DNA BARCODING AND PBL IN AN AUSTRALIAN POSTSECONDARY COLLEGE

Joseph Cross, Helen Garard, and Tina Currie

INTRODUCTION

Project-Based Learning (PBL) PBL is an approach to pedagogy which was developed in the 1960s by McMaster University Medical School, and has since spread to educational institutions around the world.

PBL design elements can be defined as:

- **Key Knowledge, Understanding, and Success Skills** — The project is focused on student learning goals, including standards-based content and skills such as critical thinking/problem-solving, communication, collaboration, and self-management.
- **Challenging Problem or Question** — The project is framed by a meaningful problem to solve or a question to answer, at the appropriate level of challenge.
- **Sustained Inquiry** — Students engage in a rigorous, extended process of asking questions, finding resources, and applying information.
- **Authenticity** — The project features real-world context, tasks and tools, quality standards, or impact — or speaks to students' personal concerns, interests, and issues in their lives.
- **Student Voice & Choice** — Students make some decisions about the project, including how they work and what they create.
- **Reflection** — Students and teachers reflect on learning, the effectiveness of their inquiry and project activities, the quality of student work, obstacles and how to overcome them.
- **Critique & Revision** — Students give, receive, and use feedback to improve their process and products.
- **Public Product** — Students make their project work public by explaining, displaying and/or presenting it to people beyond the classroom.

("What is Project Based Learning (PBL)?", 2017. Retrieved from https://www.bie.org/about/what_pbl).

Some of the goals of PBL, in addition to factual learning, are: "to take learning one step further by enabling students to transfer their learning to new kinds of situations and problems and to use knowledge more proficiently in performance situations" (Barron & Darling-Hammond, 2008, p.1–3).

In Australia, PBL is used in many schools and universities, covering almost all disciplines (Briggs, 2015).

DNA BARCODING

"Just as the unique pattern of bars in a universal product code (UPC) identifies each consumer product, a short "DNA barcode" (about 600 nucleotides in length) is a unique pattern of DNA sequence that can potentially identify any living thing." ("DNA Barcoding at the DNA Learning Center", 2017).

DNA barcoding is increasingly being introduced into biological science educational curricula worldwide. The technique has a number of features that make it ideal for science curricula and particularly for PBL. These include: the ability to identify specimens from a wide variety of taxa; a relatively simple set of techniques, which science students can master following a basic training period; the ability to generate real scientific data; and a well-organised series of bioinformatics platforms, which allow data analysis, storage and sharing.

In the USA, two major education and barcoding projects exist. These are the Urban Barcode Project (UBP) (http://www.urbanbarcodeproject.org/), and those conducted by Coastal Marine Biolabs' Barcoding Life's Matrix Project (http://www.studentdnabarcoding.org).

These two projects have been highly successful and have slightly different emphases. The UBP takes the form of a mentored competition involving high school student groups from the New York City area. Students are judged at a final seminar and winners receive prizes. The Barcoding Life's Matrix Project involves student groups working alongside scientists to create a reference barcode library of commercially and ecologically important marine taxa.

Henter et al. (2016) have recently published an important paper summarising and comparing five different DNA barcoding and education projects in the USA. They found that:

> *These projects are successful because the scientific content is authentic and compelling, DNA barcoding is conceptually and technically straightforward, the workflow is adaptable to a variety of situations, and online tools exist that allow participants to contribute high-quality data to the international research effort. (p. 1).*

Although barcoding and its integration with PBL has been in existence and utilised in US institutions for a number of years, its use in Australian postsecondary education is in its infancy.

This report outlines the development of a DNA barcoding project in an Australian TAFE college, which also combined a PBL approach. Students enrolled in the Diploma of Laboratory Technology at Holmesglen have used DNA barcoding to identify and record species in the Rubicon State Forest area in rural Victoria. This was part of a PBL-based course, which incorporated cross-discipline teaching and curriculum.

The project was evaluated via observational methods and via an online survey of course satisfaction and student self-reporting. Some of the pedagogical benefits noted were:

- a major boost to student engagement and motivation;
- learning to do real science;
- resumé building;
- using a range of skills (molecular biology, microbiology, mathematics); and
- independence and problem-solving.

METHODS AND MATERIALS

The students completed this project to meet the learning objectives for the elective units Perform Molecular Biology Tests and Procedures, and Apply Electrophoretic Techniques, part of the two-year Diploma of Laboratory Technology at Holmesglen in Moorabbin, Victoria. This course typically leads to employment as laboratory technicians or research assistants, or students can receive credits for higher degrees.

Students' ages ranged from 19–45 years. In total, there were 11 students, organised into two groups of 4 and 1 group of 3. The project was carried out over 8 weeks at the Rubicon State Forest in rural Victoria. Each student received a printed project manual. This manual contained the basic molecular biology protocols for each section of the project (microbiology, and mathematics aspects of the project were conducted using separate instructions in class).

In addition to the basic laboratory techniques outlined in the manual, students incorporated the internet and used cell phones for various aspects, including: learning basic facts; mapping sample collection areas using Google Earth; and recording field data using the DNA Barcoding Assistant (Version 1.2) [Apple iPhone app]. Available from http://www.dnabarcodingassistant.org/.

Students were assessed by presentation at a departmental seminar and a printed report in scientific report format.

Faculty met initially a number of times to coordinate the integration of the individual components of the course, as per our PBL format. Each faculty had individual expertise in microbiology, mathematics, or molecular biology.

Week 1–2, Brainstorming: Students decided on a question they would like to answer and drew up a plan for how to do this. Examples of typical investigational questions student groups developed were, "What species can be found in a rainforest habitat area in the Rubicon state forest?" and "How do we calculate the requirements for a representative sample from a given area?".

During this time, students also developed a hypothesis. Hypotheses ideally flowed logically from the investigational question. For example: "There are a variety of species typically found in temperate rainforest areas which grow in the Rubicon State Forest".

Week 3–4: Students collected samples and conducted laboratory bench work. Briefly: DNA was extracted as described (Kress & Erickson, 2012); PCR of samples was conducted using primers specific for a specific section of either the rbcl gene (Ribulose-1,5-bisphosphate carboxylase oxygenase) for plants, or the COI gene (cytochrome c oxidase subunit I) for fish (Kress & Erickson, 2012). PCR conditions were as described with minor variations (Kress & Erickson, 2012). Successful PCR samples (those showing the presence of bands of expected sizes following agarose gel analysis) were sent for bi-directional sequencing at the Micromon facility, Monash University, Clayton Campus Victoria. It is expected during this process there will be some loss of samples due to failure to extract genomic DNA or PCR failure. For this project, approximately 50% of the original samples collected were lost during either the DNA extraction or PCR stages.

Week 5: Bioinformatics analysis and species identification. Students received sample DNA sequence data electronically. Forward and reverse trace sequences were then input into either the DNA subway (part of the UBP) or the Student Data Portal (SDP) (http://v3.boldsystems.org/index.php/SDP_Home), part of the Barcode of Life Data System (BOLD) (www.barcodinglife.org). It was found that each site offered slightly different advantages dependent on the species being analysed.

Week 6: Students uploaded plant species data into the Atlas of Living Australia (https://www.ala.org.au/), which has links with the international BOLD site. The fish sample data was uploaded directly to BOLD through the SDP due to a technical difficulty with the Atlas that prevented its use for fish samples.

Week 7: Seminar presentation production. Students wrote reports in correct scientific format and produced and practiced verbal seminar presentations.

Week 8: Seminar presentations. The seminar was advertised throughout Moorabbin campus and to other colleges via an email contact list. The seminar was well attended by students from the course, by students from other courses, and by educators and scientists from other institutions.

Student project results and presentations were also published online. "Holmesglen DNA Barcoding", (2014).

RESULTS

The results of this project demonstrated a number of pedagogical benefits compared with more traditional biological science courses involving didactic lectures and laboratory-based exercises.

These included:

- A major boost to student engagement and motivation

  All faculty reported an increase in student engagement. The most commonly reported in this regard was student enthusiasm in general. This was observed by the authors in student group conversations and in a willingness to put in extra effort (For example, meeting as a group outside of class hours to discuss and plan projects). Comments made by other teachers and managers, which were made directly to the authors, also supported this, and included: "I've never seen our students so motivated before", and "I heard students discussing the project outside of class time". Student comments in support of this included: "It was an awesome experience which laid down great foundations for my future". We also monitored attendance for the period of this project, which was almost 100%, a significant increase on the course prior to introducing DNA barcoding.

- Learning to do real science

  Students were aware that they were conducting research that was not just an "exercise", and that their results, if successful, would be uploaded to databases containing worldwide scientific data, and that this had potential for assisting in managing ecosystems and dealing with the effects of climate change, among other benefits. Comments made by students supported this, including: "I really like this project because I am interested in conservation, and I think our work is helping with that".

- Resumé building

  Students have their names listed as contributors in the database records for their samples. This was a real achievement that could be added to their resumés. The final seminar was attended not just by our department faculty, but by scientists, educators and students from other institutes. They could therefore also add this seminar presentation to their resumés. Student comments in support of this included: "The barcoding project provided students with great practical experience, which gives students confidence to get a job in the workplace", and "I feel the skills I learnt during this project really added something to my resumé".

- Using a range of skills

  These projects developed skills in molecular biology techniques (e.g., PCR), mathematics (e.g., statistical significance measures), bioinformatics (e.g., database searching), presentation of scientific results, collaboration and team work, research skills (e.g., searching for peer-reviewed sources), protocol troubleshooting, and public speaking. This was apparent from the protocols associated with the project, from the final student reports, and from teacher comments such as: "It was good to see students being able to put it all together to achieve an outcome."

- Independence and problem-solving

  Perhaps related to the increased engagement noted above, students showed a willingness to spend effort to independently overcome obstacles rather than passively relying on teachers. Despite a number of fairly challenging technical problems, students overcame all issues, and positive results were obtained by all student groups. These challenges included, for example, troubleshooting PCR protocols, and using Google Earth to divide a sampling region into one metre square segments. Progress in this area was seen as an essential part of the learning outcomes for this course, as troubleshooting protocols is a central aspect of a laboratory technician's and scientific researcher's skill set. All teachers involved in the project observed evidence of this during class. A comment by one teacher, "Your students don't just wait for the teacher to solve the problem", supports this.

- General quality of learning outcomes

  Overall, the final learning products, a seminar presentation of student group results and a written scientific report, were of a high quality. This was supported both by teacher grading using a rubric and also by attendees at the seminar. Academics from other institutions who attended the seminar commented to the authors that they were, "Impressed by the quality of the presentations", and that "We would like to emulate what you have done at our school".

A video summarising the project was also produced (Holmesglen TAFE, 2014). Further student and teacher comments in support of these results can be found in the video.

DISCUSSION

Students at Holmesglen have used DNA barcoding as part of a PBL-based educational program. Students surveyed various ecosystems in rural Victoria, Australia; collected samples; and extracted genomic DNA. Samples were identified via DNA barcoding and the results were uploaded to databases at the Atlas of Living Australia and BOLD sites. Finally, students presented a publicly advertised seminar on their findings. This was the first use of DNA barcoding in any postsecondary educational context in Australia that we are aware of.

Our results demonstrate a number of important points. Firstly, the cross-disciplinary nature of PBL proved very well suited to this project, allowing adequate coverage of curriculum-mandated learning objectives across a number of disciplines and specialised skills. Secondly, it permitted multiple facets of a single focus, the ecosystem of the Rubicon State Forest, to be investigated simultaneously. This finding was consistent with those of previous studies (Briggs, 2015). DNA barcoding was also found to be uniquely adaptable to educational contexts. The technique was technically well within the capabilities of students with relatively limited molecular biology knowledge. This was again consistent with previous studies (Henter et al., 2016).

From a pedagogical point of view, the results demonstrate that PBL, combined with DNA barcoding, proved to be an effective strategy for increasing student engagement in the learning process, concomitant with an increase in student motivation and independence. Teacher comments made to the authors, such as, "I've never seen our students so motivated before" bear this out. Student comments included: "It was an awesome experience, which laid down great foundations for my future."

We believe this is related to two important factors. The first of these was student awareness that they were involved in a genuine process of scientific enquiry ("authenticity"), and that their results were in fact new discoveries, as shown by student comments such as: "I really like this project because I am interested in conservation, and I think our work is helping with that". The second important factor was student group ownership of their projects, through being given the opportunity to devise their own topic ("student voice & choice"). Although there also were some problems related to this, noted below, overall we believe this positively impacted student engagement in the project.

Lastly, we believe the combination of PBL and DNA barcoding as used in this study provided for excellent learning outcomes in terms of skills development. For instance, all student groups obtained valid scientific data, which was confirmed by acceptance for inclusion at the BOLD site. Also, the final seminar presentations were of high standard, as judged by attendees and grading. A register of student destinations maintained in the Holmesglen Biosciences Department showed that including the project on student resumés added value in job interviews.

Limitations and Challenges

Student Voice & Choice

One of the more difficult pedagogical tasks noted by all faculty was allowing student ownership of their projects (through allowing topic choice for example) and a degree of autonomy in how they chose to work, but still giving enough guidance to achieve meaningful scientific results and meet the learning objectives for the course. This was not just a matter of providing the basic skills, for example, training in the polymerase chain reaction (PCR). Faculty noted they needed to provide a basic framework, and then allow a degree of uncertainty in student activity. In general, getting the balance right for this was seen as a matter of experience and judgment, but it was found that faculty with more years of teaching experience were better able to do this than those who were relatively newer to teaching.

Group Dynamics

The faculty's previous experience with this student cohort made it possible to ensure a mix of abilities and skills in each group. Generally, group cohesion and cooperation was observed to be at a high level. Some groups, for example, would meet outside class to brainstorm and work out other details of their project. It was also thought to be important to ensure a mix of competencies and skills in assigning group members. Again this was possible through faculty already having some familiarity with the students.

Depth of Knowledge

One of the criticisms of PBL in general, is that it comes at the expense of depth of learning (Guido, 2014). This view suggests students may miss the broad detailed factual information that may be obtained from traditional methods such as didactic lectures.

One approach to assess whether this criticism is valid for our project would be to set up two student groups, one which experienced a more traditional curriculum, as a control, and one which did the barcoding course. A detailed assessment of knowledge and skills using examinations and questionnaires could then be conducted and results compared.

The logistics of doing this at Holmesglen at this time makes such a study design impossible. For example, there are not sufficient faculty or laboratory space for two student groups. However, we also note that some of the skills imparted via a PBL approach, such as the so-called 21st century employment skills of cooperation and innovation, are very difficult to assess as a series of discrete skills or pieces of knowledge.

Also, as the nature of our course was particularly slanted towards bench laboratory skills, we feel the approach outlined here is particularly suitable. We note that graduates from our course are preferred hires for a number of major institutes, suggesting perhaps that our students are high performers on the real-work tasks and problem-solving required in employment.

Future directions

Feedback from some students (e.g., "We feel that not everybody in our group put in the same effort.", and "Our group got a high grade, but some of our group don't deserve it."), indicated that they would prefer to also be assessed on an individual basis, in addition to the group assessment. This was also seen to be important as the project counts for a large proportion of the final grade for the course, and students who perhaps did not put in the same effort as other group members may still have received an equally high grade for the course.

In future, individual group participation will be assessed by development of an individual assessment rubric (in addition to the group assessment), and by allowing such things as recording of individual contributions to the final product report, and by student self-assessment of other group members.

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Joseph Cross *has a PhD in genetics and has experience in prostate cancer research and marsupial genomics. He is currently a visiting assistant professor at Texas A and M University, USA.*

Helen Garard *teaches science-related and communications subjects from the Diploma of Laboratory Technology and Certificate IV in Laboratory Techniques, at Holmesglen Institute (Moorabbin campus)*

Tina Currie *is a teacher of laboratory programs at Holmesglen Institute (Moorabbin campus). She specialises in teaching mathematics units that encompass processing, interpreting and statistically analysing scientific data.*