# eDNA Sequencing Proves Accuracy in the Analysis of the COX1 Gene Coding Region of Red Abalone

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## Summary

- Red abalone with withering disease metabarcoded for *COX1* gene
- Compared accuracy of eDNA sequencing against tissue DNA & results from NCBI RefSeq with phylogenetic trees, sequence alignment, and gel electrophoresis
- eDNA sampling is an accurate tool to detecting *COX1* gene coding region & withering disease

## Abstract

Withering disease is a fatal disease in abalone caused by a Rickettsiales-like organism which occurs along the *eastern Pacific margin of North America, USA and Baja California Mexico.*
We investigated the similarities of *COX1* sequences between different organisms such as red abalone, fruit fly, medaka, worms, and zebrafish by designing phylogenetics trees to analyze the differences between *COX1* sequences from tissue DNA, eDNA, and the NCBI database. Through collecting eDNA samples of abalone, medaka, and zebrafish and contrasting it with the positive control, we've been able to analyze the similarities through *Clustal Omega* phylogenetic trees, gel electrophoresis, and BLAST Sequence Alignment to conclude that sequences from eDNA yield accurate results to detecting the *COX1* gene.

## Introduction

Hypothesis
Results collected from eDNA water sampling will yield less accurate results than DNA collected from tissue samples corresponding to the data of sequences found on the NCBI database..

Haliotis cracherodii (black abalone)
-Population listed as endangered by NOAA's NMFS due to overfishing & withering syndrome

Haliotis rufescens (red abalone)
-Largest abalone population out of 8 species [UCSC 2021]

Candidatus xenohaliotis californiensis
-Withering syndrome
-Bacterium restricts digestive enzyme production
-Foot withers, loses ability to attach to rocks, increased vulnerability to predators & starvation [NOAA 2022]

*COX-1*
-Commonly used for metabarcoding
-High mutation rate across species & lower variation in species
-Mitochondrially encoded

Investigation Methods
-Tissue samples of humans, fruit fly, worms
-eDNA samples of zebrafish
-Tissue & eDNA samples for abalone, medaka
-Undergoes extraction, PCR, gel electrophoresis, sequencing
-Analysis of phylogenetic tree

## Methodology

Collection of Sample
-Tissues collected from medaka, red abalone, c.elegans, fruit fly, and human for DNA extraction
-eDNA water samples of red abalone, medaka, and zebrafish for eDNA sampling

eDNA
1 - 3L sample from tank
-filtered through .45 uM MCE filter
-processed with Mo Bio Power Water kit
-product= 100 ul of
-frozen at -80C for storage
-samples of red abalone, medaka, zebrafish

DNA
-tissue sample collected from abalone living in tank
-additional samples from human, fruit fly, c.elegans, medaka

DNA Extraction
Homogenization: DNA broken into identical parts to yield high DNA amount while maintaining integrity of nucleic acids
**1) Disruption of cellular structure to create a lysate**
**2) Separation of soluble DNA from cell debris**
**3) Binding of DNA to purification matrix**
**4) Washing using buffers to remove contaminants**
**Elution buffer applied to membrane**

PCR
-GoTaq 2X protocol
-PCR conducted using 5 uL of extracted DNA
**1) Denaturation at 95 degrees Celsius**
**2) Annealing at 55 degrees Celsius for general, 65 degrees Celsius for abalone**
**3) Elongation at 72 degrees Celsius**

**Primers**
***COX1 General:***
FWD:GGTCAACAAATCATAAAGATATTGG
RVS:
TAAACTTCAGGGTGACCAAAAAATCA

***COX1 (abalone friendly and only used on abalone):***
FWD:TGATCCGGCTTAGTCGGAACTGC
RVS:GATGTGTTGAAATTACGGTCGGT

Gel Electrophoresis
10ul of PCR rxn used + 2ul loading dye
Agarose gel (1.5% gel) for 1Kb DNA Ladder used (12ul)
-90V for 55Minutes
-SYBR Safe Stain and UV Box use

Sanger Sequencing
-Single strand reverse primer
-Low concentration of fluorescently labelled ddNTPs
-Loaded into capillary array for capillary gel electrophoresis
-Data stored and interpreted by chromatogram
-Sequence obtained post data cleanup

Data Analysis
-Phylogenetic trees constructed using Clustal Omega
-NCBI Blast Alignment tool

## Results


**Fig. 1 Phylogenetic tree of tissue DNA for COX1 gene** collected from the following species: *H. sapiens* (human), *D. melanogaster* (Fruit fly), *C elegans* (Worms), *H. rufescens* (Abalone), *O. latipes* (Japanese medaka), *D rerio* (zebrafish), (*sequence collected from NCBI, not lab tests), sequences were aligned with Clustal Omega


**Fig. 2 Phylogenetic tree constructed with entire COX1 genome sequence from NCBI RefSeq.** Sequences from *H. sapiens* (human), *D. melanogaster* (Fruit fly), *C elegans* (Worms), *H. rufescens* (Abalone), *O. latipes* (Japanese medaka), *D rerio* (zebrafish), sequences were aligned with Clustal Omega

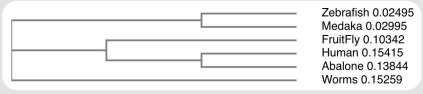


**Fig. 3 Phylogenetic tree constructed with COX1 genome sequence collected through tissue & eDNA sampling** from the following species: *H. sapiens* [Human (T)], *D. melanogaster* [Fruit Flies (T)], *C. elegans* [Worms (T)], *H. rufescens* [Abalone (e)], *O. latipes* [medaka (e)], *D. rerio* [zebrafish (e)], shows abalone in the same clade as human & fruit fly, sequences were aligned with Clustal Omega

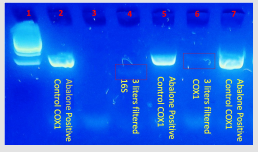


**Fig. 4 Agarose gel electrophoresis results** showing the abalone positive control *COX1* (tissue samples) in comparison to eDNA sampling results running to 500 bp

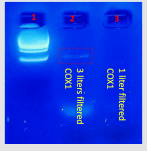


**Fig. 5 Agarose gel electrophoresis results** comparing the positive control with filtered samples with red squares signifying faint bands camera couldn't pick up

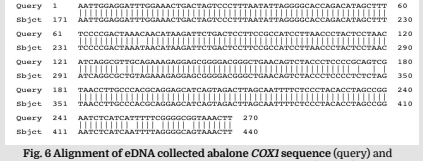


**Fig. 6 Alignment of eDNA collected abalone *COX1* sequence** (query) and most similar sequence from NCBI database: red abalone (subject)

## Discussion/ Conclusion

-Phylogenetic trees values show low levels of variance in the branch formation that reveal eDNA samples will yield similar results to tissue and the NCBI database (Fig. 1, 2 and 3)
-Greater DNA concentration in PCR product from red abalone tissue DNA samples, compared to eDNA (Fig. 4)
-eDNA *COX1* sequence of red abalone has high percent identity to NCBI's red abalone *COX1* sequence (Fig. 5)
-eDNA proves accuracy in barcoding COX1 gene as compared to tissue sequences (positive control)

## Limitations/Future Directions

Limitations:
- Unable to test H1 due to lack of black abalone samples & efficacy of eDNA in detecting black abalone cannot be confirmed
- Inability to collect samples from a natural marine environment

Future directions:
- Utilizing various primers to see which one is more accurate for PCR amplification

## References