Successful *Haliotis rufescens* cytochrome c oxidase I gene detection in environmental DNA samples when using DNeasy PowerSoil™ Pro Kit compared to DNeasy PowerWater™ Kit

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Summary

- DNeasy PowerSoil Pro Kit resulted in higher nucleic acid yields (ng/µl) of extracted eDNA from marine samples than the DNeasy PowerWater Kit.
- DNeasy PowerSoil Pro kit method led to successful detection of red abalone COX1 gene in eDNA samples.

Abstract

Non-invasive eDNA sampling is a tool for scientists to monitor endangered species. The goal of this study is to determine if the DNeasy PowerSoil Pro Kit or the DNeasy PowerWater Kit is more effective in eDNA isolation and subsequent detection of black abalone, a critically endangered species, from sea water samples. A sample of water from the Channel Islands was processed through the two kits, and the PowerSoil Pro method led to eDNA samples with higher nucleic acid yields. The extracted eDNA samples were PCR amplified with universal and abalone-specific COX1 primers. The PCR results were checked with gel electrophoresis, and amplified products were processed with Sanger sequencing. Nucleotide BLAST analysis reveals that the sequence of the amplified DNA aligns best to the red abalone COX1 gene. We conclude that the PowerSoil Pro method resulted in successful eDNA detection of red abalone from sea water samples.

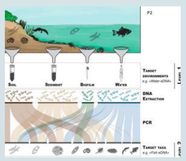
Introduction

Hypothesis: The DNeasy PowerSoil Pro method leads to successful detection of the red abalone COX1 gene when using abalone-specific COX1 primers in marine samples.

Black Abalone:

- Suffered from a severe population bottleneck due to Withering Syndrome caused by *Candidatus Xenohaliotis californiensis* in 1995[1]
- Plays a key role in the prevention of oceanic algal blooms[2]

eDNA



- Nuclear or mitochondrial DNA shed by organisms to the environment[3]
- Sources include cell shedding, fecal matter, or gametes

COX1 gene

- Encodes a subunit of electron transport chain's complex IV, which catalyzes the final step of mitochondrial oxidative phosphorylation
- COX1 is commonly used as a target gene for DNA barcoding for identifying organisms, due to the variability and conservation across species[4]

Materials and Methods

Sampling and Filtration

- A 3 L marine sample was collected from Little Scorpion Anchorage in Channel Islands
- Replicates of 350 ml of the 3 L water were vacuum-filtered

DNeasy PowerWater Kit

- Four replicates were processed
- Each filter was added to a PowerBead tube

DNeasy PowerSoil Pro Kit

- Three replicates were processed
- ⅔ of each filter was cut into thin strips and placed in PowerBead tube

eDNA Sample Quality Check

- 2 µl of each sample was analyzed on NanoDrop for both yield and purity (as A260/A280 and A260/A230 ratios)

Primer Sequences

universal COX1 primers
Forward: 5'- GGT CAA CAA ATC ATA AAG ATA TTG G -3'
reverse: 5'- GAT GTG TTG AAA TTA CGG TCG GT -3'

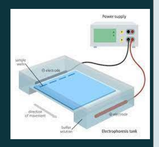
abalone-specific COX1 primers
Forward: 5'- TGA TCC GGC TTA GTC GGA ACT GC -3'
reverse: 5'- GAT GTG TTG AAA TTA CGG TCG GT -3'

Polymerase Chain Reaction (PCR)

- BSA was added to PW samples
- Taq polymerase

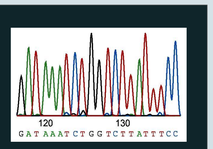
Gel Electrophoresis

- 1.5 % Agarose gel
- SybrSafe dye used
- Quick-Load® 1 kb Plus DNA Ladder
- 110V for 50 minutes
- UV used to visualize gel



Sanger Sequencing and nucleotide BLAST

- Sequence was visualized as chromatogram, then exported as FASTA
- FASTA sequence was aligned to database sequences using Nucleotide BLAST



Results

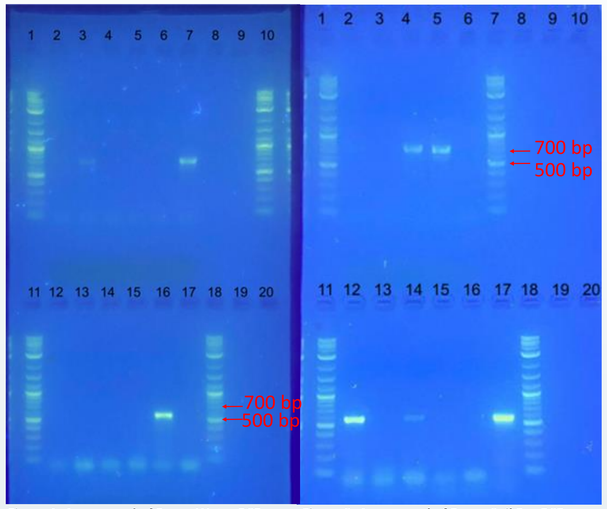


Figure 1. Agarose gel of PowerWater PCR products using universal and abalone specific COX1 primers. Lane 1, 10, 11, and 18 are ladder lanes. Lanes 7 and 16 are red abalone positive control samples. Lanes 6 and 17 are the negative controls. Lanes 2-5 are PowerWater Samples 1-4 with the universal COX1 primer used. Lanes 12-15 are PowerWater samples 1-4 with the abalone specific COX1 primer used.

Figure 2. Agarose gel of PowerSoil Pro PCR products using universal and abalone specific COX1 primer. Lanes 1, 7, 11, 18 are ladder lanes. Lanes 5 and 15 are red abalone positive control samples. Lanes 6 and 16 are negative controls. Lanes 2-4 are PowerSoil Pro samples 1-3 with the universal COX1 primer. Lanes 12-14 are PowerSoil Pro samples 1-3 with the abalone specific COX1 primer used. Lane 17 is red abalone tissue DNA.

Figure 3. PowerWater and PowerSoil Pro sample nucleic acid yields. The y-axis represents the yields of each sample were measured using the Nanodrop in nanograms per microliter. The x-axis represents the PowerSoil Pro (PS) and PowerWater (PW) samples. Each sample is identified by name on the plot. Averages for both kits (PS = 34.3, PW = 22.85) are shown in blue.

Description	Scientific Name	Query Cover	E value	Percent Identity	Accession
Haliotis rufescens isolate H16 cytochrome oxidase subunit I (COI) gene	*Haliotis rufescens*	90%	0	100	MH304614.1
Haliotis rufescens isolate H_22_1 cytochrome oxidase subunit I (COI) gene	*Haliotis rufescens*	90%	0	99.02	MH304621.1
Haliotis rufescens isolate H11_OCT cytochrome oxidase subunit I (COI) gene	*Haliotis rufescens*	90%	0	99.02	MH304601.1

Figure 4. NCBI nucleotide BLAST for PS1 sequence. The table includes the description, scientific name, query coverage, E value, percent identity, and accession of the top 3 matches for the sample sequences matching the PS1 sample sequence when sorted by the E value.

Discussion and Conclusion

- The PowerSoil Pro method yielded samples with a higher average nucleic acid yield when compared to PowerWater (Figure 3).
- A previous study reported that marine samples processed with the PowerWater method produced consistently higher yields compared to the DNeasy Blood and Tissue Kit and was successful in the detection of eDNA.[6] Their nucleic acid yields were higher than ours, this may be due to the larger pore size of our filter (0.3 µm compared to the 0.2 used in the study) and the amount of water filtered (350 ml compared to 1 liter in the study).
- Universal COX1 primers led to amplification product in PW2, but no other PW samples (Figure 1). No product was observable in PW samples when using abalone-specific COX1 primers (Figure 1).
- Universal COX1 primers led to amplification product in PS3 (Figure 2). Abalone-specific COX1 primers led to amplification product in PS1 and PS3 (Figure 2).
- PowerSoil Pro method reportedly led to higher qPCR amplification of eDNA for fish species compared to PowerWater from marine samples.[5] The relatively lower yield combined with potential contamination by PCR inhibitors may have contributed to the low performance of PowerWater.
- Our results show the eDNA extracted through the PowerSoil Pro method successfully led to identification of red abalone.

Study Limitations

- None of the PS nor PW samples had values of the A260/A280 and A260/A230 ratios close to the ideal values (1.8 and 2.1, respectively), indicating contamination likely confounded the relationship between yield and PCR success.
- We used only one sample, while additional replicates could possibly lead to better evaluation of the methods.

Future Directions

- Design and use a black abalone COX1 specific primer.
- Increase amount of water per sample filter to maximize the yield.
- Utilize literature supported changes in our methods, such as filtering more water per sample or using a filter with a different pore size (.2µm).

Bibliography