FASTFISH-ID: A multi-centre evaluation of a novel method for rapid non-targeted seafood identification

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Introduction

Mislabelled fish products hiding lesser-value or lower-quality species are a risk to the economic welfare of food companies and can damage brands and consumer trust. DNA analysis readily authenticates species in fish products, but the high costs and time required in sending specimens out for sequencing is a barrier to the large-scale testing needed to protect seafood industries. FASTFISH-ID (Thermagenix, Natick, MA, thermagenix.com) offers a novel solution for rapid and cost-effective on-site authentication of commercial fish species, which can be carried out anywhere along the supply chain in ~3 hours, including DNA extraction and amplification. Standardized DNA identification of animal species uses the sequence of a region of the mitochondrial COI gene as a universal molecular identity tag (DNA barcode). FASTFISH-ID enables convenient COI-based species DNA identification using this same region in a simple, one-step protocol without DNA sequencing.

Methods

- 75 blind samples from 18 commercially important fish species were tested by two independent laboratories to evaluate the FASTFISH-ID method
- The identity of each species was independently validated by conventional DNA barcode sequencing

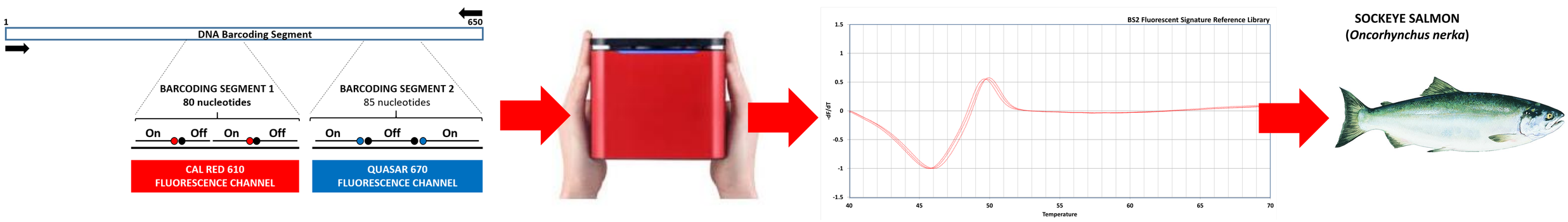

Figure 1. To run the test, DNA from a specimen is simply added to the test reagents. The test amplifies the entire COI DNA barcode and then interrogates two segments within the amplicon using two sets of fluorescent probes, each labeled with a unique color. Amplification and fluorescence measurements are done using a portable Mic (Magnetic Induction Cycler; Bio Molecular Systems Upper Coomera QLD, Australia; biomolecularsystems.com), and completed in ~2 hours. This results in fluorescent melt curves in two colors, one for each interrogated segment. The shape of the first derivative of these melt curves (the fluorescent signature) correlates to the DNA barcode sequences at each of the two targeted segments and is therefore unique for each fish species. Fluorescent signatures from a test specimen are uploaded to a website where an online algorithm automatically compares the signatures to a reference library and reports the species identity

Results

- Fluorescent probes generated unique sets of fluorescent signatures for each species
- Differences in target sequence produced different fluorescent signatures in either or both of the two interrogated segments within the amplified COI DNA barcode; species were identified based on these fluorescent signatures using an online database
- **75 blind samples from 18 commercially important fish species were tested by two independent laboratories with >96% accuracy**
- Failures were not incorrectly identified, but were due to low fluorescent signal which will be addressed with improved DNA extraction methods

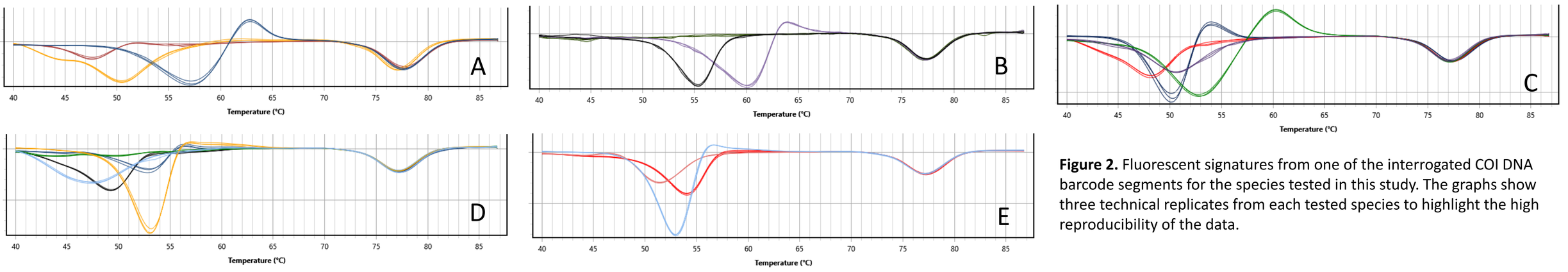

Figure 2. Fluorescent signatures from one of the interrogated COI DNA barcode segments for the species tested in this study. The graphs show three technical replicates from each tested species to highlight the high reproducibility of the data.

A: Red Snapper (*Lutjanus campechanus*), Atlantic Salmon (*Salmo salar*) and Pandora (*Pagellus erythrinus*); **B:** Pacific Ocean Perch (*Sebastes alutus*), Tilapia (*Oreochromis niloticus*), and Pacific Red Snapper (*Lutjanus peru*); **C:** Monkfish (*Lophius americanus*), Pacific Cod (*Gadus macrocephalus*), Channel Catfish (Ictalurus punctatus), Atlantic Cod (*Gadus morhua*), Swordfish (*Xiphias gladius*); **D:** Coho Salmon (*Oncorhynchus kisutch*), Haddock (*Melanogrammus aeglefinus*), Sockeye Salmon (*Oncorhynchus nerka*); and **E:** Atlantic Halibut (*Hippoglossus hippoglossus*), Gilt-Head Seabream (*Sparus aurata*) , Yellowtail Snapper (*Ocyurus chrysurus*) and King Salmon (*Oncorhynchus tshawytscha*).

Conclusion

The FASTFISH-ID method was shown to be highly accurate across technical and biological replicates by two independent laboratories for the 75 samples tested in this study. The method is non-targeted and portable, and could be used for species identification of fish fillets using the same set of reagents in a single-tube test. The simple protocols and automated analysis allows for use by anyone at any point in the supply chain when paired with existing rapid DNA extraction methods suitable for the field. In addition, species that are not currently in the reference database or that happen to be mislabelled can be sent for conventional DNA barcode sequencing without additional amplification. This can provide confirmation of results, or be used for regulatory compliance with agencies that use DNA barcoding as their official identification method (e.g. the U.S. FDA), as the full COI DNA barcode region is amplified. The expansion of the species-specific library of fluorescent signatures is on-going as is evaluation by additional laboratories. **FASTFISH-ID offers a portable, turnkey solution for rapid and cost-effective on-site authentication of commercial fish species**

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