STANDARD OPERATION PROCEDURE – KELP GAMETOPHYTE ISOLATION

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1. SCOPE AND APPLICATION

The purpose of this Standard Operation Procedure (SOP) is to establish a uniform procedure for isolating gametophytes from a 6-welled plate to start a strain collection of single celled individuals.

2. SUMMARY OF METHODS

Kelp meiospores left to develop in 6-welled plates for ~2 weeks under ideal growth conditions will be ready to be transferred into individual wells for creating a monoclonal strain. Using a pulled pipette, and an inverse microscope, look for individual sexually differentiated cells to pick up and transfer into an empty well with seawater + growth media.

3. DATA AND RECORDS MANAGEMENT

Once isolation is confirmed by reviewing the single celled wells, a new accession can be input into our database following the established nomenclature. Data to be entered will include parental collection metadata, as well as all process stages since meiospore release.

4. HEALTH AND SAFETY

All microbiologically contaminated media and kelp tissues in the laboratory shall be bleached (10%) prior to disposal.

Laboratory equipment and benches shall be disinfected with Ethanol (70%) prior and after working with any kelp material.

Sharps, such as razorblades must be discarded in proper sharps containers, care must be taken when handling such instruments.

All accidents, particularly those which may result in infection, shall be reported to the Laboratory Supervisor.

SDS sheets detailing the hazards of media and reagents are available in the laboratory.

5. QUALITY CONTROL

Use different pipettes for different sexes, to minimize failed isolation risk to have a mixed sex culture.

6. EQUIPMENT AND SUPPLIES

6-well plate with gametophytes	24-well plate
Inverted Microscope	5 mL culture tubes
Pulled Pasteur pipettes	25 mL Sterile serological pipette
Nalgene tubing	Pipette Pump
Growth Media	Growth Media + GeO_2
Parafilm	Labels
Permanent Marker	Laminar flow hood

Alcohol lamp	Metal Forceps
Scissors	Sterile 250 mL Beaker
Nitrile gloves	Laboratory coat

7. PROCEDURE

1. After a week under low light (<5 µmol m^{-2} s^{-1}) and 10°C has passed, check on meiospore release 6-well plates, using an inverted microscope with red light. Check if the meiospores have developed into gametophytes and check for size, 5-10 cells are ideal for transferring into 24-well plates, and for differentiation between male and female gametophytes.
2. Prepare pulled pipettes by flaming them over an alcohol lamp using metal forces to slowly pull apart to leave a small opening. If it is closed all the way, it can be reopened by clipping it with forceps.
3. Cut a ~0.5 m piece of Nalgene tubing, and attach the pulled pipette over one end, and use cotton or Kimwipe on the other end to avoid sucking in any seawater.
4. Using a dissection or inverted microscope, use the pulled pipette to mouth-pipette up selected gametophytes.
5. Prepare a 24-well plate with appropriate labeling, following a general nomenclature of noting Species, collection site, sex, and date.
6. Transfer the gametophyte by blowing it gently into a 24-well plate filled with Growth Media seawater + GeO_2. Repeat this step for all wells with different individual gametophytes.
7. Once all the wells are filled, wrap the plate with parafilm and keep it cold (~12 °C) and dark (<5 µmol m^{-2} s^{-1}).
8. Check the gametophyte status after 1 month, to ensure your isolation was successful, in terms of catch, and correct identification. If isolation was successful, transfer the strain into a 5 mL culture tube. Repeat this step for all successfully isolated gametophytes.
9. Create a label for the strains as they are put into the 5 mL culture tubes. Strain ID at time of confirmed isolation will follow the following nomenclature: "KA#-#####" following serial number increases.
10. Once the strain is established, add it to the database, and all culture related spreadsheets.

8. BIOSECURITY

8.1 WASTE MANAGEMENT & DISPOSAL

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.

9. TRAINING AND COMPETENCY REQUIREMENTS

• Personnel must complete lab and aquaculture safety training before handling kelp materials.

• Training on the use of microscopes, hemocytometers, and sterile techniques is required.

• Annual competency assessments should be conducted for all personnel performing this protocol.

10. FIGURES

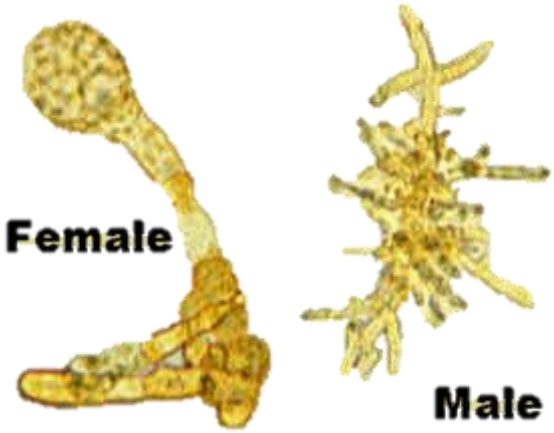

Figure 1. Female and Male gametophyte as viewed under a microscope.