Lab. No. 3
Protocols for Isolation and Purification of a single Algal Species

In order to explore any algal species it may be necessary to grow this algae as a pure culture. That is, a culture of only a single species (called a unialgal culture). To obtain a unialgal culture you will need to "isolate" one species from all the rest. We can borrow some techniques from microbiology to help us with this isolation. The photo below shows all the equipment you will need.

Three methods to purify contaminated algal cultures and/or to produce single cells are described below: the agar plate method, successive dilutions of the original contaminated culture, and picking up of single cells from the original culture by using the capillary method. All techniques are also applied when new algal species are isolated from the wild.

1. Isolation of pure algal strain by the agar plating technique
A solid medium can grant more stable conditions for the growth of the desired species. This technique needs some sterile equipment like Petri dish and platinum hooks.

Materials
- Agar Agar
- Plastic Petri dish bag
- Bunsen burner
- 2 liter conical flask
- 2 l-liter flasks
- Natural seawater
- 1 ml or 0.1 ml pipette box
- inoculating loop
- Para film

1. Prepare several "isolation plates". These are simply agar plates prepared with the growth medium you are using to grow the algae.

To prepare 1 liter of solid medium follow the following instructions

- Prepare a 1.5% agar medium by weighing out 15 g of agar powder and placing it into a 2 l conical flask to which 0.5 l of dist water was added.
• Boil the agar while stirring to dissolve the agar. If available a microwave oven works very well for this.
• Add 0.5 l of hot, **double strength seawater** prepared by boiling the natural seawater until its salinity reach to 50‰ (this is of course if the desired final salinity is 25‰).
• Combine the contents of both flasks together into one flask.
• Mix well and cover the flask
• Autoclave at 125 °C for 30 minutes at 1 atm.
• Cool to about 50 °C.
• Add the nutrients of F/2 medium as previously described in the last lab to the medium while mixing by gently rotating the flask to ensure mixing of the nutrients and avoid bubble formation
• Aseptically pour the warm medium into the sterile Petri dish, cover and leave them to cool and solidify
• Store them in plastic bags in a refrigerator until needed.

2. **Streak plates.**
• Using a sterilized 1 ml-pipette take 0.1 ml from the initial contaminated strain culture prepared in the previous lab and place it on the agar plate.
• Your instructor will provide you with test tubes containing contaminated algae in order to purify them by the same procedure.

• Sterilize the inoculation platinum loop by heat until it turned red.
• Cool the loop and spread the algal sample by streaking onto the agar. This will spread out the algal cells floating in the water drop.

• Now place the lid back on the isolation plate and place masking tape (Para film) along the edge of the plate. The masking tape will prevent the agar from drying out, since it may take several days for the individual algal cells to grow and produce visible colonies.

• Incubate the Petri dishes at desired environmental conditions (light and temperature) placing the dish upside down, so that water drops will not form on the lid and then fall on the culture.

• Don’t forget to label the dish!

• Depending on the density of the inoculum, the algal cells will grown enough to form colonies on the surface after 5 - 21 days

• Once algal colonies are observed, take a sample by means of a sterile platinum hook and check under the microscope

Since different colonies on this first streak plate often tend to grow together, it is difficult to pick off colonies with only one species. You may have to scrape some
colonies off the first plate and re-streak them on a second plate in order to get good separation.

3. Select and transfer a small portion of the monospecific pure colony together with some agar using a sterile platinum loop into a test tube filled with 5-10 ml of culture medium and shake it regularly during incubation on an illuminated glass rack.
• When a colour change is observed in the tube, check under the microscope the isolated algal strain
• Subculture these cells about once a week in order to keep them healthy.

2. Dilution method
Materials
• 20 screw capped test tubes filled with 9 ml of algal culture medium.
• Safety cabinet.
• Bunsen burner
• Sterilized 1-ml tip box
Procedure
1. Use the test tubes filled with 9 ml of F/2 medium prepared in the last lab.
2. Number the tubes from 1 to 10.
3. Put the test tube containing the strain to be diluted in the biological safety cabinet, remove the cap and flame its neck.
4. Using a sterilized 1 ml-pipette take 1 ml from the initial contaminated strain culture prepared in the previous lab. and add it to the tube No. 1, then stir gently.
5. Follow up the same procedure with the contaminated algal sample by your instructor.
6. Using a new sterile 1 ml-pipette repeat the previous step by taking 1-ml inoculum from tube No. 1 and inoculate it into tube No. 2;
7. Repeat the same procedure with the remaining tubes, each time pipetting 1 ml from the previous tube (gently stirred) into the next one; flame necks and caps them);
8. Keep under controlled environmental conditions.

When cell growth reappears, check samples of the tubes under the microscope and get rid of the tubes that are still contaminated, typically the initial ones, and keep only the purified cultures, usually in the more dilute tubes. Repeat the process using the last dilutions if necessary, and in any case at least every three months to always have a safe amount of purified cultures ready at hand.

3. Picking up method (Capillary method):
This technique follows the dilution method, but the inoculum is obtained by selecting single cells of the desired species by means of a capillary pipette handled under a microscope.
The isolation or purification of cultured strains should be repeated as many times as required to produce contaminant-free cultures.

Materials
- Pasteur pipette
- Inverted and/dissecting microscopes
- Microscopic slides
- Bunsen burner
- Forceps
Preparation of a micropipette from a Pasteur pipette.

- The Pasteur pipette is held in the hottest region of the flame, supported on the left by a hand and on the right by forceps. The pipette should be rotated as the glass is heated to a soft, pliable condition.
- When the glass is soft, the pipette is quickly removed from the flame with a gentle pull to produce a thin tube.
- The forceps is then relocated to the appropriate region of the thin tube.
- The forceps is used to gently bend the thin area so that it breaks, forming a micropipette.
- An enlarged tip of a micropipette, showing a jagged break; this tip is not suitable for use.
- An enlarged tip with a very smooth break; this tip is suitable for use. Note that the diameter of the tip is larger than the flagellate cell (bearing microscopic scales), thus reducing the probability of shearing as the cell enters the micropipette during isolation.