eDNA extraction of marine water samples with the DNeasy PowerSoil™ Pro Kit but not DNeasy PowerWater™ Kit , leads to successful detection of *Haliotis rufescens* COX1 gene

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Summary

- PowerSoil Pro method yields successful detection of the COX1 gene of *Haliotis rufescens* (red abalone) in marine eDNA samples.
- Although the PowerWater samples have comparable purity (A260/A280), abalone COX1 DNA detection was unsuccessful.

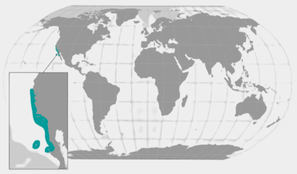
Abstract

Haliotis cracherodii is a critically endangered marine mollusk species found on the coastlines of California. With populations plummeting by over 90% since the mid-1980s due to bacterial disease, rising sea surface temperatures, and human overfishing, there is a pressing need for effective monitoring methods. We used environmental DNA (eDNA) detection, a previously successful technique in monitoring other marine species, to detect black abalone. We compared two different eDNA extraction kits, DNeasy PowerSoil™ Pro, and DNeasy PowerWater™, using water sample from the coast of Channel Islands; isolated DNA was used for PCR amplification with primers targeting the cytochrome c oxidase gene of abalones. While both kits resulted in relatively pure eDNA samples, only samples isolated with the PowerSoil™ Pro kit led to the successful detection of *Haliotis rufescens*.

Introduction

Black Abalone

- *Haliotis cracherodii* is a critically endangered intertidal marine mollusk species.[1]
- Since the mid-1980s, their populations have declined over 90% [2], due to the fatal withering syndrome, rising sea surface temperatures, and human overfishing.[3]
- Black abalone inhabit coastal environments and range from Point Arena, California, to Bahia Tortugas and Isla Guadalupe, Mexico. [4]

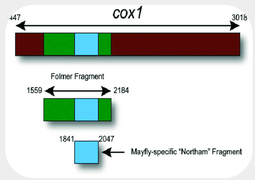


Environmental DNA (eDNA)

- eDNA is environmentally available DNA released from an organism. [5]
- Sources include feces, shed skin, and hair [5]
- Previous studies have used eDNA to monitor other marine and freshwater species such as vulnerable manatee populations[6], endangered endemic cave crayfish[7], and potamodromous fish species[8].

DNA Barcoding

- Using the sequence of one or more segments of genes to identify species. [9]
- The cytochrome c oxidase subunit 1 (COX1) gene, is a mitochondrial gene commonly used for DNA barcoding. [10]



Methodology

Tissue DNA Extraction

Used Lamba Biotech Column-Pure™ Animal Genomic DNA Kit for extraction of DNA from 30 mg of tissue sample from red abalone

eDNA Extraction

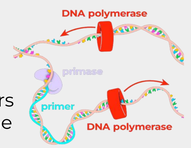
3 L Water sample collected off the coast of Channel Islands. 350 ml replicates filtered and extracted through PowerWater (n=4) and PowerSoil Pro kits (n=3)

eDNA Quality Check

2µl of DNA samples were placed on a NanoDrop spectrophotometer to measure yield (ng/µl) and purity (A260/A280)

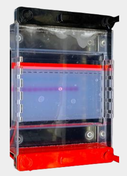
Polymerase Chain Reaction (PCR)

PCR used universal COX1 primer for all tissue samples (except for abalone) and in eDNA samples to confirm eDNA presence. We used abalone-specific COX1 primers in eDNA samples and abalone tissue samples to detect abalone eDNA.



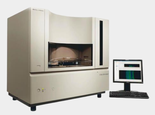
Agarose Gel Electrophoresis

We used 1.5% agarose gels stained with SYBR Safe DNA Gel Stain. The Quick-Load® 1 kb Plus DNA Ladder was used for reference. All gels were visualized using UV light.



Sanger Sequencing

Sanger sequencing was done on the PCR product to determine the nucleotide sequence. We received our results as a chromatogram. We then exported those files into a FASTA file format.



Nucleotide BLAST

Nucleotide BLAST was used to determine the percent identity, query coverage, and the E value of the sequence alignment.

Results

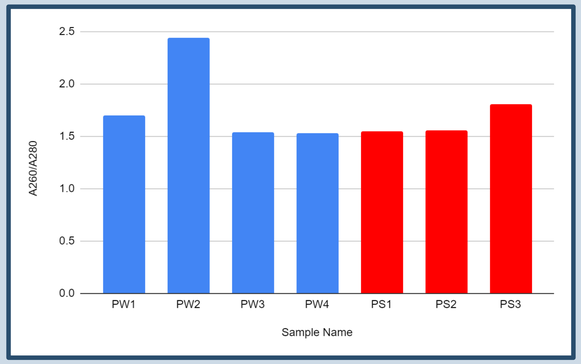


Figure 1. Comparison of DNA purity between eDNA samples. The y-axis represents the A260/A280 ratios. The blue columns are PowerWater samples while the red columns are PowerSoil Pro samples. The mean value for PowerWater samples was 1.8025, while the mean value for PowerSoil Pro samples was 1.64.

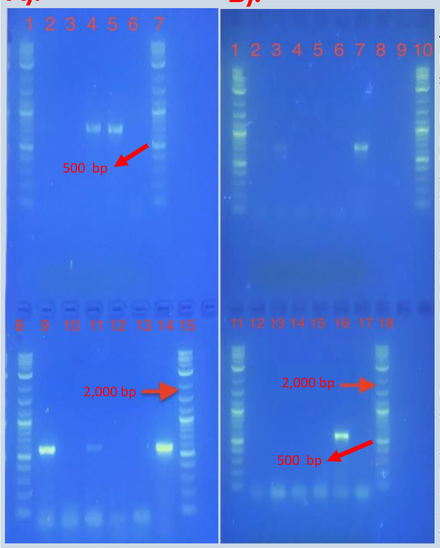


Figure 2. Agarose gel visualization of A) PowerSoil Pro and B) PowerWater sample PCR products. A) Lanes 1, 7, 8, and 15 are the DNA ladder. Lanes 2-4 contain PCR products PS1-PS3 using universal COX1 primers. Lane 5 contains a positive control. Lane 6 contains a negative control. Lanes 9-11 are PCR products PS1-PS3 using abalone-specific COX1 primers. Lane 12 is a positive control. Lane 13 is a negative control. Lane 14 is a red abalone tissue sample. **B)** Lanes 1, 10, 11, and 18 are the DNA ladder. Lanes 2-5 contain PCR products PW1-PW4 using universal COX1 primers. Lane 6 contains a negative control. Lane 7 contains a positive control. Lanes 12-15 contain PCR products PW1-PW using abalone-specific COX1 primers 4. Lane 16 contains a positive control. Lane 17 contains a negative control.

Description	Accession	Query Cover	E Value	Percent Identity
(COI) gene, partial cds; mitochondrial	MH304614.1	90%	0.0	100.00%
(COI) gene, partial cds; mitochondrial	MH304621.1	90%	0.0	99.02%
(COI) gene, partial cds; mitochondrial	MH304601.1	90%	0.0	99.02%
(COI) gene, partial cds; mitochondrial	EU636201.1	90%	0.0	99.02%
(COI) gene, partial cds; mitochondrial	MH304607.1	90%	0.0	98.82%

Table 1. Top 5 BLAST results for PS1 PCR product sequence. Gene description and accession number give in columns 1 & 2. Query coverage as a percentage given in column 3. E value given in column 4. Identity as a percentage is given in column 5.

Discussion and Conclusion

eDNA Isolation Methods Comparison

- Both PowerWater™ and PowerSoil™ Pro kits were successful in extracting eDNA from all field water sample replicates.
- The purity of the eDNA is similar across the samples, except for PW2 (Figure 1).
- The PowerSoil™ Pro eDNA extraction method yielded two samples, PS1 and PS3, that had successful PCR amplification with both universal and abalone-specific COX1 primers (Figure 2A).
- The PowerWater™ yielded one sample, PW2, with successful PCA amplification using universal COX1 primers. None of the PW samples amplified with abalone-specificCOX1 primers (Figure 1B).
- The PowerSoil™ Pro kit is reportedly more efficient and consistent in capturing bacterial DNA than the PowerWater™ kit when processing turbid water samples such as pond, sewage [11], and wastewater [12] samples. Similarly, our samples were collected from tidepools near a kelp forest and likely contain a lot of organic debris.
- Our results suggest that the PowerSoil™ Pro kit is better than the PowerWater™ kit at isolating eDNA from turbid marine water samples for PCR.

Species Identification

- BLAST analysis on the PS1 sequence revealed our amplification product was the red abalone (*Haliotis rufescens*) COX1 (Figure 3).
- While we did not detect the black abalone, our method enabled successful eDNA detection of an abalone species.

Limitations and Future Directions

- Only one field replicate sample was used, and the results might not be representative of the site.
- We will process more field samples with the PowerSoil™ Pro kit to test its consistency.
- The primers used were not specific to black abalones, which resulted in the amplification of red abalone COX1 gene.
- We'll compare genomes of different abalone species in the future to develop black abalone-specific primers for eDNA detection.
- Once the method is fully developed, we'll use eDNA detection to determine the presence and genetic diversity of black abalones across various geographic locations.

References