DNA Barcoding as an Educational Tool: Case Studies in Insect Biodiversity and Seafood Identification

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Abstract

High school students may find it difficult to understand the application of molecular biology laboratory methods like gel electrophoresis, PCR and DNA sequencing and teaching these concepts can be challenging. Demonstrating a link between protocols and applied questions of socio-economic importance can make these techniques more accessible and more interesting, facilitating science and technology learning objectives. This article summarizes two case studies conducted to engage students in biodiversity and molecular biology through applications of DNA barcoding. These projects were conducted in collaboration with a research lab at the University of Guelph in Canada and with Let's Talk Science, a not-for profit organization dedicated to science outreach. Two parallel case studies were undertaken to illustrate the implementation of DNA barcoding by high school teachers to provide a service learning opportunity for students. By participating in these case studies, students learned about DNA barcoding and the associated laboratory and bioinformatics tools as a way to identify food fraud at local restaurants and supermarkets and to explore insect biodiversity of their high-school campuses. The resources used in both case studies have been deposited within available online portals to allow for their continued use and improvement when teaching concepts in molecular biology through applications of DNA barcoding.

Introduction

Molecular biology has become a standard part of high school science, particularly in upper-year biology classes. It is a rapidly changing field, involves abstract concepts and straddles multiple traditional disciplines such as biology, chemistry, ecology, and bioinformatics [1]. As such it can be difficult for educators to teach in a relevant way. Although curricula often cover individual topics like gel electrophoresis, DNA extraction and Polymerase Chain Reaction (PCR), it can be difficult to demonstrate the relationships between these methods. Teaching each concept individually does not give students an adequate understanding of how molecular biology can be used to address socio-economically relevant issues. This gap between science in the classroom and the application of science can leave students uninterested in and/or underprepared for post-secondary science. To address this, students need concrete examples of the scientific process from start to finish to understand how individual

[1] Lena A.E. Tibell and Carl-Johan Rundgren. "Educational Challenges of Molecular Life Science: Characteristics and Implications for Education and Research." *The American Society for Cell Biology Life Science Education*, 9 (2010) 25-33.

laboratory methods are used in concert. Hands-on activities can encourage interest and understanding in science, especially for complex issues or methods. By using DNA barcoding as a classroom tool, teachers are provided a coherent framework with which to introduce students to basic concepts in molecular biology. The hands-on aspect of the case studies described here help students to engage and promotes an interest in the topic, and we hope, of science in general.

What is DNA Barcoding?

DNA barcoding is a Canadian innovation that uses molecular genetic methods for species identification, based on taxon-specific differences in the nucleotide sequence (barcode) of a standard gene region[2] (Figure 1), which only require small tissues samples from the organism to be identified. Gene regions have been identified for barcoding animals[3], plants[4] and fungi[5] . It is particularly useful for identifying small organisms (e.g. insects) that are difficult to identify with morphology, and for specimens where diagnostic morphological features that are typically employed for species identification have been lost or removed (e.g. fish fillets). Once a DNA barcode sequence is generated from an unidentified specimen, it can be matched to a database of barcode sequences derived from expert identified reference specimens. This database, the Barcode of Life Data Systems[6] houses over 2.8 million barcode sequences and corresponding metadata from approximately 200,000 different species (as of January 2014). Data is entered into BOLD as a project where sequence information as well as metadata such as collection location is organized. BOLD projects can be contributed by anyone. The International Barcode of Life Initiative (iBOL.org) is a multinational campaign to add DNA barcodes from species of the highest socio-economic importance around the world to BOLD, creating a digital library for species identification. This library is publically accessible and supports a number of analytical tools used for validating the reference library.

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[2] Mark Y. Stoekle, and Paul D.N. Hebert. "Barcode of Life" *Scientific American*, 299 no. 4 (2008): 82-88.

[3] Paul D. N. Hebert, Alina Cywinska, Shelly L. Ball and Jeremy R. deWaard. "Biological identifications through DNA barcodes" *Proceedings of the Royal Society. London, B,* 270 (2003): 313–321

[4] CBOL Plant Working Group. "A DNA barcoding for land plants." *Proceedings of the National Academy of Sciences*106, no. 31(2009): 12794-12797.

[5] Conrad L. Schoch, Keith A., Sabine Huhndorf, Vincent Robert, John L. Spouge, C. Andre Levesque, Wen Chen and Fungal Barcode Consortium. "Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi." *Proceedings of the National Academy of Sciences*, 109 no. 16 (2012): 6241-6246.

[6] Sujeevan Ratnasingham and Paul D.N. Hebert. "BOLD: The barcode of life data system (www.barcodinglife.org)". *Molecular Ecology Notes*, 7 (2007): 355-364.

Figure 1. DNA barcode sequences can be represented visually with four colours representing the four different nucleotide bases from which DNA is composed. Different species have more differences in their DNA barcode sequences than individuals within the same species. These differences can be used to tell species apart. These species have a total of 138 differences in their DNA barcodes, 6 of which are shown in detail.

DNA Barcoding in the Classroom

As a classroom tool, DNA barcoding is particularly useful for illustrating the links between these laboratory methods for three reasons: (1) DNA barcoding has been used to investigate a number of socio-economic issues, such as food authenticity[7]

[7] Mark Y. Stoeckle. Catherine C. Gamble, Rohan Kirpekar, Grace Young, Selena Ahmed, and Damon P. Little."Commercial teas highlight plant DNA barcode identification successes and obstacles." *Scientific Reports.* 1no. 42 (2011): 1-7; Eugene H.K Wong and Robert H. Hanner. "DNA barcoding detects market substitution in North American seafood." *Food Research International*, 41 (2008): 828-837.

conservation[8] , epidemiology[9] and in general is useful to explore the linkages between science and society, (2) it involves a standardized workflow that includes sample collection, DNA extraction, PCR amplification, agarose gel electrophoresis, Sanger DNA sequencing, and bioinformatics (Figure 2) – all the basic tools of molecular biology – therefore it covers all the necessary curriculum points and also shows students how these exact laboratory methods are integrated and used by research scientists, and (3) a public reference library of DNA barcode sequences already exists to aid in sample identification which means that students can easily assign identifications to unknown specimens from DNA barcode sequences. Though the library is not complete, it has reached a stage where many species of socio-economic importance are represented, something that just five years ago would not have been possible. The existence of this database allows these kinds of projects to be undertaken.

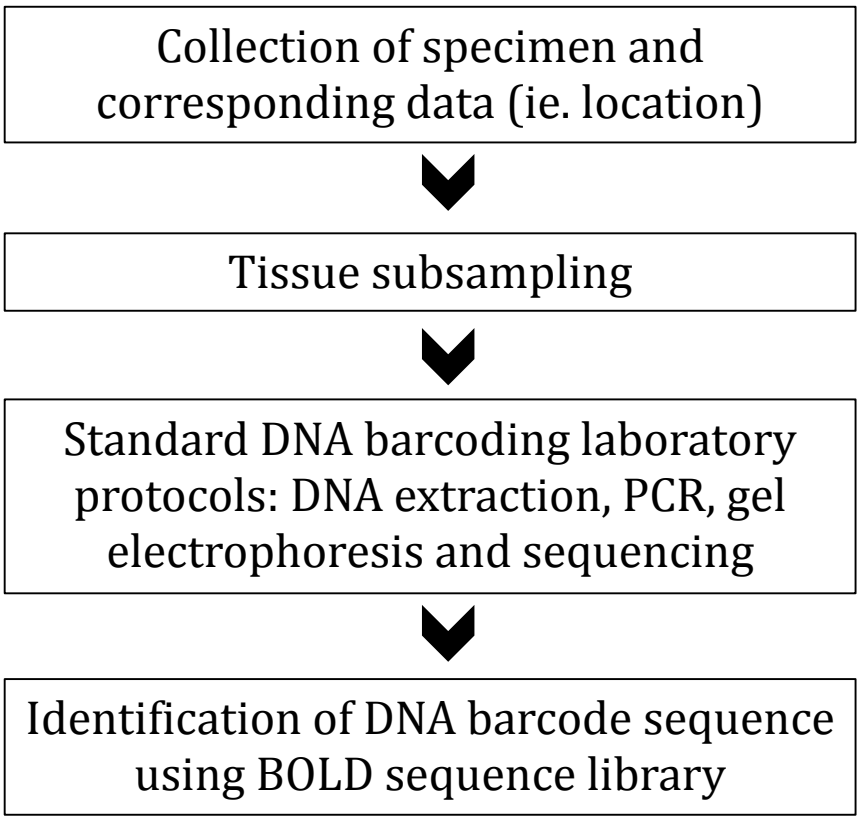


Figure 2. Standard protocols for obtaining an identity from an unknown specimen using DNA barcoding.

[8] Iffat Parveen, Hemant K. Singh, Saurabh Raghucanshi, Udai C. Pradhan and Shashi B. Babbar. "DNA barcoding of endangered Indian Paphiopedilum species." *Molecular Ecology Resources,* 12 no 1. (2012): 82-90.

[9] Golding, Nick, Miles A. Nunn, Jolyon M. Medlock, Bethan V. Purse, Alexander G.C. Vaux and Stefanie M. Schafer "West Nile virus vector *Culex modestus* established in southern England." *Parasites and Vectors,* 5 no. 32 (2012): 1-5.

Using DNA barcoding as a focus, molecular biology techniques can be taught in the context of broader projects looking at biodiversity, food fraud, epidemiology and other questions of socioeconomic importance. This allows students to see how the individual molecular techniques can be integrated to address other related topics in genetics and evolution. The benefits of DNA barcoding as an educational tool have been reviewed recently[10]. We have had success incorporating DNA barcoding into undergraduate ichthyology labs at the University of Guelph to accompany visual (e.g. morphological) identification of fish, and to conduct seafood market surveys at Wilfred Laurier as a means for students to practice their laboratory methods. However, many of the basic techniques used in molecular biology such as DNA extraction, gel electrophoresis and PCR are taught in high school and we sought to extend this learning opportunity to younger students who stand to gain even more from a hands-on experience.

Some opportunities already exist for high school students to get involved with DNA barcoding in the United States, including the Barcoding Life's Matrix program offered by Coastal Marine Biolabs (http://coastalmarinebiolabs.org/) and the Urban Barcode Project offered by Cold Spring Harbor Laboratory (http://www.urbanbarcodeproject.org/). In collaboration with Let's Talk Science, a Canadian national not for profit organization dedicated to science outreach and literacy, we developed resources for high school teachers and students in Canada. These resources were used in different ways in two case studies undertaken with high school students in Ontario. The first case study addresses the application of DNA barcoding to identify insects collected on school campuses as a way to gain appreciation for local biodiversity. This is the first example of student involvement in insect biodiversity surveys using DNA barcoding. The second case study focuses on the identification of seafood collected at local markets. This paper provides a short summary of these case studies and their results as well as a brief review of the resources generated. As these types of projects continue to be developed, they can only improve with increased community collaboration and discussion. By making these resources openly accessible online, we hope to encourage continued use and improvement of educational resources and projects related to DNA barcoding.

Malaise Trap Case Study

This case study was designed to work within the existing Let's Talk Science outreach program (http://www.letstalkscience.ca/), in which graduate student volunteers from universities across Canada visit classrooms for hands-on science demonstrations or lessons. Resources, including discussion forums, presentations,

[10] Linda Santschi, Robert H. Hanner, Sujeevan Ratnasingham, Michelle Riconscente, and Ralph Imondi. "Barcoding Life's Matrix: translating biodiversity genomics into high school settings to enhance life science education." *PLoS Biology*, 11, no. 1 (2013): e1001471. doi:10.1371/journal.pbio.1001471.

assignments and videos were also made available to students and teachers online at http://www.explorecuriocity.org/community/actionprojects/barcoding.aspx).

Malaise traps are tent-like insect traps, commonly used to conduct insect biodiversity assessments (Figure 3). We sent malaise traps to three participating high-school science teachers along with instructions for set up, including a step-by-step video of how to set up the trap properly. Teachers deployed the traps for five days in September on the campuses of their respective schools. We also deployed a trap for the same time period on a woodlot on the University of Guelph campus. All the insects collected were preserved in 95% ethanol, and shipped to the University of Guelph for analysis.

In the lab, insects from each site were sorted into groups based on morphology. We selected one individual from each group at each site for DNA barcoding. All results were deposited in a BOLD project (Let's Talk Science Malaise Traps; http://www.boldsystems.org/index.php/MAS_Management_OpenProject?code=LTSM). Over 200 samples were submitted for DNA barcoding. This represents the number of unique groups of specimens, and therefore the number of specimens submitted for DNA barcoding. The actual number of specimens collected is much higher as multiple individuals were caught from many of these groups.

Figure 3. The use of malaise traps supports a standardized approach to insect sampling that enables meaningful comparisons among and between sites when simultaneously deployed.

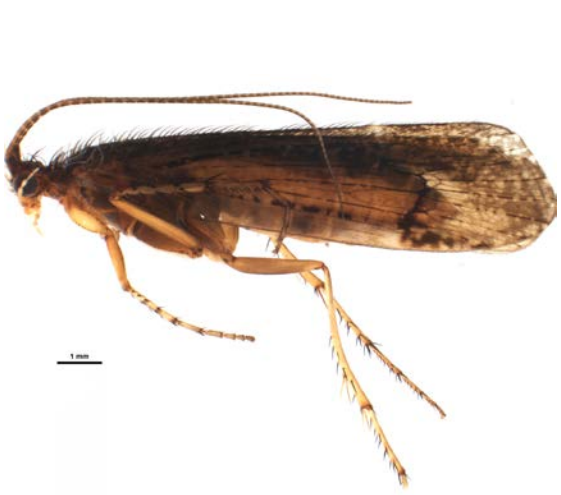
A Let's Talk Science volunteer from the University of Guelph visited each class twice, either in person or via videoconference in the case of remote sites. On the first visit, the volunteer gave an instructional presentation about DNA barcoding to introduce the topic to students. This presentation is available online, and explains why DNA barcoding is important to society, and how the process works including an overview of the laboratory methods and applications. The presentation also briefly reviewed

traditional classification of organisms using taxonomic keys, emphasizing the difficulty of morphological identification as well as some background on DNA barcoding before finally moving on to a brief introduction to BOLD.
During the second half of the first visit, students went online – either at a computer lab or via laptops in their classroom. The volunteer then provided students with an activity to illustrate how species identifications can be made using a DNA barcode sequence and BOLD. Students used BOLD to identify unknown DNA sequences from food products, one of the common applications of DNA barcoding. The activity concluded with a discussion of the socioeconomic impacts of any substitutions in the food products, including pork substituted for beef, tilapia substituted for red snapper, and cow's milk substituted for sheep's milk, among others. All examples were taken from publications or media coverage of actual DNA barcoding studies.

At a second visit a volunteer reviewed highlights from the insect DNA barcoding results. Of the almost 200 DNA barcodes generated, 84% could be identified using BOLD. This included examples of agricultural pests, and species used in forensic entomology and environmental monitoring. Students were surprised at the biodiversity on their campuses. Examples were discussed in more detail during this class including integration of online supplementary articles and videos (Figure 4). Topics of discussion included the complexity of insect taxonomy and classification, insect life cycles and feeding strategies and ecology.

The remaining 30 samples could not be identified using BOLD, meaning that these DNA barcode sequences were new to the database. This was startling as two of the four sites were in Guelph, Ontario a region where insect diversity has been extensively sampled as part of the iBOL project and where barcoding began. These results demonstrated a continuing need for biodiversity assessment and alerted students to the wealth of biodiversity in their own backyards. It also illustrated how citizen science can make meaningful contributions to an international research initiative like iBOL, and the potential for the use of DNA barcoding in monitoring urban biodiversity, which has recently gained increased support [11].

[11] Richard Blaustein. "Urban Biodiversity Gains New Converts." *BioScience,* 63 no. 2 (2013), 72-77.



Limnephilus submonili

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Lucilia illustris

The green bottle fly c forensic entomologists time of death du investigation.

Lygus lineolaris

The tarnished plant bu most serious agricultura fruit and vegetables in N

Figure 4. Examples of insects found in this study, and information shared with students.

Market Survey Case Study

The market survey case study utilized another Let's Talk Science resource – the CurioCity website (www.explorecuriocity.org). This website houses articles and other resources for science educators and students, including career information and discussion forums. To alleviate the constraints of relying on volunteers, we wanted to provide a tool kit for teachers to be able to implement DNA barcoding into their existing classroom curriculum themselves. For this case study, a series of learning modules were created to cover DNA barcoding-related topics. These were designed based on the Ontario Grade 12 "Molecular Genetics" curriculum, but would be useful, in part or as a whole, for other courses and in other provinces and territories as well. All learning modules and supplemental resources are available at:
http://www.explorecuriocity.org/Community/ActionProjects/MarketSurvey.aspx.

The eight learning modules were designed to introduce the DNA barcoding market survey, and also to illustrate common laboratory and informatics methods in molecular biology. They are available on the project web page along with other information for students and teachers including external resources and short articles about DNA barcoding. Lessons were intended to support the DNA barcoding market survey, as well as remain available and relevant if a full DNA barcoding project is not possible due to time or resource shortage. Lessons did not need to be carried out in any specific order. Educators were free to use the modules throughout the year where they felt they best fit with the curriculum.

The following is a brief overview of these learning modules: (1) As an introduction to this project, students read two articles about how DNA Barcoding has been used to detect mislabeled seafood. Students discuss the two articles and the issues raised. (2) Students view a PowerPoint presentation that describes the history, process and applications of DNA barcoding and then answer questions using a Think-Pair-Share strategy.(3) Students learn about the first laboratory step of DNA barcoding— DNA extraction — by extracting the DNA from a banana. An assignment to accompany the activity is provided. (4) Students create a paper-based model of the process of DNA sequencing using the method established by Frederick Sanger in 1977 to understand how DNA is sequenced for procedures such as DNA barcoding. (5) Students are introduced to the Barcode of Life Data System (BOLD), which is used to identify specimens. They will be able to enter DNA sequences from the assignment page into the BOLD database to determine if the samples were labeled correctly. Examples are taken from published DNA barcoding studies. (6) This module describes the sample and related information collection protocol for the DNA barcoding market survey. It reviews how students should collect samples of fish from local grocery stores or fish markets and how to prepare the samples for

shipment to the Canadian Centre for DNA Barcoding in Guelph, Ontario. (7) Students view a presentation summarizing the results of the market survey. They may then aggregate and discuss their class results and/or log into BOLD to view their class results directly. The results presentation and BOLD project will remain online for use by other classes. (8) Students learn about the seafood supply chain, how and why fish may be mislabeled, and ways to minimize mislabeling. Resources, and assignments related to this projects can continue to be accessed by students and teachers at any time through their personal accounts on the CurioCity website.

Once the learning modules were designed, teachers were invited by Let's Talk Science to sign up to take part in a DNA barcoding market survey and to use the DNA barcoding lesson plans to teach about DNA barcoding. Those participating in this case study attended a 2-day training session at the University of Guelph in August 2012 to gain familiarity with DNA barcoding methods and to review each of the learning modules in detail. The workshop provided an opportunity for teachers to gain an understanding of DNA barcoding pedagogy and the resources provided for them to apply it in their own institutions. In addition to presentations and lab tours by the authors, Let's Talk Science staff members demonstrated each activity and representatives from BioRad demonstrated the laboratory processes involved in DNA barcoding using equipment which they supply for this purpose.

Figure 5. Students from Donald A. Wilson Secondary School in Durham, Ontario collecting data from seafood market samples.

Sampling kits were mailed to teachers in September. Online instructions for class sampling plans, and tissue sub-sampling were provided online. All samples and corresponding data were shipped to the University of Guelph for analysis and upload to BOLD. In total 295 DNA barcodes were generated. Using the Canadian Food Inspection Agency's Fish List (http://www.inspection.gc.ca/food/fish-and-

seafood/product-inspection/fish-list/eng/1352923480852/1352923563904) to navigate between scientific names derived from barcoding and acceptable market names for those species, 67/295 (22%) samples were suspected of mislabeling. The full results and analysis have been submitted for publication in a scientific journal and revealed continued mislabeling of seafood in North America as previous studies had similarly found[12]. This is another example of citizen science contribution.

Once analysis was complete, results and a corresponding presentation were provided to teachers as a presentation available for download on the project website. Teachers were able to use this presentation, as well as the other DNA-barcoding-related learning modules, to enhance the learning experience for their students.

Comments from Participants

Comments from teachers and students were collected informally from the malaise trap project. Optional surveys were provided to teachers who participated in the market survey project and comments were collected from these surveys as well as informal discussion with teachers. Teachers and students from both of these case studies had positive feedback for us. Students commented on their increased interest levels and enhanced understanding of basic molecular biology when the subject matter was presented in the context of DNA barcoding. The use of online components helped stimulate interest for students and they enjoyed the applied nature of the project, commenting that *"Things that are happening on our own school grounds actually happen in the real world"*, and *"…it's not some phony little project – it's really real science"*. Teachers valued the fact that students were *"seeing how science can come to life and how molecular genetics can be applied to everyday life."* To our knowledge, all the teachers that participated continue to incorporate DNA barcoding into their teaching of molecular biology.

Conclusion

High school students played a role as citizen scientists in assessing the insect biodiversity of their campuses or in identifying seafood market samples using DNA barcoding. Notably, they revealed insect species new to BOLD and detected cases of seafood market substitution. Table 1 summarizes the number of participants, number of specimens collected and outcome of these two case studies. Teaching molecular biology through DNA barcoding can help to connect the subject with other fields, including ecology and biodiversity, evolution, ethics, the food supply chain, and more. As a central project it can set the stage in any biology classroom for discussion of other scientific concepts in the context of molecular biology and

[12] Eugene H.K Wong and Robert H. Hanner. "DNA barcoding detects market substitution in North American seafood." *Food Research International*, 41 (2008): 828-837.

provide opportunity for debate of socioeconomic issues and their relation to science. The DNA barcoding process gives students the opportunity to see the combination and application of molecular biology tools to these real-world problems and questions. As an added benefit, students can make a real contribution to the scientific community. In the case of the malaise trap case study, new sequences were added to the online database of DNA barcodes and in the market survey, students identified suspected food fraud. Both examples provided an authentic sense of discovery. This type of hands-on, experiential learning provides a sense of ownership to students, and service-centered-learning in science improves student engagement, and can even affect students' life-long goals[13]. In combination with the new eBOL initiative, a means for sharing resources and projects related to DNA barcoding, (www.educationandbarcoding.org) the instructions for and data from similar projects can be shared with students and teachers around the world.

Table 1. Summary of number of participants, number of samples submitted, and case study outcomes.

	Number of Students	**Number of Educators**	**Number of Samples**	**Outcome**
Malaise Trap Case Study	200	3	212	New DNA barcode sequences were added to BOLD, some representing species not yet catalogued in the database.
Market Survey Case Study	1000	19	332	Students uncovered cases of continued seafood market substitution using DNA barcoding.

These projects have both been submitted to the eBOL database of DNA barcoding projects. The BOLD Student Data Portal (http://www.boldsystems.org/index.php/SDP_Home) will allow even more hands-on opportunities for students participating in DNA barcoding projects, including sequence editing. Educational kits offered by companies like BioRad (http://www.bio-rad.com/en-ca/product/fish-dna-barcoding-kit) allow classes to conduct their own DNA extraction, PCR and gel electrophoresis, further increasing student involvement in the process. Continued efforts to create new opportunities for students to become engaged in molecular biology through DNA barcoding is warranted for the scientific benefits, as exemplified in the results from the two projects described here and also for the improvement of science education for students. The continued availability of these resources on online portals (CurioCity and eBOL) will support their re-use as educational tools. Continued contribution of resources to these databases will help to build an online educational community

[13] Steven Newmaster, Carole Ann Lacroix and Chris Roosenboom. "Authentic Learning as a Mechanism for Learner Centredness." The International Journal of Learning, 13 no. 6 (2006): 103-112

involving barcoding and its applications that will facilitate contemporary science and technology learning objectives.

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