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Protocol status: Working
We use this protocol and it's working

Sanger Tree of Life Sample Homogenisation: Covaris cryoPREP® Automated Dry Pulverizer

In 1 collection

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ABSTRACT

This protocol describes the procedure for cryogenic homogenisation of tissue samples using the Covaris cryoPREP® Automated Dry Pulverizer CP02. The protocol is designed for DNA and/or RNA extraction aimed at long-read sequencing or RNA-Seq applications. The method is particularly effective for disrupting tissues with a mass exceeding 25 mg across all taxonomic groups studied in the Tree of Life Programme. However, it is not advisable for samples smaller than this, due to inherent tissue loss during processing. Additionally, the technique has limitations when applied to fibrous plant tissues and samples rich in polysaccharides (e.g., macroalgae and molluscs), where it may result in either insufficient disruption or excessive tissue loss. The resultant sample is compatible with any Sanger Tree of Life DNA and RNA extraction protocols as well as Hi-C library preparation.

IMAGE ATTRIBUTION

Photograph by David Levene.

GUIDELINES

- This method is not recommended for samples with mass less than 25 mg.
- It is not recommended to cryoPREP samples >200 mg if preparing for multiple downstream procedures, as tissue disruption may be incomplete.
- Expect to lose up to 15 mg of tissue. Disrupted samples sometimes may become trapped in the TissueTUBE bag corners, or adhere to the inside surface.
- Samples must remain as cold as possible during disruption without introducing liquidised gas into the TissueTUBE.
- Place into liquid nitrogen immediately prior to and following each impact. Dry ice

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is not sufficiently cold for this procedure.

Table 1: Suggested settings for cryoPREP disruption of samples

Sample type	Initial Setting 1 impact	Subsequent impacts (Setting × number of impacts)
Algae	Yes	S6 × 2
Annelids	Yes	S4 × 1
Arthropod	Yes	S6 × 1
Plant	Yes	S6 × 6
Fungi	Yes	S6 × 6
Protist	Yes	S6 × 1
Sponges	Yes	S2 × 2
Soft tissue: Vertebrates, Molluscs and others	Yes	S4 × 1
Hard tissue: Corals and others	Yes	S6 × 6

Table 2: Tissue weight required for CryoPREP

Downstream process	Tissue weight required before CryoPREP
DNA Extraction - MagAttract/Plant MagAttract	Metazoa: 30–40 mg Plants/Algae/Fungi: 60–70 mg Protists: 55–100 mg
DNA Extraction - Plant Organic Extraction (POE)	Plants: 80–90 mg
RNA Extraction	Metazoa: 20–30 mg Plants/Algae/Fungi: 20–30 mg Protist: 55–100 mg
HiC	Metazoa: 60–70 mg Plants/Algae/Fungi: 60–70 mg

Additional Notes

- It is good practice to check the TissueTUBE bag after each impact to ensure the bag is not damaged, to prevent sample loss. If the TissueTUBE bag appears damaged during disruption, replace the damaged TissueTUBE bag.
- Occasionally discs of compressed material can form during disruption; break these up using chilled forceps after each impact.
- If the sample is very small, the smaller Covaris cryoPREP TissueTUBE can be used, for larger samples use Covaris cryoPREP TissueTUBE TT1 extra thick.

- FluidX tubes are used throughout the Tree of Life program to track samples, custom adapters have been 3D printed (on-site) in order to pair them with Covaris TissueTUBE bags.

MATERIALS

- Cotton glove liners (recommended)
- Dry ice
- Frozen tissue sample
- Covaris TissueTUBE TT1 Extra Thick (Cat. no. 520007)
- Covaris TissueTUBE Extra Thick TT05M (Cat. no. 520140)
- Cleaning materials (e.g. Azowipe, blue roll & 80% ethanol spray)
- Cryogenic gloves
- Liquid nitrogen
- Virkon or similar
- Sample tube (FluidX or equivalent)

Equipment:

- Cool rack Corning® CoolRack CF45 Product number 432051 (or equivalent)
- Covaris CryoPREP CP02 (Cat. no. 500000)
- Adapter: Custom made (reusable) adapter that fixes sample tube to the CryoPrep TissueTUBE
- Dewar flask
- Rubber ice bucket(s)
- TissueTUBE TT1 insertion Tool

Protocol PDF: Sanger Tree of Life Sample Homogenisation_ CryoPREP.pdf

SAFETY WARNINGS

- 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 also strongly recommended.
- Before decanting liquid nitrogen, the operator should have undergone liquid nitrogen decanting training.
- While decanting liquid nitrogen, in addition to PPE listed above, the operator must wear a face shield and cryogenic gloves.
- When using liquid nitrogen in a room without an oxygen depletion alarm, a personal oxygen depletion alarm must be worn.
- When operating the cryoPREP, the door to the room must be closed and all room occupants must be wearing ear defenders.
- When operating the cryoPREP, put a warning sign on the door to notify that the cryoPREP is in operation.
- Waste needs to be collected in a suitable container (e.g. plastic screw-top jar) and disposed of in accordance with local regulations.
- Safety spectacles must be worn to avoid accidental splashing of liquid nitrogen in eyes.
- To reduce cold exposure and ergonomic risks, it is recommended that operators work in pairs to allow for operator rotation.

BEFORE START INSTRUCTIONS

- Ensure an adequate amount of sample has been prepared according to weight recommendations provided in Table 2 of the Guidelines section.
- Decant a suitable amount of liquid nitrogen into the liquid nitrogen Dewar flask for the number of samples being processed.

Transfer sample to TissueTUBE

1 Place the sample into the tissueTUBE TT1 and seal using the adapter and attaching the sample tube on the top.

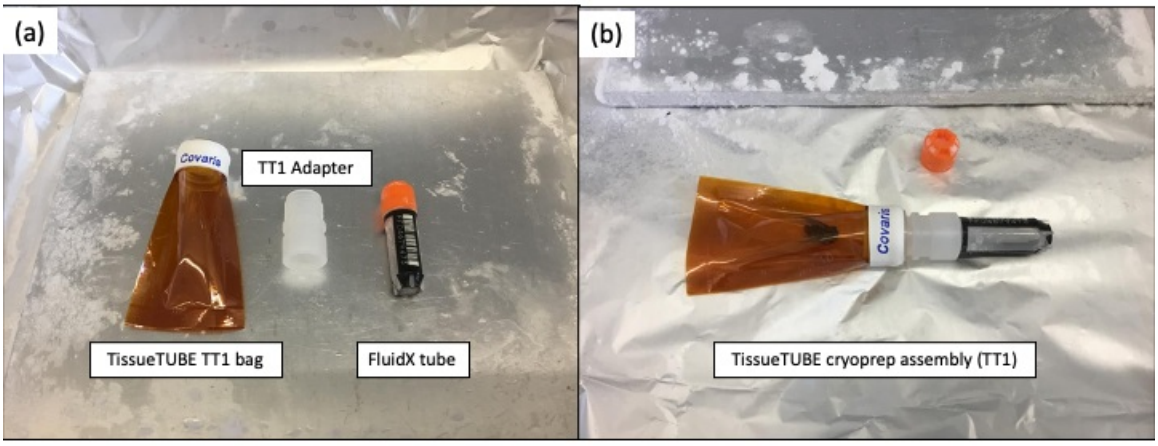

Figure 1. TissueTUBE assembly. (a) Left to right: Covaris TT01 TissueTUBE, TT01/1.9ml FluidX adapter and 1.9ml FluidX tube. (b) assembly with sample in place.

2 If processing multiple samples, bury the bag containing the sample in the dry ice whilst processing the others. Try to keep the samples as cold as possible.

Disruption

3 Power on the cryoPREP instrument. Ensure all doors to the room are closed and all room occupants are wearing ear defenders. Set the setting dial to “1”.

4 Hold the sample tube such that the tissue remains in the TissueTUBE bag.

5 Loosen the tube by ¼ to ½ turn to allow the air inside the tube to escape during disruption.

6 Place the TissueTUBE assembly so the bag is immersed in liquid nitrogen and hold for 1 to 2 seconds after the boiling subsides. Do not submerge the collar or any part above to prevent liquid nitrogen entering the bag.

7 Remove the TissueTUBE assembly from the liquid nitrogen and check for liquid nitrogen or liquidised air inside the bag. Allow the gas to evaporate if present.

8 Insert the TissueTUBE assembly into the cryoPREP and close the lid.

9 Check cryoPREP settings and press the green “Activate” button. For recommended disruption settings, check Table 1.

10 Place the bag in liquid nitrogen (as per step 4) before and after each impact.

11 Additional impacts can be performed if the sample is not fully disrupted and the TissueTUBE bag remains intact.

12 Tighten the adapter and submerge the TissueTUBE bag in liquid nitrogen one last time. Take the sample out and invert so the disrupted tissue leaves the bag and enters the sample tube, quickly and vigorously agitate/flick the bag to transfer the disrupted sample into the tube.

13 Immediately put the inverted TissueTUBE assembly in dry ice or a cold rack if available.

14 Remove the TissueTUBE bag and adapter and replace the lid on the tube. The disrupted tissue can be transferred to a new tube if required for downstream processing or if aliquots have to be made.

Decontaminate adapters for reuse

15 Prepare an appropriate quantity of Virkon disinfectant or alternative, following manufacturer's instructions.

16 Dispose of the TissueTUBE bag as biological waste.

17 Check the adapter for large pieces of material. If material is present, remove with an Azowipe or forceps and dispose of as biological waste.

18 Place adapters in disinfectant solution and leave overnight.

19 Dispose of solution following local guidance.

20 Rinse adapters thoroughly with ultrapure sterile water.

21 Allow adapters to dry in a sterile environment, under UV light if possible.