STANDARD OPERATION PROCEDURE – MEIOSPORE RELEASE

Author: _Michael Marty-Rivera Date: _____
Collection Curator

Approval: _____ Date: _____
Director of Operations

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1. SCOPE AND APPLICATION

The purpose of this Standard Operation Procedure (SOP) is to establish a uniform procedure for inducing the release and capture of meiospores from kelp sporophyll/sorus tissue with the purpose of isolating offspring strains for seedbanking purposes or seeding on a line for farming applications.

2. SUMMARY OF METHODS

Kelp reproductive material is cleaned and desiccated to promote meiospore release. After 24 hours in darkness and low temperatures (10°C), the tissue is resubmerged in sterile sea water to stimulate spore release. Cell count estimates are used to determine cell density, and appropriate volume is used depending on the end goal.

3. HEALTH AND SAFETY

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.

Safety Data Sheets (SDS) detailing the hazards of media and reagents are available in the SDS Binder located in the laboratory.

4. ROLES AND RESPONSIBILITIES

Collection Curator – Oversees meiospore release procedures, ensures compliance with the SOP.

Laboratory Technicians – Perform meiospore release following this SOP.

Director of Operations – Approves SOP and ensures alignment with regulatory requirements.

Quality Control Personnel – Monitor and document procedure accuracy and updates database.

5. DATA AND RECORDS MANAGEMENT

Success and failure of release from sporophyll/sorus tissue will be recorded in accordance with parental tissue ID.

In the case of a successful release, motile and non-motile meiospores will be accounted for and data logged into cloud-based spreadsheets.

Subsequent accession of collected material offspring (i.e. Gametophytes) into the culture collection will reference this data and be necessary for future work.

6. QUALITY CONTROL

Prior to starting meiospores release, look for spore-print on paper towel wrapping (Figure 1).

Ensure all epiphytes and debris has been removed from collected material.

Ensure all materials used in this protocol are sterile, as contamination at this stage can complicate final use of the released meiospores.

Work in a clean room environment, using laminar flow hood as much as possible.

Never return a taken sample back into beakers to avoid potential contamination.

Important to note, if releasing meiospores for isolation purposes, you must not use the same materials for all the samples as doing so can introduce cross-contamination between samples.

Ensure you have low mucous sorus tissue before attempting this protocol, as mucous will trap most meiospores.

7. EQUIPMENT AND SUPPLIES

1. Ripe sorus tissue material	2. Beakers for spore release
3. Iodine solution 3%	4. Shade cloth
5. Scraping tool (razor blade, cotton balls)	6. Hemocytometer
7. Paper towel	8. Micropipette + tips
9. Ziplock bag	10. Transfer pipette
11. Sterile filtered Seawater	12. PES seawater
13. Beakers for rinsing	14. Recipient (6-welled plate, etc)
15. Sharpie markers	16. Ethanol 70%

8. PROCEDURE

Phase 1. Reproductive material cleaning.

1. Cut out all unhealthy and unreproductive tissue from the sporophyte.
2. Clean with paper towel or cotton balls; if the tissue is not rugged (i.e., *S. latissima*), you can use a blunt razor blade to scrape epiphytes and other organisms from it.
3. Cut the further down to small pieces (5 cm^2).
4. Dip for 5 seconds in a 3% iodine solution, then sequentially rinse in two different sterile seawater beakers to ensure to remove all iodine from the tissue.

5. Move the sporophyte tissue into clean paper towel and lightly dampen it with sterile seawater, while taking care that no tissues are touching, and put inside a Ziplock bag. Keep in darkness and 10°C for 6-24 hours. This will dessicate the sporophylls and stress them.

Phase 2. Meiospore release.

6. After incubation, check for a spore print (figure 1.B) on the paper towel, this usually serves as an indicator for meiospore release.
7. Move the sorus tissue into a 600 mL beaker with at least 100 mL of sterile seawater.
8. Adjust seawater volume as needed to maintain appropriate meiospore density.
9. Let the sorus tissue sit in the beaker for up to 30 minutes on a shaking table, otherwise stir occasionally.
10. Count meiospores in solution using a microscope (10x magnification) and a hemocytometer (figure 2) to measure cell density.

density calculation formula:

$$\frac{Average\ \#\ of\ cells\ per\ square \times Dilution\ Factor\ (if\ any)}{Volume\ of\ the\ square} = Cell\ Density$$

11. Once you establish your desired density, pour the spore solution into a recipient like 6-welled plate, petri dish, or tube following naming conventions (Figure 2).
12. Desired density depends on application. We have found that for gametophyte isolation a density of at most 1,000 cells/mL is ideal; for 35-40 meters of spool seeding a range of 5,000 – 10,000 cells/mL is desirable.
13. Once meiospores are poured into recipient, keep them away from light, to prevent any sexual interaction before being moved into desired conditions (isolation, or proliferation).
14. Check on the plates after a week under darkness and 10°C, using an inverted microscope, use red light if available. Check if the meiospores have developed into gametophytes and check for size, 5-10 cells are ideal for transferring into culture tubes, and for differentiation between male and female gametophytes. Wrap the plate with parafilm and keep in cold (~12C°) and dark (<5 µmol quanta m^{-2} s^{-1}).

9. 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.

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.

10. TRAINING AND COMPETENCY REQUIREMENTS

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

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

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

11. FIGURES

Figure 1. sorus tissue (Dark) on kelp blade, Spore print on paper towel.

Figure 2. Hemocytometer for cell counts.

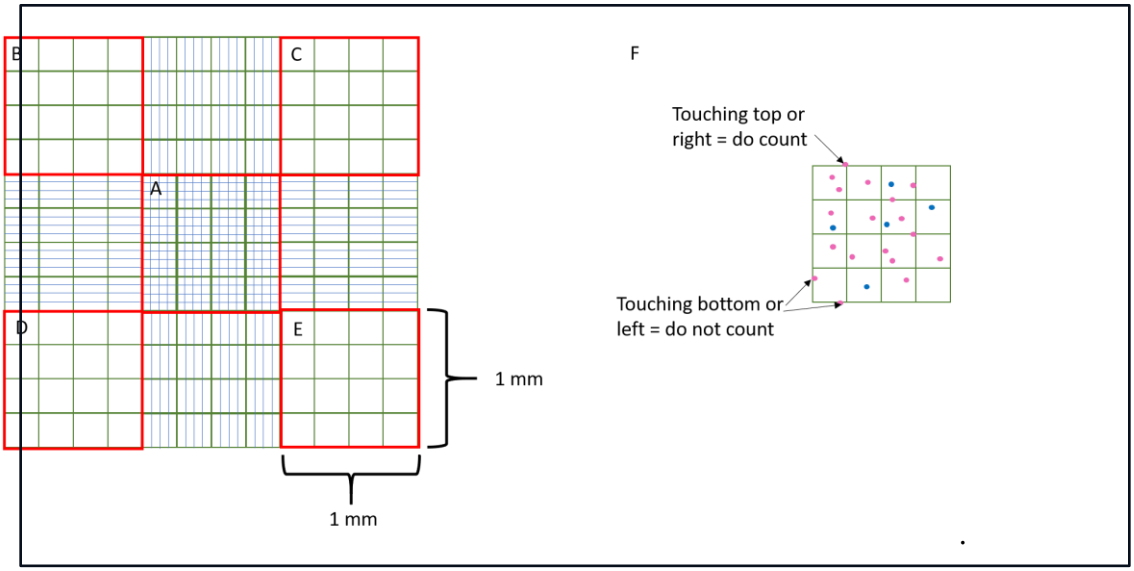

Figure 3. Six-Welled plate