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

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A “Squeeze” method to enrich for intact cells (eukaryotic and prokaryotic) prior to DNA Extraction from marine sponge tissues

Most marine demosponge species possess a siliceous skeleton and spicules which can compose a high volume of the total biomass. This skeleton can be a priori removed before standard DNA extraction procedures (with CTAB, proteinase K, and various standard DNA extraction kit protocols).

The cell pellets derived from this protocol can be applied in other enrichment protocols prior to standard DNA extraction methods. For example, sponge cells may need to be separated from the large bulk of microbial symbionts by Percoll gradients.

https://works.bepress.com/jose-lopez/about/

Joe Lopez 2022. Squeeze” enrichment of intact cells (eukaryotic and prokaryotic) from marine sponge tissues prior to routine DNA extraction . **protocols.io** https://protocols.io/view/squeeze-enrichment-of-intact-cells-eukaryotic-and-canfsdbn

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Sterile petri dish
Sterile forceps,
Scalpels
Sterile L buffer

1 Collect live or frozen sponge specimens, and have ready with all materials on the bench.

2 With sterile scalpel and forceps cut a piece of sponge mesohyl (tissue) approximately 1 - 2 cm3 place in a sterile petri dish. This can also be done on small fresh or frozen sponge pieces. If frozen, do not let sponge thaw completely but only enough to slice, since cell lysis may begin during thaw.

3 Soak sponge section by dripping 1- 3 ml "L buffer" (10 mM Tris, ph. 7.6, 100 mM EDTA, 20 mM NaCl) on top of sponge tissue in the petri dish. L buffer is derived from from the Sambrook and Russell, Molecular Cloning: A Laboratory Manual (2001).The high EDTA concentration will protect DNA integrity.

4 Mince the tissue as small as possible with the sterile scalpel and forceps

5 Squeeze or press the mesohyl pieces with forceps and against the petri dish bottom, expelling the contents of the cellular interior into the L buffer.

6 Collect the cells (~ 3ml) into microcentrifuge tubes

7 Centrifuge at top speed (10,000g) for 30 secs, or at half the speed if live cells are to be used downstream besides DNA extractions.

8 Decant the supernatant; SAVE THE PELLET– these are the cells both sponge and microbial that will be the source of DNA or other downstream procedures, such as cell fractionations via centrifugation. The cell pellets can now be frozen for longer term storage.

9 Add an additional 1ml L buffer to rinse any remaining cells from the dish, mix with original

tubes, and re-centrifuge

10 Decant supernatant, and save pellet

11 Spin 30sec more and remove remaining supernatant with a pipet; leave 10-20 ul of L buffer on pellet before frozen storage.