[A blue background with white text

Description automatically generated](https://nam12.safelinks.protection.outlook.com/?url=http%3A%2F%2Fresearch.tamucc.edu%2F&data=04%7C01%7Clarisa.ford%40tamucc.edu%7Cf1e4fed7f64e4b073b0908d8ea236fd6%7C34cbfaf167a64781a9ca514eb2550b66%7C0%7C0%7C637516785207162120%7CUnknown%7CTWFpbGZsb3d8eyJWIjoiMC4wLjAwMDAiLCJQIjoiV2luMzIiLCJBTiI6Ik1haWwiLCJXVCI6Mn0%3D%7C1000&sdata=Urv65o%2B14XRj149aIToqR%2FT0ondlyaVPzS%2BZl8Uf%2BoE%3D&reserved=0)

# Student Research Competition Cover Page

Please submit this form, along with your internal funding proposal as a **single PDF document** to **researchdevelopment@tamucc.edu by September 30, 2023**. If you do not receive an email confirmation of your submission within 3 business days, please reach out to the Student Research Competition Point of Contact (POC). The POC for the Student Research Competition is **Dr. Garth Clayton (garth.clayton@tamucc.edu)**.

# Date of Submission: 9/29/2023

|  |  |  |
| --- | --- | --- |
| **Project Title: DNA Profiling of 40-Year-Old South Pacific Coral Skeletons** | | |
| **Student Name** | **Department** | **Signature** |
| Joseph Garza | Life Sciences | *Joseph Garza* |
|  |  |  |
|  |  |  |
|  |  |  |
|  |  |  |

|  |  |
| --- | --- |
| **Faculty Mentor Name** | **Faculty Mentor Signature** |
| Chris Bird, Ph.D. | A close-up of a signature  Description automatically generated |

Does this project involve human subjects, animals, and/or biological agents/recombinant DNA?

Yes No

DNA Profiling of 40-Year-Old South Pacific Coral Skeletons

Joseph Garza

Department of Life Sciences, Texas A&M University – Corpus Christi

**Abstract**

Coral reefs, which are built from accumulated layers of calcium carbonate (CaCO3), are found in tropical and subtropical warm shallow sea water environments around the world. These reef ecosystems support an estimated 25 percent of all known marine species, and the variety of aquatic life living around coral reefs is greater than anywhere else on the planet. However, coral reefs are projected to be entirely under threat within the next 20 years and the need for standardized protocols to assess biodiversity at the gene level are essential. Corals, in particular, have highly variable phenotypes and DNA is necessary to determine taxonomic identity. This project explores the feasibility of extracting and sequencing DNA from 87 processed coral skeletons that were collected from the South Pacific Ocean before 1989. The bulk of the samples have not been identified down to the genus level effectively providing an opportunity to quantify genetic diversity and to optimize DNA extraction from coral skeletons. I plan on taking advantage of the experience in Dr. Bird’s Lab and the Genomics Core-Lab by processing sensitive low biomass DNA specimens and employing these techniques to quantify DNA embedded in coral skeletons. Sequence data from the recovered DNA will be analyzed to gain a better understanding of the relationship between coral skeletons and genotype, which will address one of NSF’s 10 Big Ideas: Understanding the Rules of Life. If we are successful, this will open up opportunities to sequence coral skeletons maintained in museum collections world-wide.

**Project Description**

Statement of Research

This project presents an exciting opportunity to advance our understanding of corals and genetic diversity. By successfully extracting DNA from both known and unknown coral samples, I aim to contribute valuable data to the field of coral research. Historically, extracting DNA has been of great difficulty because of the low yield, constrained genetic diversity, and community of microorganisms living in close association with coral (Thompson et al., 2015). However, I hypothesize that the established methods described in (Cartier et al., 2018) will yield sufficient amounts of DNA for downstream sequencing of our coral specimens. These 87 coral skeletons are significant because they were collected from islands spanning the South Pacific ranging from the Marshal Islands to Tutuila Island, American Samoa. The majority of the samples were collected from Kwajalein Atoll (Fig. 1), which serves as a unique and pristine environment in whereby research can be conducted on a habitat that has been minimally touched. This project seeks to test and develop laboratory techniques to isolate and sequence DNA from previously processed coral skeletons taken from the South Pacific.

A map of a body of water with green points

Description automatically generated An aerial view of a small island

Description automatically generated

Figure 1 | Kwajalein Atoll, Republic of the Marshal Islands.

Upon initial collection the corals were chemically bleached to better preserve and enhance skeletal features with the ultimate goal of taking these specimens on exhibition. Our approach to investigating these processed corals will build upon the DNA analysis method described by (Cartier et al., 2018), to distinguish species, assuming that coral DNA molecules have be trapped in the organic material or adhered to the (CaCO3) crystals during the formation of the skeleton (Lendvay et al., 2019). The phases of the project will occur in the order described as follows.

**DNA Extraction and Purification from Known Coral Samples:** The initial phase of the project involves working with known coral samples to refine and optimize DNA extraction and purification techniques. These samples will serve as a benchmark for my methods and will allow for the validation and effectiveness of the chosen protocol.

**DNA Extraction and Purification from Unknown Samples:** Following successful DNA extraction from known coral samples, I will proceed to extract DNA from the 87 coral specimens collected prior to 1989. These specimens have not been identified to the species level, making this a critical aspect of the project.

**PCR Amplification & Quantification:** Amplification of purified DNA with two sets of primers for a large ribosomal RNA gene subunit (LR gene) and the putative mismatch repair protein (MSH gene) will be conducted. Quantification of DNA yield will be analyzed using qPCR techniques.

**Bioinformatic Analysis:** Upon successful DNA extraction, purification, and quantification of unknown coral samples; cross referencing of sequencing/barcoding data will be executed. The goal here is to identify the species and gather genetic information from these previously unidentified corals.

**Optimization of Coral DNA Extraction Methods:** The final stage of the project will encompass optimizing experimental design and protocols used for the extraction of coral DNA from coral skeletons. Optimization of materials and reagents, instrumentation, standard operating procedures, variables and controls, data collection, and methods of statistical analysis will be evaluated.

Control III

Control II

Control I

Treated

Known Coral Skeleton

Known Coral Skeleton

Known Coral Tissue

Treated

Unknown Coral Skeleton

Figure 2 | Experimental Design

The illustrated workflow (Fig. 2) shows the different controls that will be used to validate the proposed extraction method used in this experiment. The initial phase of our approach will be to extract, amplify, and sequence known controls to determine the optimum set of procedures to be used for the treated unknown coral skeletons. After successful processing and sequencing of known coral skeletons and tissues, the same set of procedures will be used on the processed corals taken from the South Pacific.

Intellectual Merit or Significance

This significant of this project encompasses a wide range of aspects all that which gesture at the conservation and restoration of coral reefs. By building upon the limited collective knowledge of coral DNA extraction, my aim is to accelerate the rate at which genomic research of coral can take place (Solomon et al., 2019). In addition, this research will establish methods which seek to recover DNA from other organisms which excrete calcium carbonate including but not limited to oysters, clams, snails and even algae. By effectively establishing DNA extraction protocols for coral, this study inherently seeks to explore new methods for quantifying genetic diversity in various natural populations (Moll, 2008).

Broader or Societal Impacts

The broader and societal impacts regarding coral research gesture not just at the ecological aspect of saving coral reefs. Coral reefs in addition to being harbors of genetic diversity also play a direct role in socioeconomical phenomena. These ecosystems serve as habitats for numerous commercially valuable fish and shellfish species and are major attractors of tourism contributing significantly to coastal economies (Wilkinson, 1996). Lastly, the unfortunate situation that coral have been placed in is providing an ongoing case study as to measure the effects certain scientific approaches have at ecological restorative efforts. Coral reefs are important to maintaining the health of our coastal ecosystems but also play an important role in the collective effort to synthesize a sustainable approach to live in the world we all find ourselves in.

**BIBLIOGRAPHY**

Cartier, L. E., Krzemnicki, M. S., Lendvay, B., & Meyer, J. B. (2018). DNA fingerprinting of pearls, corals and Ivory: A brief review of applications in Gemmology. *The Journal of Gemmology*, *36*(2), 152–160.

Lendvay, B., Cartier, L. E., Gysi, M., Meyer, J. B., Krzemnicki, M. S., Kratzer, A., & Morf, N. V. (2019). *DNA Fingerprinting: An Effective Tool for Taxonomic Identification of Processed Precious Corals*.

Moll, R. (2008). Faculty opinions recommendation of coral reefs under rapid climate change and ocean acidification. *Faculty Opinions – Post-Publication Peer Review of the Biomedical Literature*.

Solomon, E., Martin, D., & Berg, L. (2019). Ecology and the Geography of Life. In C. Martin (Ed.), Biology (10th ed., pp. 1224–1225). essay, CENGAGE Learning.

Thompson, J. R., Rivera, H. E., Closek, C. J., & Medina, M. (2015). Microbes in the coral holobiont: Partners through evolution, development, and Ecological Interactions. *Frontiers in Cellular and Infection Microbiology*, *4*.

Wilkinson, C. R. (1996). Global change and coral reefs: Impacts on reefs, economies and human cultures. *Global Change Biology*, *2*(6), 547–558.

**BUDGET**

The budget illustrated (Fig. 1) consists of all the supplies that will be needed for successful completion of this project.



Figure 1 | Tabulated Supplies

**BUDGET JUSTIFICATION**

The total cost for this project is (**$797.77)**. The line items and justifications are provided and summarized below.

**Contamination Control: $0.00**

Our contamination control will consist of Personal Protective Equipment (PPE) designed specifically for the task of ensuring safety of laboratory personnel and to prevent cross contamination of coral samples. The types of PPE we will utilize in this experiment consist of protective barriers made for the respiratory tract and skin. Bleach will be needed to decontaminate our workstations and samples by destroying foreign DNA and to kill microbes. The coral skeletons are expected to have a very low amount of DNA, and as a consequence, we will employ ultra clean laboratory techniques to ensure the genetic purity of our samples.

**DNA Isolation: $62.10**

DNA Isolation from coral specimens will be executed using an Omega Bio-Tek Cycle Pure Kit for PCR cleanup. We are requesting support for one purification kit (1 Kit at 150rxns = $62.10). This kit is essential for the clean-up stage of our DNA extractions.

**Library Preparation: $559.67**

The supplies needed for library preparation will encompass the use of PCR kits, bead cleanup kits, AccuClear quantification, sequencing prep, primers, fragment analysis and qPCR. We are requesting funding for 575 PCR reactions for two specific genes (150rxns per locus, 300rxns per gene at a total of 600rxns). Conducting 150rxns per locus will give us the opportunity to amplify a single gene twice. This is imperative because conducting more PCR reactions per locus will allow for increased replicates and controls; thereby improving the reliability and reproducibility of our experimental results. Lastly, because coral historically yield low amounts of DNA, this will ensure sufficient material for downstream analysis without depleting our original samples.

**Sequencing: $176.00**

Our project will be utilizing a *NovaSeq X Plus* platform to profile the DNA from our coral extracts ($176.00 at 100,000,000 read pairs). This project represents an opportunity to utilize industry-leading sequencing with unparalleled accuracy. Sequence preparation for all collected coral, lab space, instrumentation and upkeep associated with processing these samples is made possible by Dr. Bird’s research lab and the Genomics Core-Lab, Texas A&M - Corpus Christi. The coral samples in our analysis will be provided by the Bahr Marine Ecology Lab, Texas A&M - Corpus Christi.