# Genetic Approaches for Identifying Shark Fins and Other Products:

## A TOOL FOR INTERNATIONAL TRADE MONITORING AND ENFORCEMENT

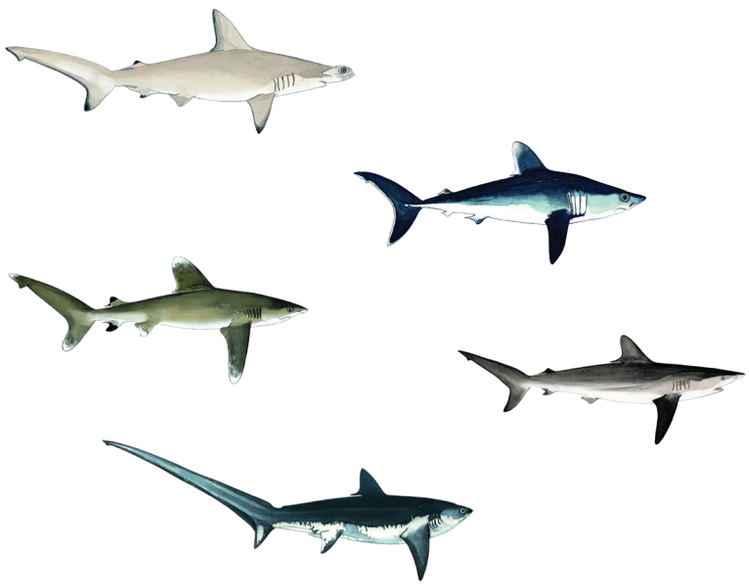


**Table 1:** The shark species included in Appendix II of CITES as of 2016.

| Species | Scientific Name | Effective Date in Appendix II* |
|---|---|---|
| **Basking shark** | *Cetorhinus maximus* | 2003 |
| **Whale shark** | *Rhincodon typus* | 2003 |
| **Great white shark** | *Carcharodon carcharias* | 2005 |
| **Oceanic whitetip shark** | *Carcharhinus longimanus* | 2014 |
| **Porbeagle shark** | *Lamna nasus* | 2014 |
| **Great hammerhead shark** | *Sphyrna mokarran* | 2014 |
| **Scalloped hammerhead shark** | *Sphyrna lewini* | 2014 |
| **Smooth hammerhead shark** | *Sphyrna zygaena* | 2014 |
| **Silky shark** | *Carcharhinus falciformis* | 2017 |
| **Bigeye thresher shark** | *Alopias superciliosus* | 2017 |
| **Common thresher shark** | *Alopias vulpinus* | 2017 |
| **Pelagic thresher shark** | *Alopias pelagicus* | 2017 |

* Convention on International Trade in Endangered Species of Wild Fauna and Flora Secretariat. “History of CITES listing of sharks (Elasmobranchii)”, https://www.cites.org/eng/prog/shark/history.php.

**Between 63 and 273 million sharks are killed every year** in the world's commercial fisheries (Worm *et al*. 2013), primarily caught for their fins and meat (Dent & Clarke 2015).

## Background

Due to drastic population declines and their value in international trade, a number of shark species have been included in Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) in recent years (Table 1). Several of these species are considered to be commercially important, traded in large numbers primarily for their fins and meat. In fact, the CITES listings for sharks that have been adopted since 2013 are considered landmark decisions for global shark conservation because they mandate substantially increased monitoring and regulation of the international trade in shark products, including fins. Prior to 2013, no regularly commercially-traded shark species were afforded international trade protections.

International trade of Appendix II listed sharks requires the CITES Management Authority of exporting countries to issue export documents certifying that the trade in each specimen is legal, traceable, and not detrimental to the survival of the species. Customs personnel of both exporting and importing nations, therefore must be able to recognize the traded products of these species and be able to readily identify illicit trade (i.e., trade across international borders without corresponding CITES documentation) in order to effectively implement and enforce their CITES obligations.

Some of the traded products of CITES listed shark species can be visually identified, particularly unprocessed, frozen or dried fins (e.g., www.identifyingsharkfins.org; Abercrombie & Hernandez 2017; Abercrombie *et al.* 2013). However, species identification or verification of products that have been modified or heavily processed throughout the supply chain (i.e., fins, meat, liver oil, personal care products, skin and teeth) becomes more difficult. In many of those cases DNA testing will be required, either to screen products randomly to detect illicit trade or to confirm or refute the identity of a product suspected to be derived from a CITES listed species from other types of evidence. There are DNA tools currently available to readily identify species-of-origin for shark fins, meat and other traded products at various points along the supply chain — from harvest to consumption — that will be used by laboratories that conduct genetic testing for CITES compliance and enforcement contexts.

This manual synthesizes all of the DNA protocols available in the published literature as of 2018 and outlines a useful process to decide which protocols are best to use under different scenarios. All CITES listed sharks can be reliably identified using these genetic protocols. These protocols will help ensure any continued international trade of these shark species is *legal*, *sustainable* and *traceable*. Any shark product can be genetically tested using these techniques. However, this manual focuses primarily on shark fins and meat because they are the most commonly traded products in high volumes across international borders and thus, fall under the purview of CITES controls.

**Monitoring and traceability of traded shark products** throughout the supply chain is crucial for ensuring effective implementation and enforcement of CITES provisions.

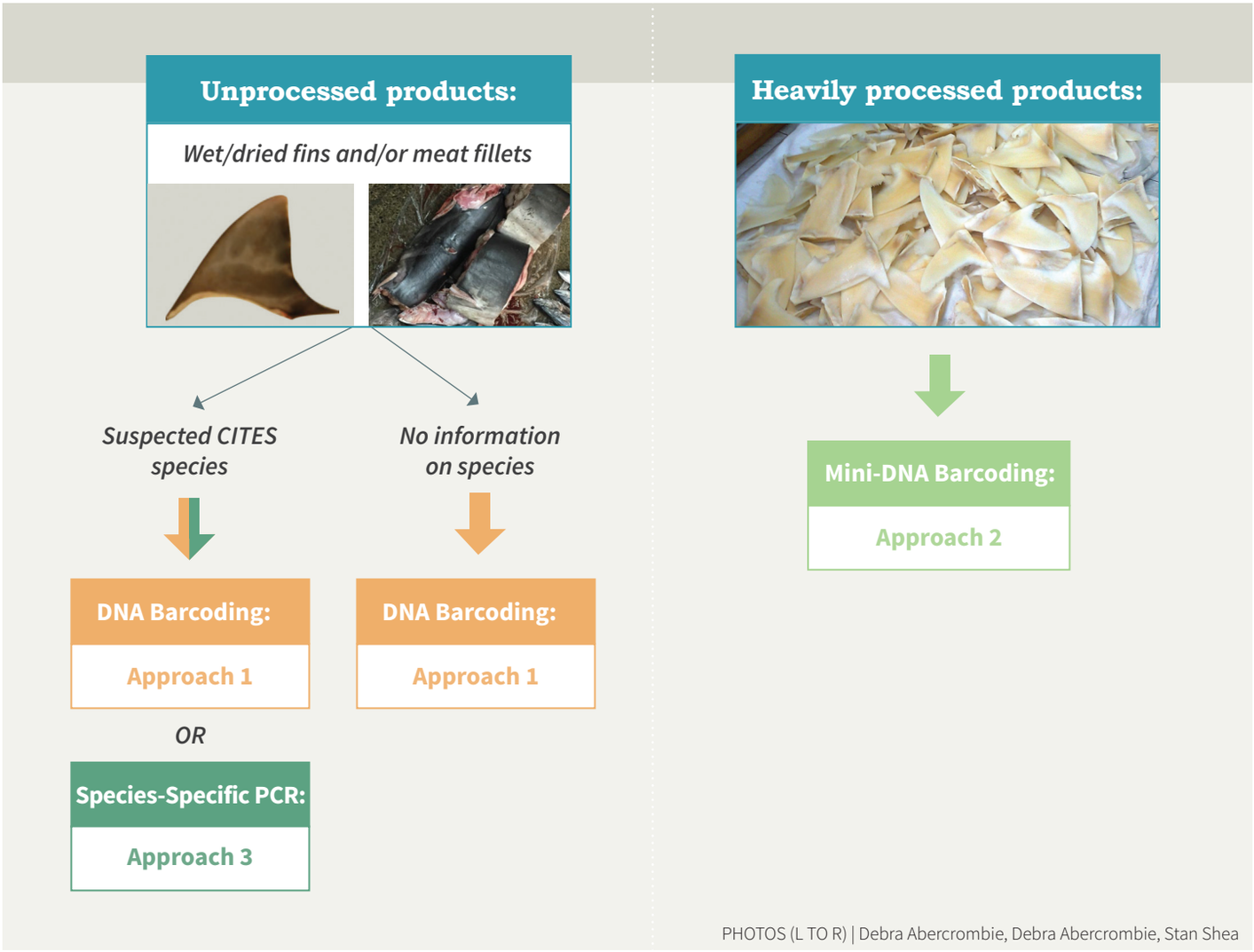
## Purpose Of This Manual

This manual was created for use by professional DNA analysts charged with the genetic testing of shark fins, meat fillets and other traded shark products in CITES-enforcement contexts. It applies to all shark[1] species listed in the Appendices of CITES as of October 2016 at the 17th meeting of the Conference of the Parties. In general, the process for streamlining and reducing costs associated with genetic testing of shark products collected as evidence can be summarized in three basic criteria.

**1** The number of samples from fins, meat fillets or other products that enforcement/border control personnel submit for DNA testing (ones, tens or hundreds);

**2** The level of processing of the fins, meat fillets and other products; and

**3** Whether or not the analyst has any prior evidence that the product(s) originated from CITES listed species.




PHOTOS (L TO R) | Debra Abercrombie, Debra Abercrombie, Stan Shea

**Figure 1:** Determining which genetic assay to use for species identification.

[1] While this manual is focused on the shark species listed in Appendix II of CITES, the methods would apply to the CITES listed ray species as well.

## How To Use This Manual

### **1:** How many samples need to be tested?

The number of samples taken as evidence from products (e.g., fins, meat fillets) within a detained shipment that will need to be genetically tested is going to vary from location to location and will also depend on how processed the product is. It is also likely to be a field agent (not the DNA analyst) collecting evidence from detained shipments. However, many labs equipped to screen wildlife products will have personnel trained in morphology who can assist with the visual identification of shark fins from CITES listed sharks and potentially reduce the amount of product for screening. While costs for using molecular techniques are continually decreasing, DNA testing takes time and funding, so it is important to select the approach that is the most efficient and accurately balanced against these potential costs. In some cases, shipments may be detained that contain only a small number of shark products. In larger detained shipments, DNA testing of tens or hundreds of fins, meat or other products may be required. This manual is meant to compliment established laboratory protocols for screening wildlife products (which will vary by location/country) and is meant to be used to help choose the most cost-effective protocol for testing shark products found in international trade.

### **2:** How processed is the shark product in question?

The most important step in choosing which genetic assay to employ is determining how processed the fins, meat fillets or other shark products are (Figure 1). The level of processing will determine how degraded the genomic DNA is going to be and therefore, will have implications for selecting the genetic approach that will be most successful at amplifying the genomic DNA present. Typically, heavily processed products at the end of the supply chain will have highly degraded DNA.

Once a shark has been harvested, the meat (Figure 2A) is kept on ice, frozen, or dried and salted. The quality of the DNA in fillet form can range from very good to degraded.

Wet fins (Figure 2B) are those that have been removed from a recently harvested shark but not dried or further processed. This includes frozen fins. These fins still have their skin on and are pliable.

Dried, unprocessed fins (Figure 2C) are rigid and still contain both skin and cartilage. Most fins entering international trade are in this condition. Both wet and dried, unprocessed fins typically contain high-quality genomic DNA that can be amplified using polymerase chain reaction (PCR).

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PHOTO | Demian Chapman

PHOTO | Stan Shea

**Figure 2:** Examples of shark products typically found in international trade: minimally processed shark meat (2A); wet, unprocessed shark fins (2B); dried, unprocessed shark fins (2C); and processed shark fins (2D).

Processed fins (Figure 2D) are dried fins that have been chemically treated to remove the skin and are a yellow or golden color. The genomic DNA of processed fins is often degraded, meaning the molecules are broken down into very small nucleotide fragments that are often incompatible with the use of standard genetic identification techniques.

While meat fillets and fins are the primary focus of this guide, it is worth noting that screening other forms of evidence may be required periodically. There may be circumstances where dried and salted meat products will be collected as evidence during routine inspections. Raw shark liver oil is traded internationally and is found in processed form in personal care products (e.g., cosmetics and skin care). Typically, deepwater sharks are targeted for liver oil, and often more than one species can be detected when screening for species identification purposes. Additionally, the DNA present in heavily processed meat and liver oil products will likely be highly degraded.

### **3:** Is there prior evidence the product was derived from a CITES listed species?

Once the number of samples taken as evidence for genetic testing has been determined, and also how processed these samples are, having prior evidence that the meat, fin(s) or other products are derived form a certain CITES listed shark species will be useful.

Fins from CITES listed shark species that are common in trade are visually distinctive based on their characteristic shape and/or markings. For example, the primary fins (dorsal, pectorals, lower caudal lobe) from CITES listed whale sharks (*Rhincodon typus*) and basking sharks (*Cetorhinus maximus*) are typically very large (>1-2 m tall). Border control personnel may therefore be able to visually identify the fin to species-of-origin and present a DNA analyst with a fin or fins that they already suspect comes from a CITES listed species. This would streamline the process for employing the appropriate technique based on the currently available published protocols highlighted in this manual.

For more information on how to visually identify CITES listed species, visit www.identifyingsharkfins.org.

### Summary

The protocols for three basic genetic approaches that have been developed for identifying shark products in trade are included in this manual. These protocols are from peer-reviewed literature and have been well validated. The details for each protocol are provided below:

- **Approach 1: DNA Barcoding (Page 5)**
- **Approach 2: Mini-DNA Barcoding (Page 7)**
- **Approach 3: Species-Specific PCR (Page 9**)

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## Approach 1: DNA Barcoding 1

DNA barcoding is the most widely used genetic approach and involves sequencing ~650 bp of the cytochrome c oxidase I gene and then using the resulting sequence as a query in the Barcode of Life Data System (BOLD) and/or using diagnostic bases (Wong *et al.* 2009) to identify species-of-origin. This is the **most expensive** and time consuming of the genetic approaches available (at $2.50-$5.00 per sample in reagents in most labs), **works best on products that have been well-preserved** and identifies species-of-origin for all tested samples. It is also **considered the strongest evidence in court**.

DNA barcoding is a sequence-based assay based on the mitochondrial cytochrome c oxidase I (COI) gene that yields an amplicon of ~550– 650 bp belonging to a particular species. Fins from all of the CITES listed sharks can be confidently identified using a 652-bp fragment from the 5' region of the COI and/or a 574 bp region of the NADH2 gene.

**This approach nearly always works on wet or dried, unprocessed fins but can suffer a higher rate of reaction failure on dried, processed fins.** The primers used to amplify and sequence these fragments are universal, so it is not necessary to have prior information on what the species-of-origin is to obtain a species identification.

1

## DNA Barcoding Protocol:

- COI PCR can be conducted using one of two sets of forward and reverse primer cocktails: "C_FishF1t1/ C_FishR1t1" or "C_VF1LFt1-C_VR1LRt1" (Ivanova *et al.* 2007, Wong *et al.* 2009; Table 2).
- All of these primers are appended with M13 tails to facilitate sequencing (Messing 1983).
- PCR can be performed in a volume of 50 uL, which includes 50–100 ng of DNA of genomic DNA extracted from ~10-25 mg of tissue, 10 pmol of each forward and reverse primer, 1X PCR buffer, 200 uM dNTPs, and 1 unit of HotStar© *Taq* Polymerase (Qiagen, Valencia, California).
- The thermal cycling program for PCR using the C_FishF1t1/ C_FishR1t1 primers (marked with * in Table 2) consist of 2 min at 94°C, followed by 35 cycles of 30 s at 94°C, 40 s at 52°C and 1 min at 72°C and concluding with 10 min at 72°C.
- The reaction program for samples using the C_VF1LFt1- C_VR1LRt1 primers consists of 2 min at 94°C, followed by 5 cycles of 30 s at 94°C, 40 s at 50°C, and 1 min at 72°C, followed by 35 cycles of 30 s at 94°C, 40 s at 54°C, and 1 min at 72°C, concluding with a 10 min at 72°C.
- NADH2 amplification and sequencing can be performed with the primers ASNM59 (5'- AAC GCT TAG CTG TTA ATT AA-3') and ILEM 59 (5'-AAG GAG CAG TTT GAT AGA GT-3').
- PCR is composed of 50 and 100 ng of template genomic DNA in a 25 µL volume containing 0.3 µM primers, 2.5 mM MgCl2, 200 µM dNTP, 10X buffer and 0.25 U *Taq* Polymerase.
- The thermal cycling program initiates with denaturation at 94 °C for 3 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 48°C for 30 s and extension at 72°C for 90 s (Naylor *et al.* 2012).
- Amplified fragments (~700 base pairs) can be resolved on a 2% agarose gel and visualized using ethidium bromide and UV transillumination to verify successful amplification.
- PCR products can be purified with ExoSAP-IT (Affymetrix, Inc., Santa Clara, CA, USA) and sequenced using the Big Dye Terminator v3.1 cycle sequencing kit following manufacturer instructions (Applied Biosystems, Foster City, CA, USA).
- The resulting products are precipitated with 125mM EDTA and 100% ethanol and run on a DNA Analyzer. Resulting sequences can then be validated by eye, trimmed for quality and any primer sequence present removed.

Confirmation that a full barcode sequence is from a CITES listed species is a two-step process. The sequence is entered in the searchable databases of (1) the Barcode of Life Data System (COI only; BOLD http://www.boldsystems.org/index.php/IDS_OpenIdEngine) and (2) National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (COI or NADH2; BLAST http://blast.ncbi.nlm.nih.gov/Blast.cgi), both of which will return the closest matching sequences to the unknown. All of the CITES listed species are identifiable in this manner alone. The COI-based species identification can also be checked by using the diagnostic bases presented for each of these species in Wong *et al.* (2009).

**Table 2:** Primer sequences for DNA barcoding elasmobranchs.†

| Primer Name | Sequence |
|---|---|
| ***VF2_t1** | 5'TGTAAAACGACGGCCAGTCAACCAACCACAAAGACATTGGCAC3' |
| ***FishF2_t1** | 5'TGTAAAACGACGGCCAGTCGACTAATCATAAAGATATCGGCAC3' |
| ***FishR2_t2** | 5'CAGGAAACAGCTATGACACTTCAGGGTGACCGAAGAATCAGAA3' |
| ***FR1d_t1** | 5'CAGGAAACAGCTATGACACCTCAGGGTGTCCGAARAAYCARAA3' |
| **VF1_t1** | 5'TGTAAAACGACGGCCAGTTCTCAACCAACCACAAAGACATTGG3' |
| **VF1d_t1** | 5'GTAAAACGACGGCCAGTTCTCAACCAACCACAARGAYATYGG3' |
| **VF1i_t1** | 5'TGTAAAACGACGGCCAGTTCTCAACCAACCAIAAIGAIATIGG3' |
| **VR1_t1** | 5'CAGGAAACAGCTATGACTAGACTTCTGGGTGGCCRAARAAYCA3' |
| **VR1d_t1** | 5'CAGGAAACAGCTATGACTAGACTTCTGGGTGGCCAAAGAATCA3' |
| **VR1i_t1** | 5'CAGGAAACAGCTATGACTAGACTTCTGGGTGICCIAAIAAICA3' |

† The forward and reverse primers marked with * are each mixed into a forward or reverse primer cocktail at a 1:1 ratio, which is then used for PCR (the "C_FishF1t1/ C_FishR1t1" cocktails). The remaining primers are part of the "C_VF1LFt1- C_VR1LRt1" cocktails in which the forward and reverse primers are mixed prior to PCR in a 1:1:3 ratio with three times the amount of the primers VF1i_t1 and VR1i_t1 than the other two primers in each cocktail.


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## Approach 2: Mini-DNA Barcoding 2

Mini-DNA barcoding involves using a shorter fragment of the mitochondrial COI (<200 bp) to identify species-of-origin when the product being tested contains **highly degraded DNA** (Fields *et al*. 2015; Cardeñosa *et al.* 2017). Time and costs are comparable to that for DNA barcoding, and this approach provides robust identifications for CITES listed sharks and identifies non-CITES listed sharks to the genus or species level.

There is one mitochondrial cytochrome c oxidase I (COI) gene mini-barcode assay currently available in the literature that can identify most sharks in trade (Cardeñosa *et al.* 2017). The primers used to amplify and sequence this fragment are universal for sharks, so it is not necessary to have prior information on what the species-of-origin is to achieve identification of all CITES listed shark species. The mini-barcode multiplex PCR assay yields two short COI fragments (~150 bp amplicon and ~200 bp amplicon) and in some cases, the full COI sequence (~650 bp amplicon) simultaneously. After the initial amplification the analyst can sequence either one of the short fragments (or the full sequence, if it amplified) and attempt identification using BLAST, BOLD, and the diagnostic bases presented by Fields *et al.* (2015; for the ~150 bp amplicon) and Cardeñosa *et al.* (2017; for the ~200 bp amplicon). The sequences from the 150 and 200 bp amplicons combined, or the full COI sequence, allows for the identification of nearly all shark species in trade regardless the product (e.g., from cosmetics to processed shark fins) and from all CITES listed shark species. **While this assay does require additional primers and up to two sequencing reactions (depending on species), the accuracy may outweigh any associated costs.**

2

***Note:*** *The oceanic whitetip shark (Carcharhinus longimanus) requires a full barcode assay (as described in Approach 1) or the Cardeñosa et al. (2017) mini-barcode assay for definitive identification. Further visual inspection of any genetically identified fin of this species is recommended. Oceanic whitetip fins have distinct rounded rather than a pointed apex (or distal portion of the fin; see also www.identifyingsharkfins.org for more information regarding shark fin landmarks), which is visible regardless of fin condition.*

**Multiplex Mini-DNA Barcoding PCR Protocol** (taken from Cardeñosa *et al.* [2017]):

- DNA can be extracted using 200 µL of 10% Chelex resin in a PCR tube with a processed shark fin tissue sample of ~2 $mm^2$.
- Thermal cycling conditions for the Chelex extraction consist of a 20 min step at 60°C, followed by a 25 min step at 99-100°C (depending on maximum thermocycler settings) and storage at 4°C. PCR can be performed in a volume of 25 µL, which includes 0.5 µL of extracted DNA, 12.5 µL of GoTaq® Hot Start Green Master Mix (Promega) and five primers (sequences and volumes listed in Table 3).
- All reactions should be run with a positive (i.e., shark genomic DNA previously confirmed to amplify with other primers) and negative (i.e., no DNA).
- Thermal cycling conditions consist of an initial denaturation at 94ºC for 2 min, followed by 35 cycles at 94ºC for 1 min, 52ºC for 1 min, and 72ºC for 1 min, with a final extension of 72ºC for 10 min.
- Multiplex PCR reactions can be checked on a 3% agarose gel and all products cleaned using ExoSAP-IT (Affymetrix, Inc., Santa Clara, CA, USA) and sequenced using Big Dye Terminator v3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA, USA).
- Sequencing can be performed on an ABI 3730 DNA Analyzer (Applied Biosystems) using the M13 forward primer to sequence the Shark150 and M13 reverse primer to sequence the Shark474 (Table 3). If the whole COI barcoding region amplified, then it would sequence with either the M13 forward or M13 reverse primer, yielding a longer sequence.
- Resulting sequences can then be validated by eye, trimmed for quality and any primer sequence present removed. All trimmed sequences can be entered in BOLD (FISH-BOL) and BLAST (GenBank) databases to identify them to the lowest taxonomic category possible (e.g., genus and/or species).

Confirmation that a mini-barcode sequence is from a CITES listed species is a two-step process. The sequence is entered in the searchable databases of (1) the Barcode of Life Data System (COI only; BOLD http://www.boldsystems.org/index.php/IDS_OpenIdEngine) and (2) National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (COI or NADH2; BLAST http://blast.ncbi.nlm.nih.gov/Blast.cgi), both of which will return the closest matching sequences to the unknown.

**Table 3:** Primer sequences used in the mini-barcode PCR assay.

*(All primers were used with a concentration of 10 µM).*

| Primer Name | Sequence |
|---|---|
| **VF2_tl** | 5'-TGTAAAACGACGGCCAGTCAACCAACCACAAAGACATTGGCAC-3' |
| **FishR1_tl** | 5'-CAGGAAACAGCTATGACACTTCAGGGTGACCGAAGAATCAGAA-3' |
| **FishR2_tl** | 5'-CAGGAAACAGCTATGACACCTCAGGGTGTCCGAARAAYCARAA-3' |
| **Shark150R** | 5'-AAGATTACAAAAGCGTGGGC-3' |
| **Shark474F** | 5'-CHATTTCCCAATATCAAACACC-3' |

PHOTO | Demian Chapman

## Approach 3: Species-Specific PCR 3

Species-specific polymerase chain reaction (ss-PCR) is an identification technique that does not involve DNA sequencing but instead **uses previously optimized primers that only amplify DNA from target species to provide a positive or negative test for that species**. Multiple species-specific primers can be combined in one reaction to simultaneously screen for more than one species at once. This approach typically has the **lowest per sample cost**.

This molecular approach uses multiple species-specific primers in a single reaction tube (multiplex PCR) to produce species-diagnostic amplicons designed to amplify only a specified portion of the genome from the shark species of interest. It also incorporates an internal positive control consisting of universal primers that amplify a larger region and serves as a positive control for the reaction. For example, amplification of only the positive control would indicate that genomic DNA was present in the reaction, but it did not come from a target shark species of interest. **This approach is most efficient when there is prior information that the fin or fins comes from a CITES listed shark species.** Otherwise multiple PCR reactions may have to be run and the costs and time involved can exceed the time employed to do DNA barcoding or mini-barcoding.

Fins from all but two of the CITES listed shark species (oceanic whitetip [*C. longimanus*] and whale shark [*R. typus*], explained in more detail) can be accurately identified using species-specific PCR of the nuclear internal transcribed spacer 2 (ITS2) or the mitochondrial cytochrome c oxidase I (COI) gene coupled with agarose gel electrophoresis. **This is the quickest and least expensive genetic approach for CITES species detection if the first ss-PCR test detects the target species. If the first test fails to detect the target species, additional ss-PCR tests will need to be employed and the costs and time will quickly reach or exceed barcoding costs.**

3

Universal primers and species-specific primers can be used in one PCR reaction to produce either:

**1)** From the "target species" the simultaneous amplification of a positive control amplicon (generated from the two universal primers) and a smaller species-specific amplicon of a diagnostic sized (generated from the species-specific primer and the universal primer); or

**2)** From "non-target species" the amplification of the positive control amplicon only due to failure of the species-specific primer to anneal to genomic DNA from non-target species.

There are multiplexing options available that allow for samples to be screened for multiple species simultaneously in one PCR when the amplicons produced are of sufficiently different sizes to allow for the discrimination of the samples to the species level. **This approach nearly always works on fins regardless of the level of processing.**

**However, this technique is extremely sensitive to contamination.** For this reason it is recommended that samples that test positive for a CITES listed species be reexamined visually (when practical) or using DNA barcoding for confirmation of species-of-origin. Additionally, it is also recommended that a negative control (where no shark genomic DNA is added) be used during the reaction to further verify that cross-contamination of one or more reagents has not occurred.

## Species-Specific PCR Protocol:

- PCR and thermal cycling protocols vary by multiplex, and users should consult the original publication describing each one (Table 4). Laboratory-specific optimization may also be required.
- The PCR products can be resolved by electrophoresis on 1.2% agarose gels and visualized using any commercially available DNA stain to verifiy amplification.
- The positive control does not always amplify when target DNA is present, which results in the presence of a lone species-specific amplicon on the gel.
- When a lone band is present on the gel, it is important to carefully assess its size to ensure that it is derived from the species-specific primer and not the universal primers because sharks exhibit ITS2 locus size variation, and some species have smaller positive control amplicons (e.g., the hammerhead ITS2 is ~860 bp [Abercrombie *et al.* 2005]).

Species-specific PCR assays have been published in the primary scientific literature for the great white shark (*Carcharodon carcharias*; Chapman *et al.* 2003), the basking shark (*C. maximus*; Magnussen *et al.* 2007), the porbeagle shark (*Lamna nasus*; Shivji *et al.* 2002) the silky shark (*Carcharhinus falciformis*; Shivji *et al.* 2002), three of the common, large-bodied hammerhead shark species — scalloped hammerhead (*Sphyrna lewini*; Abercrombie *et al.* 2005), smooth hammerhead (*S. zygaena*; Abercrombie *et al.* 2005), great hammerhead (*S. mokarran*; Abercrombie *et al.* 2005) and the three species of threshers — common thresher (*Alopias vulpinus*; Caballero *et al.* 2011), bigeye thresher (*A. superciliosus*; Caballero *et al.* 2011) and pelagic thresher (*A. pelagicus*; Caballero *et al.* 2011). It should be noted that for the three hammerhead species-specific primers, there is a possibility that one or more of these primers will create false positive results with the two, as yet untested, congener hammerhead species, *S. media* and *S. corona*. A species-specific primer developed for the dusky shark (*Carcharhinus obscurus*) co-amplifies genomic DNA from the Galapagos shark (*Carcharhinus galapagensis*) and the CITES listed oceanic whitetip shark (*C. longimanus*). It is recommended that samples that test positive using this primer be reexamined visually when practical, (Figure 3) or using DNA barcoding for species-of-origin confirmation. Currently, no species-specific primer has been published for the whale shark (*R. typus*). [2]


PHOTOS | Debra Abercrombie

**Figure 3:** Comparison of the shark fins from oceanic whitetip, *C. longimanus* (3A-dorsal fin, 3B-pectoral fins) and dusky shark, *C. obscurus* (3C-dorsal fin, 3D-pectoral fins).

[2] Currently, there are no species-specific primers for the CITES listed ray species (Family Pristidae and Family Mobulidae).

**Table 4:** Species-specific primer sequences and amplicon sizes.

| **ITS2 Universal Primers** - (These two primers produce a positive control ~670-1500 (depending on taxon) and can be used in conjunction with all species-specific primers listed below.) | | |
|---|---|---|
| **FISH5.8S-F primer sequence:** | 5'-TTAGCGGTGGATCACTCGGCTCGT-3' | |
| **FISH28S-R primer sequence:** | 5'-TCCTCCGCTTAGTAATATGCTTAAATTCAGC-3' | |
| **Hammerhead Shark ITS2 Multiplex** Abercrombie *et al.* (2005) | | |
| **Great hammerhead, *S. mokarran*** | 5'-AGCAAAGAGCGTGGCTGGGGTTTCGA-3' | 782 bp amplicon |
| **Scalloped hammerhead, *S. lewini*** | 5'-GGTAAAGGATCCGCTTTGCTGGA-3' | 445 bp amplicon |
| **Smooth hammerhead, *S. zygaena*** | 5'-TGAGTGCTGTGAGGGCACGTGGCCT-3' | 249 bp amplicon |
| **Pelagic Shark ITS2 Multiplex** Shivji *et al.* (2002) | | |
| **Oceanic whitetip, *C. longimanus**** | 5'-GTGCCTTCCCACCTTTTGGCG-3' | 480 bp amplicon |
| **Porbeagle, *L. nasus*** | 5'-GTCGTCGGCGCCAGCCTTCTAAC-3' | 554 bp amplicon |
| **Silky, *C. falciformis*** | 5'-ACCGTGTGGGCCAGGGTC-3' | 1085 bp amplicon |
| **Basking Shark ITS2 Multiplex** (two species-specific primers) Magnussen *et al.* (2007) | | |
| **Basking, *C. maximus*** | 5'-TCTCGGCCTCCGGGCGAACGAATGAGA-3' | 1100 bp amplicon |
| **Basking, *C. maximus*** | 5'-AAGATGCGGCACGCTGTTGGGCACGC-3' | 900 bp amplicon |
| **Thresher Shark CO1 Multiplex** Caballero *et al.* (2011) | | |
| **Universal thresher, *Alopias* sp.(Forward primer) **** | 5'-AGCTGGRGTTGAAGCYGGAG-3' | |
| **Common thresher, *A. vulpinus*** | 5'-TCCAGCATGTGCTAGATTTCCC-3' | 76 bp amplicon |
| **Bigeye thresher, *A. superciliosus*** | 5'- TTGATGAGATACCTGCTAAATGAAGC-3' | 129 bp amplicon |
| **Pelagic thresher, *A. pelagicus*** | 5'- GTTTGATATTGGGAGATTGCAGGG-3' | 198 bp amplicon |
| **Great White Shark Bi-Organelle Multiplex** (two species-specific primers) Chapman *et al*. (2003) | | |
| **Great white, *C. carcharias*** | 5'- GCTGGAGTTCATTCTCCGTGCTG-3' | 580 bp amplicon |
| ******* | 5'-AGTCAGAACTAGTATGTTGGCTACAAGAAT-3' | 511 bp amplicon |

*Primer designed for dusky shark, *C. obscurus,* but also amplifies oceanic whitetip and Galapagos shark, *C. galapagenis.*
**No universal thresher 'reverse' primer means no positive control will be produced when no target DNA is present in the reaction.
*** Generates amplicon with another primer (LAM499F 5'- GCTTCTCAGTAGACAACGCCACCCT-3').

3

## Genetic Identification Of Additional Shark Species

Shark species that are common in the international fin trade, but not currently listed under CITES, can also be identified using one or more of the genetic methods of the approaches detailed in this document. Examples of several of these species are presented below in Table 5.

**Table 5:** Additional shark species that can be identified using one or more of the approaches detailed in this document.

Y= Yes, that species can be identified conclusively using the designated approach, N= No, the species cannot be identified conclusively using the designated approach at present (i.e., further development is needed).

| Species | DNA Barcode | DNA Mini-Barcode | Species-Specific PCR | References |
|---|---|---|---|---|
| **Blue, *Prionace glauca*** | Y | Y | Y | Wong *et al.* (2009), Cardeñosa *et al.* (2017), Shivji *et al.* (2002) |
| **Shortfin mako, *Isurus oxyrinchus*** | Y | Y | Y | Wong *et al.* (2009), Cardeñosa *et al.* (2017), Shivji *et al.* (2002) |
| **Longfin mako, *Isurus paucus*** | Y | Y | Y | Wong *et al.* (2009), Cardeñosa *et al.* (2017), Shivji *et al.* (2002) |
| **Bull, *Carcharhinus leucas*** | Y | Y | N | Wong *et al.* (2009), Cardeñosa *et al.* (2017) |
| **Sandbar, *Carcharhinus plumbeus*** | Y | * | Y | Wong *et al.* (2009), Cardeñosa *et al.* (2017), Pank *et al.* (2001) |
| **Dusky, *Carcharhinus obscurus*** | Y | * | Y | Wong *et al.* (2009), Cardeñosa *et al.* (2017), Shivji et *al.* (2002) |
| **Tiger, *Galeocerdo cuvier*** | Y | Y | N | Wong *et al.* (2009), Cardeñosa *et al.* (2017) |

*= to genus only

## Conclusion

This manual provides information from the peer-reviewed literature on genetic approaches for identifying shark products to the species-of-origin. It is intended to assist DNA analysts charged with the collection of genetic evidence to support enforcement operations associated with the requirements of CITES. This document focuses on the screening of detained meat and fins, as an example, because these are the most commonly traded commodities across international borders derived from sharks. However, any shark product can be tested as needed along various points of the supply chain. Professional DNA analysts can decide which protocol(s) to use for rapid and efficient identification of shark derived products (either confiscated or randomly sampled) based on three criteria: (1) the number of samples from shark products that need to be DNA tested (ones, tens or hundreds); (2) the level of processing; and (3) whether or not the analyst has any prior evidence that the products were derived from CITES listed shark species.

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