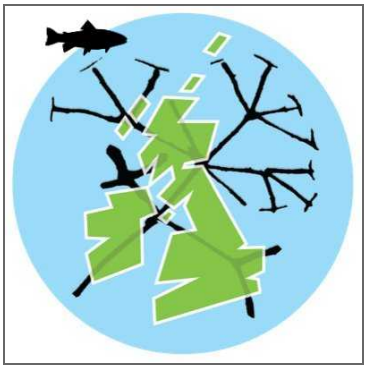

DEC 04, 2023

DOI:
dx.doi.org/10.17504/protocols.io.14egn3y6pl5d/v1

Protocol Citation: Inez Januszczak, James Maclaine 2023. DToL Specimen Tissue, Blood and Voucher Sampling Standard Operating Procedure: Chordata: Vertebrata: Fish . **protocols.io** https://dx.doi.org/10.17504/protocols.io.14egn3y6pl5d/v1

Protocol status: Working
This is a working protocol and may be subject to change.

DToL Specimen Tissue, Blood and Voucher Sampling Standard Operating Procedure: Chordata: Vertebrata: Fish

Inez Januszczak[1], James Maclaine[1]

[1]Natural History Museum, London

Darwin Tree of Life

Inez Januszczak

ABSTRACT

This Standard Operating Procedure (SOP) covers how fish specimens are to be sampled and preserved for vouchering at the Natural History Museum (NHM) in London, in association with the Darwin Tree of Life project (DToL).

Please note this SOP covers sampling tissue and blood either during an appropriate veterinary/scientific procedure or post-mortem, with assumed veterinary knowledge. It is applicable if the submitter/centre in question has opportunities to sample tissue from animals that are being either being euthanized for clinical or scientific purposes, undergoing veterinary procedures or eligible biobanked carcasses (storage dependent).

NHM prides itself on maintaining the highest quality specimens for vouchering purposes, enabling samples to be used as a reference for any future scientific study.

This SOP is covers fish in general - other available Chordata SOPs include DToL Chordata: Vertebrata: Aves and DToL Chordata: Vertebrata (for an overview).

This is version one of this SOP, and may be subject to change in the future.

Acknowledgements
Thank you to Oliver Crimmen at the Natural History Museum for reviewing this SOP.

ATTACHMENTS

DToL Specimen submission form template MARINE VERSION for external submitters_Marine.xlsx

GUIDELINES

Created: Dec 04, 2023

Last Modified: Dec 04, 2023

PROTOCOL integer ID: 91775

Keywords: fish, vouchering, sampling, SOP, dissection, Darwin Tree of Life, Natural History Museum, Wellcome Sanger Institute, biobanking, whole genome sequencing, voucher, museum

This SOP covers how fish specimens are to be sampled and preserved for vouchering at the Natural History Museum (NHM).

NHM prides itself on maintaining the highest quality specimens for vouchering purposes, enabling samples to be used as a reference for any future scientific study.

Subsequently, how samples are handled before any form of scientific process, for example dissection for tissue removal, is very important in ensuring acceptance as vouchers.

Note

Please ensure each submitted specimen has available all its relevant metadata (Date of Collection, Collector, Identifier, Collection location, Species, Specimen ID, Collection Depth (if applicable)).

The Darwin Tree of Life project will **not** accept specimens which don't have this information.

Prior to submission

It is important to check whether DToL would require the sample.

The submitter/centre in question would be shared a list, which states the species required. As this is opportunistic sampling, the list should be regularly checked to see what species are needed.

Regulatory compliance considerations

Animal Welfare - the samples must only come from animals undergoing veterinary care, euthanized for scientific or health reasons or biobanked carcasses that are eligible for dissection/ tissue preparation.

Permissions may be requested depending on the species in question.

Note

Please note the ideal storage conditions for a biobanked carcasses will be at -20 degrees or lower. Carcasses that have gone through multiple types of preservation should be handled on a case by case basis - please contact the Natural History Museum (NHM) Sampling Coordinator and Sanger Sampling teams, listed at the end of the SOP.

Carcasses stored in ethanol (at room temperature) will only be considered in exceptional circumstances, for example if it is a rare species.

Logistical Considerations

A -80 freezer is required for tissue sampling and storage (suggested brand Fryka B35-85 under bench, ETA 2-3 weeks 80kg. 58x76x54cm). Preserving the tissue directly on dry ice is also an option.

Aside from the specimen itself, labelled cryotubes that are able to withstand -80 degrees cold storage will be required (for the tissue) as well as ziplock/ plastic bags to store the tubes and carcasses.

NHM is able to provide cryotubes with barcodes (FluidX) and ziplock bags if required.

Specimen IDs

Each specimen requires two identical ID numbers; one will be used to label the ziplock bag of tubes containing the samples, the other to label the remaining carcass (if applicable).

The NHM is able to provide unique ID numbers, otherwise any matching ID will be appropriate, preferably a series of numbers.

Before Dissection

Ideally, any euthanasia method required should still preserve the entire morphology of the specimen.

a. Anaesthesia/euthanasia methods that cause minimal damage to the specimen e.g. using clove oil or MS 222 are ideal. If cranial damage is absolutely necessary, please ensure to minimise skeletal damage as much as possible, and to minimise the amount of blood leeching through the specimen.

b. Please take a high-quality photo of the specimen, lateral right and left, with any corresponding ID given to the specimen placed next to it, clearly visible in the photo (not covering the specimen itself). Additional close-ups of diagnostic features would be highly appreciated.

Dissection + preservation

a. Any tissue removed from the specimen MUST be removed from the RIGHT side only.

This is a standard vouchering procedure and ensures that all NHM specimens have the same visual consistency throughout the collection and makes comparative studies easier. This protocol also complies with the convention for imaging the left lateral view.

b. Preferably, the fish is dissected through small ventral slit on the right side.

c. The tissue sample should be minimal in size, sufficient only to accommodate modern analysis techniques.

d. Dissect out multiple (ideally minimum of 3, up to ~6) lentil-sized samples per tissue below, as is practical from the size type and condition of specimen: The tissues required for the Darwin Tree of Life project are:

Muscle
Heart
Liver
Gills
Brain (if possible)

These are listed in order of priority.

e. Please ensure the tissue is frozen and stored immediately at -80 degrees (on dry ice or in a freezer). Please ensure that the tissue and corresponding tube is accurately recorded.

f. After dissection, please ensure the whole fish is then frozen for storage (for the rest of the carcass, -80 degrees is not necessary. -30 to -20 is appropriate). The carcass must NOT be placed in any preservative (eg. formalin or ethanol). The carcass must also remain frozen throughout its transportation to the museum.

g. Email the DToL NHM Sampling Coordinator regarding the samples obtained.

Carcass and Tissue submission

Once the submitter/centre has collected at least 5 sets of samples from 5 specimens, and thus potentially 5 carcasses, they should contact NHM and Sanger DToL Sampling team who will arrange for their collection.

Note

Please note - an Excel spreadsheet with Specimen ID, Species, tube ID and tissue type of all submitting samples must also be kept (including whether a carcass was kept or not).

Each tube ID must match the tissue sampled.

Ensure that samples from separate animals are kept separate.

This spreadsheet must also be emailed to the NHM and Sanger DToL Sampling team. Any photographs of the specimens must also be saved and emailed to the DToL NHM Sampling coordinator.

A draft spreadsheet of this kind is attached to this SOP.

When submitting/ considering a submission, please contact:

Inez Januszczak, DToL NHM Sampling Coordinator: Inez.januszczak@nhm.ac.uk
The DToL NHM Sampling team: darwintreeoflife@nhm.ac.uk
Molly Carter, DToL Sanger/Tree of Life Coordinator: mc39@sanger.ac.uk

SAFETY WARNINGS

Please note - it is assumed all appropriate zoonotic disease checks have been carried out prior to any post-mortem or veterinary procedure.

ETHICS STATEMENT

Regulatory Compliance Considerations

Regarding animal Welfare - samples will only be accepted if they come from animals undergoing veterinary care. euthanized for health or scientific reasons or from a valid biobank collection.