Short protocol for mitochondrial CO1 gene analysis of shark

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Shark Tissue Sampling

1 Shark Tissue Sampling

Prepare sampling tools and equipment: camera, ruler or scale band, gloves, scissors or cutter and tweezers, tube, ethanol 96%, ID label, and sampling form for writing sample information details.

2 Start with take a photograph of shark in full length, with scale.

3 Then, measure shark total length (TL) in centimetre and total weight in kilogram if possible.

4 After that, collect shark tissue sample. Tissue sample can be collected from any part of the shark, usually taken from the part where the fin has been cut. Cut tissue into small piece (~1-3 cm) using scissor or cutter.

5 Put shark tissue into a tube containing ethanol 96%.

6 Put label for each tube, label include number, location, and date of collection.

7 Store samples safely and brought to the lab for DNA extraction process.

8 The last step, collect sample information details, including shark landing site, date-month-year, species name, sex, total length, total weight, fishing ground and other necessary information. These information also can be collected by interviewing the fishermen.

DNA Extraction

9 DNA Extraction

This DNA extraction short protocol was performed by following the gSYNC^{TM} DNA Extraction Kit Quick Protocol [1]. There are 5 steps to extract tissue sample of shark: tissue sample dissociation, cell lysis, DNA binding, wash, and DNA elution.

10 - Tissue sample dissociation

Transfer up to 25 mg of shark tissue to a 1.5 ml microcentrifuge tube. Add 200 µl of GST Buffer and 20 µl of Proteinase K then vortex thoroughly. Incubate at 60ºC overnight or until the sample lysate becomes clear.

During incubation, transfer the required volume of Elution Buffer (200 µl/sample) to a 1.5 ml microcentrifuge tube and heat to 60ºC (for DNA elution step).

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11 **- Cell lysis**

If insoluble material remains following incubation, centrifuge for 2 minutes at 14-16,000 x g then carefully transfer the supernatant to a new 1.5 ml microcentrifuge tube. Add 200 µl of GSB Buffer and shake vigorously for 10 seconds. NOTE: It is essential that the sample and GSB Buffer are mixed thoroughly to yield a homogeneous solution.

12 **- DNA binding**

Add 200 µl of absolute ethanol to the sample lysate and mix immediately by shaking vigorously for 10 seconds. If precipitate appears, break it up as much as possible with a pipette. Place a GS Column in a 2 ml Collection Tube. Transfer all of the mixture (including any insoluble precipitate) to the GS Column. Centrifuge at 14-16,000 x g for 1 minute. Following centrifugation, if the mixture did not flow through the GS Column membrane, increase the centrifuge time until it passes completely. Discard the 2 ml Collection Tube containing the flow-through then transfer the GS Column to a new 2 ml Collection Tube.

NOTE: It is important that the lysate and ethanol are mixed thoroughly to yield a homogeneous solution.

13 **- Wash**

Add 400 µl of W1 Buffer to the GS Column. Centrifuge at 14-16,000 x g for 30 seconds then discard the flow-through. Place the GS Column back in the 2 ml Collection Tube. Add 600 µl of Wash Buffer (make sure absolute ethanol was added) to the GS Column. Centrifuge at 14-16,000 x g for 30 seconds then discard the flow-through. Place the GS Column back in the 2 ml Collection Tube. Centrifuge again for 3 minutes at 14-16,000 x g to dry the column matrix.

14 **- DNA elution**

Transfer the dried GS Column to a clean 1.5 ml microcentrifuge tube. Add 100 µl of pre-heated Elution Buffer the CENTER of the column matrix. Let stand for at least 3 minutes to allow Elution Buffer to be completely absorbed. Centrifuge at 14-16,000 x g for 30 seconds to elute purified DNA. After finish, store DNA extraction products for amplification process.

DNA Amplification, Electrophoresis and Sequencing

15 This section explains short protocol for DNA amplification in a Polymerase Chain Reaction (PCR), electrophoresis and sequencing of DNA fragment which targeted mitochondrial DNA Cytochrome Oxidase subunit-I gene (CO1) of shark.

16 **DNA Amplification**

Start with preparing amplification material: PCR tube, DNA primer fish-BCL (forward) (5'–TCA ACY AAT CAY AAA GAT ATY GGC AC–3') and fish-BCH (reverse) (5'–ACT TCY GGG TGR CCR AAR AAT CA–3') [2,3], MyTaq HS Red Mix, ddH2O, shark DNA template (DNA extraction product), and electrophoresis material: Agarose 1.5%, Ethidium bromide and 100 bp DNA ladder.

17 Mix all PCR material into a PCR tube, each reaction consists of 12.5 µL of MyTaq HS Red Mix, 9 µL of ddH_2O, 1.25 µL fish-BCL, and 1.25 µL fish-BCH.

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18 Add 3 µL of DNA template (extraction product) into mixing tube. Centrifuge mixing tube in centrifuge or vortex machine for 5-10 seconds to make sure all material mixed thoroughly.

19 Put mixing tube into thermocycler PCR machine and set reaction at these conditions: pre-denaturation at 94°C for 15 seconds; followed by 38 cycles of denaturation at 94°C for 30 seconds, annealing at 50°C for 30 seconds, and extension at 72°C for 45 seconds, and a final extension at 72°C for 10 minutes [4,5]. After that, continue with electrophoresis step.

20 **Electrophoresis**

Perform electrophoresis process with 1.5% agarose gel electrophoresis added with ethidium bromide. Fill the first hole in agarose gel with 100 kb DNA ladder as marker, and another hole with 3 µL PCR product, then electrified at 100 V for 20 minutes in electrophoresis machine. After finish, observe the DNA band.

21 Observe DNA bands under UV transilluminator machine to identify the presence of DNA fragments or band.

22 **Sequencing**

Perform sequencing process for PCR product with confirmed DNA band using sequencing machine with an optimized protocol of Sanger method [6].

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23 **References**

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