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Biodiversity Research in Undergraduate Lab Courses

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Extended Abstract

Introduction

As part of our effort to bring authentic research into large undergraduate Biology labs at UCSD, we have initiated a DNA barcoding project that seeks to document biodiversity in San Diego and to use this data to answer a variety of research questions. DNA barcoding uses a specific region within the cytochrome c oxidase (CO1) gene on mitochondrial DNA as a genetic marker. This region of the CO1 gene has been designated as the standard barcode sequence for animals by the Consortium for the Barcode of Life (CBOL) and can be used to verify the identity of known species and to identify potentially new species. The Consortium for the Barcode of Life (CBOL) is an international collaboration whose mission is to compile DNA barcodes of all taxa and to establish a public library of vouchered sequences.

At UCSD, students in Ecology lab classes collect specimens from the UCSD Natural Reserve, protected lands that are set aside for conservation, education, and research. Our goal is to create an inventory of species, particularly the poorly known invertebrate fauna. Students from Recombinant DNA Techniques labs purify DNA from the specimens and then use PCR to generate a partial sequence of the CO1 gene. This year we generated data for two different organisms, honeybees (*Apis mellifera*) and marine bristle worms (*Polychaete* species). Evaluation and comparison of the DNA sequences from different samples allows students to address research questions related to species diversity in the San Diego region. The power of this general approach is that methodology needed to generate barcodes is relatively inexpensive and straightforward, any organism can be barcoded, and these methods could be adapted to any lab course that has reagents for DNA purification, a PCR machine, access to a DNA sequencing facility, and the Internet.

Methods

Ecology students collect specimens in the field, document the collection location, date of collection, and tentatively identify the genus and species if possible. They also preserve the samples for DNA isolation. These samples are passed to the Recombinant DNA lab students who purify DNA using a Qiagen DNeasy kit. Using validated primer sets from the literature or from BOLD, the students set up a CO1 gene PCR and run an agarose gel to verify that the PCR was successful. If successful, the PCR products are cleaned up and sent to Eton Bioscience for DNA sequencing. BLAST and ClustalW bioinformatics programs are then used to evaluate the DNA sequences and to determine the species id or closest match.

Results

Does more than one species exist within the putative T. mucronata population of polychaetes at the Scripps Coastal Reserve?

Polychaetes, or bristle worms, are a common and diverse class of primarily marine worms with over 10,000 species described so far. *Thoracophelia mucronata* is a polychaete species found in abundance on San Diego beaches, particularly the beach adjacent to campus. Within this worm population, significant morphological differences have been observed and *T. mucronata* can be found in different locations within the intertidal zone. These differences call into question whether more than one species exists within the putative *T. mucronata* population. To address this question, DNA was isolated from 110 specimens from the Scripps Coastal Reserve and students generated 100 high quality CO1 sequences. ClustalW alignment of over 40 CO1 sequences of *T. muraconata* were compared and students determined that all specimens, regardless of size or intertidal location, were members of the same species based on the very low frequency of CO1 polymorphisms (see alignment of 8 typical sequences

on poster). Students also discovered that the CO1 sequence for *T. mucronata* is not in the Genbank or BOLD databases; their novel sequence data will be entered into a new biodiversity database at UCSD.

Do Africanized bees carry a marker for Africanization in their CO1 gene?

Until recently, subspecies of the honeybee *Apis mellifera* have been separated geographically but human-assisted introductions have caused the mixing of large populations of African and European subspecies in South and Central America. There is evidence that the subspecies are cross breeding. Bees in the African subspecies are more aggressive, and reports have shown that the Africanized bees have moved into San Diego. There is a known assay that distinguishes Africanized bees from non-Africanized using the cytochrome B gene but no one has ever identified a marker for Africanization in the CO1 gene. To identify a possible marker, DNA was isolated from legs of 60 local bees and using a previously published assay, it was determined that 69% of local bees from the samples tested are Africanized. Students generated 50 high quality CO1 sequences and were able to identify a SNP (single nucleotide polymorphism) within the CO1 barcode sequence that is consistently correlated with Africanization (see ClustalW alignment on poster). While this data suggests that the CO1 gene does carry a marker for Africanization, further studies will determine if this SNP is evolutionarily meaningful.

Conclusions

According to our Learning Goals, after this class students should be able to do the following: explain how species are identified and differentiated from one another using molecular biology techniques and bioinformatics; explain why and how the cytochrome C oxidase gene sequence is used to identify animals in barcoding studies; give examples of polymorphisms that can occur in DNA sequences; isolate DNA and set up a PCR reaction, including designing primers; explain the theoretical basis of Sanger sequencing and assess the quality of a sequencing run; use a variety of bioinformatics databases and tools to analyze and compare DNA and amino acid sequences, including Blast and ClustalW; communicate their research results in a written report. While we have informal data showing that our learning goals were achieved, we are introducing a formal learning assessment module to the course this year.

Our overall goal is to involve students enrolled in large undergraduate laboratory courses at UCSD in authentic research. The methodology needed to generate barcodes is relatively inexpensive and straightforward, and requires only basic molecular lab equipment. As barcoding can be used to address many different types of research questions and to generate novel, useful, and potentially publishable scientific data, it is an ideal project for undergraduate laboratory courses.

Keywords: biodiversity research, student projects

Link to Original Poster: http://www.ableweb.org/volumes/vol-34/poster?art=59

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