

# **S** **T**ANDARD **O** **P**ERATING **P** **P**ROCEDURE

## **for Collection and Preservation of DNA Tissue Samples**



SWEDEN

SOUTHEAST ASIAN FISHERIES DEVELOPMENT CENTER



# **STANDARD OPERATING PROCEDURE for Collection and Preservation of DNA Tissue Samples**

Prepared by  
Marine Fishery Resources Development and Management Department  
(MFRDMD)

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**SOUTHEAST ASIAN FISHERIES DEVELOPMENT CENTER**

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## 1. INTRODUCTION

This Standard Operating Procedure (SOP) serves as a guideline and main reference for those involved in tissue sample collection at identified sampling sites (Table 1) and tissue preservation in the field or at laboratory. Collected and preserved samples from the respective countries are to be sent to SEAFDEC/MFRDMD in Malaysia for analysis.

## 2. OBJECTIVE OF THE SOP

The main objectives of this SOP are to ensure that all collected tissues are prepared and preserved according to the standard methods and procedures. The steps outlined in the SOP will ensure that sufficient high quality DNA could be obtained from the sampled tissues. High quality DNA is required to produce reliable and comparable data for stock/population identification in the Southeast Asian region.

## 3. TARGET SPECIES

The key to genus *Thunnus* of any taxonomic book would serve as a good reference. The scanned page on “Tuna Identification Sheet” poster published from *Project on Information Collection of Highly Migratory Species in Southeast Asian Waters: Tuna (2008 – 2012)* is given for quick reference (Figure 1).

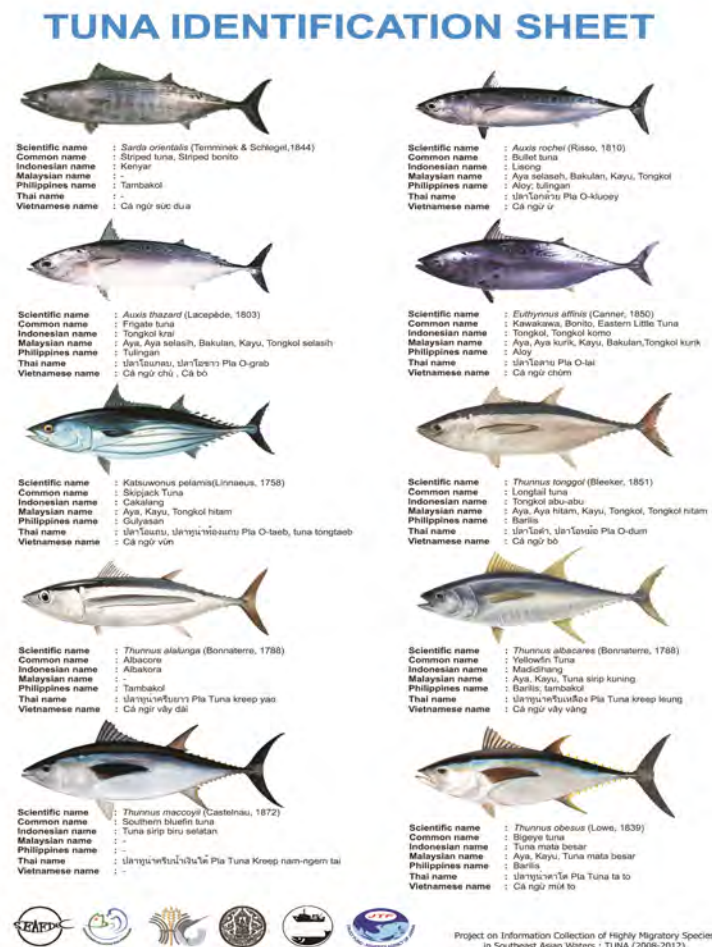


Figure 1: Tuna species in the Southeast Asian Waters

#### 4. IDENTIFIED SAMPLING SITES

Sample of the sampling sites as shown in Table 1 and Figure 2.

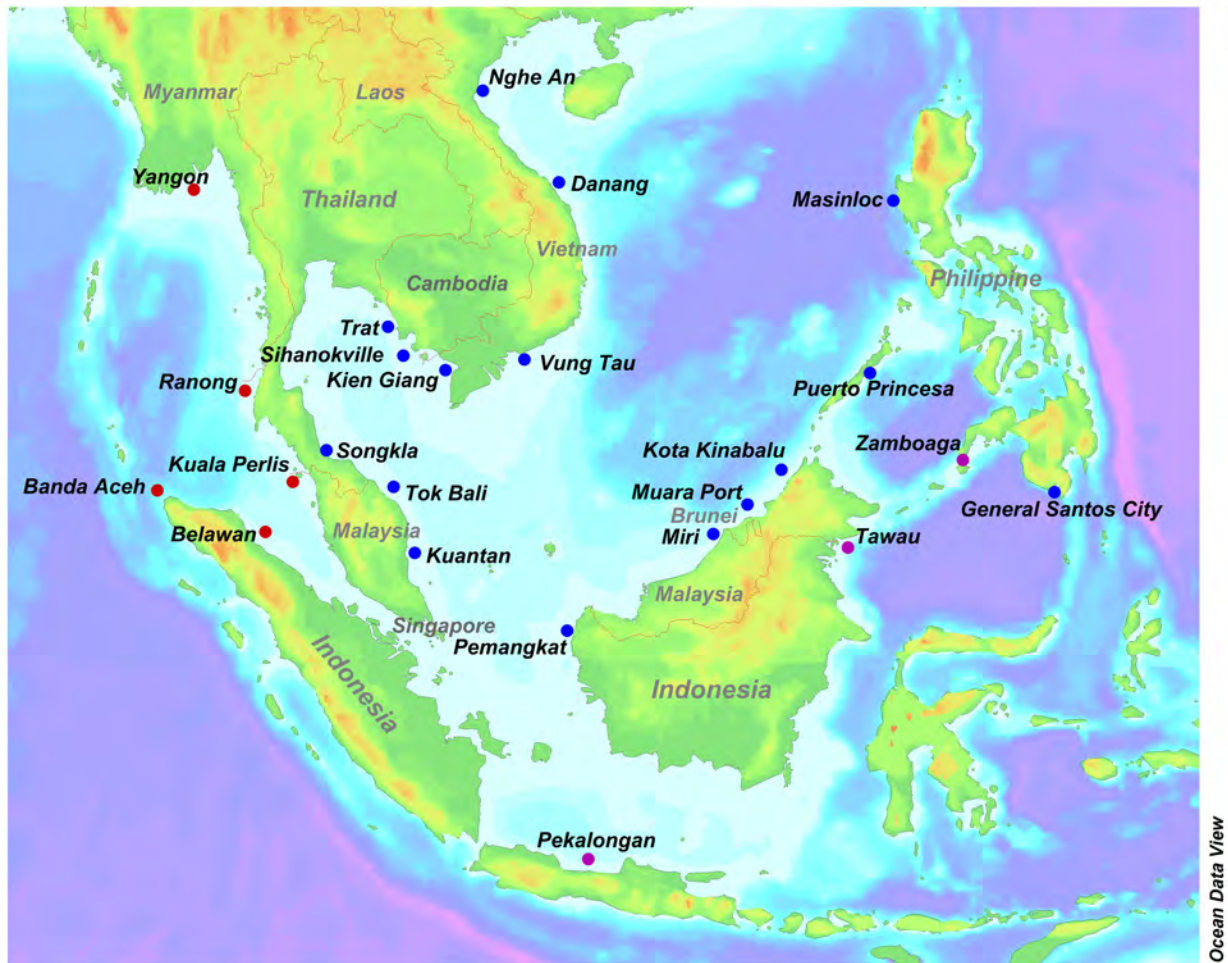


Figure 2: Map showing the distribution of the sampling sites in the Southeast Asian Region

**Table 1: Sampling sites by country, sampling sites' code, and number of samples to be collected for genetic stock study in the Southeast Asian Waters**

Country	Sampling Site/s	Country & Sampling Site Code	No. of Sample
<b>Andaman Sea Sub-Region</b>			
➤ Indonesia	1. Banda Aceh	INBA	50
	2. Belawan	INBW	50
➤ Malaysia	3. Kuala Perlis	MYKP	50
➤ Myanmar	4. Yangon	MMYN	50
➤ Thailand	5. Ranong	THRG	50
<b>South China Sea and Gulf of Thailand Sub-Region</b>			
➤ Brunei	6. Muara Port	BRMP	50
➤ Cambodia	7. Sihanokville	CBSV	50
➤ Indonesia	8. Pemangkat	INPT	50
➤ Malaysia	9. Tok Bali	MYTB	50
	10. Kuantan	MYKN	50
	11. Miri	MYMR	50
	12. Kota Kinabalu	MYKK	50
	13. Masinloc (Zambales)	PHMC	50
➤ Philippines	14. Puerto Princesa (Palawan)	PHPP	50
	15. General Santos City	PHGS	50
	16. Trat	THTR	50
➤ Thailand	17. Songkla	THSK	50
➤ Viet Nam	18. Nghe An	VTNA	50
	19. Danang	VTDG	50
	20. Vung Tau	VTVT	50
	21. Kien Giang (GoT)	VTKG	50
<b>Outgroup</b>			
➤ Indonesia	22. Pekalongan	INPN	50
➤ Malaysia	23. Tawau	MYTU	50
➤ Philippines	24. Zamboaga	PHZA	50



## 5. SAMPLING AT PORTS/LANDING SITES

### 5.1 Points of concern

- a. Fish samples must be collected from the landing sites listed in Table 1. Information on where the fish are caught is crucial to ensure that the samples represent the fishing area as intended.
- b. Fresh sample gives better DNA extraction. Samples collected must be from only properly preserved catch onboard fishing vessels. This is to ensure the freshness of the fish sampled. Information on fishing location (latitude and longitude) and catching gear type must be obtained and recorded.
- c. Avoid other than targeted tuna species in the 50 sampled tissues.  
Note: Country is required (if possible) to take photo for every fish sampled and send the photos to Laboratory. Photos of the samples must be taken together with the Identification Code No.
- d. Need to maintain the freshness of fish until the tissue is sampled and preserved. Sampled fishes at the sampling site should be kept in a container with ice or dry ice to maintain the freshness of the samples prior to tissue collection and preservation. This is particularly important in the case when tissue collection and preservation activities cannot be carried out *in situ* (at the sampling site) (Refer to 5.2).
- e. Sampled spawner fish gives better indication in population study. Spawning individuals will preferably be collected. Where possible biological data for each sample such as weight, standard length, sex, and gonad development stage (Appendix IV) should be recorded during sample collection.
- f. Possible cross-contamination when fish are sampled from mix-species container. Ensure sampled fish are properly wiped clean of slime before tissue sampling.

### 5.2 Materials and tools required for tissue sample collection



**Figure 3: Materials and tools used for sampling at port/sampling sites**

**Table 2: List of materials and tools for sampling at port/sampling sites**

	NAME	DESCRIPTION
1	Plastic bag *	This is used for packaging the sample, the size is depending on the fish size to be collected
2	Container * (optional)	This is for transportation of samples from port/sampling site to the laboratory; its size depends on the number of samples
3	Disposable gloves	To be used for handling fish during sampling process to minimize contamination
4	Data Form 1	Each sample (in a plastic bag) must be attached with a proper identification label (Data Form 1 as in Appendix I)
5	Crushed ice or Dry ice *	This is important to maintain the freshness of the fish for genetic sample collection; ample amount should be prepared for the sample collection

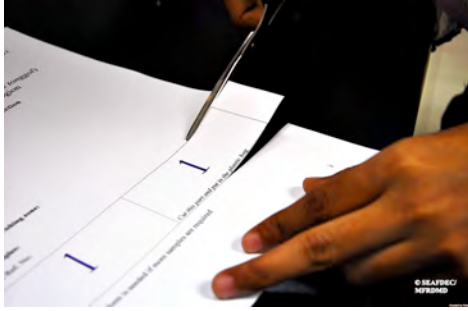
### 5.3 Procedure for tissue cutting and preservation



1. Fill up the information in Form 1



2. Write the reference number twice in Form 1



3. Cut one of the numbers in Form 1



4. Place it in a plastic bag containing the sampled fish.



5. Transfer the plastic bag containing samples into container, then cover the samples with crushed ice or place dry ice in container



6. The fish samples should be kept in the container until the next step for tissue collection and preservation, where tissue preservation could be done either at the landing site (*in situ*) or after the samples are brought back to laboratory  
\*Please proceed to 6.0 if tissue is decided to be preserved *in situ* (at the same landing site)



7. At the laboratory, fish samples should be kept in a freezer preferably at  $-20^{\circ}\text{C}$  until tissue collection and preservation procedure are carried out

## 6. TISSUE SAMPLE COLLECTION AND PRESERVATION PROCEDURES

### 6.1 Points of concern

- a. Need to maintain the freshness of the sample. Fin clip tissue should be taken immediately after the sample fish are taken out from the storage. The remaining ice/water must be wiped away from the sampling area (the second dorsal fin).
- b. Avoid contamination of the sample. Surgical gloves should be worn at all times during tissue sampling. Forceps and scissors must be washed with clean water and ethanol every time before use.
- c. Avoid mixing of samples. The vials should be labeled clearly according to the format (Year/Country&SamplingSiteCode/SampleNumber), e.g. MALAYSIA, Tok Bali: 15/MYTB/01, THAILAND, Songkhla: 15/THSK/01, MYANMAR, Yangon: 15/MNYN/01. Please refer to Table 1 for country and sampling sites code.
- d. Sample storage temperature is no longer an issue. The vials containing tissue sample in buffer (ethanol) can be stored at room temperature. Once preserved in ethanol, samples can be stored for many years. Ethanol should be checked periodically for any evaporation. Therefore, storage in fridge or freezer will reduce ethanol evaporation.
- e. Tissue should be fully preserved. Each tissue sample should be placed in individual vials, approximately 20 mg (1 cm<sup>2</sup>) of tissue in 1.5 ml of ethanol and ensure that tissue sample is fully submerged.
- g. Sample without proper label is problematic. Vials should be labelled with a non-dissolving ethanol resistant marker or printed labels or pencil to avoid possible loss of labels.

### 6.2 Materials and tools required for tissue sample collection

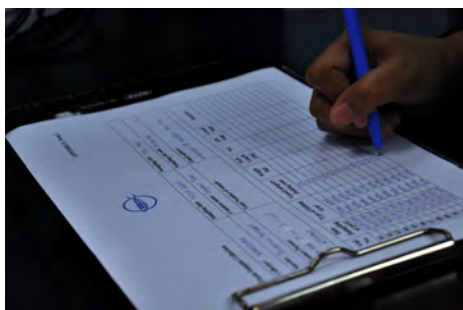


Figure 4: Materials and tools for tissue collection

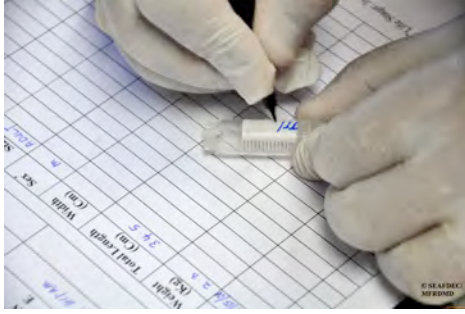
**Table 3: List of materials and tools for tissue collection**

NAME	DESCRIPTION
1. Set of forceps and scissors	For cutting tissue samples from fish
2. Washed bottle filled with ethanol (95%)*	For washing forceps and scissors
3. Washed bottle filled with clean water	For rinsing forceps and scissors
4. Tray*	For placing specimen during tissue collection
5. Vials filled with preservation buffer	For preserving tissue samples with 95% non-denatured ethanol*
6. Tissue paper*	For wiping away water and any organics from forceps and scissors
7. Disposable gloves	To be worn during sampling process
8. Permanent marker and pen	For labeling the vials containing samples and filling up forms
9. Weighing balance*	For weighing samples
10. Data Form 2 ** (Appendix II)	Information on all sampled tissues must be filled in this form (information from Form I will also be copied into Form II)
11. Measuring tape	For measuring the standard length of the fish sampled

### 6.3 Procedure for tissue cutting and preservation



- 1 Transfer information about the samples from Form 1 into Form 2  
(Note: Form 1 contains information on fish/fishes sampled at the same time and place, while, Form 2 contains information for the 50 sampled fishes)



2 Label the vials with the format given  
(Year/Country&SamplingSiteCode/SampleNumber):  
Example:

Year: 2015 = 15

CountryCode: Malaysia = MY

SamplingSiteCode: Tok Bali = TB

Sample number: 01 (Ref. Form 2)

Code: **15/MYTB/01**



3 Fill up each vial with 95% non-denatured ethanol (approximately 1.5 ml)



4 Wipe the sample fish with tissue paper



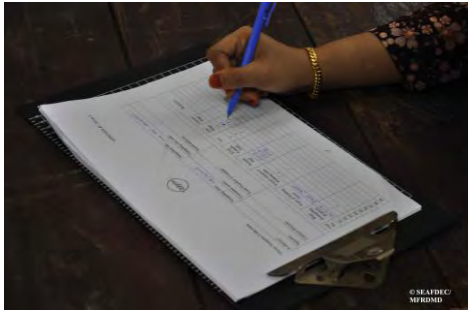
5 Weigh each of the sample fish



6 Measure the fork length of the sample fish



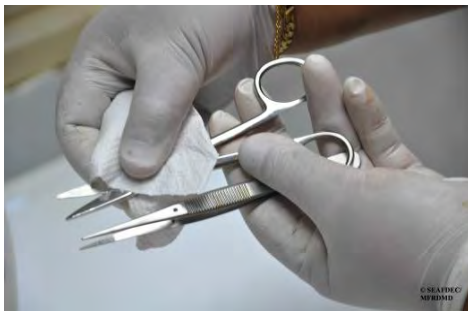
7 Capture fish photo with the vial's reference number and date



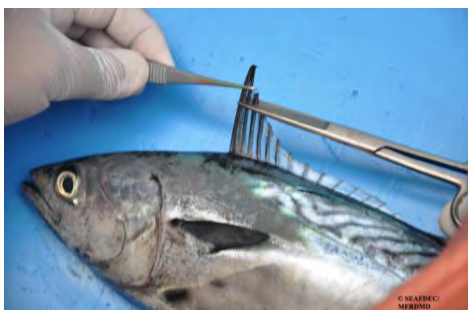
8 Fill up the information on weight and fork length (and if possible sex and gonad stage) (SEAFDEC/MFRDMD, 2015) into Form 2



9 Wash forceps and scissors with clean water and then 95% non-denatured ethanol every time before use



10 Wipe the forceps and scissors with tissue paper every time after washing



11 Cut approximately 30 mg ( $1 \text{ cm}^2/1.5 \text{ cm} \times 0.5 \text{ cm}$ ) fin clip from second dorsal fins of the sample fish with sterilized scissors

Note: Wipe fin with ethanol before cutting



12 Immediately, using forceps place the cut fin into a labelled vial (containing 1.5 ml ethanol)

Note: Always handle the tissue using sterilized tools to avoid any contaminations



13 Close the vial cap tightly and place it in a safe container

14 Repeat steps 2 to 13 for the next sample



## 7. TRANSPORTATION OF VIALS TO LABORATORY

Once 50 samples are collected from one sampling site, they must be securely packed into a box for shipment to Laboratory as follows:

- i). Shipping requires draining of ethanol from vials (**please ensure that the tissue is still maintained in a wet form**), or alternatively replace ethanol with non-combustible DMSO solution – if available
- ii). Place the vials in a sample box and wrap the sample box with air bubble plastic provided and seal the air bubble plastic with cellophane tape and in a postage box (provided)

A technical officer is required to send all the samples to Laboratory using courier service (*e.g.* DHL, FEDEX, etc.).

## REFERENCES

**SEAFDEC/MFRDMD. 2015.** Standard Operating Procedure for data collection and analysis of the neritic tunas.

“Tuna Identification Sheet” poster published from *Project on Information Collection of Highly Migratory Species in Southeast Asian Waters: Tuna (2008 – 2012)*



Genetic Study for Longtail Tuna (*Thunnus tonggol*) Stock in the Southeast Asian Region

**Form 1: Fish Samples Collection**

**Country:**

**Sampling site:**

**Date:**

**Species:**

**Type of fishing gear:**

**Vessel category/ Fishing zone:**

**No. of Samples:**

Plastic Ref. No:

	<p><i>Cut this part and put in the plastic bag</i></p>
--	--

\*Additional form is needed if more samples are required



Tissue Samples Collection

<b>Collector:</b>	<b>Sampling date:</b>	<b>Sampling site:</b>
<b>Species:</b>	<b>Common name:</b>	<b>Sampling site code:</b>
<b>Contact Person:</b>	<b>Total Number of sample:</b>	<b>Email address:</b>

±

Vial No.	Date (Sampling at port)	Type of Fishing Gear	Vessel category/ Fishing zone	Weight (kg)	Fork Length (cm)	Sex*	Life** Stage	Gonad Stage	Remarks
01									
...									
...									
...									
49									
50									

\*Sex: Male (M) / Female (F) / Unidentified (U)

\*\*Life Stage: Juvenile (J) / Adult (A) / Unidentified (U)

Label the sample vials accordingly based on the format given (refer 6.1.c)

APPENDIX II : Flow Chart

Flow Chart for Tissue Sample Collection Procedure

