Starting Algal Cultures in Flasks from Algal cultures on Agar Plates

(Based on Methods developed by Biology Program Staff)

1. SCOPE AND APPLICATION

1.1. This procedure is used to help maintain mono-cultures of test algae by starting new cultures from plated-cultures. New cultures are typically started when problems are noted in the regular cultures by toxicology or algal taxonomy staff.

2. SUMMARY OF METHOD

2.1. Culture media is placed into culture flasks. Plated cultures are aseptically transferred to flasks. Flasks are placed in appropriate culture areas.

2.2. Interferences:

2.2.1. Cross-contamination can occur if aseptic techniques are not used during the culture transfers.

2.2.2. Incorrect incubation of the cultures can interfere with growth and viability of the cultures.

2.3. Definitions

2.3.1. ***Pseudokirchneriella subcapitata*:** freshwater algae; formerly known as *Selenastrum capricornutum* (labeled as P. sub)

2.3.2. ***Dunaliella tertiolecta*:** saltwater algae (labeled as Dun)

3. EQUIPMENT AND SUPPLIES

3.1. Two 250 mL Erlenmeyer flasks with foam plugs, sterilized per DEP SOPs BG-08 and TA-04.08

3.2. Labeling tape, red and green

3.3. Two 100 mL Graduated cylinders

3.4. Shaker rack, maintained at 25 ± 3 °C with 24-hour fluorescent lighting (360-440 foot-candles), rotating at 100 ± 10 rpm

3.5. Incubator, maintained at 20 ± 2 °C with 24-hour fluorescent lighting (360-440 foot-candles), flasks shaken by hand daily

3.6. Disposable sterile inoculation loops, Fisherbrand® Steriloops™

3.5.1. If single-use inoculation loops are not available, use a Bunsen burner and flint striker (or piezoelectric sparker) to flame sterilize a platinum-inoculation loop.

3.8. Black, fine-tip, permanent marker

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4. REAGENTS AND STANDARDS

4.1. *Pseudokirchneriella subcapitata* agar plate culture, 2-4 weeks old, prepared per SOP TA-04.18 and verified for purity by the Algal Taxonomy Group (refer to the Quality Control section of this SOP).

4.2. *Dunaliella tertiolecta* agar plate culture, 2-4 weeks old, prepared per SOP TA-04.18 and verified for purity by the Algal Taxonomy Group (refer to the Quality Control section of this SOP).

4.3. Culture media

4.3.1. *P. subcapitata*—PAAP, ≥90 mL, prepared in advance per SOP TA-03.03.

4.3.2. *D. tertiolecta*—30 ppth ASW with nutrients (NEPT), ≥90 mL, prepared in advance per SOP TA-03.04.

CAUTION: Do not confuse with basal media which does not contain nutrients (NEPT).

5. CULTURE PREPARATION

5.1. *Pseudokirchneriella subcapitata* and *Dunaliella tertiolecta*

5.1.1. When needed, gather the materials for the new algal cultures and place them in the laboratory hood.

5.1.2. Using a black marker and the label tape (green for P. sub and red for Dun), label a 250 mL flask with the following:

a. Culture name
b. From plate batch #
c. Media (PAAP or 30 ppth ASW)
d. Transfer date
e. Analyst initials

5.1.3. In a clean graduated cylinder, measure 90 mL ± 10 mL of PAAP media. Pour into the appropriately labeled 250 mL flask.

5.1.4. In a second clean graduated cylinder, measure 90 mL ± 10 mL of ASW media. Pour into the appropriately labeled 250 mL flask.

5.1.5. Place the flasks containing the media in the UV sterilizer (in the microbiology lab) and sterilize for 2 minutes.

5.1.5.1. Warm up the UV sterilizer by turning it on and letting it sit for 15 minutes.

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5.1.5.2. UV sterilize PAAP and ASW media separately. Do not sterilize at the same time. Sterilize PAAP media first then ASW media. (Refer to the Quality Control section of this SOP.)

5.1.6. Under the Tox hood, remove the top from the P. sub agar plate.

5.1.7. Use a sterile disposable inoculation loop to gently scrape ½ of the surface of the agar covered by the algae, taking care not to go more than the diameter of the plastic forming the loop below the surface of the agar.

5.1.7.1. If a disposable inoculation loop is not available, prepare platinum wire-inoculation loop. **Use proper protective equipment and keep flammable materials away when using the Bunsen burner.**

5.1.8. Remove the foam plug from an appropriately labeled 250 mL flask. Immerse the loop in the media and agitate the loop to dislodge the algae. Remove the loop from the flask. Reinsert the foam plug in the flask, leaving half of the plug above the rim of the flask.

5.1.8.1. Do not put the foam plug down on any surface. If the foam plug is put down it will need to be re-sterilized, or a new sterile foam plug will need to be used.

5.1.9. Repeat Steps 5.1.6. through 5.1.8. using the Dun algae and ASW medium.

5.1.9.1. This method (Steps 5.1.6 through 5.1.9) is for starting new algae cultures. For weekly (Thursday) transfers, the plates are poured into the flasks labeled “From Plate”. Pour about 4 mL into the flask making sure there is still green left on the plate.

5.1.10. Place the “Dun” flask in a Tox lab incubator maintained at 20 ± 2 °C. Swirl the flasks by hand at least once daily and noted on the algae swirl sheet located outside incubator 7. Place the “P. sub” flask on shaker platform “B” in the Tox lab, maintained at 25 ± 3 °C. If necessary, turn the shaker off before placing the flask on it. Turn it back on when the flask is in place. Both species should be exposed to 24-hour fluorescent lighting (360-440 foot-candles).

5.1.11. Record the flask set up in the *Algae Culture Record* logbook.

6. DATA ARCHIVAL

6.1. Use a black, waterproof pen for all bench sheets and logbooks.

6.2. Record preparations in the *Algae Culture Record* logbook.

6.3. To correct raw data entries, place a single line through the incorrect or illegible entry. Write the corrected entry near the error, date and initial the correction. Do not overwrite entries.

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6.3.1. The corrected entry is written as near as possible to the error or in the comment box on the appropriate bench sheet.

6.3.2. If the correction requires an explanation, write a comment, and reference the error with a letter. Initial and date the explanation. Do not attempt to put all information (correction and comments) into a small space.

6.4. Use sequential alphabetical annotation to identify errors and corresponding comments (use A for first error and use A for corresponding explanatory comment; use B for second error, etc.).

7. QUALITY CONTROL

7.1. 2-4 week-old agar plate cultures are used to ensure healthy, viable continuing cultures.

7.1.1. Nutrients in the agar are not depleted as quickly as they are in solution, so older agar plate cultures can be used to start new algae cultures (unlike weekly algae culture transfers which use 6-8-day old algae).

7.2. Slides are prepared from cultures and are examined under a microscope by the Algal Taxonomy Group to verify the cultures are not contaminated.

7.3. Perform the freshwater transfer before the saltwater transfer.

7.3.1. A new, disposable sterile loop is used for each transfer to prevent cross-contamination. If a platinum-inoculation loop is used, it is flame-sterilized before and after each use.

7.4. The flasks are put through the standard Biology Section glassware washing procedure (see SOP BG-05), phosphate detergents are avoided because of the potential of skewed test results from phosphate-contaminated glassware. Use only non-phosphate detergent.

7.5. Temperatures are monitored daily.

7.5.1. If the shaker rack temperature (Rm B246 temperature) deviates more than ±2 °C from the preferred range of 25 ± 3 °C for any sustained length of time (i.e. more than 2 hours at one time or 4 total hours during a 24-hour period):

7.5.1.1. Check the thermostat and record the room temperature and setting from the thermostat. Include the date and time of the observation. The thermostat units will need to be converted from degrees Fahrenheit to degrees Celsius.

Degrees Celsius (°C) = (°F – 32) / 1.8

Degrees Fahrenheit (°F) = (1.8)(°C) + 32

7.5.1.2. If the temperature of Rm B246 continues to fall out of range or does not improve, send a report via LIMS: LIMS\General\Help Desk.

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Contact **Nhon Vo** (FDEP Scientific Support Services—245-8137, Lab Building A 416E) is another option via email. Do **NOT** report it to him verbally. All issues must be tracked in the Lab Support Help Desk.

7.5.1.2.1. Until temperature is consistently within range, P. sub-cultures can be moved into an incubator if necessary (lighting and temperature adjusted as needed). Refer to the incubator manual for instructions on how to program the incubator with the correct temperature and light settings.

7.5.1.2.2. The portable lights can be used to increase light if necessary.

7.5.2. If the Dun incubator has a sustained temperature deviation of ±2 °C from the preferred temperature range of 20 ± 2 °C:

7.5.2.1. Check and record the incubator setting. Include the date and time of observation.

7.5.2.2. Notify supervisor(s) that the temperature is out of range and give them your observations.

7.5.2.3. Adjust the incubator setting and monitor the temperature for one hour, upon supervisor's approval.

7.5.2.3.1. Refer to the incubator manual to adjust the temperature setting.

7.5.2.4. If the temperature does not improve, the supervisor should submit a purchase request for repair. Dun cultures can be moved to another incubator (lighting and temperature adjusted as needed).

8. SAFETY/HAZARDOUS WASTE MANAGEMENT

8.1. Proper laboratory protection—including lab hood or ventilation system, lab coat, gloves, and safety glasses—is required when working with chemicals and unprocessed samples.

8.2. Refer to the DEP Laboratory Quality Manual for sample disposal.

8.3. Refer to the DEP Laboratory Health and Safety Plan and Hazardous Waste Management.

8.4. Read MSDS information for reagent handling and disposal.

9. REFERENCES

9.1. DEP Laboratory Quality Manual

9.2. DEP Laboratory Health and Safety Plan

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9.3. DEP SOP TA-03.03, *Preparation of Preliminary Algal Assay Procedure (PAAP) Medium for Freshwater Algae Cultures*

9.4. DEP SOP TA-03.04, *Preparation of Artificial Seawater (ASW) Medium for Culturing Dunaliella tertiolecta*

9.5. DEP SOP TA-04.18, *Culture of Algae-on Agar Plates*

9.6. DEP SOP TA-04.08, *Culture of Algal-Assay Inoculation Stocks*

9.7. DEP SOP TA-08.02, *Algal Growth Potential (AGP) and Limiting Nutrient (LN) Algal Assay Set-Up*

9.8. DEP SOP TA-09.09, *Preparation of PAAP-Medium Stock Solutions*

9.9. DEP SOP TA-03.02, *Preparation of Artificial Seawater (ASW)*

9.10. DEP SOP BG-08, STERIS AMSCO Series 250LS Autoclave Calibration and Use

9.11. DEP SOP BG-05, Biology Wash Party Procedures

9.12. MSDS information sheets

APPENDIX OF CHANGES

12/12/2007 Added Scope and Application; added Summary of the Method

07/26/2013 Reformatted SOP

06/12/2014 Corrected spelling error

01/29/2015 Formatted for 508 compliance

06/01/2015 Removal of references to *Isochrysis galbana* and *Tetraselmis sp*.

06/08/2016 Updated links

07/03/2017 In Equipment and Supplies, removed “milk dilution bottle” from the list and added temperatures and light intensities for shaker rack and incubator; added verification of algae cultures by the Algal Taxonomy Group to the Reagents and Standards section. Moved sentence “New cultures are typically started…” from 5.1.1. to 1.1. In Culture Preparation, split the steps for P. sub and Dun cultures and clarified which should be transferred first. Added Quality Control steps that are performed. Added SOP TA-4.08 to the reference list and removed SOP MB-03.1.

06/04/2018 Minor edits for clarity; minor formatting corrections; changed font to Calibri.

05/31/2019 Added section 5.1.9.1 for clarity

05/14/2020 Updated wording for 6.3 and 7.5.1.2. Updated links.

04/13/2021 Updated section 5.1.10 for clarity of where swirl documentation should be made.

05/24/2022 Checked for grammar, changed shake to swirl, and updated links.

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05/19/2023 Checked for readability and grammar.

05/09/2024 Size of tape does not matter so deleted it. Added more information about UV sterilizer, information about the foam stopper, and to use portable light if needed. Biology no longer has/uses dishwasher so it is unnecessary. Updated all links and added missing references to the Reference section. Edited for grammar, clarity, and word choice.

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