Sanger Tree of Life Sample Homogenisation: Sponge Squeezing

Authors

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Abstract

This protocol is for the homogenisation of sponge samples for HMW DNA extraction and/or HiC, intended for long read sequencing. In the phylum Porifera, most marine demosponges (Demospongia) species possess a siliceous skeleton and spicules which can compose a high volume of the total biomass. Silica deposition is a fundamental process in sponges allowing sponges to form complex three-dimensional structures connected by spongin. However, these hard siliceous spicules can hinder the mechanical lysis of tissue and restrict the access of reagents like lysis buffer and Proteinase K to the cells, which are needed for DNA release and purification. Standard powermashing of these tissue types is often not sufficient to extract a sufficient yield of HMW gDNA. A “squeeze” method is described here to enrich for intact cells (eukaryotic and prokaryotic) prior to DNA extraction from marine sponge tissues. This protocol was adapted from that designed by Jose Lopez.

This process is highly effective for the disruption of flash frozen sponges 150-200 mg in mass from Demospongia covered by the Tree of Life Programme. This protocol can also be used on glass sponges Hexactinellida, but alterations should be made depending on morphology. The output of this protocol is a sponge cell pellet that can be directed towards the Sanger Tree of Life HMW DNA extraction: Manual MagAttract or Manual Plant MagAttract protocols.

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.
- Users should have training appropriate for the handling of all hazardous equipment used in this procedure (e.g. sharps).

Guidelines:

- Keep tissues on dry ice prior to transfer into the petri dish and buffer addition in order to maintain a low temperature and prevent nucleic acid degradation.
- An input mass of 150–200 mg of flash frozen sponge tissue is generally recommended, exact input requirements will vary depending on the downstream protocol to be performed, the sponge species used, tissue type and sample quality.
- Ensure an adequate volume of ‘Sponge Squeezing Lysis Buffer’ has been prepared prior to starting the protocol, 2 mL per sample required (recipe below).

- It is not recommended to ‘squeeze’ <20 mg of tissue. Extraction yields will be considerably lower.
- Glass sponge tissues may dissolve once the buffer is added. Continue to squeeze as per step 6 and omit the second tissue wash. Powermashing of these samples is also recommended before extraction.
- Different manual squeezing techniques are often required for different tissue types.

Additional Notes:

- Following disruption, the cell pellets derived from this protocol can be used immediately for appropriate downstream procedures, or can be stored long term at –70 °C with no detrimental effects observed in the mid-term (up to 1 year; longer periods have not been tested).

Before starting:

- Prepare ‘Sponge Squeezing Lysis Buffer’ (see the recipe below).

Laboratory Protocol:

1. Prepare all necessary equipment prior to starting and place any applicable items (e.g. cold blocks, tools) onto dry ice.
2. Weigh 150–200 mg of the selected flash frozen sponge tissue on a cold block on dry ice, transferring into a fresh 2 mL microcentrifuge tube before returning to the cold rack. Repeat for each sample.
3. Once all samples have been prepped, samples can be prepared to undergo ‘squeezing’. Move one sample onto the petri dish, which should be placed on the lab bench.
4. Using a dropper pipette, add 2 mL of the prepared lysis buffer directly onto the sponge tissue in the petri dish to soak the sample.
5. Using sterile forceps and scalpel, carefully slice the tissue into smaller sections and pull apart the skeleton. For more fibrous tissue, more cuts and gentle pulling will be required.
6. Carefully tilt the petri dish towards you, so that it is held at an angle. With the other hand, use wide forceps to squeeze the sponge tissue and break it down, further expelling the contents of the cellular interior into the buffer. It can also be useful to use the scalpel to carefully press the tissue against the sides or bottom of the petri dish. The buffer will most likely change colour and become more cloudy as cells are extracted. Squeezing should be done for 5–8 minutes per tissue but can vary widely between types.
7. Using a wide-bore P1000 pipette tip, collect the lysis buffer (~2 mL) into the original 2 mL microcentrifuge tube. Label this tube as ‘A’.

8. Add a further 2 mL of prepared lysis buffer directly onto the remaining sponge tissue pieces and repeat the ‘squeezing’ technique described in step 6.
9. Using a wide-bore P1000 pipette tip, collect the lysis buffer (~2 mL) into a new 2 mL microcentrifuge tube. Label this tube as ‘B’.
10. Repeat steps 3 to 9 for all sponge tissue samples until all tissues have two 2 mL microcentrifuge tubes labelled ‘A’ and ‘B’.
11. Centrifuge all tubes at 10,000 *g* for 1 minute at room temperature to pellet the cells at the bottom of the tube.
12. From tubes labelled ‘A’, carefully remove all the supernatant using a standard P1000 pipette tip and discard into a waste bottle leaving only the pellet.
13. From tubes labelled ‘B’, use a wide-bore P1000 pipette tip to remove 1 mL of supernatant and discard. Using the same tip, gently resuspend the pellet using the remaining supernatant and transfer into matching tube ‘A’. The empty ‘B’ tubes can be discarded.
14. Centrifuge all remaining ‘A’ tubes at 10,000 *g* for 1 minute at room temperature to re-pellet the cells at the bottom of the tube.
15. With the final pellet at the bottom of the tube, carefully remove the supernatant using a standard P1000 pipette tip and discard. Leave 10-20 µL remaining on the pellet. Take care not to disturb the final pellet, as it contains the sponge cells that will be used downstream in DNA extraction or HiC protocols.
16. The cell pellet can now be frozen for long term storage or used directly in the appropriate protocol.

Materials:

- Dry ice
- 2 mL DNA Lo-Bind microcentrifuge tubes (Eppendorf Cat. no. 0030108078)
- Sponge Squeezing Lysis Buffer (see recipe below)
- Pipette for 1000 µL, and filtered tips
- Wide bore pipette tips (1000 µL, filtered if available)
- 250 mL beaker (for waste)

Equipment:

- Corning® CoolRack CF45 (Cat. no. 432051) or equivalent
- Insulated ice bucket (Cat. no. CLS432101-1EA)
- Sterile glass petri dish (Cat. no. BR455742-10EA)
- Sterile scalpel
- Sterile forceps
- Dropper pipette
- Eppendorf Centrifuge 5425/5425 R (Cat. no. 5405000263)

Below recipes should be prepared as a stock prior to starting the protocol

Sponge Squeezing Lysis Buffer

Reagent	Target concentration	Stock concentration	Input from stock (100 mL total)	Input from stock (500 L total)
Ultra-pure H2O	-	-	87.9 mL	200 mL
Tris pH 7.6	10 mM	2 M	12.5 mL	25 mL
EDTA pH 8.0	100 mM	0.1 M	50 mL	100 mL
NaCl	20 mM	-	(up to 500 mL)	(Up to 1 L)
Store stock at room temperature for up to 6 months				

References:

Squeeze" enrichment of intact cells (eukaryotic and prokaryotic) from marine sponge tissues prior to routine DNA extraction- J. Lopez 2022