Bocas ARTS

Sponge Spicule Preps

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1. Locate the specimens to be processed – you can process multiple specimens at once, just keep track of which is which in your laboratory notebook.

2. Using a razor blade and forceps, cut a roughly 4mm X 2mm piece of tissue from the specimen and place the remainder back into the specimen's container.

3. Rinse forceps and blades with ethanol and flame after each use to reduce cross-contamination.

4. Place a small piece of sponge in a well of a 24-well culture plate. Record the location of each specimen.

5. Add bleach to dissolve the sponge; allow 30-60 min or even overnight for digestion.
    - Can vary concentration of bleach to vary speed of dissolution
    - Some sponges require full concentrations; some will not completely digest
    - Can also move sponge to different wells of culture plate as it dissolves
    - Can stop dissolution at various stages to examine skeletal structure

6. Cut the tips of as many transfer pipettes as samples. Cutting the tip makes a larger diameter pipette, which can be useful if pipetting larger spicules. Make sure to use the same pipette for each specimen throughout the procedure.

7. You can examine spicules under the microscope at this point, but the bleach is not good for you or the microscope.

8. Let the spicules settle to the bottom of the well (wait about 10-15 minutes).

9. Remove the bleach with a glass pipet or a disposable plastic pipet.

10. Using a new pipet, add Milli-Q water to the culture well and wash the spicules by pipetting up and down. You can cut the tip off of a disposable plastic pipet if you are concerned about breaking large spicules.

11. Let spicules settle for 10-15 minutes.

12. Remove water. Repeat washing with water 3 more times to get rid of ALL of the bleach.

13. While waiting for the spicules to settle in each wash, label 3 or 4 microscope slides for each sponge specimen.

14. Remove as much water as possible, then wash once with 95% ethanol.

15. Remove ethanol wash, then add new ethanol for a second wash. Pipet up and down, then let spicules settle for 10-15 minutes.

16. Carefully remove about half of ethanol at top of well (without spicules). Draw the remaining ethanol into the pipette. Hold the pipette vertically for 30-60 seconds so that the spicules will float to the bottom of the pipette.

17. Place about 2 drops of the ethanol containing spicules onto an appropriately labeled microscope slide labeled (with the date and the specimen number). Always try to make several slides for each specimen.

18. Evaporate the ethanol on a warm-to-touch hot plate.

19. Add 1-2 drops of Permount to the dry spicules and carefully cover with a coverslip.

20. Allow the slide to dry (approximately 2 days).

21. Now you have a permanently mounted spicule slide.

22. Examine the slide carefully at 40x, 100x, 200x, and 400x power.

23. Draw, photograph, and measure all types of spicules found. For reference when naming types of spicules, use the *Thesaurus of Sponge Morphology, available here*: https://repository.si.edu/handle/10088/5449

This protocol is adapted from previous work by the Porifera Tree of Life project (www.portol.org).