Genetic Sequencing for Measuring Biodiversity in Recent and Ancient Marine Sediments

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Genetic sequencing for measuring biodiversity in recent and ancient marine sediments

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Background

Taxonomic biodiversity, measured by counting the number of species present in a given area, is the most common method of capturing ecosystem biodiversity in recent and ancient environments. While this method is widely accepted, it is limited by poor preservation and identification of many individuals, making it impossible to include every species within an ecosystem and resulting in the loss of some diversity information.

Research Objectives

To address the above issue, we attempted to measure the genetic biodiversity (in which species are determined based on sequencing of their DNA) of shallow marine ecosystems by extracting and sequencing the 18S ribosomal gene from bulk carbonate sediment samples taken from several locations around the Caribbean island of Grand Cayman. By comparing genetic and taxonomic biodiversity, we hope to provide a more reliable, or at least supplementary, measure of molluscan biodiversity in this ecosystem. Our first objective was to develop a protocol for extraction and PCR to determine the best region of the genome to target for molluscan biodiversity.

Study Area and Sample Collection

The island of Grand Cayman, located in the Caribbean Sea, just south of Cuba. Samples were taken from two onshore locations (SR-F and HQ-F) from the Ironshore Formation (Pleistocene), and three offshore locations from recent carbonate sediments (RP-S, SS-S, and EB-S).

Figure 1. The island of Grand Cayman, located in the Caribbean Sea, just south of Cuba. Samples were taken from two onshore locations (SR-F and HQ-F) from the Ironshore Formation (Pleistocene), and three offshore locations from recent carbonate sediments (RP-S, SS-S, and EB-S).

Results

DNA Quality

Figure 8. DNA quality of samples after extraction. 260nm is the wavelength at which DNA absorbs UV light. 260/230 ratio shows the amount of salt in the sample, <1.5 preferred. 260/280 ratio shows DNA purity, 1.7-2.0 preferred.

Figure 9. Concentration of DNA was read on a spectrophotometer using 1ul of sample. Most PCR reactions require around 100ng of DNA total, so a concentration greater than 20ng/ul is preferred.

DNA Concentration

Figure 10. Grain size analysis showing the differences in grain size distribution across sampling sites. South Sound and Rum Point had more fine grain sediment than East Bay.

Figure 11. The weight percent of samples left after heating them to 1,000°C. The organics were burned off, while the calcium carbonate sediment was left behind.

Lab Methods

Extraction

- Ground samples with pestle and mortar
- Created extraction buffer with EDTA, SDS, and Proteinase K
- Combined 0.5g of sample with 1 mL of buffer
- Placed in shaking incubator for a two day cycle
- Used QiAquick PCR Purification Kit to clean and elute DNA
- Samples were run on a 1% agarose gel to determine if PCR concentration and quality (SS-4 and RP-2)

Amplification

- Polymerase Chain Reaction (PCR) amplifies the amount of DNA in a sample
- We targeted region V9 to test its accuracy in determining biodiversity
- We tested two different mastermixes with these primers: Ampliqon Gold and Phusion High Fidelity
- Mastermixes were tested on the samples with the highest concentration and quality (SS-4 and RP-2)
- Samples were then run on a 1% agarose gel to determine if PCR had amplified the quantity of DNA

Discussion

DNA concentration and quality after extraction seems very promising for most samples. The two samples with the lowest concentration were from East Bay, which was rockier and harder to sample from in the field. Because of this, the grain size analysis was performed to see if there was any correlation between grain size distribution and DNA concentration. EB-2 and EB-4 had the lowest concentration of DNA, and also seemed to have higher percentages of coarse grains. RP-4 and SS-4 had high concentration levels, and higher percentages of fine grains. We decided to perform an LOI experiment to see if the concentration data we were getting matched the amount of organics in the samples, in order to rule out contamination during extraction. However, there does not seem to be much of a relationship between concentration and the LOI results. We hope to test different target regions in the future to determine the best protocol to determine biodiversity.

Conclusions

- Extracting high concentrations of DNA from bulk sediment is feasible.
- PCR protocol for bulk sediment extractions needs to be optimized.
- PCR failed at amplifying target region V9 in our samples, as shown in Figure 12.
- Future work should target V4 or V7 instead of V9.

References


Acknowledgments

I would like to thank Dr. K. Arkle and Professor J. Arkle for the opportunity to do research in Grand Cayman and for all the help since. Thank you to Dr. Murphy for giving up countless hours to be with me in the lab. I would like to thank Dr. Wolf, Dr. Strasser, Dr. Hammer, Susan, Jenn, and the entire Augustana Geology Department for constantly supporting me and inspiring me to be curious and confused at a higher level. Thank you to the Augustana Student Research Grant and Augustana Department of Geology for funding this research.