Economy Matters: Mislabeling and *Pseudomonas* Contamination of Salmon sold in Seattle and New York

Authors: Naomi Alam[1] and Sofia Kashtelyan[2] Mentor: Christine Marizzi, Ph.D.[3]

[1]Manhattan Center for Science and Mathematics; [2]High School of American Studies; [3]Cold Spring Harbor Laboratory, DNA Learning Center[3]

Cold Spring Harbor Laboratory DNA Learning Center

Funded by: The Pinkerton Foundation

URBAN BARCODE RESEARCH PROGRAM

Manhattan Center for Science and Mathematics

Introduction

Aim: In our project we focused on the mislabeling and contamination of salmon products sold in New York City (NYC) and Seattle.

Intended Outcomes: We hypothesize that: 1) Restaurants will serve more mislabeled fish in comparison to supermarkets. Restaurants are not required to provide consumers with species-specific labels, thus increasing the chance of seafood fraud. 2) We also searched for the existence of parasites such as Japanese broad tapeworms in our samples, as recent CDC studies reported that wild pink salmon caught in Alaska had been infected by this parasite.

Abstract: Our research team tested 12 samples of fresh and smoked salmon from the Pacific and Atlantic coasts to test the validity of proper fish labeling in grocery stores, fish markets and restaurant by utilizing DNA barcoding (1). Furthermore, we tested each piece of salmon for the presence of parasites such as Japanese broad tapeworms and bacteria. At first, we used more generic primers to test the salmon samples, but we then used more specific primers to more accurately identify the species. The result of our research was that salmon mislabeling is not prevalent in NYC nor Seattle. Only one of our samples was inaccurately labeled: Atlantic salmon *Salmo Salar* was sold as Pacific Red (Sockeye) salmon *Oncorhynchus nerka*. However, we found *Pseudomonas* species in all of our samples, including *Pseudomonas fluorescence* that can be potentially harmful for vulnerable immunocompromised patients.

What is DNA Barcoding?

Like a unique pattern of bars in a universal product code (UPC) identifies each consumer product, a short "DNA barcode" (~700 nucleotides) is a unique pattern of DNA sequence that can potentially identify each species.

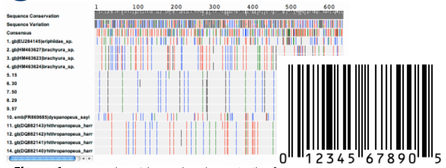


Figure 1: DNA and UPC barcodes share similar features.

Materials & Methods

Our project workflow is depicted in Figure 2. We obtained 12 samples of salmon from 2 locations - NYC and Seattle. In NYC we obtained 8 samples in the Bronx and 1 sample in Manhattan (9 total). In Seattle all three samples came from the same location. The samples we collected ranged in price (2.75 to 15.79 $ per sample) and type (50 % fresh/25% frozen/25% smoked.) Additionally, 50% of samples were purchased in grocery stores while 50% of came from restaurants.

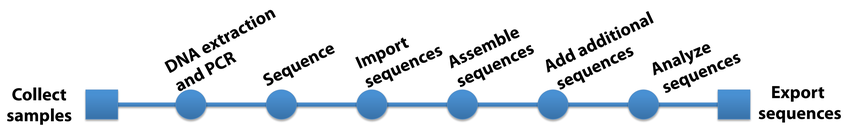
Following the standard DNA barcoding protocol we extracted genomic DNA from sample tissue using the silica DNA extraction method (2). We performed polymerase chain reaction (PCR) to amplify the *cytochrome oxidase 1 (CO1)* barcode for fish and parasites, and tested and compared the two different primer sets for fish-specific DNA barcoding. In addition, we employed PCR to amplify the 16S rRNA gene variable regions for bacteria using universal and *Pseudomonas* genus specific primers (Table 1).

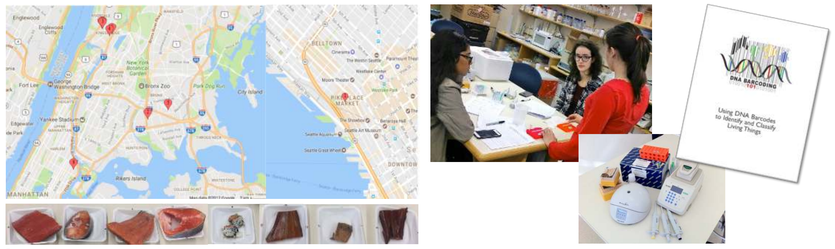
PCR success was verified through gel-electrophoresis (Figure 3). Samples that showed sufficient amplification were sent off for PCR cleanup using ExoSAP-IT™, and Sanger cycle-sequencing using Big Dye on an ABI 3730 Automated Sequencer through GENEWIZ. After obtaining the sequences we used the DNA Subway interface to assemble, trim and edit the sequences, then aligned the sequences using the program MUSCLE. Results were verified through independent search on Basic Local Alignment Search Tool, nucleotide work suite (BLASTn) and The Barcode of Life Datasystems (BOLD) (2).

Table 1: Primers used in this study.

Primer Name	Sequence 5' -> 3' (with M13 tags)	DNALC Name	Reference
Fish			
VF2_t1-M13F	TGTAAAACGACGGCCAGTCAACCAACCACAAAGACATTGGCAC	Fish Cocktail	Ivanova et al. 2007 (2)
FishF2_t1-M13F	TGTAAAACGACGGCCAGTCGACTAATCATAAAGATATCGGCAC	Fish Cocktail	Ivanova et al. 2007 (2)
FishR2_t1-M13R	CAGGAAACAGCTATGACACTTCAGGGTGACCGAAGAATCAGAA	Fish Cocktail	Ivanova et al. 2007 (2)
FR1d_t1-M13R	CAGGAAACAGCTATGACACCTCAGGGTGTCCGAARAAYCARAA	Fish Cocktail	Ivanova et al. 2007 (2)
FISHCOILBC_ts-M13F	TGTAAAACGACGGCCAGTCAACYAATCAYAAAGATATYGGCAC	FishBOL	Baldwin et al. 2009 (2)
FISHCOIHBC_ts-M13R	CAGGAAACAGCTATGACACTTCYGGGTGRCCRAARAATCA	FishBOL	Baldwin et al. 2009 (2)
Invertebrate			
mlCOIint-M13F	TGTAAAACGACGGCCAGTGGWACWGGWTGAACWGTWTAYCCYCC	Updated DMI	Leray et al. 2013 (2)
mlCOIintR-M13R	CAGGAAACAGCTATGACGGRGGRTASACSGTTCASCCSGTSCC	Updated DMI	Leray et al. 2013 (2)
Bacteria			
Bac1492Runiv-M13R	CAGGAAACAGCTATGACCGGTTACCTTGTTACGACTT	16S rRNA Bacteria	Jiang et al. 2006 (2)
Bac27Funiv-M13F	TGTAAAACGACGGCCAGTAGAGTTTGGATCMTGGCTCAG	16S rRNA Bacteria	Jiang et al. 2006 (2)
PA-GS-M13F	TGTAAAACGACGGCCAGTGACGGGTGAGTAATGCCTA	Pseudomonas Specific	Spilker et al. 2004 (2)
PA-GS-M13R	CAGGAAACAGCTATGACCACTGGTGTTCCTTCCTATA	Pseudomonas Specific	Spilker et al. 2004 (2)

The DNA Barcoding Workflow





Representative photographs of the fish samples collected.

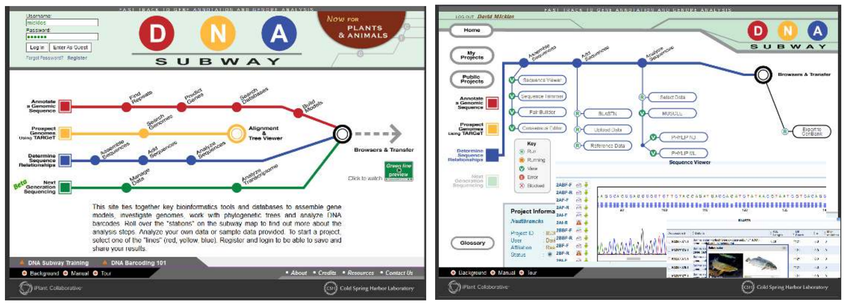


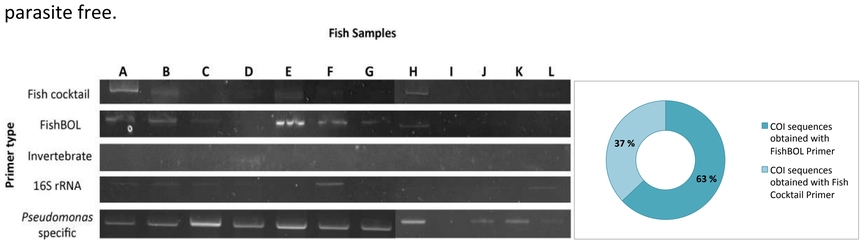
Figure 2: DNA barcoding workflow.

Results

DNA extraction, PCR and sequencing:

Of the 12 samples we extracted, 75% amplified and sequenced for both fish *CO1* and 16S rDNA (Figures 3 and 4). Fresh samples amplified with a success rate of 90%, in contrast to frozen (66%) and smoked samples (66%).

Updated Baldwin primers (FishBOL) had higher efficacy in both amplification and species discrimination compared to standard Fish cocktail primers (63% vs. 37% success rate, respectively). Interestingly, standard Fish cocktail primers amplified weakly and returned several sequencing results that matched *Pseudomonas* species on GenBank. We verified this result with an additional PCR using 16S rDNA and *Pseudomonas* genus specific primers. None of the samples showed positive PCR amplification with invertebrate specific *CO1* primers, and we considered them parasite free.



Figures 3 (left) and ***4*** (right) show the efficacy of primers in DNA amplification and sequencing.

Species Identification:

9 out of 12 salmon samples were identified to the species level (Table 2); and represented four different salmon species: *Oncorhynchus nerka* (sample A; 8%), *Oncorhynchus tshawytscha* (sample H; 8%) , *Oncorhynchus keta* (samples I,J; 16%) and *Salmo salar* (samples B,C,E,F,G; 41%).

Sample	Origin	Description	Price/Sample	Fish sp. Per Label	*COI* Barcoding ID	Common Name
A	Grocery Store	Frozen Fillet	$15.79	Sockeye Salmon	*Oncorhynchus nerka*	Sockeye Salmon
B*	Grocery Store	Frozen Fillet	$7.99	Red (Sockeye) Salmon	*Salmo Salar**	Atlantic Salmon*
C	Grocery Store	Fresh Fillet	$9.99	Atlantic Salmon	*Salmo salar*	Atlantic Salmon
D	Grocery Store	Fresh Fillet	$9.99	Not declared	No sequence obtained	No sequence obtained
E	Grocery Store	Fresh Fillet	$10	Farmed Salmon	*Salmo salar*	Atlantic Salmon
F	Restaurant	Sushi	$2.75	Atlantic Salmon	*Salmo salar*	Atlantic Salmon
G	Restaurant	Sashimi	$3	Scottish Salmon	*Salmo salar*	Atlantic Salmon
H	Restaurant	Sashimi	$4	King Salmon	*Oncorhynchus tshawytscha*	King Salmon
I	Fish Market	Smoked Salmon	$5.59	Keta Salmon	*Oncorhynchus keta*	Keta Salmon
J	Fish Market	Smoked Salmon	$11.98	Keta Salmon	*Oncorhynchus keta*	Keta Salmon
K	Fish Market	Smoked Salmon	$11	Keta Salmon	No sequence obtained	No sequences obtained
L	Grocery Store	Frozen Fillet	$5.49	Canadian Salmon	No sequence obtained	No sequence obtained

Table 2: Sample analysis and fish species with their common names and scientific names. * Indicates substitution.

The 16S rDNA sequences generated with *Pseudomonas* specific primers allowed to identify 4 *Pseudomonas* species in our salmon samples (Table 3): *P. fragi, P. fluorescence, P. psychrophila,* and *P. parafulva*, based on 100% of similarity with other reference sequences of those species included in GenBank. 58 % of fish samples contained *Pseudomonas* but GenBank was unable to resolve it down to the species level, therefore they were classified as *Pseudomonas sp*.

Sample	*Pseudomonas Spp.*	Characteristics	Pathogenesis reported for humans
A	*Pseudomonas sp.*		Depending on species
B	*P. fragi*	Opportunistic microbiota (3), Food spoilage (4)	None
C	*P. parafulva*		None
D	*P. parafulva*		None
E	*P. fluorescens*	Opportunistic pathogen in fish (5), Seafood spoilage (6)	Immunocompromised patients (7)
F	*Pseudomonas sp.*		Depending on species
G	*Pseudomonas sp.*		Depending on species
H	*Pseudomonas sp.*		Depending on species
I	*Pseudomonas sp.*		Depending on species
J	*Pseudomonas sp.*		Depending on species
K	*P. fluorescens*	Opportunistic pathogen in fish (5), Seafood spoilage (6)	Immunocompromised patients (7)
L	*P. psychrophila*		None

Table 3: DNA characteristics of the *Pseudomonas* species detected in commercial fish and potential risk for humans.

Discussion

We conclude that out of the 12 samples one was mislabeled. One grocery store sold Atlantic salmon as Pacific Red salmon. Scottish salmon is generally farm-raised Atlantic salmon and we conclude sample G as correctly labeled. We were unable to detect parasite DNA via PCR, however, we identified bacteria of the *Pseudomonas* genus in all samples tested. This is not surprising, as the microflora that spoils fresh, ice-stored fish consists largely of *Pseudomonas sp.* (4,6.) Bacteria from the *Pseudomonas* genus cause psychotroph spoilage and do not survive heat processing, so their presence in processed food suggest postprocess contamination through biofilms on equipment (4,6). Although we did not find the most dangerous species of *Pseudomonas* for humans, *P. aeruginosa*, the *P. fluorescence* species found in our study could be potentially harmful for vulnerable or immunocompromised consumers. This is due to the capacity of *P. fluorescence* to adhere to nerves, and it was reported to have caused outbreaks in oncology patients (7). While PCR is rapid and sensitive, it does not distinguish living from dead bacteria. *Pseudomonas sp.* are considered a health hazard for consumers only when their number exceeds 10^6- 10^7 CFU/g of product (4,6). Suggestions for further research include additional testing using Standard Plate Count or fluorescence-based techniques to measure the count of total viable microorganisms in the samples.

References

(1) http://usa.oceana.org/press-releases/oceana-reveals-mislabeling-americas-favorite-fish-salmon
(2) www.dnabarcoding101.org
(3) Gram, L.; Huss, H.H. Fresh and Processed Fish and Shellfish. In *The Microbiological Safety and Quality of Foods*; Lund, B.M., Baird-Parker, A.C., Gould, G.W., Eds.; Chapman and Hall: London, UK, 2000; pp. 472-506.
(4,6) Food Microbiology: An Introduction, Third Edition. ASM Press, 2012
(5) Bruno, D.W.; Ellis, A.E. Salmonid Disease Management. *Dev. Aquac. Fish. Sci.* 1996, 29, 729-824.
(7) Hseuh, P.R.; Teng, L.J.; Pan, H.J.; Chen, Y.C.; Sun, C.C.; Ho, S.W.; Luh, K.T. Outbreak of *Pseudomonas fluorescens* bacteremia among oncology patients. *J. Clin. Microbiol.* 1998, 36, 2914-2917.
(8) All fish pictures are retrieved from Wikipedia.org, May 14 2017.

Acknowledgements

This project was supported by the *Urban Barcode Research Program*, funded by the Pinkerton Foundation. We thank Melissa Lee, Alison Cucco and Jenna Dorey (DNA Learning Center, Cold Spring Harbor Laboratory) for their continuous assistance.