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The Ocean Genome Legacy Center

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Fish Forensics: two strategies for fish identification

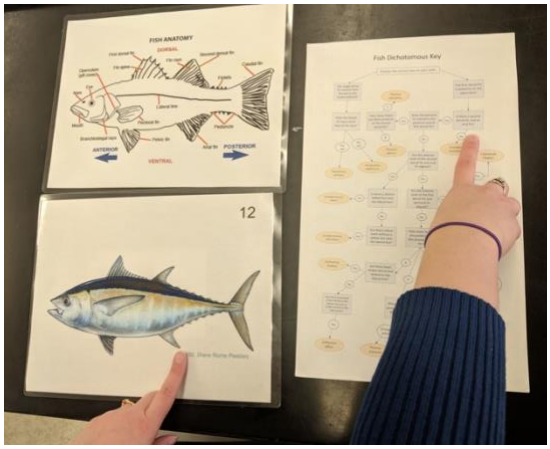
Purpose: The purpose of this activity is to learn about two different strategies for identifying fish. One is using a dichotomous key based on physical characteristics and the second is DNA barcoding. We typically present this activity as a 10- to 15-minute exercise, but it can be easily modified to discuss the process of DNA barcoding more in depth, or to even perform these laboratory protocols. Please feel free to reach out to Ocean Genome Legacy if you have any questions or would like guidance about completing any protocols in a laboratory.

Part 1: Why is it important to be identify fish?

1. Have each student, or pair of students, chose a fish. We often have students pretend they are 'fishing' when they select their fish.
    a. Fish images are in the "Fish" folder.
2. Discuss why it is important to know the identity of this fish.
    a. fisheries management
    b. food safety
    c. conservation
    d. others?

Part 2: Learning to use a dichotomous key

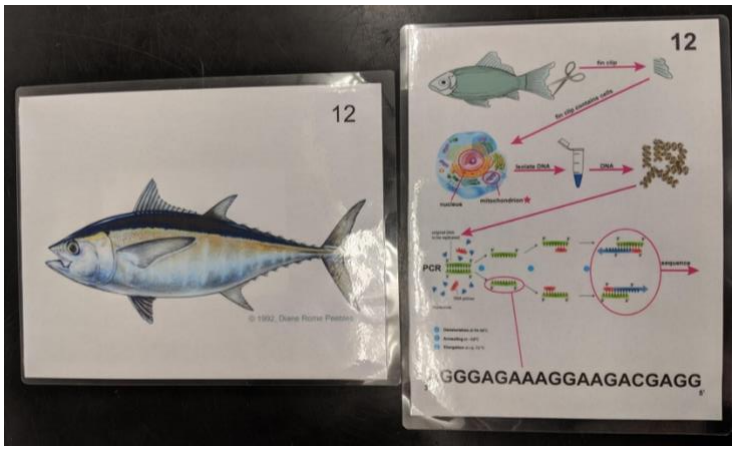
1. One way to identify an organism is to use morphological features. Using the flow chart, answer each of the questions based on the *image* with which the student is working.
    a. Flow chart is DichotomousKeyFlow_10.11.17.pptx in the "Dichotomous Key" folder.
    b. The key is alternately displayed as a traditional dichotomous key with a series of questions. It can be found in the same folder.
    c. Anatomy diagrams and definitions can be found in the "Fish Anatomy and Website Sheets" folder.



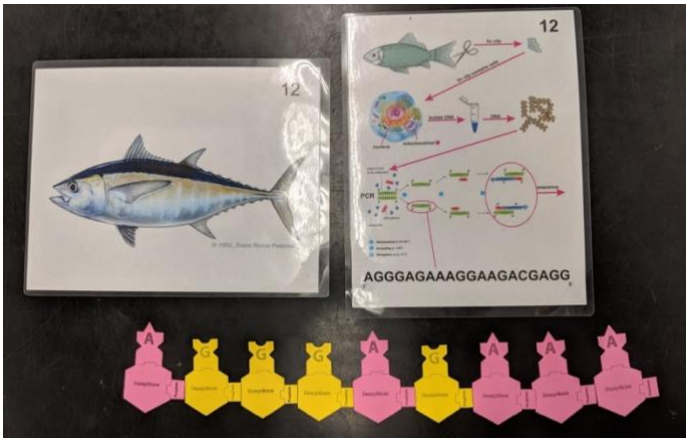
2. Once students have reached an identity for their fish based on morphological characteristics, discuss how using the flow chart went. Was it easy to ID these fish? What happens if you only have a part of the fish such as a fillet? What happens if it's been injured and is missing fins?
3. In these other circumstances, it is important to have additional methods for identification.

Part 3: Learning about DNA barcoding

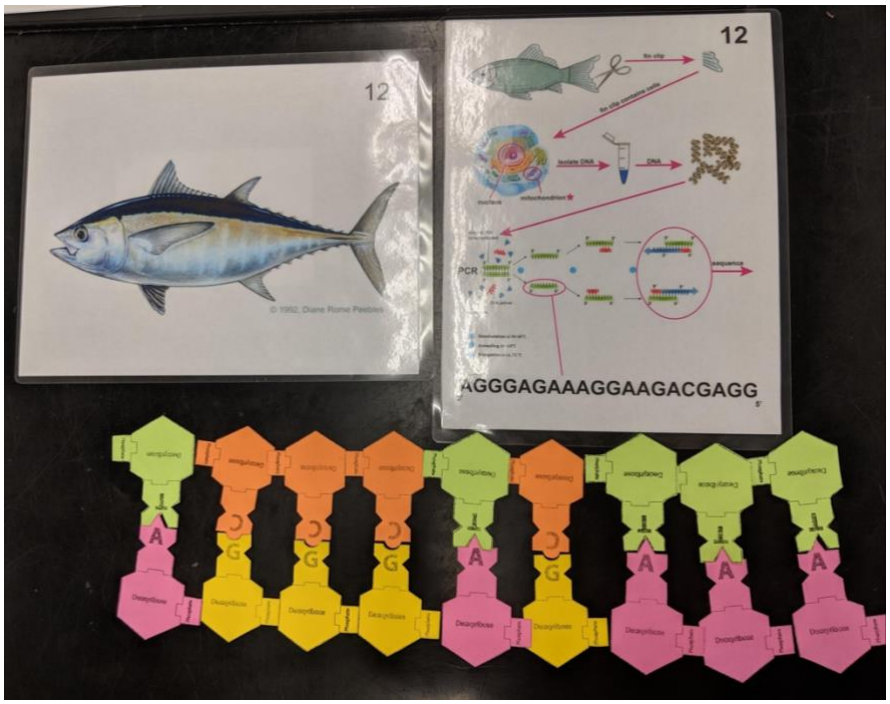
1. Pass out the appropriate DNA sheet to each student; the number on the DNA sheet corresponds to the number on the Fish with which the student is working.
    a. These DNA sheets are found in the folder "DNA sheets."



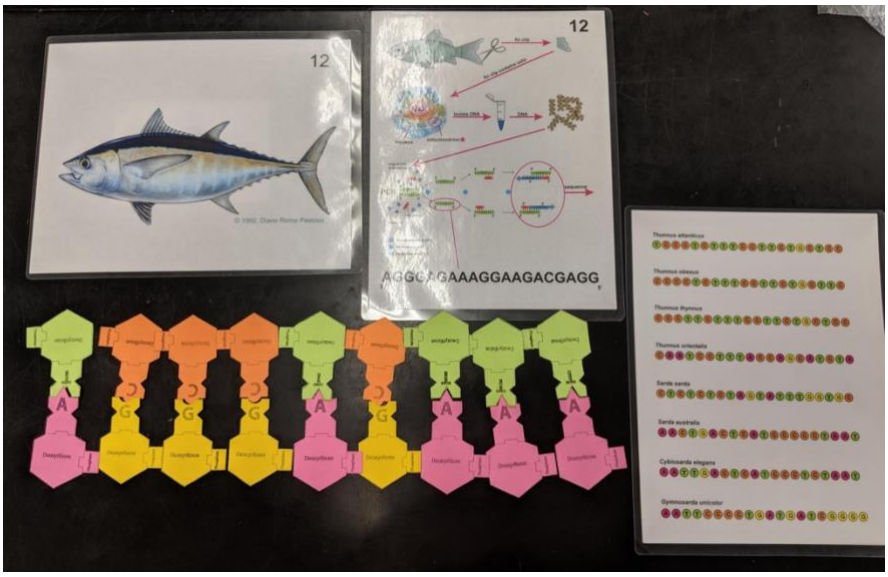
2. In DNA barcoding, we take a small piece of tissue, such as a fin clip, and isolate the DNA. We then use polymerase chain reaction (PCR) to make a lot of copies of the barcoding gene. For fish we use the mitochondrial COI gene as the barcoding gene because every species of vertebrate has a different version of this gene and so we can use it to identify the fish. [We use different genes for other taxonomic groups like bacteria, fungi or plants.]
3. On each student's DNA sheet, there is a unique DNA sequence which matches the COI sequence for that specific species of fish. We need to compare this COI sequence to a series of known sequences. In real life, these known sequences are hosted on online databases by scientists around the world (i.e., NCBI, BOLD) or regulatory agencies (i.e., the FDA RSSL). In this case, we are going to compare that sequence to sequences found in the folder "Barcode Key."
    a. Websites for examples of these databases can be found in "Fish Anatomy and Website Sheets."
4. Using the bases in the folder "DNA Puzzle" have students first build the DNA sequence that is given to them on the DNA sheet.
    a. We suggest printing the bases on colored paper (A on pink, C on orange, G on yellow and T on green). This will make it easier to find the correct sequence on the Fish Barcode Key.



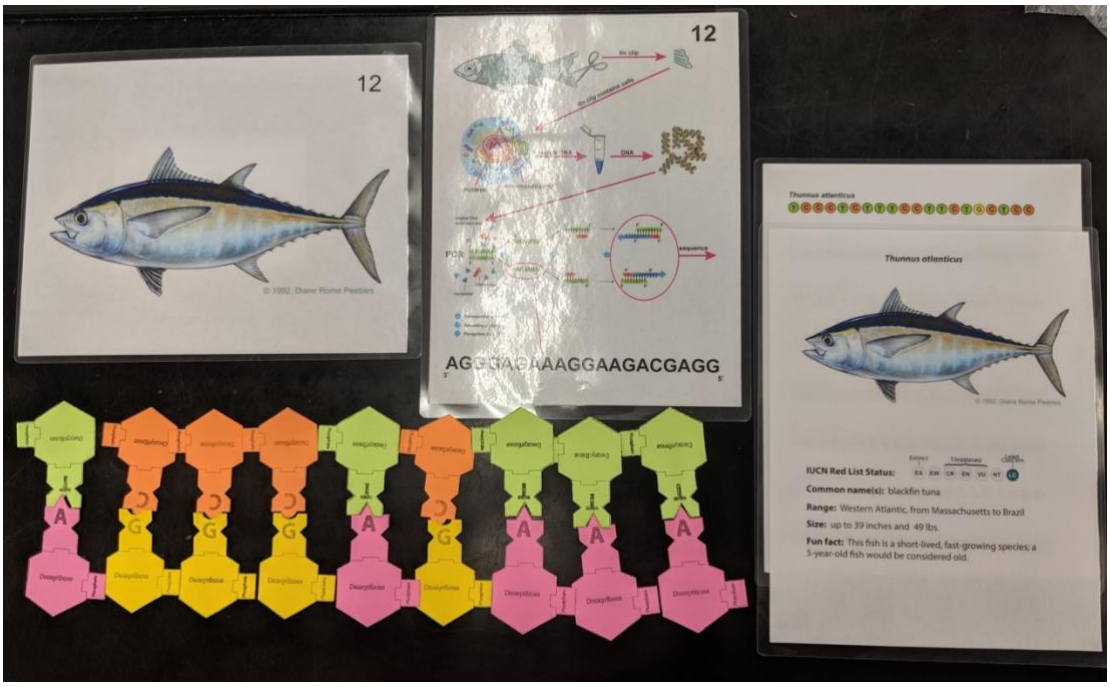
5. However, the Barcode Key is a library made up of the **complementary** sequence of the COI sequence listed on the DNA sheet. Therefore, have students build the reverse complementary strand of DNA for the given DNA sequence.



6. Use this complementary strand to compare to the known sequences on the Barcode Key.



7. Once a student has barcoded their fish, you can present them with a fact sheet about the fish.
    a. Can be found in “Fish Info Sheets.”



8. Did your students id the fish correctly both ways? Discuss the pros and cons of each method of identification.

Fish by #
1 – *Cybiosarda elegans* (leaping bonito)
2 – *Euthynnus affinis* (Kawakawa)
3 – *Euthynnus lineatus* (black skipjack)
4 – *Gymnosarda unicolor* (dogtooth tuna)
5 – *Paralichthys californicus* (California halibut, California flounder)
6 – *Paralichthys dentatus* (Summer founder, fluke)
7 – *Sarda australis* (Australian bonito)
8 – *Sarda sarda* (Atlantic bonito)
9 – *Scomber scombrus* (mackerel, Atlantic mackerel)
10 – *Scomberomorus maculatus* (Atlantic Spanish mackerel)
11 – *Scomberomorus regalis* (Cero, pintado kingfish, painted mackerel)
12 – *Thunnus atlanticus* (blackfin tuna)
13 – *Thunnus obesus* (bigeye tuna)
14 – *Thunnus orientalis* (Pacific bluefin tuna, bluefin tuna)
15 – *Thunnus thynnus* (bluefin tuna, Atlantic bluefin tuna)