

Oct 01, 2025

Sanger Tree of Life Sample Homogenisation: Sponge Squeezing



Forked from Squeeze enrichment of intact cells (eukaryotic and prokaryotic) from marine sponge tissues prior to routine DNA extraction



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DOI

<https://dx.doi.org/10.17504/protocols.io.kqdg317qpl25/v1>

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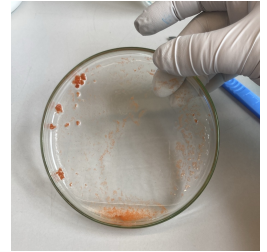
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Protocol Citation: Elizabeth Sinclair, Graeme Oatley, Caroline Howard 2025. Sanger Tree of Life Sample Homogenisation: Sponge Squeezing. protocols.io <https://dx.doi.org/10.17504/protocols.io.kqdg317qpl25/v1>

**Manuscript citation:**

On the path to reference genomes for all biodiversity: lessons learned and laboratory protocols created in the Sanger Tree of Life core laboratory over the first 2000 species | bioRxiv

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Protocol status: Working

We use this protocol and it's working

Created: September 26, 2025

Last Modified: October 01, 2025

Protocol Integer ID: 228292

Keywords: marine sponge tissue, sponge cell, marine demosponge species, various standard dna extraction kit prot..., cell pellet, enrichment of intact cell, microbial symbiont, intact cell, total biomass, cell, dna, spicule, sample homogenisation, sponge squeezing, sponges, marine sponges, reference genome, long read sequencing, sanger tree of life, tree of life programme, demosponges, Demospongia, glass sponges, Hexactinellida, dna extraction from marine sponge tissue, marine sponge tissue, homogenisation of sponge sample, sponge sample, sanger tree of life hmw dna extraction, life hmw dna extraction, fundamental process in sponge, sponge cell pellet, dna extraction, allowing sponge, hmw dna extraction, frozen sponge, flash frozen sponge, sponge, sanger tree of life sample homogenisation, hard siliceous spicule, dna release, glass sponges hexactinellida, most marine demosponge, phylum porifera, life sample homogenisation, sufficient yield of hmw gdna, siliceous skeleton, mechanical lysis of tissue, dna, extraction, spicule, purific

Funders Acknowledgements:

Wellcome Trust

Grant ID: 218328

Wellcome Trust

Grant ID: 206194

Gordon and Betty Moore Foundation

Grant ID: GBMF8897

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.



Guidelines

- 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).

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).

Materials

- Dry ice
- 2 mL DNA Lo-Bind microcentrifuge tubes (Eppendorf Cat. no. 0030108078)
- Sponge Squeezing Lysis Buffer (see recipe below)
- Pipette for 1000 μ L, and filtered tips
- Wide bore pipette tips (1000 μ L, 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 H ₂ O	-	-	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				

Protocol PDF:  Sanger Tree of Life Sample Homog... 150.7KB

Before start

Prepare 'Sponge Squeezing Lysis Buffer' (see the recipe in Materials).



- 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 μ L 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.

Protocol references

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