Identifying Species of Fish Through DNA Barcoding and Phylogenetic Analysis
Daniel Launudy, Fatemah Sarsak, Gisel Medina, Gordon Mosher, Khine Thinn, Michael Samaan, Patricia Koenig, Raul Gonzalez, Syed Haque, Shea Martinez, Salama Sheren, Sunny Trivedi and Zia Hussaini
University of California Riverside (UCR) and Norco College

Abstract
One can identify the species of fish based on the sequence of DNA nucleotides in a specific region. To accomplish that, however, one must extract the DNA in the fish, cut the targeted gene with specific enzymes, simplify that gene using PCR (Polymerase Chain Reactions), and finally sequence the DNA and upload the sequence to a database where the species could be confirmed. Based on the data of this experiment, the analysis of the DNA of some fish mismatched the label on the fish when bought.

Method

1. Pour a 1.5% agarose gel.
2. Put 0.8 g of agarose in a boiling flask.
3. Fill the flask with 1X TAE to the red line.
4. Microwave for 1.5 mins.
5. Add 2.0 µl of Ethidium Bromide.
6. Load 5.0 µl of ladder into the first well
7. Load 12.0 µl of each reaction into the other wells.
8. Run the gel at 170V for 20 min.
9. Photograph the gel using the gel imager.

DNA Sequencing
1. To sequence the DNA, use the Big Dye Cycle.
2. The strands to be sequenced are copied using chemically altered bases.
3. The process is carried out for all four bases and then put together like a jigsaw revealing the sequence of the DNA.

DNA Subway
1. To analyze the sequence, use DNA Subway.
2. Create a project on the Blue Line, which will have all the necessary steps.
3. Run the “Sequence Trimmer.”
4. After trimming, the software needs to be told the forward and reverse pairs. Open the Pair Builder tool and select the two members of the pair.
5. Run the Consensus Builder tool, that will create one sequence from each pair.
6. Move on to “Add Sequences” and run BLAST for each consensus sequence, when the data is ready it will show as “View Results”.
7. Use this information to determine which species your sequence came from.

Results

TOTAL SPECIES: 20
- Ictalurus punctatus
- Pangasianodon-hypophthalmus
- Coryphaena Hippos
- Osmerus Mordax
- Oncorhynchus Mykiss
- Colobissaria
- Nemipterus Hexodon
- Thunnus Albacares
- Salmo Salar
- Seriola Quinqueracliata
- Dissostichus eleginoides
- Ictiobububalus
- Xiphias gladus
- Oreochromis Aureus Niloticus
- Trachinotus Blochi
- Fiadus Macrocephalus
- Sebastis Crameri
- Hoplostethus Atlanticus
- Parophys Velulus
- Ictalurus Punctorus
- Rastrelliger Kanagurta

TOTAL MISMATCH: 16

Analysis
There were a wide variety of fish used in this experiment including Catfish, Cod, Mahi Mahi, Ahi, Red Snapper, Orange Roughy, Swai, Tilapia, Salmon, Chilean seabass, Dole, Salmi, Atlantic Salmon, Albacore, Yellowfish, Indian mackerel, Alaskan code, Whitefish basa, Basa filet, River smelt, Smelt lake, Mackerel pike, Tuna, Golden thread, Norwegian saba, Buffalo Fish, and Swordfish. There was no general trend in terms of which type of fish’s PCR sample was able to amplify. In this experiment, students were able to collect DNA samples from local fish and amplify the 16 S region of the DNA in order to analyze the region’s amplicons. The DNA was then sequenced, converted to a .ab1 file, and compared with known databases using the DNA Subway. Based on the initial PCR data collected, the Swai fish and the Catfish provided polarizing results as there were cases of no amplification and decent amplification of DNA.

Conclusion
In the experiment the DNA of 77 different samples of fish were isolated and amplified using PCR. The target DNA sequence was analyzed in the DNA Subway to determine what species each sequence came from. Out of the 77 samples, it was determined that there were 20 different species of fish.

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References
- Paul Hebert’s research group at the UoG
- Rastrelliger Kanagurta
- Ictalurus Punctatus
- Parophrys Vetulus
- Sebastis Crameri
- Dissostichus eleginoides
- Ictalurus Punctorus
- Rastrelliger Kanagurta

Inland empire has great variety of ranging from small to big fishes in reservoir and lakes. The prominent fishes are Trout, Black bass, Panfish, Catfish and rest are in local fish market. Some of the big and small bites are also caught in Big Bear, Corona Lake,Elsinore, and Lake Perris. Inland empire has great variety of ranging from small to big fishes in reservoir and lakes. The prominent fishes are Trout, Black bass, Panfish, Catfish and rest are in local fish market. Some of the big and small bites are also caught in Big Bear, Corona Lake,Elsinore, and Lake Perris. Inland empire has great variety of ranging from small to big fishes in reservoir and lakes. The prominent fishes are Trout, Black bass, Panfish, Catfish and rest are in local fish market. Some of the big and small bites are also caught in Big Bear, Corona Lake,Elsinore, and Lake Perris. Inland empire has great variety of ranging from small to big fishes in reservoir and lakes. The prominent fishes are Trout, Black bass, Panfish, Catfish and rest are in local fish market. Some of the big and small bites are also caught in Big Bear, Corona Lake,Elsinore, and Lake Perris.