# Species relatedness to *Haliotis cracherodii* (black abalone)

**Thontepu T.[1], Lewis E.[2], Jiang C.[3]**

[1]Canyon Crest Academy, [2]Francis Parker School, [3]Scripps Ranch High School

## Abstract

Black abalone are an endangered species of marine sea snails native to San Diego. This project seeks to understand new methods of discovering species relatedness by testing specific primers in order to further develop methods of detecting eDNA, an important method for detecting the population of endangered species in water samples. To prove our methods, we will be genetically sequencing the mitochondrial cytochrome C oxidase 1 (*Cox-1)* gene and analyzing the mutation rates.

## Introduction

- Black Abalone = marine sea snails from the Haliotis family[3]
  - *Haliotis cracherodii*
- Diet consists of algae, kelp, bacteria, and plankton [3]
  - Can live up to 30 years [3]
- Were once extremely common off the coast of California, but are now endangered [1]
  - There is a high mortality rate for "withering disease" [1]
    - The abalone epidemic began in the 1980s [1]
    - Affects the digestive system of the abalone [1]
    - Exacerbated in warmer waters [4]
  - Overfishing and poaching have also killed off a lot of the population [1]
- Utilizes muscular "foot" for movement and attachment to rocks and hard shell for protection [3]
- Long-term monitoring of black abalone populations since the 1970s [1]
  - Conservation efforts must come from multiple angles: preventing and treating diseased abalone, protection from fishing and poaching, as well as maintaining water quality [1]
- Cytochrome C Oxidase Subunit 1 gene (*cox1*) is often used for DNA barcoding or identification of species based on specific DNA sequences [2]
  - *cox1* is favorable due to species specificity [2]
- The genetic identification of Black Abalone using the *cox1* gene allows for phylogenetic mapping, or analyzing the genetic relatedness of the Black Abalone to its closely related species [5]
  - Increases awareness of genetic makeup of the species [5]
  - contributes to conservation efforts, especially important for endangered species like the Black Abalone [5]

## Materials and Methodology

In order to do DNA barcoding, we had to do these following steps.

**DNA extraction**
Using Black Abalone (*Haliotis cracherodii*), fruit fly (*Drosophila melanogaster*), roundworms (*Caenorhabditis elegans*), zebrafish (*Danio rerio*), and medaka (*Oryzias latipes*) tissue samples using NucleoSpin Tissue silica column extraction kit.

**Nanodrop spectrophotometer**
Verify DNA purity & approximate quantity.

**PCR**
Twelve samples were tested and divided into two batches. For each batch, 7x of the PCR buffer was created twice, one for each batch, with 2.5uL of DNA at 50 ng/uL. Primer concentration at 10uM.

Forward primer: TGATCCGGCTTAGTCGGAACTGC
Reverse primer: GATGTGTTGAAATTACGGTCGGT

**Gel electrophoresis**
Performed to separate DNA strands by size and verify PCR with a 1.5% gel.

**Sequencing**
DNA sanger sequencing after PCR Amplification

**Data Analysis**
Data visualization in order to generate the tree and analyze the evolutionary patterns and characterize the black abalone to a specific position on the tree using Clustal Omega and NCBI database and BLAST on NCBI.

## Results

Figure 1: Real Phylogenetic tree generated with Clustal Omega showing 5 species from the NCBI database. The branches on the tree represent where lineages evolve and diverge from each other. Red abalone is closely related to japanese medaka because the branches are closest together.

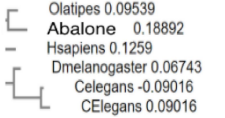



Figure 2: Real Phylogenetic tree generated with Clustal Omega using dna from samples extracted in the lab.

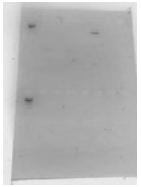
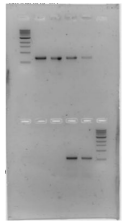


Figure 3: Gel electrophoresis image 1 amplifying *cox 1* gene

Figure 4: Gel electrophoresis image 2 amplifying *cox 1* gene. Top: Medaka, C. Elegans, C. Elegans w/ E. coli, Fruit Flies, Zebrafish Bottom: Human, abalone R1, abalone R2, abalone R3, Abalone R4



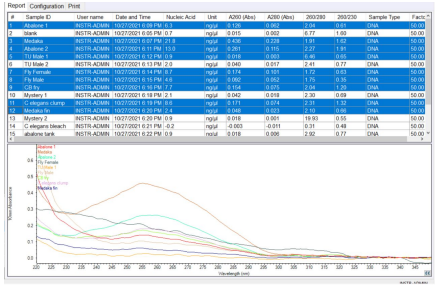


Figure 5: Student-collected Nanodrop graph of absorbance + Nanodrop readings of DNA

## Discussion & Conclusion

- Black Abalone is most closely related to the Japanese Medaka fish
- Red abalone (therefore Black Abalone) is the closest branch to the Japanese Medaka fish
- Increases awareness about genetic information for Black Abalone
- Helps with conservation efforts
- Understanding the similarities between Medaka and abalone
- Can now use information about each species to promote the other
- Phylogenetic trees are very similar – methods were successful
- Black abalone is a positive control
- Categorized phylogenetically based on specific primers
- Methods should work for future eDNA research.
- Gel indicated DNA amplification was successful
- Nanodrop data indicated purity and concentration of DNA samples
- Phylogenetic trees show correct DNA was successfully retained for each species and compared to each other genetically
- Trees help understand species relatedness
- Leads back to conservation efforts for above reasoning

## Future directions

More careful DNA extraction – if the Nanodrop data showed that our samples weren't pure enough, we should have continued to purify our samples or started over to ensure that our data was even more readable

Having the ability to compare the Cox1 gene of the Black Abalone rather than the Red Abalone would likely not have changed our main conclusion BUT would have made for less margin of error and more accurate results.

## References

[1] Fisheries, NOAA. "Black Abalone." NOAA www.fisheries.noaa.gov/species/black-abalone.

[2] "Cytochrome C Oxidase Subunit I." Cytochrome C Oxidase Subunit I (COX1), Wikimedia Foundation, 26 Aug. 2021, en.wikipedia.org/wiki/Cytochrome_c_oxidase_subunit_I.

[3]https://wildlife.ca.gov/Conservation/Laboratories/Shellfish-Health/Abalone-Diseases

[4] Assessing the species composition of tropical eels (Anguillidae) in Aceh Waters, Indonesia, with DNA barcoding gene *cox1*
Assessing the species composition of tropical... | F1000Research

[5] Loh, Jin Phang, et al. "Utility of COX1 Phylogenetics to Differentiate between Locally Acquired and Imported Plasmodium Knowlesi Infections in Singapore." *Singapore Medical Journal*, Singapore Medical Association, Dec. 2016, www.ncbi.nlm.nih.gov/pmc/articles/PMC5165177/.

# Species relatedness to *Haliotis cracherodii* (black abalone)

**Thontepu T.[1], Lewis E.[2], Jiang C.[3]**

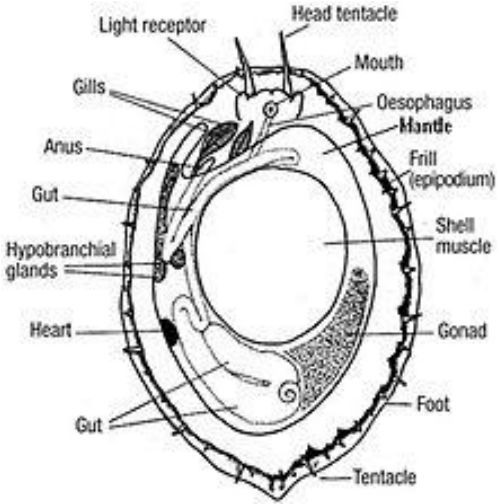
**[1]Canyon Crest Academy, [2]Francis Parker School, [3]Scripps Ranch High School**

# Abstract

- Black abalone = endangered species of marine sea snails
- DNA extraction, PCR
- DNA barcoding, phylogenetic tree generation
- Confirming basic methods for future research and conservation efforts

# Introduction - Black Abalone

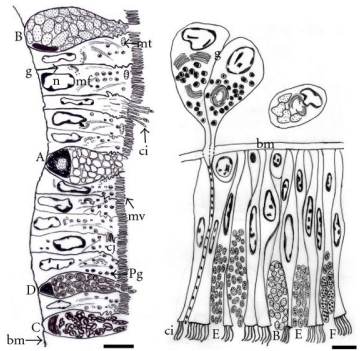
- Black Abalone = marine sea snails
  - *Haliotis cracherodii*
- Endangered on California's coast
  - "Withering Disease"
  - Overfishing and poaching -> conservation efforts since '70s



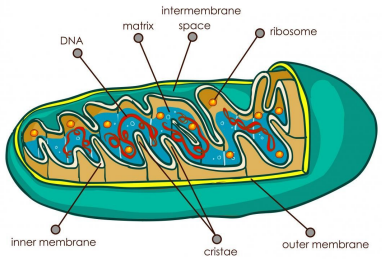
# Introduction - *Cox1* Barcoding

- DNA barcoding → Cytochrome C Oxidase Subunit 1 gene (*Cox1*)
- Cox 1 gene is the best biomarker used for barcoding thus far
  - Has a sequence variation that's relatively low across species, but relatively high between species
  - Cox 1 gene has high mutation rates between species, and has enough variation within species to associate them with similarities
  - Can categorize how species fit along a phylogenetic tree
- Cox1 barcoding → phylogenetic mapping
  - Increases awareness of genetic makeup of the species
  - Conservation efforts

https://www.fisheries.noaa.gov/species/black-abalone




https://www.hindawi.com/journals/tswj/2012/960159/fig1/




https://www.medicalnewstoday.com/articles/320875

# Materials and Methods ★

1 — 2 — 3

**DNA Extraction**

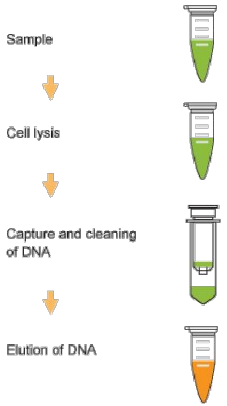
Multiple species' samples

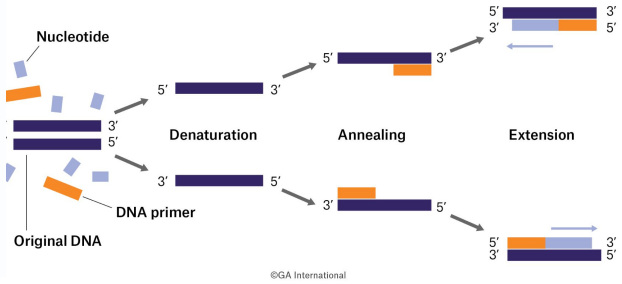
**Nanodrop Spectrophotometer**

DNA quality check

**PCR**

Amplify





# Materials and Methods ★

4 — 5 — 6

**Agarose Gel Electrophoresis**

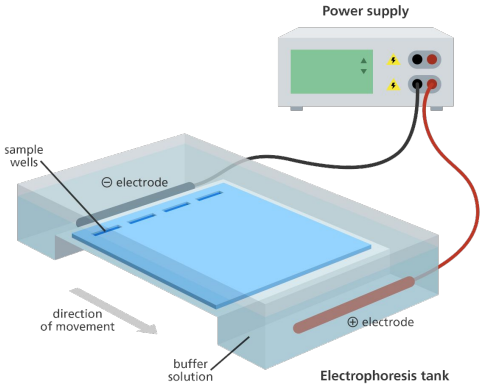
Verify PCR products

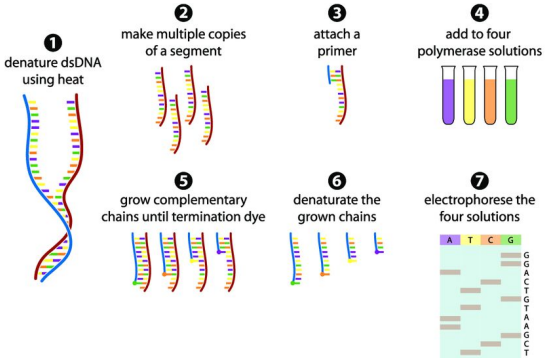
**Sequencing**

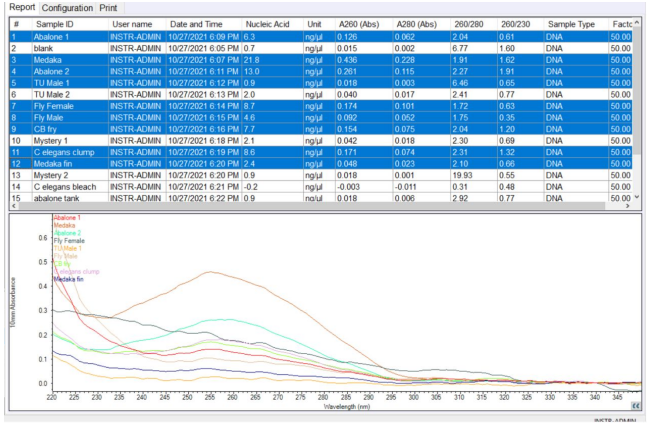
Determine the sequence of the 6 dna samples that were extracted in the lab

**Data Analysis**

Nanodrop, AGE, phylogenetic tree



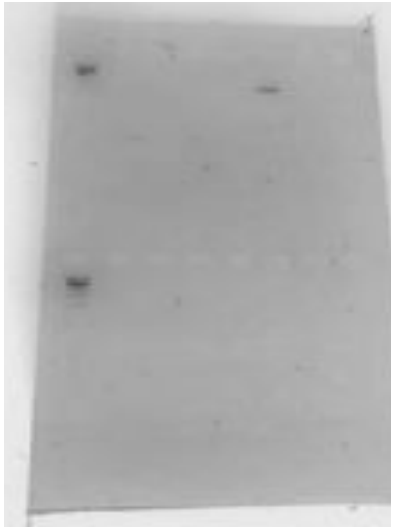



Report | Configuration | Print

| # | Sample ID | User name | Date and Time | Nucleic Acid | Unit | A260 (Abs) | A280 (Abs) | 260/280 | 260/230 | Sample Type | Factc |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Abalone 1 | INSTR-ADMIN | 10/27/2021 6:09 PM | 6.3 | ng/µl | 0.126 | 0.062 | 2.04 | 0.61 | DNA | 50.00 |
| 2 | blank | INSTR-ADMIN | 10/27/2021 6:05 PM | 0.7 | ng/µl | 0.015 | 0.002 | 6.77 | 1.60 | DNA | 50.00 |
| 3 | Medaka | INSTR-ADMIN | 10/27/2021 6:07 PM | 21.8 | ng/µl | 0.436 | 0.228 | 1.91 | 1.02 | DNA | 50.00 |
| 4 | Abalone 2 | INSTR-ADMIN | 10/27/2021 6:11 PM | 13.0 | ng/µl | 0.261 | 0.115 | 2.27 | 1.91 | DNA | 50.00 |
| 5 | TU Male 1 | INSTR-ADMIN | 10/27/2021 6:12 PM | 0.9 | ng/µl | 0.018 | 0.003 | 6.46 | 0.65 | DNA | 50.00 |
| 6 | TU Male 2 | INSTR-ADMIN | 10/27/2021 6:13 PM | 2.0 | ng/µl | 0.040 | 0.017 | 2.41 | 0.77 | DNA | 50.00 |
| 7 | Fly Female | INSTR-ADMIN | 10/27/2021 6:14 PM | 8.7 | ng/µl | 0.174 | 0.101 | 1.72 | 0.63 | DNA | 50.00 |
| 8 | Fly Male | INSTR-ADMIN | 10/27/2021 6:15 PM | 4.6 | ng/µl | 0.092 | 0.052 | 1.75 | 0.35 | DNA | 50.00 |
| 9 | CB fly | INSTR-ADMIN | 10/27/2021 6:16 PM | 7.7 | ng/µl | 0.154 | 0.075 | 2.04 | 1.20 | DNA | 50.00 |
| 10 | Mystery 1 | INSTR-ADMIN | 10/27/2021 6:18 PM | 2.1 | ng/µl | 0.042 | 0.018 | 2.30 | 0.69 | DNA | 50.00 |
| 11 | C elegans clump | INSTR-ADMIN | 10/27/2021 6:19 PM | 8.6 | ng/µl | 0.171 | 0.074 | 2.31 | 1.32 | DNA | 50.00 |
| 12 | Medaka fin | INSTR-ADMIN | 10/27/2021 6:20 PM | 2.4 | ng/µl | 0.048 | 0.023 | 2.10 | 0.66 | DNA | 50.00 |
| 13 | Mystery 2 | INSTR-ADMIN | 10/27/2021 6:20 PM | 0.9 | ng/µl | 0.018 | 0.001 | 19.93 | 0.55 | DNA | 50.00 |
| 14 | C elegans bleach | INSTR-ADMIN | 10/27/2021 6:21 PM | -0.2 | ng/µl | -0.003 | -0.011 | 0.31 | 0.48 | DNA | 50.00 |
| 15 | abalone tank | INSTR-ADMIN | 10/27/2021 6:22 PM | 0.9 | ng/µl | 0.018 | 0.006 | 2.92 | 0.77 | DNA | 50.00 |

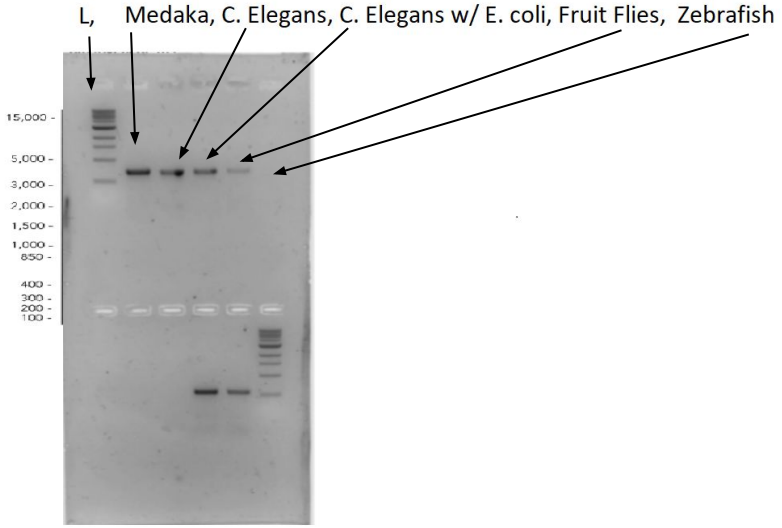
Figure 3: Gel electrophoresis image 1 amplifying *cox 1* gene



# Results

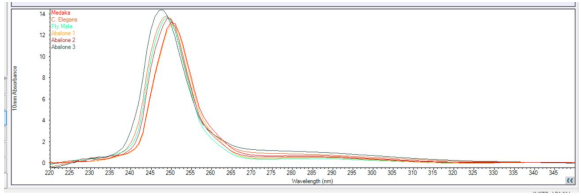
- Successfully amplified the *Cox1* gene

Figure 4: Gel electrophoresis image 2 amplifying *cox 1* gene.

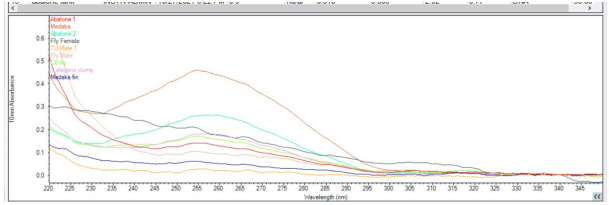



L, Human, abalone R1, abalone R2, abalone R3, Abalone R4

Instructor values:



Student values:



# Results

Figure 1: Real  Phylogenetic tree generated with Clustal Omega showing 5 species from the NCBI database.  The branches on the tree represent where lineages evolve and diverge from each other. Red abalone is closely related to japanese medaka because the branches are closest together.

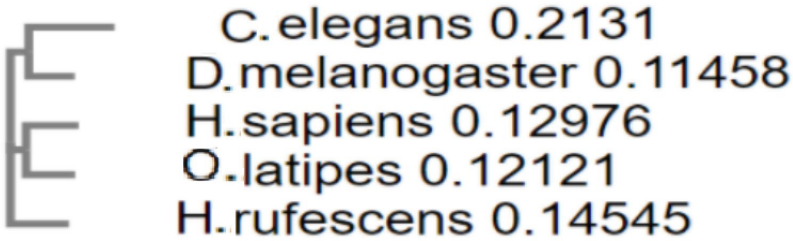
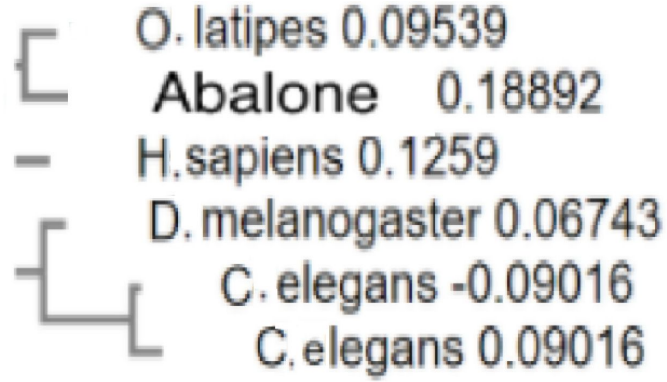



Figure 2: Real Phylogenetic tree generated with Clustal Omega using dna from samples extracted in the lab.



# Discussion

- Our conclusions help with conservation efforts and future research!

Figure 3: Gel electrophoresis image 1 amplifying *cox 1* gene

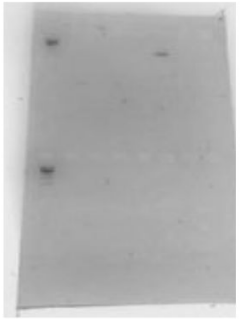
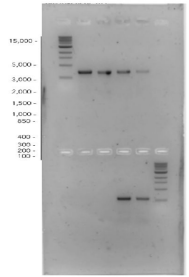
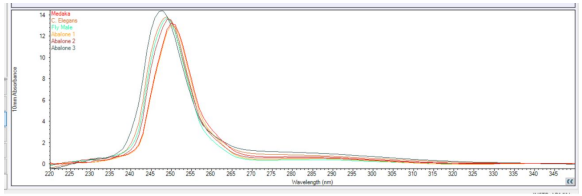


Figure 4: Gel electrophoresis image 2 amplifying *cox 1* gene.

L, Medaka, C. Elegans, C. Elegans w/ E. coli, Fruit Flies, Zebrafish




L, Human, abalone R1, abalone R2, abalone R3, Abalone R4



- Our nanodrop and gel data indicate that the DNA was successfully extracted

# Discussion

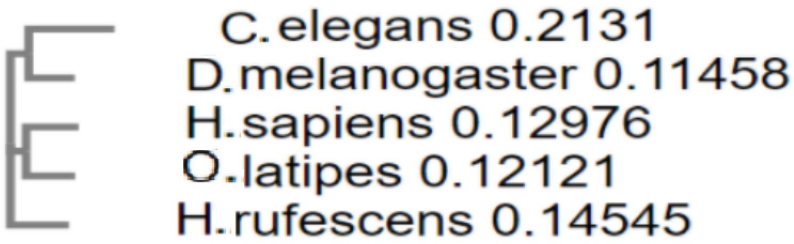



Figure 1: Real Phylogenetic tree generated with Clustal Omega showing 5 species from the NCBI database. The branches on the tree represent where lineages evolve and diverge from each other. Red abalone is closely related to japanese medaka because the branches are closest together.

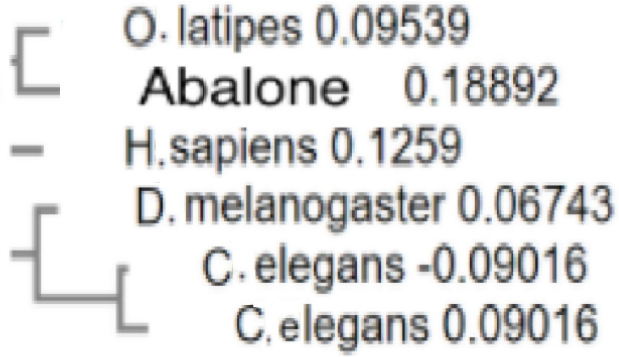



Figure 2: Real Phylogenetic tree generated with Clustal Omega using dna from samples extracted in the lab.

- Black Abalone are most closely related to Japanese Medaka fish (according to species' sampled)

# Study Limitations

- More careful DNA extraction
  - Especially after assessment of Nanodrop graphs for purity
- Ability to compare gathered gene sequences with the Black Abalone data rather than Red Abalone
  - May not have changed our conclusion
  - Would have improved accuracy and ability to conclude confidently

# Future Directions

Go to ocean → extract eDNA-> extract DNA from abalone

# References


[1] Fisheries, NOAA. “Black Abalone.” NOAA www.fisheries.noaa.gov/species/black-abalone.

[2] “Cytochrome C Oxidase Subunit I.” Cytochrome C Oxidase Subunit I (COX1), Wikimedia Foundation, 26 Aug. 2021, en.wikipedia.org/wiki/Cytochrome_c_oxidase_subunit_I.

[3]https://wildlife.ca.gov/Conservation/Laboratories/Shellfish-Health/Abalone-Diseases

[4] Assessing the species composition of tropical eels (Anguillidae) in Aceh Waters, Indonesia, with DNA barcoding gene *cox1*
Assessing the species composition of tropical... | F1000Research

[5] Loh, Jin Phang, et al. “Utility of COX1 Phylogenetics to Differentiate between Locally Acquired and Imported Plasmodium Knowlesi Infections in Singapore.” *Singapore Medical Journal*, Singapore Medical Association, Dec. 2016, www.ncbi.nlm.nih.gov/pmc/articles/PMC5165177/.

https://www.fisheries.noaa.gov/species/black-abalone