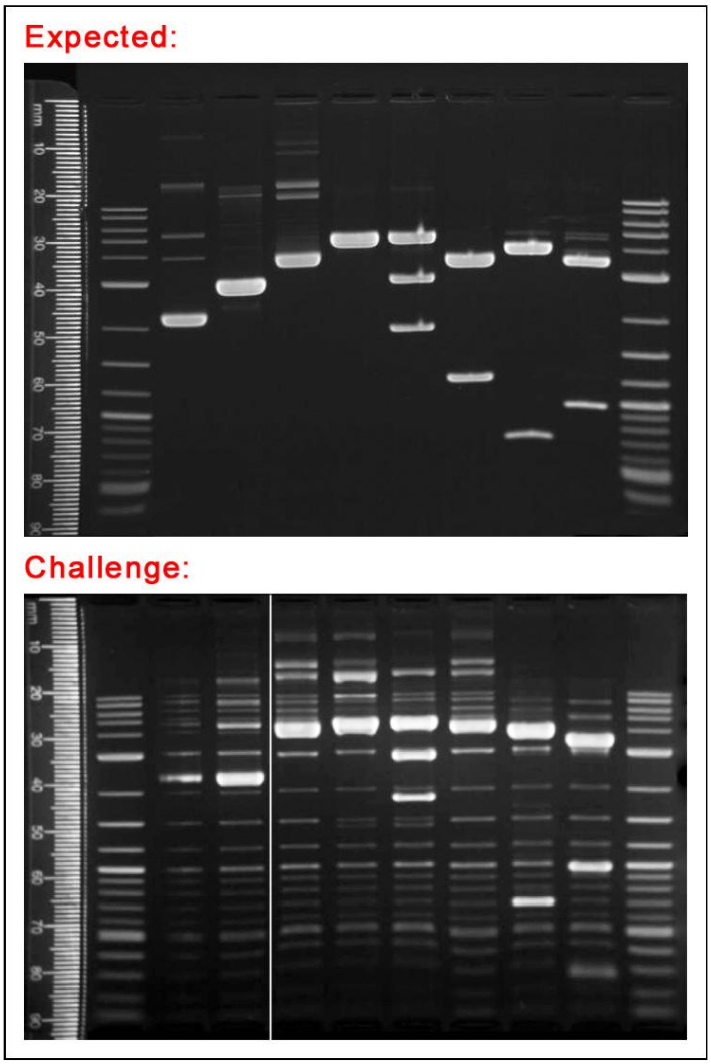

Figure 1. Two allegedly identical endonuclease digests produced by former students. For the gel labeled "Expected," the protocol produced predictable results. For the gel labeled "Challenge," things went awry. In the "Challenge" gel, the students were supposed to load the Molecular Weight DNA Ladders in Lanes 1 & 10 but instead loaded the ladder in all the lanes, superimposing the ladder on top of the bands for the endonuclease digests. [While we did not include this particular "Challenge" gel in the set packaged for other instructors, it is provided here as an effective illustration.]

MATERIALS AND METHODS

Subjects The endonuclease digest task was used in teaching an upper division laboratory class at UCLA. UCLA students consisted of 11 males and 11 females; 12 students identified themselves as Asian, two African American, four White, and four other. The PCR task was used in a Human Genetics course at Quinnipiac University (QU). QU students consisted of six males and 13 females; 18 students identified themselves as White and one identified themself as African American. Besides grades, quantitative data were acquired via a pre-module test ("pretest") and post-module test ("posttest") that tapped the material presented in the module as well as critical thinking. Questions on the pretest and posttest were the same and therefore a paired two-tailed t test was used to assess statistical significance. Participation was voluntary, and not all students in the course participated, although the majority did. Additionally, affective measures were taken on both populations as well as an open-ended qualitative item.

Web Resources The entirety of this module's resources can be found on the Gel Scramble web page at http://mdcune.psych.ucla.edu/modules/gel.

Table 1 lists all of the files available for free for all users. These are the files that students download to execute the module. Table 2 lists additional files available to faculty who intend to teach the module. These files are also available for free but require a faculty account to access. Faculty accounts are granted at no cost to individuals who teach at accredited institutions. Table 3 lists external resources for better understanding PCR. These video animations are useful but not required for teaching.

Table 1: MDCUNE Resources, Student

File Description	Category
Image Database	Images
Student Tutorial	Tutorials
Lecture Slides, Week 01 Class	Lectures
Lecture Slides, Week 01 Class, VO	Lectures
Lecture Slides, Week 01 Lab	Lectures
Lecture Slides, Week 02 Class	Lectures
Lecture Slides, Week 03 Class	Lectures
Lecture Slides, Week 03 Lab	Lectures
Mystery cDNA 'A' Digestion Protocol	Protocols, RE Digest
Mystery cDNA 'B' Digestion Protocol	Protocols, RE Digest
Mystery cDNA 'C' Digestion Protocol	Protocols, RE Digest
Mystery cDNA 'D' Digestion Protocol	Protocols, RE Digest
Mystery cDNA 'E' Digestion Protocol	Protocols, RE Digest
Mystery cDNA 'F' Digestion Protocol	Protocols, RE Digest
Mystery cDNA 'A-F' PCR Protocol	Protocols, PCR
PCR Animation	Protocols, PCR
PCR Lab Slides by Lani Keller	Protocols, PCR
PCR Lab Assignment Sheet	Protocols, PCR
Mystery cDNA 'A' Sequence	DNA Sequences
Mystery cDNA 'B' Sequence	DNA Sequences
Mystery cDNA 'C' Sequence	DNA Sequences
Mystery cDNA 'D' Sequence	DNA Sequences
Mystery cDNA 'E' Sequence	DNA Sequences
Mystery cDNA 'F' Sequence	DNA Sequences
Mystery cDNA Information	DNA Sequences
Mystery cDNA Schematic	DNA Sequences

Table 1. Gel Scramble web resources available for free download to student (or all) users.

Table 2: MDCUNE Resources, Instructor	
File Description	**Category**
Student Tutorial, Editable	Tutorials
Instructor Tutorial, Editable	Tutorials
Mystery cDNA Digestion Product Key	Keys
Mystery cDNA PCR Product Key	Keys
Plasmid Vector & Mystery cDNA PCR Product Key, Detailed	Keys
Mystery cDNA PCR Gel Problems	Keys
Grading Rubric	Keys

Table 2. Gel Scramble web resources available for free download to instructor (or faculty) users.

Table 3: External Web Resources, PCR Video Guides
http://www.dnalc.org/resources/animations/pcr.html
http://learn.genetics.utah.edu/content/labs/pcr
http://www.sumanasinc.com/webcontent/animations/content/pcr.html
http://highered.mheducation.com/sites/0072556781/student_view0/chapter14/animation_quiz_6.html
http://www.lifetechnologies.com/us/en/home/life-science/pcr/elevate-pcr-research/pcr-video-library/pcr-animation.html
http://www.promega.com/resources/multimedia/pcr/introduction-to-pcr/

Table 3. PCR external web resources available for free to student (or all) users.

The DNA Sequences Six unlabeled, or "Mystery," cDNA sequences are employed in this module, and are all initially isolated from a library of rat hypothalami. These cDNAs code for: (a) Jag1 protein—involved in development and ligand for the NOTCH receptor, (b) nicotinic Ach receptor α7 subunit, (c) Delta1—ligand for the notch receptor, (d) NOTCH2 receptor—involved in cell signaling, (e) nicotinic Ach receptor α9 subunit, and (f) NOTCH1—homologue of *Drosophila* notch. DNA Sequences for these proteins were subjected to an endonuclease digest and were used in the PCR task as well. Each endonuclease digest or PCR was usually replicated several times by different groups of students.

Notch, Delta, and Jagged are all cell surface proteins with membrane spanning domains. Delta and Jagged are ligands for Notch, which is their receptor (Weinmaster, 1997). Interactions between Notch and Delta/Jagged ligands are involved in cell-cell signaling (Weinmaster, 1997). Delta, Jagged, and Notch have epidermal growth factor-like repeats in their extracellular domains (Lindsell et al., 1996; Thurston et al., 2007). Notch has an extracellular ligand-binding domain, which is cleaved upon binding, releasing an intracellular domain that transduces the ligand signal (Weinmaster, 1997, Thurston et al., 2007). These Notch/Jagged/Delta interactions are believed to be important in development such as cell-fate selection and differentiation (Weinmaster, 1997; Lathia et al., 2008) and may also be important in neurite remodeling and pathological states such as stroke and Alzheimer's in adulthood.

The ACh receptor α7 and α9 subunits also have a membrane spanning domain (Elgoyhen et al., 1994; Dineley et al., 2015). The α9 receptor has an unusual pharmacological profile: both nicotine and muscarine can reduce its response to acetylcholine (Elgoyhen et al., 1994). The ACh receptor α9 can be found in the pituitary, in the olfactory epithelium, and is involved in cochlear innervation during development (Elgoyhen et al., 1994; Vetter et al., 1999). Both the α7 and α9 subunits can form ACh-gated channels when expressed as a single subunit, and they also form ACh receptors sensitive to nicotine and α-bungarotoxin (McGehee and Role, 1995). The α7 receptor has been implicated both in schizophrenia and in Alzheimer's disease (Dineley et al., 2015).

Task 1: Endonuclease Digest Goals for this portion of the module are five-fold:

Goal 1: Determine the band sizes for a given protocol. When predicting DNA band sizes, students need to consider the size of the plasmid vector alone, whether the plasmid will be linearized or not in the digest, and whether this will matter to the migration of the molecules during electrophoresis. The endonuclease digests all examined cDNAs inserted into a plasmid vector, PBluescript (GenBank # X52330.1), which is a 2961-base pair (bp) plasmid. Whether or not a given enzyme will cut in the multiple cloning site is thoroughly described in the materials available on the website, part of which is displayed in Figure 2.

Secondly, students need to consider the size of the insert and whether the enzyme cut within the sequence of the cDNA insert (the gene of interest) in order to predict the size of digestion products. For the cDNA insert, students can use NEBcutter, a free web tool provided by New England Biolabs (NEB) at http://tools.neb.com/NEBcutter2/index.php (Vincze et al., 2003). See Figures 3 and 4 for a look at NEB's user interface. Students were given a "prelab" assignment between lab sessions 1 and 2, which had them at least attempt to list the expected digestion fragments in each lane for each of the protocols. Further, they were assigned to sketch a restriction map for each of the protocols as a part of the prelab exercise.

Goal 2: Identify all of the relevant bands on a given gel. Students need to learn to read and interpret DNA agarose gels. Initially, they need to learn which bands are meaningful and which are extraneous to analyses (see Figure 5). The loading wells will sometimes have residual DNA but are irrelevant. Similarly, when plasmid DNAs are not linearized, they can become high molecular weight forms, usually linking up with other plasmids to make chain-like molecules or concatemers, which are also irrelevant to the analyses.

Goal 3: Determine which gel matches which protocol. Students should be able to predict the size of the bands in each lane. In all of our protocols, Lane 1 is the molecular weight standard ladder, a picture of which is available at the New England Biolabs website http://www.neb.com/nebecomm/products/productN3200.asp.

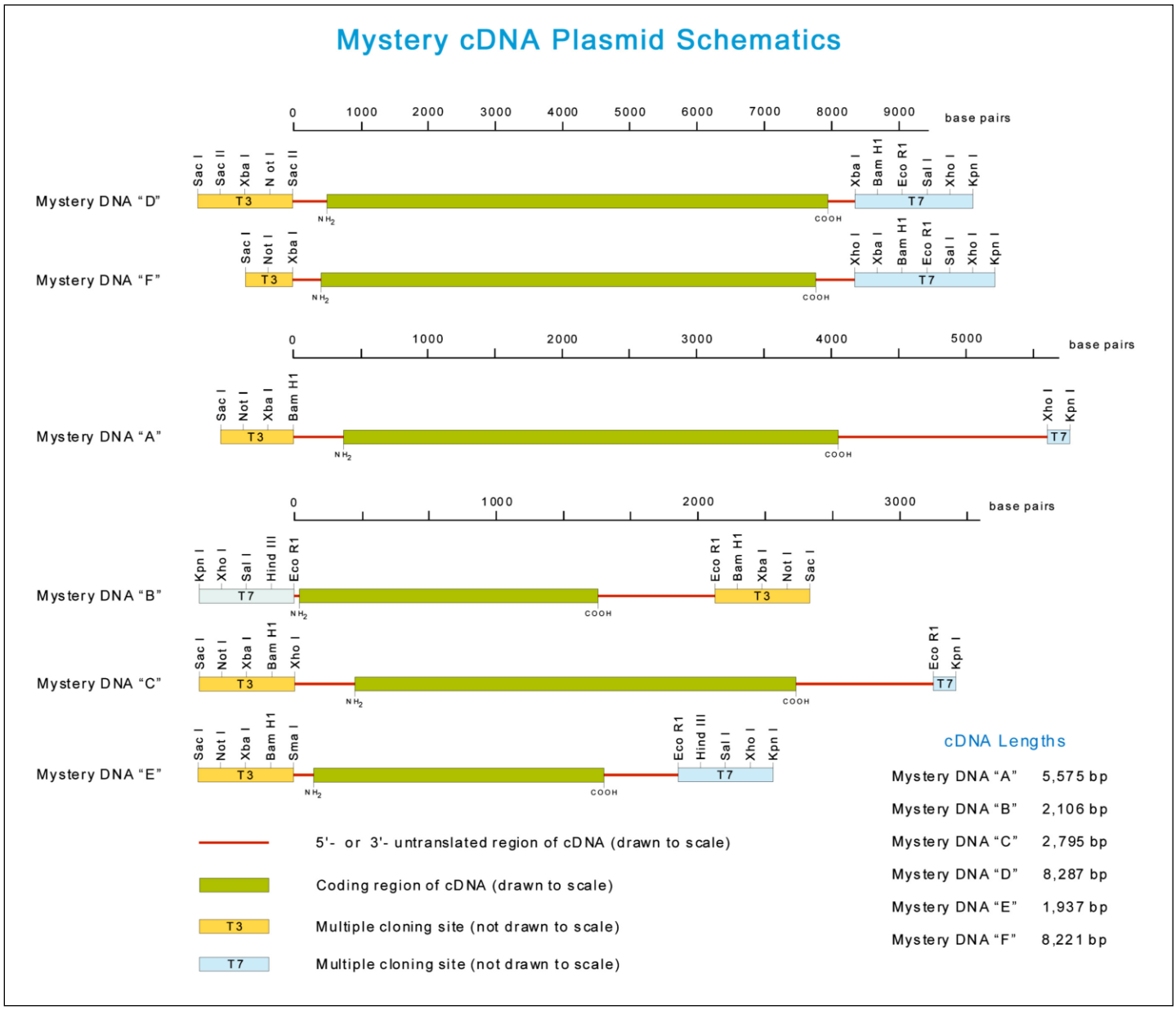

Figure 2. Schematic detailing which enzymes will cut in the multiple cloning site for each of the Mystery cDNAs employed.

Lane 2 is supposed to be the uncut plasmid, Lane 3 is supposed to be the linearized plasmid, Lane 4 is supposed to be the uncut plasmid with insert, Lane 5 is supposed to be the linearized plasmid with insert. The remaining lanes contain various digests except Lane 10, which is another lane with a molecular weight standard ladder. Students should use the protocols available to obtain information about the enzymes used in the various digests to aid in their predictions and matching.

Instructors are provided with an opportunity to explain why one would want lanes with the cut and uncut plasmid as well as lanes devoted to linearized and unlinearized plasmid with insert.

Goal 4: Identify when bands do not match the expected results. The module was designed so actual results do not always match the expected results. Students should be encouraged to check their predictions and have the instructor approve them if necessary. (Instructor keys are available for free by first going to the Gel Scramble web page at http://mdcune.psych.ucla.edu/modules/gel, then clicking on the links in the Faculty section and setting up an account.)

Goal 5: Identify what happened when the bands on the gel do not match the expected. The critical thinking challenge for students is not only to correctly identify what digest goes with which sequence but also to ascertain what mistakes were made to explain the aberrant examples. Sometimes this discrepancy will be due to errors—wrong DNA in a given lane, "swapped" lanes, failing to add DNA and/or enzyme to the digest for a given lane, etc. Other times, this discrepancy may be due

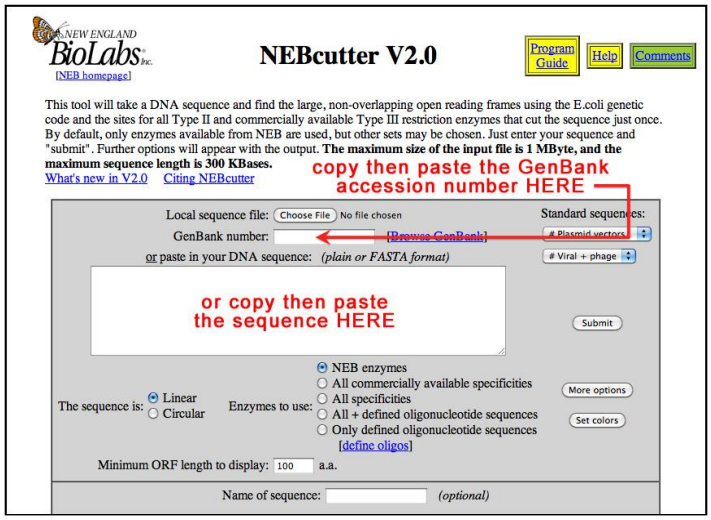

Figure 3. Screen capture of the NEBcutter V2.0 home page. NEBcutter V2.0 is a free online tool that describes where enzymes would cut in a known DNA sequence. The red labels indicate where students could enter data to use the tool.

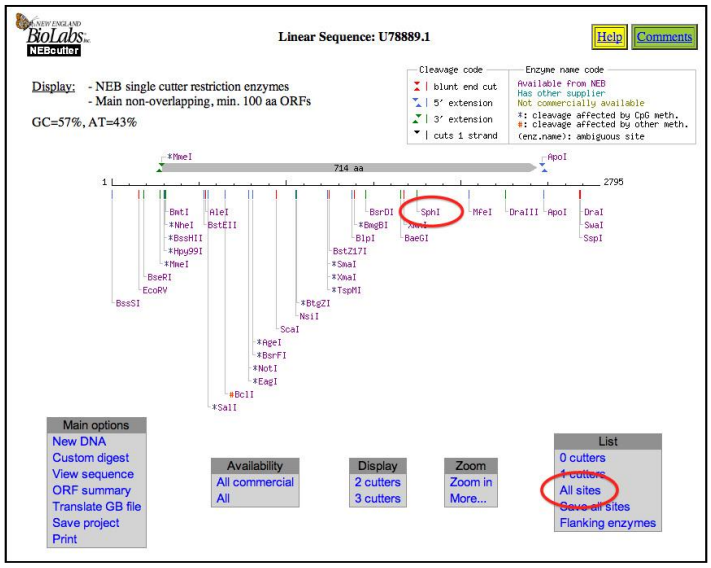

Figure 4. Screen capture from the NEBcutter V2.0 website. This figure of a linear DNA sequence, also seen in the student tutorial, is used as an example to guide students in predicting bands in an endonuclease digest. Here, all the enzymes that would cut this sequence one time are displayed. The red circles indicate links where students could further obtain further information.

to characteristics of the DNA—a methylated cut site, for example. Still other times it could be that the bands were run off of the gel. Additionally, there could be partial digests—when the DNA was not fully digested by the enzyme resulting in some uncut DNA remaining. Students should provide some plausible explanation for the various discrepancies that they encounter. (Again, instructor keys that provide the most likely explanations are available at http://mdcune.psych.ucla.edu/modules/gel.)

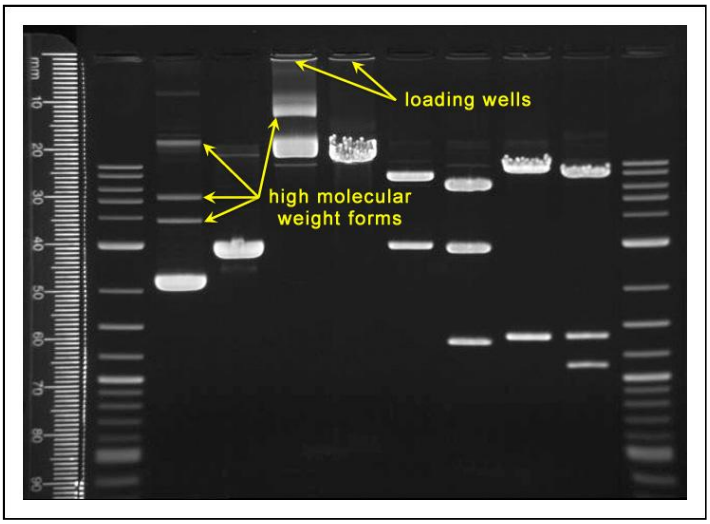

Figure 5. Picture of a gel from the Gel Scramble Image Database used for the endonuclease digest task. Certain aspects that are irrelevant to the analysis and interpretation of results—loading wells and high molecular weight forms—are indicated.

Task 2: Polymerase Chain Reaction (PCR) Goals for this portion of the module are three-fold:

Goal 1: Perform an in silico PCR. In this phase, students initially do an *in silico* PCR. Then they download a rich-text format copy of each of the Mystery cDNA sequences as well as a spreadsheet of associated BLAST information (cDNA description, accession numbers, and primer sequences) on the Gel Scramble web page at http://mdcune.psych.ucla.edu/modules/gel. Students use the NCBI Basic Linear Alignment Search Tool (BLAST) at http://blast.ncbi.nlm.nih.gov/Blast.cgi to individually BLAST their primer sequences (see Figures 6 and 7) as well as their cDNA accession numbers (see Figure 8). By marking where the upstream and downstream primers would bind on the cDNA sequence, students should be able to predict the size of the corresponding PCR product. (Students are sometimes baffled because the downstream primer is the complement of the gene sequence and reads right to left. This gives the instructor a good opportunity to review PCR with students.)

Goal 2: Compare the expected PCR product size to the empirical product size. Since the PCR procedures had been well-optimized, all PCR protocols included only negative controls (Lanes 2-4). These negative controls provide instructors with an opportunity to discuss each specific control with students. A PCR product is only expected in Lanes 5 and 6—the only difference between Lanes 5 and 6 is that a 100-fold more template was used in Lane 5 than in Lane 6. Students should be able to use the DNA size ladder in Lane 1 to determine the size of the PCR product. Students *may* be able to identify which protocol led to which gel but since some of the PCR products are close in size between the different cDNAs, complete accuracy may not always be possible. The PCR results are usually straightforward with only one surprise: one of the primers has two binding sites and so the PCR produces two products, and hence two bands (see Figure 9).

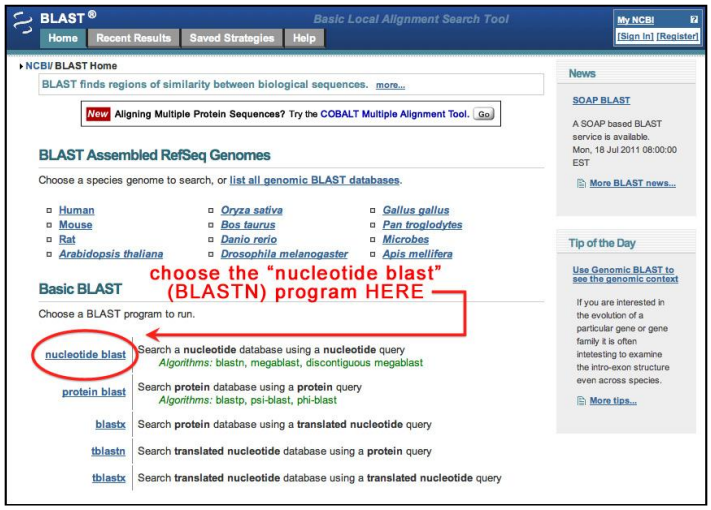

Figure 6. Home page of the NCBI BLAST tool. The red label indicates a link to "nucleotide blast," the specific BLAST program where students will enter their primer sequences.

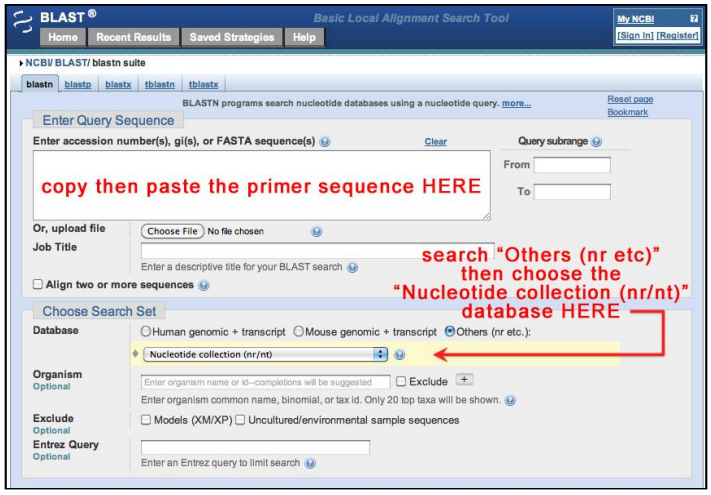

Figure 7. Web page of the BLASTN program, which searches nucleotide databases using a nucleotide query. The red labels indicate where students should enter data, as well as under which database set to search.

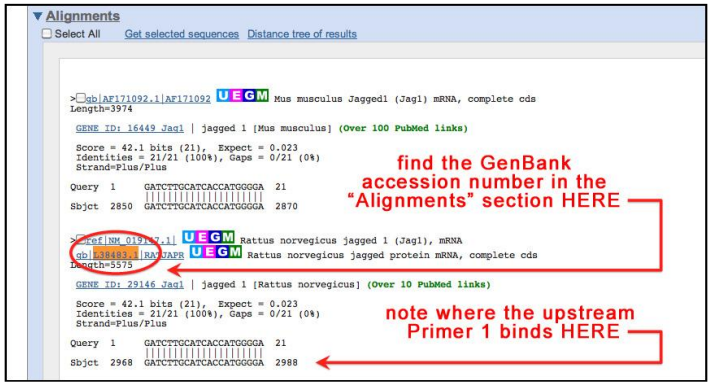

Figure 8. Web results of a nucleotide BLAST with one of the Gel Scramble primers. The red labels indicate pertinent information about the primer's behavior with the Mystery cDNA that will help students predict the size of their PCR product.

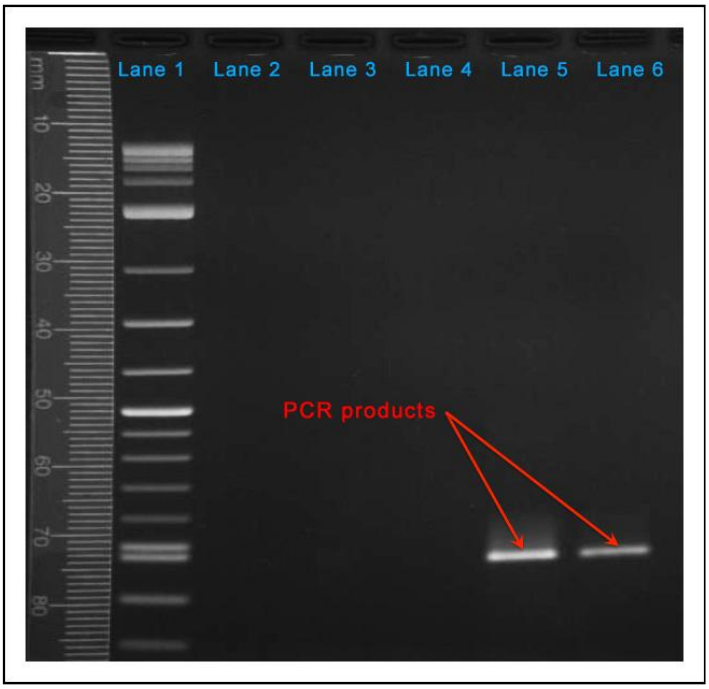

Figure 9. PCR products from one of the six Mystery cDNAs. Lane 1 contains the Molecular Weight DNA Ladder, Lane 2 is a control containing no primers but a high concentration of the cDNA template, Lane 3 is a control containing the upstream Primer 1 with a high concentration of the cDNA template, Lane 4 is a control containing the downstream Primer 2 with a high concentration of the cDNA template, Lane 5 contains both primers with a high concentration of the cDNA template, and Lane 6 contains both primers with a low concentration of the cDNA template. Here, a high concentration of cDNA is 10 ng/uL, and low concentration of cDNA is 0.1 ng/uL.

Goal 3: Identify any anomalies in expected results versus actual DNA gel results. The most challenging part of this exercise is to explain the errors that exist in the PCR products. Were there any gels in which the empirical results did not match the predicted results? Were there any other problems with the execution of the protocol? Most errors have obvious explanations, such as failure to add the template/ primers/ Taq polymerase to the reaction. Many errors simply involve complete or partial reversal of the lanes. Other errors, however, are more complex (see Figure 10).

Students need some background knowledge in order to complete the *in silico* module independently. At QU, students were provided a short PowerPoint stressing not only the technical details but also the vast utility of PCR throughout the biological sciences (i.e., sequencing, site-directed mutagenesis, forensic analysis, diagnosis of genetic diseases, etc.). Since it was a Human Genetics course, they focused on using PCR in forensic testing and in genetic disease diagnosis.

QU students also were provided with web-based resources to provide a further foundation prior to beginning the *in silico* PCR task. These web-based PCR animation resources are listed in the supplementary information (the top two most useful are the top two links). Additionally, to get students quickly engaged in the topic of

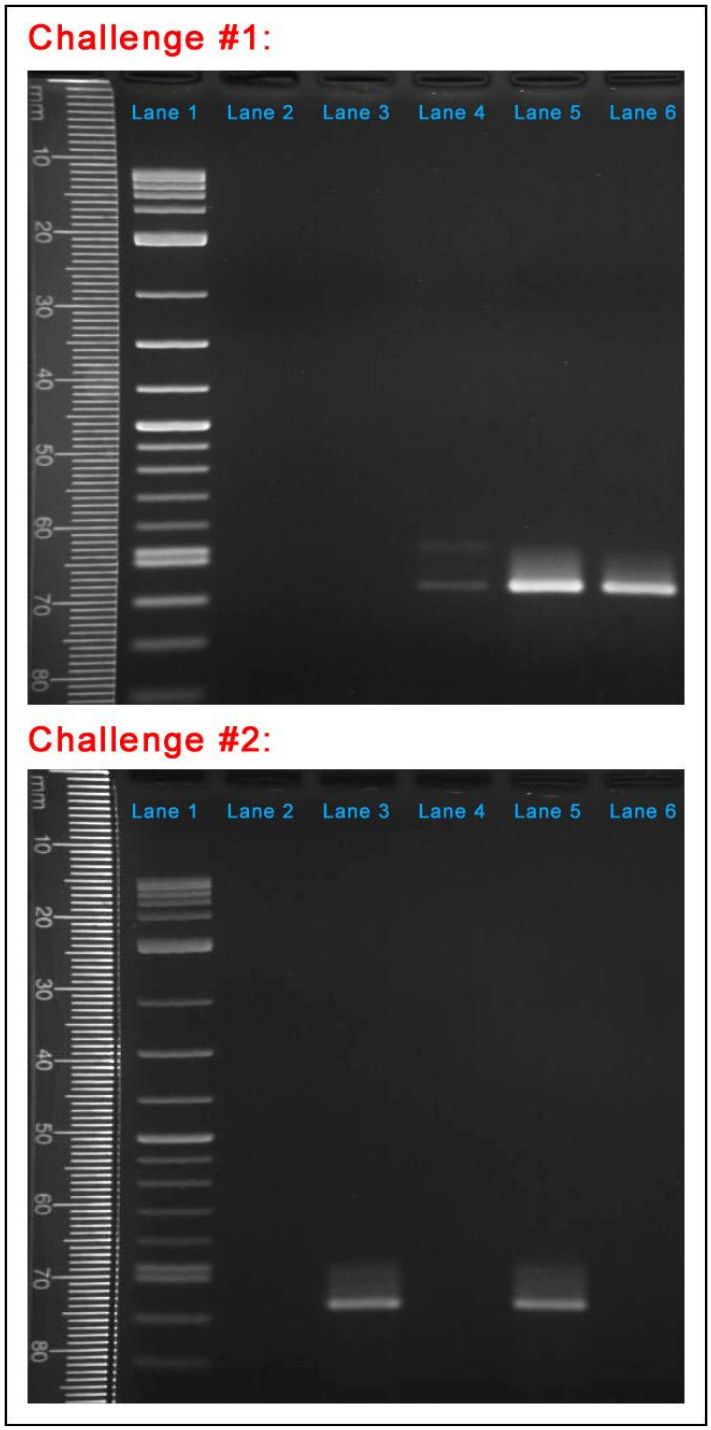

Figure 10. Two instances of PCR results that require explanation. The gel labeled "Challenge #1" has bands in Lane 4 that should not be present. Lane 4 represents a control that should only contain the downstream Primer 2 with a high concentration of cDNA template and a master mix with Taq polymerase, and so should not produce any PCR product. The lower band in Lane 4 could represent contamination by the upstream Primer 1; the upper band in Lane 4 defies explanation. The gel labeled Challenge #2 is more complex. Lane 5 should (and does) contain a PCR product. Lane 6 should contain a band of less intensity than that of Lane 5, but instead no bands are present. Lane 3 contains a band of equal intensity to that of Lane 5, but should contain no bands as it represents a control.

PCR they were presented with the following YouTube video/song as they entered: (http://www.youtube.com/watch?v=x5yPkxCLads).

RESULTS

We restricted our analyses on the pretest-posttest data to the items that were relevant to that cohort. That is to say, we analyzed the items relating to PCR for the QU students, and endonuclease digest items for the UCLA students. The QU students' performance showed improvement on the pretest-posttest (provided in supplementary material). The overall percentages for QU students increased from 38.59% in the pretest to 57.89% in the posttest showing a statistically significant change of 19.30%, $t(18) = 2.16$, $p < 0.05$, two-tailed test (Figure 11). Similarly, UCLA students showed gains from 50.00% in the pretest to 61.16% in the posttest, with an overall change in 11.16%, as measured by the pretest-posttest, $t(21) = 3.03$, $p < 0.01$ (Figure 11).

Affective response data is displayed in Figures 12 and 13. Approximately 70% of both QU and UCLA students agreed with Item 02. Approximately 35% of QU students and 65% of UCLA students agreed with Item 06. Nearly 70% of QU students and 50% of UCLA students agreed with Item 07. Approximately 60% of QU students and 70% of UCLA students agreed with Item 09. [Refer to Figures 12 and 13 for the full item descriptions.] Overall, the UCLA students responded more positively to the affective questions than did the QU students.

A free response item, "Please describe the purpose of the Gel Scramble module from a learning standpoint in the space provided below" was included as a part of the posttest. Characteristic responses obtained from both UCLA and QU students are displayed in Table 4. Nineteen of twenty responses from UCLA students were positive; nine out of the ten responses from QU were positive (refer to Table 4).

DISCUSSION

Both the UCLA and QU students showed significant gains on the pretest-posttest instrument, most of the affective responses reflected a mixed but generally positive experience. Although it seems like the UCLA students had a more positive experience, the UCLA and QU students experienced completely different aspects of the module, so their data are not directly comparable. The free response item overwhelmingly established that students at both institutions understood the pedagogical goals and saw value in the experience.

Endonuclease Digest Task The endonuclease digestion was taught as a stand-alone module as a part of a Neuroscience Laboratory class at UCLA. Students were asked to do a prelab in which they predicted the size of the fragments for each of the endonuclease digest protocols and presented these as a spreadsheet. They were also asked to draw a to-scale schematic of the plasmid with insert, including the endonuclease cut sites. This latter

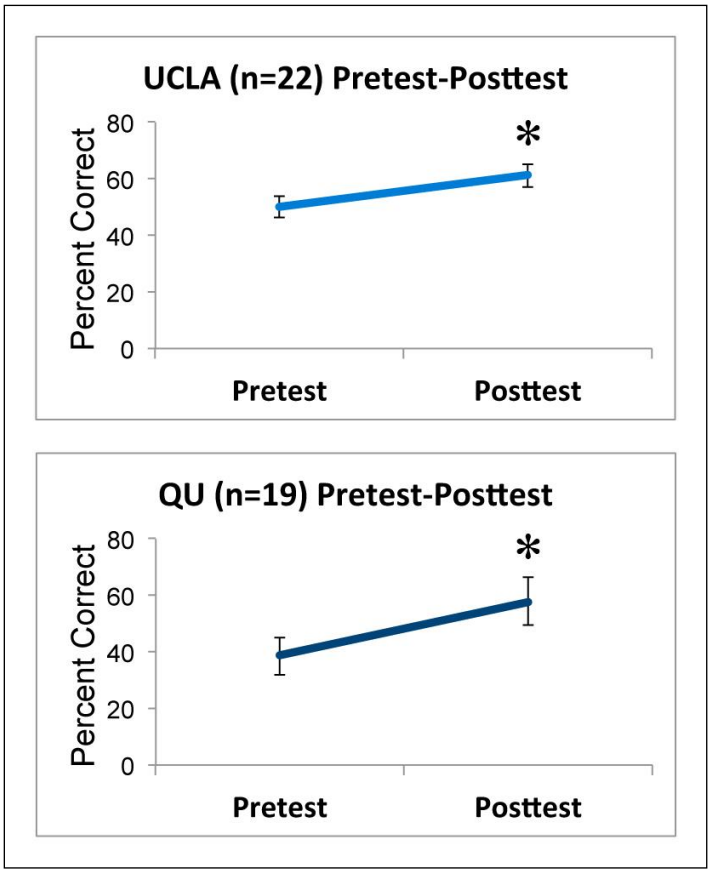

Figure 11. Statistically significant changes were demonstrated in Pretest-Posttest scores at both UCLA and QU.

exercise was included to guide students in their thinking about the potential results of the endonuclease digests. Students used lab time to match the protocols to the gels and ascertain the cause of anomalous/unpredicted results. In lectures, common problems in interpreting gels and common mistakes and their consequences were discussed.

Student and Faculty Considerations for Endonuclease Digest Task Most students successfully completed the module (grade mean = 86.57%, SD = 11.16), although one earned a failing grade. Not surprisingly, students' biggest challenges came both in matching the gels to the given digest protocols and also in explaining unexpected results. This task required guidance from the instructor, although the lectures had focused their thinking somewhat so students knew on which aspects to focus and were rarely distracted by irrelevant details. The biggest problem came when students made decisions without considering all the data—two digest protocols give similar results and only differ appreciably in one lane that allows students to distinguish between them. Students that did not consider all the data sometimes hastily confused these outcomes and mis-assigned the gels to the wrong protocols. Most students resolved any difficulties and ultimately figured out their mistakes. Nonetheless, some students remained very fixated on only one aspect of the data and thus were inflexible in their thinking. These students had to be really

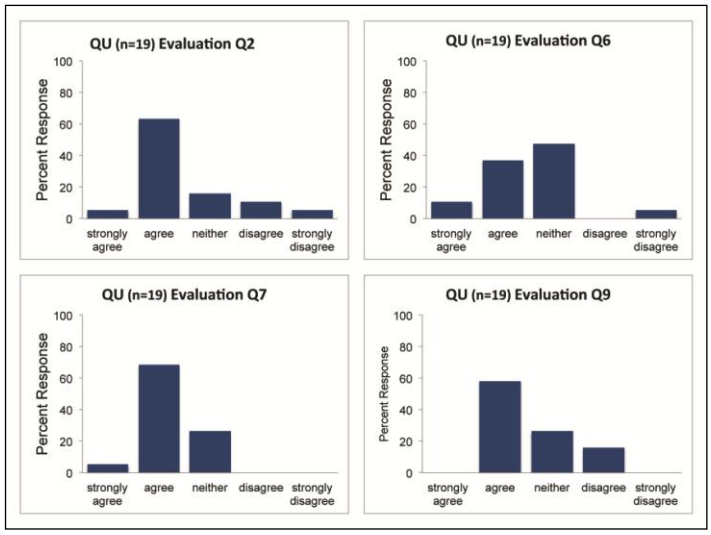

Figure 12. QU student (n=19) affective responses from to selected items included in the posttest. Item 02: "Overall, the purpose of the computer tasks in the Gel Scramble module was clear and easy to follow." Item 06: "My understanding of control procedures was enhanced by the Gel Scramble module." Item 07: "I learned something about molecular biology from the Gel Scramble module." Item 09: "I feel that the Gel Scramble module has helped me to think critically, especially when experiments did not go as planned."

pushed to consider all of the data in deciding how to assign gels to protocols. These teaching moments helped students realize that hypotheses need to fit all the relevant data rather than just a small aspect of it.

The most successful and least successful aspects of this module resulted from the prelab. Having students predict the results of the digest as a prelab was absolutely key in getting them immersed in the task and left time in lab for dealing with assigning gels to protocols as well as identifying anomalous outcomes, which is the most challenging aspect and which required the most instructor assistance. Only if there is appreciable time should students draw a to-scale schematic of the plasmid+insert with endonuclease cut sites indicated since it is quite time consuming. A simple sketch could replace this schematic and still be just as useful. The lectures discussing interpretation of gels and common mistakes were crucial—students probably would not be able to tackle the task without them.

PCR Task The Gel Scramble website provides students with the opportunity to conduct *in silico* PCR experimentation. This online activity simulated the intellectual experience of performing a PCR without the necessity of expensive reagents or equipment.

For an educator, one of the best aspects of the Gel Scramble PCR task is its adaptability. Educators can utilize individual portions of the larger module to teach specific aspects of PCR such as BLAST and prediction of DNA sizes. Then instructors may build upon these concepts by introducing additional steps of the module such as data interpretation through gel analysis of PCR products. Additionally, educators can use this online

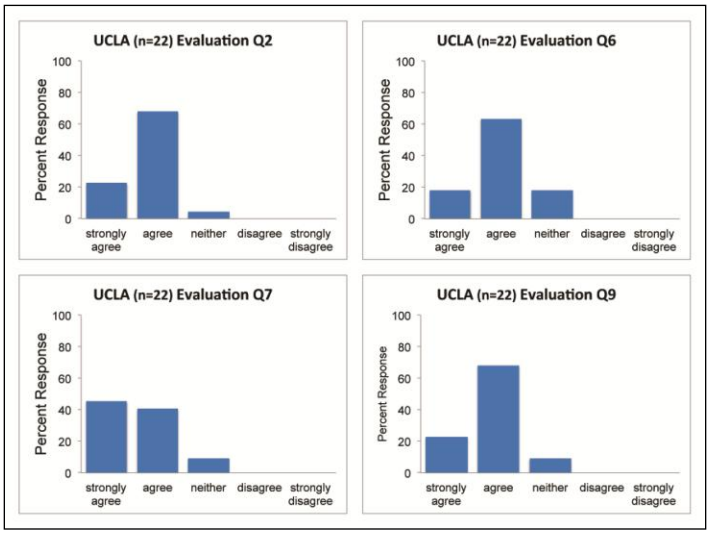

Figure 13. UCLA student (n=22) affective responses from to selected items included in the posttest. Item 02: "Overall, the purpose of the computer tasks in the Gel Scramble module was clear and easy to follow." Item 06: "My understanding of control procedures was enhanced by the Gel Scramble module." Item 07: "I learned something about molecular biology from the Gel Scramble module." Item 09: "I feel that the Gel Scramble module has helped me to think critically, especially when experiments did not go as planned."

resource unaccompanied or in preparation of a hands-on laboratory where student conduct an actual PCR experiment. QU students completed this online module prior to conducting their own PCR reactions where they had the opportunity to amplify a region of non-coding mitochondrial DNA from their own cheek cells. In my experience (L.K.), the PCR task nicely compliments hands-on experimentation and provides students with foundational knowledge necessary for understanding molecular techniques. Using the *in silico* PCR procedure as a stepping stone, educators can then go beyond this and provide students with additional self-directed investigations. For example, one of the authors had students then pick genes in which they were interested (often a disease gene) and determine the relative position of forward and reverse primers as well as the expected size of the amplified DNA resulting from PCR. Instructors could do even further experiments having the students use either cDNA or genomic DNA as a template to determine expected sizes of amplified gene products.

Student and Faculty Considerations for PCR Task It is important that the faculty member is physically around and available to help students as they go through the *in silico* PCR task. The instructor tutorial is straight-forward and easy to read and understand. The student instructions, however, were a little more difficult to interpret and there may be confusion with the plethora of items to download from the Gel Scramble website. To make things a little more clear, the instructor downloaded all necessary student material and posted it within the university's online content delivery system. Additionally, the instructor

Table 4: Posttest, Responses

Institution	Comments
UCLA	(1) The purpose was to learn how to think critically and to be able analyze data. Also the purpose was to learn how to look at data and determine if things may have gone wrong and what that may indicate in terms of the results. (2) The major goal of doing this module is to practice critical thinking when the experiment doesn't go as planned and find out why the unexpected happen; it is important to note that mistakes in science lead to great future discoveries. (3) To learn how to map out digests and predict bands of DNA and also be able to explain errors that may arise during the experiment.
QU	(1) The Gel Scramble was for students to become more knowledgeable on PCR and gel electrophoresis. When you do out a lab such as this one, you tend to absorb more information. (2) I believe that this procedure helped me to understand PCR's and how exactly they work, what materials are required, and how to complete a gel electrophoresis. I now understand what components are needed to complete a PCR such as a reaction buffer, deoxynucleotides, DNA template, primers, and the DNA polymerase. I understand that PCR's cannot be used to amplify entire genomes, entire genes, but they can however amplify entire pieces of genes with SNPs. (3) The Gel Scramble was for students to become more knowledgeable on PCR and gel electrophoresis. When you do out a lab such as this one, you tend to absorb more information.

Table 4. Characteristic answers to the free response on the posttest.

created an Excel table which students were required to turn-in, that had all the necessary information clearly stated (i.e., position of primers, estimated DNA size, gel analysis interpretations). In terms of the actual PCR task, students were a little confused on the BLAST webpages, in particular when trying to find their specific accession numbers after BLAST. It was important that the instructor was there to help out with such issues.

Surprisingly, several of the students were confused on the gel analysis section because they did not understand that the ruler shown on the side of the gel was not the DNA molecular weight markers. The gel analysis fostered critical thinking skills and was very useful for students. Informal discussion with students led me (L.K.) to determine that despite their frustration at times, they had a much better understanding of PCR as a whole. They understood how the technique worked, all the reagents necessary for the PCR, how to use NCBI databases to BLAST and determine expected DNA sizes, and perhaps most importantly how to interpret DNA gels.

Summary In 1999, Francis Crick predicted that molecular biology would have a huge impact on the future of neuroscience, which has been borne out. To fully understand any complex biological system, precise inference at the cellular and molecular level is essential. Neuroscience students must be able to understand and interpret molecular biology experiments in order to take advantages of novel techniques ranging from optogenetics to pharmacological treatments.

Well-rounded neuroscience students need to know how to read and interpret electrophoresis gels with DNA samples. These exercises provide a learning experience that provides them with this training. Students using these e-tools will have a very similar intellectual experience in molecular biology to those who are taking a "wet-lab" course. These teaching tools also allow instructors who lack equipment—such as a PCR machine—to provide such an exercise. Further, students get experience with bioinformatics tools such as NCBI BLAST.

As discussed in the Instructor Manual and above, these exercises can be altered to change both their length and complexity in both tasks. These projects can also be flexible with regard to both class and course time commitments and even order of tasks. These experiences could stand alone or be used in conjunction with "wet-lab" courses, depending on the needs of the instructor. Further, due to their digital nature, these tasks are amenable to classes of various sizes. Also because of their digital nature, the preparation time should be far less than a "wet lab" experience and easy to implement. Additional ideas for incorporating neuroscience topics into similar modules would be to have students determine DNA sequences containing either deletions or insertions based on DNA gel electrophoresis data that would directly affect neuronal function. Incorporation of DNA sequence analysis may also encourage students to determine specific structure-function relationships of particular molecules within neurological systems.

Although these exercises are completely digital, they are not really simulations. We agree that "simulations are physically unconvincing and never provide the ambiguous results that …[can]…occur with real instruments [that] promote critical questioning of cause and effect" (Jeschofnig and Jeschofnig, 2011, p. 53). Advocates of hands-on learning further argue that besides providing students with real data, they also provide "unexpected clashes"—the disparity between theory and experimental reality (Ma and Nickerson, 2006). "Unexpected clashes," however, need not be the exclusive domain of wet-lab learning. The digital experiences provided in the Gel Scramble module embrace the messiness of reality and "unexpected clashes" showing students how science can extract a comprehensible story from a messy reality. Molecular biology requires a lot of thought to properly interpret the outcomes, and this module provides the opportunity to develop such critical thinking skills. Training students' critical thinking skills should be a basic aspect of science education, and Gel Scramble provides tools to accomplish this goal.

All materials, including the student tutorial and instructor manual are available for free at http://mdcune.psych.ucla.edu/modules/gel.

REFERENCES

Crick F (1999) The impact of molecular biology on neuroscience. Philos Trans R Soc Lond B Biol Sci 354:2021-2025.

Dineley KT, Pandya AA, Yakel JL (2015) Nicotinic ACh receptors as therapeutic targets in CNS disorders. Trends Pharmacol Sci 36:96-108.

Elgoyhen AB, Johnson DS, Boulter J, Vetter DE, Heinemann S (1994) Alpha 9: an acetylcholine receptor with novel pharmacological properties expressed in rat cochlear hair cells. Cell 79:705-715.

Jeschonfnig L, Jeschofnig P (2011) Teaching lab science courses online: resources for best practices, tools, and technology. San Francisco, CA: Jossey-Bass.

Lathia JD, Mattson MP, Cheng A (2008) Notch: from neural development to neurological disorders. J Neurochem 107:1471-1481.

Lindsell CE, Boulter J, diSibio G, Gossler A, Weinmaster G (1996) Expression patterns of Jagged, Delta1, Notch1, Notch2, and Notch3 genes identify ligand–receptor pairs that may function in neural development. Mol Cell Neurosci 8:14–27.

Ma J, Nickerson JV (2006) Hands-On, Simulated, and Remote Laboratories: A Comparative Literature Review. ACM Comput Serv 38:1-24.

McGehee DS, Role LW (1995) Physiological diversity of nicotinic acetylcholine receptors expressed by vertebrate neurons. Annu Rev Physiol 57:521-546.

NSF, AAAS (2011) Vision and Change in Undergraduate Biology Education: A Call to Action (accessed 2015-05-01). http://visionandchange.org/files/2011/03/Revised-Vision-and-Change-Final-Report.pdf

Thurston G, Noguera-Troise I, Yancopoulos GD (2007) The Delta paradox: DLL4 blockade leads to more tumour vessels but less tumour growth. Nat Rev Cancer 7:327-331.

Vetter DE, Liberman MC, Mann M, Barhanin J, Boulter J, Brown MC, Saffiote-Kolman J, Heinemann,SF, Elgoyhen AB (1999) Role of alpha9 nicotinic ACh receptor subunits in the development and function of cochlear efferent innervation. Neuron 23:93–103.

Vincze T, Posfai J, Roberts RJ (2003) NEBcutter: a program to cleave DNA with restriction enzymes. Nucleic Acids Res 31:3688-3691.

Weinmaster G (1997) The ins and outs of notch signaling. Mol Cell Neurosci 9:91-102.

Received February 27, 2015; revised May 13, 2015; accepted May 19, 2015.

Acknowledgements: Gel Scramble and other digital teaching tools produced for the Modular Digital Course in Undergraduate Neuroscience Education (MDCUNE) project were developed and supported by NSF CCLI Grant DUE-0717306, as well as a grant from the UCLA Office of Instructional Development. We owe a huge debt of thanks to Dr. James R. Boulter of UCLA's Department of Psychiatry and Biobehavioral Sciences. Dr. Boulter has taught this module as a wet lab for several years for the undergraduate major. Dr. Boulter was kind enough to share materials from his module including pictures of students' gels. This resource literally would not be possible without the help and tutelage that both he and Dr. Geraldine Weinmaster provided.

Address correspondence to: William Grisham, Ph.D., Psychology Department, UCLA, 1285 Franz Hall, PO Box 951563, Los Angeles, CA 90095-1563. Email: dr.billgrisham@gmail.com