Basic Preservation for DNA Extractions and Limited Morphological Work

A) Each individual specimen should be placed into a separate container. We find that 50 ml tubes or WhirlPaks work well.

B) Be sure to label the outside of the container with your specimen ID number. If you do not already have a system in place, you could use your initials followed by a number (e.g., RT53; or in conjunction with a collection event, e.g., Bali2010-RT53).

C) Appropriately label a small piece of solvent-resistant paper with a pencil and place it inside each specimen container.

D) Before fixing each specimen, remove all dirt and contaminating macroorganisms if possible.

E) Place the specimen into the container and fill it with 95% ethanol. Again, it is best to fix a portion of the sponge that includes ectosome and choanosome. If a specimen is huge, these may need to be separate samples. We find that a 1:2 ratio of specimen: ethanol works well. Partially dissecting the specimen(or breaking it apart with your hands) can enhance preservation for thick specimens. Note that this concentration will dilute since the specimen contains water itself. For better DNA preservation, the specimen should be preserved as soon as possible after the collection.

F) After 12-24 hours, change the fixative, replacing it with fresh 95% ethanol. If pigments continue to be extracted from the specimen, an additional change can improve preservation.

G) Store at 5°C or room temperature (if possible or as soon as possible).

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Preservation for Histology: 4% paraformaldehyde (PFA) and 70% ethanol

For many morphological features, especially those observed with histological techniques, it is useful to fix specimens in 4% PFA, followed by storage in 70% ethanol. However, specimens processed in this manner yield little to no successful DNA extraction, PCR, and sequencing.

The following procedure works well to prepare specimens for histological sections used in confocal microscopy and FISH staining.

A) Prepare fixative by first filter-sterilizing seawater; then add PFA for a final concentration of 4%.

B) Incubate specimen in 4% PFA overnight.

C) Transfer specimen to 70% ethanol.

D) Store at 5°C or -20°C.

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Preservation for RNA including microRNAs
75% ethanol series at -20°C

To adequately preserve RNA, and particularly ensuring the preservation of microRNAs, multiple changes of ice-cold 75% ethanol can be used.

A) Keep materials on ice or ice-cold at all times.

B) It is best to preserve 2 “thumb-end size” pieces (or 4 1-cubic-cm pieces) in a 50 ml tube of ethanol. The larger volume ratio of ethanol:sponge, the better.

C) Put fresh tissue in 75% ethanol.

D) Change to fresh 75% ethanol after 15 minutes.

E) Change to fresh 75% ethanol after 1 hour.

F) Change to fresh 75% ethanol the next day.

G) Store at -20°C.

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Preservation for RNA/DNA (using RNAlater, no microRNAs)

This commercially available fixative is often used with success; other labs find it problematic. It is apparently not a good means for preserving microRNAs. Best practices are as follows:

A) Maintain a fixative:tissue ratio of 5:1 at least. More fixative is better.

B) Cut the tissue into very small pieces, e.g., 1-2 mm cubes.

C) Incubate overnight at room temperature.

D) Store at -20°C or -80°C.

An alternative commercially available fixative is DNA/RNA Shield. This product can be used with the same protocol as listed above.

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