| Coral Removal and Tissue Processing | |
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| Prepared by: | Bahr Marine Ecology Lab |
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| Safety Precautions: | |
| * Required PPE – Enclosed shoes, gloves | |
| Purpose: | |
| * Removal of coral tissue for analysis * Proper homogenization and aliquoting of sample for analysis | |
| Materials: | |
| * Airbrush * Two buckets of ice (one for airbrushing, one for homogenizing) * 10% bleach solution * 70% ethanol solution * Reverse Osmosis (RO) water * Phosphate Buffer Solution (PBS) solution   + PBS tablets to make more PBS solution * Two labeled 250mL tri-corner beakers:   + one to rinse ethanol into (ethanol waste)   + one to rinse RO water and biological waste into (sink waste) * Airbrushing containers with 1. PBS water 2. 70% ethanol * 50mL Falcon tubes for each sample * 1.5uL Eppendorf tubes for aliquots * 1 sliding Ziploc bag for each coral sample * Stand for homogenizer * Homogenizer (tissue master) * Vortex * Centrifuge * Timer * 1000uL pipette * 1000uL pipette tips * Pipette disposal bin * Graduated cylinder   Notes:  *Before beginning, make sure all Falcon tubes and Eppendorf tubes are prelabeled and ready to go*  *Never reach a hand into the bag of Eppendorf tubes. Wear gloves and shake tubes out of the bag onto a paper towel. Never put tubes back into the Eppendorf bag. All to eliminate contamination* | |
| Airbrush Set-up: | |
| * Place airbrush motor under the hood * Unwrap chord and attach actual airbrush from kit and screw the fat end of the cord into the motor and the smaller end of the cord into the brush * Turn airbrush motor on (button on top of motor) * Pull out knob on airbrush motor to adjust pressure. To the left removes pressure, to the right increases pressure. Twist all the way to the right until it doesn’t go anymore (maintain pressure between 3 and 4). Push down on airbrush button to increase pressure.   *To use the airbrush, if you press the airbrush button down, it’s air, if you press and hold back, it's water.*   * Clean airbrush into a beaker, first with ethanol attachment * Then attach PBS bottle and clean airbrush again into another beaker     *Always keep airbrush upright, place in a larger beaker or on falcon tube stand*  *Keep airbrush tip all the way closed (all the way to the right) – should already be like that when you set-up and break-down, don’t adjust if you don’t have to* | |
| Airbrush – Removing Coral Tissue: | |
| * Take coral sample out of whirlpack and place in sliding Ziploc bag (save whirlpack for the coral skeleton) * Place coral in bottom corner of Ziploc bag to maximize surface area being sprayed * Slider to close Ziplock should be at top so you can zip your hand inside to make sure spray isn’t coming out of bag * Don’t want to spray too much water (make sure final volume in the falcon tube is the same for every sample), so switch back and forth to just air to spray off excess water * The entire coral should turn white. Be consistent with how much you spray for each sample * Once done spraying, close bag as much as you can then pour water sample out from the corner of the bag into the 50mL Falcon tube * Place Falcon tube back on ice. Sample should ALWAYS be on ice. * Rinse Ziploc bag one last time with PBS into a tri-corner beaker for water/biological material waste. | |
| Airbrush Clean-up: | |
| * Turn airbrush motor off * Hold down toggle until air is released (at 0) * Keep cord attached to compressor but remove cord from airbrush pen and place pen in airbrush box (wipe down with Kimwipe) | |
| Airbrush Troubleshooting: | |
| * If the airbrush is spraying liquid when it should be spraying air (i.e., when you press directly down it should be air and when you press down and pull back it should be liquid from the attached bottle (PBS or ethanol)) then:   + Remove the red back piece (labeled 1)   + Unscrew the first metal piece (labeled 2)   + Slightly adjust the needle (push slightly forward if it is spraying only liquid OR pull slightly back if it is only spraying air) (labeled 3)   + **PRO TIP:** When adjusting the needle, you should be able to look through the front piece (labeled 4) and push the button down and then use the lever (labeled 5) to move the needle back and forth. The needle should have a little bit of space between it and the head of the airbrush when pulled back, allowing water to move through. When just pushed down there should be no space between the needle and head, allowing no water to move through. | |
| Homogenizing: | |
| * Homogenizer should already be vertical in stand, if not, then place it in vertical stand * Plug in homogenizer, vortex, and centrifuge      * Fill 2-50mL Falcon tubes, one with 10% bleach and one with RO water * Remove homogenizer cap * Rinse homogenizer with bleach then RO water @ level 4 for 30 seconds * Homogenize the sample in the Falcon tube at level 4 for 30 seconds (set timer). Move sample up and down when homogenizing   + If sample is greater than 40mL invert 5 times to ensure proper homogenization * Place sample in centrifuge for 5 minutes at 3000rpm (make sure sample is balanced – need the same volume in every Falcon tube) * Pipette 1000uL (1mL) of coral supernatant into Eppendorf tubes for each aliquot (e.g., protein) one for actual analysis, one for backup     *Keep pipette up-right hanging, not laying down on lab bench*  *Keep pipette tip box closed to eliminate contamination.*  *Use a new pipette tip between the supernatant and pellet samples.*   * Empty remaining liquid into biological material waste container WITHOUT emptying the algae pellet * Pipette (or use graduated cylinder) 5mL of PBS solution into 50mL Falcon tube with the algae pellet * Vortex Falcon tube for 1 minute until PBS/algae are well mixed * Pipette 1000uL into tubes aliquoted for Chl-a and zoox counts and backups of each   *Vortex between aliquots to ensure samples stay well mixed and* integrated   * Clean homogenizer with FRESH bleach and then RO water. * Unplug homogenizer (ALWAYS DO THIS FIRST).   + Disassemble generator tube and rotor shaft per instruction manual. Wipe down rotor shaft with 10% bleach and immediately follow up with 70% ethanol to remove residue. Be very careful not to bend rotor shaft.   + Clean generator tube with 10% bleach solution and follow up with 70% ethanol, using brush to clean inside of tube. Wipe dry with Kimwipe, then reassemble. Leave homogenizer vertical in stand to dry further with a beaker underneath to catch any dripping water.   *NEVER turn on homogenizer without generator tube attached as this will bend the rotor shaft.* *ALWAYS submerge probe in liquid before starting. Never run dry.* *Do not submerge drain hole or leave probe submerged for an extended period. Doing so can cause rust to build up on the shaft.*   * Unplug centrifuge and vortex * Clean up all supplies (e.g., tubes, pipettes, pipette tips and put back in correct locations) * Rinse out the used 50mL Eppendorf tubes with RO water, clean off sharpie labels with ethanol, and place in re-use, drying rack | |
| Waste Disposal Clean-up: | |
| * Bleach, RO, and biological material can go down the drain * Ethanol waste needs to be put in the waste container under the hood (labeled with ethanol) * Rinse used tubes and bottles with a 10% bleach and RO solution and dry on drying rack   *Keep bottles with bleach and ethanol under hood. Keep PBS bottles refrigerated.*  *Make sure all surfaces are wiped down and all equipment is unplugged before you leave the lab.*  *Make sure to re-fill PBS bottles at end of the day for the next user. To make more PBS, add 5g for every 500mL of water.* | |
| Quality Assurance and Control: | |
| *Proper Training*  Proper protocols and training must be implemented to ensure the quality of data generated in the laboratory. Researchers must ensure that all equipment is accurately calibrated, inspected, and maintained according to the manufacturer’s instructions.  *Data Review*  All laboratory data will be reviewed for completeness and transfer errors. Data will be reviewed by a second individual after entry into Excel spreadsheets by comparing the entered, electronic data to the original records (e.g., hand-written datasheets or laboratory notebooks). Data will be summarized as descriptive statistics and in tabular and graphical form to allow visual inspection and verification, and comparison to expected or target values.    *Data Verification*  Data will be checked for compliance with the procedures outlined in the SOPs. Any deviations from those procedures and the impact on the quality of the data will be assessed and discussed with Task Members. Any laboratory data outliers will be flagged.    *Data Validation*  Once the data has been reviewed and verified, it will be assessed to determine the overall acceptability of the objectives of the project. Blank samples, such as water quality testing, will be used to determine any biases or instrument calibration issues during the sample collection and analysis processes. Control samples will be used to determine the condition of the experimental test specimens in the absence of experimental treatments or exposures. Any errors in datasets detected will be discussed with lab members and project leads to determine the impact on the data and its use for the project. If there are any limitations to the data, they will be disclosed as part of the published literature.  *Procedure Specific QA/QC Methods* The same individual performs tissue removal to decrease differences across members. Lot numbers and expiration dates for consumables are recorded by personnel performing the testing on datasheets or logbooks, as appropriate. Reagents or standard solutions are used beyond the expiration date printed on the label. All supplies, equipment, and consumables procured for the analysis of this study are documented, inspected, and accepted in accordance with the requirements of each. All instruments used have been calibrated according to calibration procedures described in the instrument manuals. | |