Sanger Tree of Life Sample Preparation: Triage & Dissection

Authors:

Jessie Jay, Halyna Yatsenko, Juan Pablo Narvaez Gomez, Haddijatou Mbye, Maria Morra, Michelle Strickland, Clare Cornwell, Caroline Howard

Abstract

This protocol describes the process of sample preparation for the extraction of DNA and/or RNA from the wide variety of samples processed by the Tree of Life Core Laboratory as part of the Tree of Life Programme. It also describes the triage steps and recommended weight requirements for the different taxonomic groups covered by the Tree of Life programme. The output of this protocol is a sufficient amount of sample that can be directed towards any of the Sanger Tree of Life Sample Homogenisation protocols.

Health & Safety requirements

- The operator must wear a lab coat, powder-free nitrile gloves and safety specs to perform the laboratory procedures in this protocol. Cotton glove liners are strongly recommended.
- Waste needs to be collected in a suitable container (e.g. plastic screw-top jar, Biobin or sharps bin) and disposed of in accordance with local regulations.
- Liquid waste needs to be collected in a suitable container (e.g. glass screw-top jar) and disposed of in accordance with local regulations.

Guidelines

- Sample triage consists of weighing the samples, dissecting and aliquoting portions of samples as needed. Recommended weights can be found in Table 1, and are determined by sample type, downstream process and weight. More in depth decision trees can be found in the Appendix.

- The majority of samples submitted to the Sanger Tree of Life are those that have been snap frozen; these are processed as ‘standard samples’. However, samples which have not been snap frozen are also submitted; these samples include blood samples preserved in ethanol and tissues stored in preservative solutions such as RNAlater, DMSO, DESS & ethanol. These samples are referred to as ‘non-standard samples’ and they have varying degrees of success downstream of sample preparation.

- HiC processing is completed by the Long Read Sequencing Team within DNA Pipelines Operations at the Wellcome Sanger Institute, therefore within this protocol, the required tissue prep refers to the sample which is sent for further processing outside of the Tree of Life Core Laboratory.

- It is essential that samples remain frozen throughout this process for high quality extraction and sequencing results:
    - A cold rack can be used to keep samples cold and organised.
    - Tools used for dissection should be pre-chilled and kept in dry ice when not in use (tweezers, scalpel).
    - Samples should be dissected on a prechilled metal dissection plate.
    - Try to minimise the time that the sample is not on dry ice (i.e. when weighing on the balance).

Table 1. Tissue preps required by downstream process

Downstream process	Required tissue prep	Tissue input for cryoPREP
HiC	Up to 20 mg of frozen tissue for Metazoa can be sent as whole frozen tissue OR 50 mg of cryoprepped tissue for all taxonomic groups (excluding protists) If the sample is an arthropod and the head is visible, dissect and take the head only	**Metazoa:** 60-70 mg **Plants/Algae/Fungi:** 60-70 mg **Protist:** Never cryoPREP
DNA Extraction - MagAttract/Plant MagAttract	Up to 25 mg of frozen tissue for Metazoa can be powermashed OR Cryoprepped tissue weight depends on taxonomic group requirements: **Metazoa:** 25 mg **Plants/Algae/Fungi/Corals:** 50 mg **Protist:** 50 mg	**Metazoa:** 30-40 mg **Plants/Algae/Fungi/Corals:** 60-70 mg **Protist:** 55-100 mg
DNA Extraction - Plant Organic Extraction (POE)	65 mg of cryoprepped plant/fungi	**Plants:** 80-90 mg If less than 40 mg do not proceed
RNA Extraction	Up to 15 mg of frozen tissue for Metazoa can be powermashed OR Cryoprepped tissue weight depends on taxonomic group requirements: **Metazoa:** 15 mg **Plants/Algae/Fungi/Corals:** 15-20 mg	**Metazoa:** 20-30 mg **Plants/Algae/Fungi:** 20-30 mg

Additional Notes

- Protist samples can be pooled together if from the same colony in order to reach the recommended weight.
- Samples that have shells that do not weigh enough to be cryoprepped are not recommended for further processing. The shell makes the sample difficult to powermash, and tissue loss after cryoPREP will result in not not enough remaining tissue for the downstream process.

Before Starting

- Prepare at least 2 rubber buckets with dry ice (use more depending on the number of samples and other requirements/ personal preference):

    - Cover one bucket with dry ice with aluminium foil and place a metal dissection plate on top.
    - Use the second dry ice bucket to store the samples that are going to be prepared. Samples should be kept on dry ice to ensure they remain frozen.
- Clean and pre-chill materials needed for dissection (weigh boats, scalpel, tweezers, scissors etc.) by placing them on dry ice.
- Set up analytical balance.
- Retrieve frozen samples.

Laboratory Procedure

1. Select the first sample from the cold rack.

2. Inspect the sample and check the sample preservation method.
   For snap frozen samples ('standard samples'), proceed to step 3.
   For samples that have not been snap frozen ('non-standard samples'), please refer to step 7.

3. Decide how much sample is required for the desired downstream process (see Table 1 or the relevant Decision Making Tree in Appendices).

4. Weigh the sample. If taking only part of a sample, dissect the desired amount.

    4.1. Use an analytical scale to tare a new clean weighing boat.

    4.2. Use clean and pre-chilled dissection tools (scalpel, tweezers, scissors) and metal dissection block to cut the sample until the desired weight has been achieved.

    4.3. Place prepared sample into correct destination container & put back any remaining tissue into original container

    4.4. Clean the tools and dissection block with Azowipe tissue and bury the dissection tools in dry ice so that they are ready for the next sample.

5. If the total weight is 25 mg or less and it is Metazoa (excluding sponges and corals), use the 'Sanger Tree of Life Sample Homogenisation: Powermash' protocol.

6. If the total weight is greater than 25 mg for all taxonomic groups, use 'Sanger Tree of Life Sample Homogenisation: Covaris cryoPREP® Automated Dry Pulverizer' protocol.

7. The current method to prepare samples stored in preservative solutions is as follows:

    7.1. Place the tube containing the sample in wet ice to allow the liquid to defrost.

    7.2. Remove the sample from solution and transfer to a new tube. Dispose of the preservative solution in an appropriate waste container.

    7.3. Then proceed as if the sample was frozen tissue (see step 3). Keeping in mind the recorded weight will not be accurate, as the sample will have absorbed some of the preservative solution.

8. If the sample type is blood, defrost on wet ice and aliquot required amount for the desired downstream protocol. If the blood is stored in ethanol, the aliquot should be taken from underneath the layer of ethanol.

Materials

- Dry ice
- Weighing boats (SLS Cat. no. bal1820sp)
- Aluminium foil
- New and empty tubes
- Cleaning material (e.g., Azowipe or 80% ethanol & blue roll)
- Cotton and silk glove liners (recommended)

Equipment

- Sharps disposal bin
- Waste disposal bin
- Mettler Toledo Analytic Balance ME204 (Material No. 30029066)
- Insulated ice buckets
- Cool rack (Corning® CoolRack CF45 Product no. 432051) or equivalent
- Cold metal dissection plate
- Tweezers/Forceps (fine and round tips)
- Spatulas (e.g. small and medium size; concave and flat-blade)
- Scalpel
- Scissors