| Symbiont Cell Counting | |
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| Prepared By: | Bahr Marine Ecology Lab |
| Last Updated: | December 2022 |
| Prerequisite SOP: Coral Airbrushing Protocol | |
| Safety Precautions: | |
| * Required PPE – Enclosed shoes, gloves | |
| Purpose: | |
| * To count the number of symbiotic algae (zooxanthellae) in a sample | |
| Materials: | |
| * Samples (thawed with vortex or in the fridge) * Labeled dilution Eppendorf tubes * 3 pipettes (1000uL, 200uL, 10uL) * 3 pipette tip boxes (1000uL, 200uL, 10uL) * Pipette disposal * PBS * Gloves * Kimwipes * Lens Paper * 70-95% ethanol * Microscope * Hemacytometer * Cover slide * Ice bucket * Counter | |
| Preparing Hemocytometer and Microscopes: | |
| * If using a glass hemocytometer and coverslip, clean with alcohol before use.   + Moisten kimwipe with 70-95% ethanol and wipe down the hemacytometer   + Moisten the coverslip with water and affix to the hemocytometer.   + Place the pipette steadily next to the cover slip at a downward angle and slowly inject sample under cover slip.   + Always use LENS PAPER on the microscope, not Kimwipes Graphical user interface, application      Description automatically generated with medium confidence | |
| Preparing Sample: | |
| * Invert the sample/vortex to ensure the sample is well mixed (be consistent with the amount of time you mix the sample I.e., 20 seconds each time it is remixed) * Immediately after, take a subsample using a sterile pipette and place in a new Eppendorf tube. * Add filtered seawater (FSW) for appropriate dilution into the same Eppendorf tube as the subsample   + Start with 1:3 dilution (100 µL sample to 200 µL FSW)   + Conduct appropriate dilution for ~100 cells for the entire sample. If more than 300 cells, repeat with greater dilution. (Increase dilution by 100uL and re-examine cell density under the microscope)   + Make sure to write down your dilution (sample: FSW) | |
| Loading Samples: | |
| * Always clean the slide before you load the sample by rinsing the slide and cover slip with 70-95% ethanol. Air dry/gently wipe the slide and cover slip with *Kimwipes*.   + Never use paper towels or soap. * Place the clean and dry slide on your work surface and place the coverslip on top to cover the reflective surfaces. * Invert/Vortex sample in Eppendorf tube vigorously until mixed.   + Take a pipette and mix the sample within the Eppendorf tube.   + Vortex for 5 more seconds, to ensure proper mixture.   + If the sample has clumping, use a glass pipet and pipet up and down 10x to break up the cells. If you do this to one sample, make sure to do it to the rest as well for consistency’s sake * Using a pipette, take 10 µL of diluted sample and apply it to the V-shaped groove on each side of the hemacytometer. If using a glass hemocytometer, very gently fill both chambers underneath the coverslip, allowing the cell suspension to be drawn out by capillary action.   + If you have diluted your cells in a test tube, invert the tube several times to resuspend the cells.  Using a micropipette, quickly and smoothly without interruption, add 10 μl of your cell suspension (or 1 drop from a transfer pipette) to the V-shaped groove on each side of the hemacytometer., If the slide is clean, the suspension should move quickly under the cover slip covering the entire reflective surface of the hemacytometer. The suspension should not flow into the channels or gutters along the slides of the reflective surface. If your sample moves into the gutters, you may not have loaded the sample in the correct location or you may have used too large of an aliquot. Practice first and make any adjustments that are necessary. If the sample does not flow quickly across the surface the hemacytometer may not be clean or you may not have expelled the solution quickly enough. | |
| Counting: | |
| * Using a microscope, focus on the grid lines of the hemocytometer with a 10X (yellow) objective lens. * Using a hand tally counter, count the live cells in one set of 16 squares (each corner consists of 16 small squares)   + When counting, employ a system whereby cells are only counted when they are set within a square or on the right-hand or bottom boundary line. CONSISTENCY is KEY * If there are still clumps remaining, use the fine focus to focus on the different layers of the clump and count as many cells as you can. * Move the hemocytometer to the next set of 16 corner squares and carry on counting until all 4 sets of 16 corners are counted. * Finish counting all 4 corner squares (outlined in red below) (n=4 counts) for the sample. Record the total number for that replicate. * Repeat counting 4 times for each diluted sample (n=4 replicates). Average the number per colony. * Average all counts per colony with the dilution factor and standardize to the surface area.   + This is what a zooxanthellae cell should look like | |
| 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* All instruments are calibrated according to calibration procedures described in the instrument manuals.  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 not 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. | |