



STANDARD OPERATION PROCEDURE – KELP PERPETUAL MAINTENANCE

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1. SCOPE AND APPLICATION

The purpose of this Standard Operating Procedure is to establish a uniform procedure for the upkeep and maintenance of kelp gametophyte strains of various species under low growth conditions. This SOP is meant to be followed by ASC Personnel, and as a training base for workforce development.

2. SUMMARY OF METHODS

Following the database media change schedule, racks of Kelp gametophyte strains will be subject to media changes by decanting at least half of their volume into a wastewater bin and then replaced with fresh growth media. Wastewater will be mixed with bleach solution to a final bleach concentration of 10%.

3. HEALTH AND SAFETY

Safety Data Sheets (SDS) detailing the hazards of media and other chemicals are available in the laboratory at the SDS Station.

Personal Protective Equipment requirements: The operator must wear a laboratory coat, nitrile gloves, and safety glasses when performing the PROCEDURE section.

4. DATA AND RECORDS MANAGEMENT

Each event of growth media change will be noted in our database. Records of operator, and date will be recorded.

5. QUALITY CONTROL

High humidity can cause holdings to promote the growth of mold. To minimize this problem, ensure that no growth media spills on the labels or sides of the culture tubes.

Culture conditions may vary amongst strains in collection, as place of origin conditions could be different. (e.g. Certain strains use $\frac{1}{2}$ PES Seawater, or $\frac{1}{4}$ Iron Concentration). Check the Spreadsheet column [Growth Media] to ensure that the right media is being used.

Avoid work fatigue from doing media changes on more than five racks at a time. This will minimize the risk of making mistakes while working with hundreds of samples.

6. EQUIPMENT AND SUPPLIES

1. 5 mL Culture tubes
2. Culture tube rack
3. 5 Liter Carboy
4. Printed Labels
5. Sterile Artificial Seawater with appropriate growth media
6. 50 mL serological pipette
7. Pipette pump
8. 600 mL Sterile Beaker
9. Wastewater collection bin

10. 70% Ethanol spray bottle
11. Nitrile gloves
12. Laboratory coat
13. Safety glasses

7. PROCEDURE

1. Following the schedule posted under Media Change Log Spreadsheet ([LINK](#)), determining which strains require a growth media change.
2. Turn on the Laminar Flow Hood light (Red light only) and fan.
3. Spray down the Laminar Flow Hood surface area with 70% Ethanol and wipe clean with a paper towel.
4. Bring one rack of gametophyte strains into the Laminar flow hood.
5. Open a culture tube, and decant at least half of the growth media, gently tap it against the wastewater bin to break water surface tension. Replace the cap on the tube. Replace the cap without tightly sealing it, repeat this process for all the tubes in the rack.
6. Pour 250 mL of sterile appropriate media in Seawater into a sterile 600 mL Beaker.
7. Use a sterile 25 mL serological pipette to draw 25 mL of Growth media. One by one, grab the culture tubes, uncap, and add PES Seawater to each of them up to 4 mL. Recap each tube as you have finished filling them.
8. Only work with one rack at a time, this will prevent the tubes from becoming too warm, and potentially thermally shocking the strains.
9. Return the rack back to its place, and move on to the next rack, if this was the last rack needing media change, clear the Laminar Flow Hood of any instruments used in this protocol and use a paper towel to wipe the surface with 70% Ethanol. Then turn off the light and fan.
10. Sterilize all wastewater accumulated during the media changes in 10% bleach for 24 hours.
11. Upon completion of media change on the strains, update the database to reflect new operator, and date of media change.

8. BIOSECURITY

To ensure no accidental release of organisms occurs, all wastewater and instruments will be submerged in a 10% bleach solution for 24 hours prior to being disposed of into the drain connecting to the sewer system to be treated at wastewater treatment plant or solid waste bin, respectively.