



**SCIENTIFIC COMMITTEE  
SEVENTEENTH REGULAR SESSION**

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**Standard Operating Procedures for sampling (by observers and port samplers) and  
handling of tissue for genetic analyses in WCPFC fisheries**

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**WCPFC-SC17-2021/EB-IP-12**

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### **SC17 Considerations:**

- Note that SPC is developing standard operating procedures for genetic sampling and associated handling, storage and processing of sampled tissue (gSOP).
- Note that the gSOP are expected to be finalised by December 2021 and will be made available for integration into regional and national observer and port sampling programs in 2022.
- Note that adherence to the gSOP will ensure that tissue samples collected as part of the WCPFC Tuna Tissue Bank are of appropriate quality for the range of genetic analyses WCPFC is likely to have an interest in applying.
- Note that the gSOP should also provide a standardised for the curation of sequenced data for future use by WCPFC.

### **Background**

Recent expansions in the field of genetics include numerous new applications that are relevant to fisheries management in the jurisdiction of the WCPFC, including questions of stock structure, species identification and abundance estimation (see Bravington et al. 2021 [SC17-SA-IP-14] for an example). Additionally, progress also continues on developing genetic indicators to age fish, specify a region of origin, and the degree of mixing between and within populations.

The accuracy of genetic analyses hinges on proper sample handling in the field and in the lab to maintain DNA quality and avoid contamination. In an effort to standardize population genetic sampling, handling and assessments conducted in Pacific fisheries, SPC has developed a genetics standard operating procedure (gSOP) for tissue sampling with intent to conduct genetic analyses. The procedure covers details from study design to post-sequencing quality checks that will produce the best quality analysis results. The current methods are aimed to provide maximum study quality while using resources and techniques that can be replicated by onboard observers, port samplers, and private researchers. Future adjustments are anticipated to further simplify the protocols and diversify their application, some of which will stem from presently on-going field experiments.

There are many benefits to establishing standardised methodologies for genetic assessments of species of interest to Pacific fisheries. First, establishing protocols that maintain adequate sample quality will maximise return on investment; studies that incorporate samples with insufficient DNA quality for sequencing or are suspect of contamination (neither of which can be confirmed except via sequencing) are forced to exclude samples from analyses despite having already invested in their collection, processing and sequencing. If too many samples fail quality tests, the entire study can lose statistical power and confidence in data interpretation. Second, broad application of compatible protocols simplifies comparisons between studies, which will expedite regional collaboration. Third, universal adherence to a single handling protocol both at sea and in the lab will improve confidence in the use of samples accessed from repositories like the WCPFC Tuna Tissue Bank.

### **Sources of input**

All gSOP elements are either directly confirmed to be effective by SPC studies or derived from citable literature. SPC has conducted several studies that inform the current conclusions.

1. A cross-contamination experiment to compare contamination rates associated with a variety of common or proposed on-board sampling protocols.
2. A storage experiment to compare DNA quality of samples stored in a common stabilising solution (RNAlater), compared to directly frozen.
3. A laboratory handling experiment to assess the rate of contamination in the lab, and potential protocols to recover contaminated samples.

## **gSOP Table of Contents (Annotated)**

1. Sample Size  
*This section provides advice on the number of fish to be sampled on standard purse-seine and longline trips and stratification across size classes and species.*
2. Sampling procedures  
*This section provides detailed instruction on where, how and what tools to use when taking genetic samples. It includes information on alternative approaches to sampling when the ideal tools are unavailable. Instructions are provided on immediate storage on vessels and equipment cleaning protocols.*
3. Sample storage and handling  
*This section provides detailed instruction on storage and handling of samples (off vessel) and prior to transport to their final destinations.*
4. Freight and transport  
*This section provides detailed instruction on freight requirements (including sample preparation) and permitting for movement of tissue samples using domestic and international freighting services.*
5. WCPFC Tissue Bank and Laboratory handling and manipulations  
*This section provides detailed instruction on the storage, handling and withdrawals of tissue samples in the WCPFC Tissue Bank.*
6. DNA extraction, sequencing, quality checking  
*This section provides advice on extraction and sample quality checking protocols.*
7. Sequencing data handling and analysis  
*This section provides advice on the curation of sequencing data for WCPFC applications.*

Full discussion of each of these elements (and others) are collated in a document currently undergoing internal review at SPC. It is important to emphasise that the current state of the document is expected to change and expand in the near future, given input from further experiments that are ongoing at SPC and the continue evolution of discussion in the literature.

### **Next steps**

SPC has ongoing follow-up studies regarding field sampling that explore further tools and protocols that could expand or out-perform existing options. These experiments also explore additional sources of contamination in the current observer sampling protocol.

A separate and also ongoing study will further clarify degree of DNA degradation at various stages of fish storage, from fresh-caught to fresh caught but landed dead (especially relevant to longliners), to stored on ice for multiple days prior to sampling. The study also incorporates multiple species, which might demonstrate the need for additional species-specific guidelines.

### **References**

Bravington, M., et al. 2021. WCP Albacore Close-Kin Mark-Recapture: update on design. SC17/SA-IP-14.