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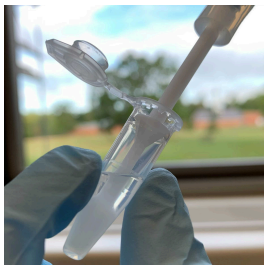
Sanger Tree of Life Sample Homogenisation: PowerMash



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We use this protocol and it's working

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Abstract

This protocol describes the homogenisation of tissue samples for DNA and/or RNA extraction intended for long read sequencing or RNA-Seq, using the Diagnocine PowerMasher II tissue disruptor. This process is highly effective for the disruption of tissue samples weighing less than 25 mg from all taxonomic groups covered by the Tree of Life Programme except for plants, fungi, protists, sponges and corals. Larger samples are not processed through this method due to difficulties in achieving homogenisation. Crustaceans and molluscs are particularly challenging samples. The output of this protocol is a sample that can be directed toward any of the Sanger Tree of Life DNA and RNA extraction protocols.

Guidelines

- Keep tissues on dry ice prior to transfer into the BioMasher tube and lysis buffer addition in order to maintain a low temperature and prevent nucleic acid degradation.
- This method is not recommended for samples that weigh more than 25 mg.
- This method is not recommended for plants, fungi, moss, protists, sponges or corals samples – these samples should be disrupted using a different method.

Materials

- 1.5 mL BioMasher II tubes and pestles (sterile) (Cat. no. 9791a)
- DNA/RNA lysis buffer
- 15 mL or 50 mL centrifuge tube
- Dry ice

Equipment

- Diagnocine PowerMasher II tissue disruptor (Product no. 891300)
- Pipettes for 0.5 to 1000 µL and filtered tips

Protocol PDF: Sanger Tree of Life Sample Homog... 66KB

Troubleshooting

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 strongly recommended when handling the samples on dry ice.
- Waste needs to be collected in a suitable container (e.g. plastic screw-top jar or BioBin) and disposed of in accordance with local regulations.

Before start

Prepare lysis buffer for the tissue's intended downstream protocol: either DNA or RNA extraction.

Laboratory protocol

1 Transfer the sample into the sterile 1.5 mL BioMasher II tube and add the lysis buffer required for the downstream protocol.

2 Attach the sterile BioMasher pestle to the PowerMasher II tissue disruptor, avoiding contact with the end of the pestle that will contact the sample to prevent contamination.

3 Disrupt the sample in the lysis buffer within the BioMasher tube using the PowerMasher II tissue disruptor and sterile BioMasher pestle; this is done by placing the pestle into the BioMasher tube and against the sample, applying some gentle pressure so the sample remains between the pestle and the wall of the BioMasher tube, and then squeezing the trigger of the PowerMasher II tissue disruptor to activate it.

4 Continue for approximately 30 seconds to 1 minute (depending on the tissue type), until no large pieces of the sample remain, or the sample cannot be disrupted any further. Gently moving the pestle up and down can encourage sample movement within the lysis buffer and prevents the sample becoming stuck at the bottom of the tube, allowing for maximum disruption.

5 Remove the pestle from the BioMasher tube and disconnect it from the PowerMasher tissue disruptor II. If any tissue remains stuck to the pestle, gently try to remove this by wiping the pestle tip on the rim of the BioMasher tube. Dispose of pestle in biological waste.

6 Proceed with the powermashed sample to the next step of the tissue's downstream protocol –
either DNA or RNA extraction.

Protocol references

BioMasher Product Catalogue: **Biomasher User's Manual (cosmobiousa.com)**