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OGL Sampling Protocol – OGLFix

Overview	
Protocol:	
1. Document the organism, including photos if possible.	
2. Cut a small piece of tissue:	
☐ Most organisms: 50-1,000 mg of tissue (ideally 200 mg, abou ☐ Small organisms (<100 mg): entire organism	at the size of a pea)
3. Place tissue in vial and screw cap on tightly.	
Notes:	
☐ Samples can be stored and shipped at room temperature.	
☐ OGL welcomes samples from a range of species, as well as up to	10 individuals of the same species.
☐ Please provide copies of any permits required for collecting, sar applicable, importing/exporting material.	npling, transferring, and, if
☐ If possible, please document specimens with voucher material that - whole organisms in ethanol (may be in another collection) - diagnostic parts (dried or in ethanol)	
Safety:	
☐ We recommend wearing laboratory gloves. OGLFix preservative contains eye and skin irritants.	
OGLFix does not require special or hazardous designation for sh	ipping.
actions for sample preparation:	
oals are to preserve the DNA contained in the tissue samples and to minimize sample degradation or contamination w	

Instru

non-source DNA. Where possible, clean the specimen, wear clean gloves, and use tools and surfaces sterilized with bleach and ethanol when sampling. (We realize that these precautions may not be practical in the field, however.)

- 1. Document the specimen: If possible, preserve voucher materials such as images, hard parts, or whole organisms (in ethanol or dried). When photographing specimens, consider including the vial in the photo. Indicate if the vouchers are housed in a museum collection.
- 2. Excise a small piece of tissue:
 - ☐ An optimal sample size is ~200 mg of tissue per tube of OGLFix (1 ml), roughly the size of a pea or an aspirin tablet. For blood samples, 100-500 µl is ideal. Smaller or larger samples (50-1,000 mg) are also acceptable, but please use additional fixative for very large samples (see below).
 - ☐ Avoid tissue from digestive organs. Muscle generally works well. OGLFix is not recommended for corals, sponges, or seaweeds.
- 3. Place the tissue in the vial of fixative provided (OGLFix): The volume of fixative should be at least 5 times the sample volume for solid tissue or 2-3 times the volume for blood. The fixative will penetrate 2-3 mm of tissue, so dice the tissue pieces before fixing, if possible.
 - ☐ For large organisms: If possible, obtain multiple ~200-mg samples representing different tissues and put them into separate tubes.
 - ☐ **For smaller organisms** (<100 mg): Preserve the entire organism if possible.
- 4. Storage and shipping: Samples can be stored and shipped in OGLFix at room temperature. Fixed samples may be stored at room temperature for months, but refrigerated storage is preferable for the long term. Please contact OGL before shipping any material.

Note: OGLFix can be shipped without hazardous designation. It does contain ingredients that are irritants and may be harmful if ingested. Therefore, please wear gloves and return any remaining fixative to OGL for proper disposal.

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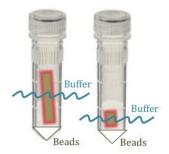
OGL Sampling Protocol – Coral & Sponge Samples (CHAOS)

Overview

Protocol:

- 1. Document the organism, including photos if possible.
- 2. Plug in and switch on the homogenizer (BeadBug). Remove the red screws from the bottom.
- 3. Cut a small piece of tissue and place in vial the vial should be half full.
- 4. Add CHAOS buffer using plastic pipette the ideal volume is 5x the volume of soft tissue, excluding the skeleton.

Examples of tissue/buffer volumes



- 5. Screw cap on tightly.
- 6. Place up to 3 vials into the homogenizer (BeadBug).
- 7. Press the START button to homogenize 2800 rpm for 60 seconds.

Notes:

- Samples can be stored and shipped at room temperature.
- OGL welcomes samples from a range of species, as well as up to 10 individuals of the same species.
- Please **provide copies of any permits** required for collecting, sampling, transferring, and, if applicable, importing/exporting material.
- If possible, please document specimens with voucher material that could include:
 - whole organisms in ethanol (may be in another collection) digital images
 - diagnostic parts (dried or in ethanol)– molecular information

Safety:

- We recommend wearing laboratory gloves. CHAOS preservative ingredients that are corrosive and harmful if ingested. In case of skin or eye contact, rinse affected area thoroughly. CHAOS is incompatible with strong acids, strong oxidizing agents, and cyanides.
- CHAOS does not require special or hazardous designation for shipping.
- Red screws must be secured into the bottom of the homogenizer (BeadBug) before shipping.



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Instructions for sample preparation:

Our goals are to preserve the DNA contained in the tissue samples and to minimize sample degradation or contamination with non-source DNA. Where possible, clean the specimen, wear clean gloves, and use clean tools and surfaces when sampling. (We realize that these precautions may not be practical in the field, however.)

- 1) **Document the specimen:** If possible, preserve voucher materials such as images, hard parts, or whole organisms (in ethanol or dried). Indicate if the vouchers are housed in a museum collection.
 - If photographing the specimen, consider including the vial in the specimen photo.
- 2) **Set up the homogenizer:** Plug it in, switch it on, and **remove the red screws**. Set the speed to 2800 rpm and the time to 60 seconds.
- 3) Excise a small piece of tissue:
 - CHAOS is only recommended for corals and sponges.
- 4) **Place the tissue in vial and add CHAOS buffer:** Use a disposable plastic dropper to add CHAOS to the vial. The volume of fixative should be at least <u>5 times the volume of soft tissue</u>, excluding the skeleton.
 - For large organisms: If possible, obtain multiple samples representing different tissues and put them into separate tubes.
 - For smaller organisms (<100 mg): Preserve the entire organism if possible.
- 5) **Homogenize samples:** Place up to three vials in the homogenizer, press the start button, and homogenize samples at 2800 rpm for 60 seconds.
- 6) Storage and shipping: Samples can be stored and shipped in CHAOS at room temperature. Fixed samples may be stored at room temperature for months, but refrigerated storage is preferable for the long term. Please contact OGL before shipping any material. The red screws must be secured to the homogenizer (BeadBug) before shipping.

Note: CHAOS can be shipped **without hazardous designation**. It does contain ingredients that are corrosive and harmful if ingested. In case of skin or eye contact, rinse affected area thoroughly. CHAOS in incompatible with strong acids, strong oxidizing agents, and cyanides. Therefore, **please wear gloves** and return any remaining fixative to OGL for proper disposal.

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OGL Sampling Protocol – Silica Gel (Seaweed Samples)

Overview **Protocol:** Ideal sample size 1. Document the organism, including photos if possible. for seaweeds 2. Cut a small piece of seaweed tissue, $\sim 1 \times 1 \text{ cm}^2$. 3. Place tissue in bag and seal tightly. cm 4. Affix appropriately numbered label to the bag. **Notes:** • Samples can be stored and shipped at room temperature. • OGL welcomes samples from a range of species, as well as up to 10 individuals of the same species. • Please provide copies of any permits required for collecting, sampling, transferring, and, if applicable, importing/exporting material. • If possible, please document specimens with voucher material that could include: - whole organisms in ethanol (may be in another collection) - digital images diagnostic parts (dried or in ethanol) - molecular information **Safety:** • Silica gel does not require special or hazardous designation for shipping.

Instructions for sample preparation:

Our goals are to preserve the DNA contained in the tissue samples and to minimize sample degradation or contamination with non-source DNA. Where possible, clean the specimen, wear clean gloves, and use tools and surfaces sterilized with bleach and ethanol when sampling. (We realize that these precautions may not be practical in the field, however.)

- 1. **Document the specimen:** If possible, preserve voucher materials such as images, hard parts, or whole organisms (in ethanol or dried). When photographing specimens, consider including the vial in the photo. Indicate if the vouchers are housed in a museum collection.
- 2. Excise a small piece of tissue:
 - An optimal sample size for seaweeds is ~1 x 1 cm².
 - Avoid tissue encrusted with other organisms. Silica gel is only recommended for collecting seaweeds.
- 3. **Place the tissue in a bag filled with silica gel:** The volume of silica gel should be at least 5 times the sample volume for solid tissue. Because silica gel dehydrates samples, it helps if access water is removed from them before collection.
 - For large organisms: If possible, obtain multiple 1 x 1 cm² samples representing different tissues and put them into separate bags.
 - For smaller organisms (<100 mg): Preserve the entire organism if possible.
- 4. Affix the appropriately numbered label to the bag.
- 5. **Storage and shipping:** Samples can be stored and shipped in silica gel at room temperature. Silica gel changes from orange to white as it becomes saturated with moisture. If the silica in a sample bag has turned entirely white, please add some charged, orange silica to the sample. **Please contact OGL before shipping any material.**

Note: Silica gel can be shipped **without hazardous designation**. Please return any remaining gel to OGL for proper disposal.

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OGL Datasheet

Detailed documentation adds scientific value to samples, although we realize that it is not always possible to know everything about each specimen. **Please provide as much data as you can.**

- 1. **Required fields** are marked with an asterisk (*). All other fields are optional but appreciated. We also value any additional notes (such as field observations or water quality data) that you wish to provide.
- 2. **Taxonomy:** If you are uncertain of the correct scientific name, report the finest level of classification that you can with reasonable certainty. Please use ID qualifiers to express uncertainty:
 - a. **sp.** Indicates that the specimen <u>likely belongs to the given genus</u> but we cannot confidently assign it to a species (e.g., *Solemya sp.*).
 - b. **cf.** We use **cf.** (Latin for confer = compares with) with a species epithet (e.g., *Solemya cf. velum*) to indicate that the specimen <u>likely belongs to the named genus and species</u> but there is doubt and further analysis should be done.
 - c. **aff.** We use **aff.** (**Latin for affinis** = **related to**) with a species epithet when the biological species is probably not the named species but appears to be closely related to it (e.g., *Solemya* **aff.** *velum*, means a species of *Solemya* which is similar to *S. velum* but is probably not *S. velum*).
- 3. **Sample:** A sample is any part or derivative of an organism (e.g., an aliquot of blood or a piece of tissue). For very small organisms, the entire specimen (whole organism) may also be considered a single sample. Each sample is assigned a unique tube number, which is printed on the provided labels.
- 4. **Organism #:** To keep track of which samples came from each individual organism, please number each individual organism, starting with 1. For example, if tubes #10, 11, and 12 each contain a sample taken from organism #1, all three entries should have 1 as the organism number.
- 5. **Date of collection/sampling:** Please provide the date when the organism was collected, and if different, the date when the sample tissue was removed from the organism.
- 6. **Collection location:** GPS coordinates are preferred. It may be possible to estimate using websites such as Google Maps to locate the collection site using nearby landmarks as a guide. Where possible, indicate the estimated accuracy of your coordinates. We also appreciate information about the collection event, e.g., a cruise, dive, trawl, or visit to a tide pool.
- 7. **Vouchers:** Traditionally, a voucher is a specimen (or diagnostic parts of a specimen) set aside to serve as a morphological reference for the remaining samples and extracts. When possible, the voucher materials should be derived from the same specimen as the samples and extracts. If your materials are related to vouchers in another publicly accessible collection, include the institution name and accession number on the spreadsheet. In addition to traditional morphological vouchers, OGL also accepts:
 - a. **Electronic vouchers:** These may include digital images, x-ray images, sound files, etc.
 - b. **Molecular vouchers:** These may include mitochondrial cytochrome oxidase I sequences (DNA barcodes) or other standard molecular biomarkers, such as ribosomal RNA genes.