



Growing RCC algal strains

SBR-RCC-PA-Information before ordering strains-v03.docx



Before ordering

Before ordering strains, please ensure that your laboratory has suitable equipment and conditions for the reception and maintenance of the cultures.

Our website (<http://www.roscoff-culture-collection.org/>) provides a range of information concerning culture conditions for each strain (temperature, medium, light intensity...).

Equipment, supplies and media

Equipment

- Laminar Flow Hood – Used to transfer cultures
- Filtration system – Used to prepare media
- Autoclave – Used to prepare media
- Culture room or Culture cabinet

Media

Each culture grows in a specific media that you can either prepare yourself or purchase as described on our web site : <http://roscoff-culture-collection.org/protocols/media-recipes>. Please note that the RCC does no sell media.

Supplies

Filter 0.22 µm	Millipore	GSWPO9000
Prefilter	Millipore	AP1507500
Neons Sylvania Daylight	Sylvania	F58W/54/765
Starter for Neon	TABUR	00007698476
Ventiled Flasks	Sarstedt ou Nunc	833910.002 or 136196
Culture tubes	CML	TCU12PS25

Health and Safety

To avoid any risk of contamination of the natural environment, all culture residues must be sterilized (by autoclaving or by treatment with bleach) before being discarded.

Micro-organisms transfer should have be done in an axenic way (work under a flow laminar hood or near flame).

Culturing protocol

Before receiving live cultures

You need to make sure :

- you have prepared culture media
- you have set an environment with proper temperature and light
- you have containers (flasks, tubes or erlenmeyers) to grow the cultures
- you have a way to monitor culture growth

Media (not supplied by the RCC)

The media used by the RCC are most often prepared from seawater collected off Roscoff (salinity ca. 33‰), stored for at least two months in darkness, then filtered on 0.22 μ m filters (Millipore filter GSWPO9000 plus Millipore prefilter AP1507500) and autoclaved.

If your laboratory does not have access to natural seawater, you can try using artificial seawater made from mixtures of salts (consult the "culture media" section on our website). Please note that we cannot guarantee growth of culture in artificial seawater based media (except for cyanobacteria for which it is recommended to use Red Sea Salt artificial seawater).

Sterile (autoclaved and/or 0.22 μ m filtered) nutrient and trace metal stocks must be added to the sterile seawater under a laminar flow hood. You should not autoclave vitamins (sterilization by 0.22 μ m filtration and storage at -20°C).

Temperature and Light intensity

You have to check on the RCC web site what is the RCC growth temperature. We are using one of 3 conditions usually:

- 4°C for Arctic and Antarctic strains
- 15°C for temperate strains
- 20 or 22°C for tropical strains

You can grow the cultures either in a special dedicated room with temperature control or a culture cabinet. **Strains will not grow on a bench in the lab, you need a temperature-controlled environment.**

All RCC strains are exposed to a 12H/12H day/night light cycle. We use "daylight" neon tubes (Sylvania Daylight F58W/54/765 ref : 0001440+ starter ref :00007698476). Light intensity for culture maintenance rarely exceeds 100 μ Einsteins.m⁻².s⁻¹. In most case you can maintain them at lower light intensities (20 μ E)

Some of our cultures (some cyanobacteria) grow under blue light (we use a blue filter « Moonlight Blue 183 » (Minet Eclairages Scéniques). However this is not really critical and they can probably grow as well in white light.

Culture flasks

RCC strains are routinely maintained in single-use sterile 50 mL polystyrene flasks with a ventilated filter cap (Sarstedt ref 831810.002 or Nunc ref 136196) or single-use sterile 10 mL polystyrene tubes (CML ref TCU12PS25). Growth is usually better and more easy to check in culture flasks. Our strains generally also grow well in Erlenmeyers or other glass flasks.

Monitoring culture growth

There are several ways to control growth

- Culture color. This will work for most easy to grow cultures but some cultures develop very slowly and you may never be able to see the color.
- Microscopy. The easiest way is to use an inverted microscope adding a drop of the culture on a slide or directly looking at the culture flask
- Flow cytometry. This is the preferred way for cyanobacteria such as *Prochlorococcus* which are impossible to see by microscopy.

Receiving live algal cultures

After receiving your order, RCC staff will contact you in order to arrange a suitable delivery date. We usually use DHL courier service. Upon pick-up of the package, we will send you the DHL tracking number by e-mail. Packages should be delivered within one to three days (depending on location). Upon delivery, **cultures should never be stored in a cold room or a freezer, this will kill them instantly**. Please warn your mail or reception-room of the imminent arrival of the package - they should store it at room temperature and inform you immediately of the arrival of the package.

After opening the package, transfer the flasks into a culture room or culture cabinet with the appropriate temperature and light conditions. Cultures are subjected to stress during transport and we therefore recommend that you wait for one to two days before transferring cultures into fresh medium. Most of our strains must be transferred every 2-3 weeks with 1/10 to 1/50 dilution in new medium.

In case the cultures are not in good shape upon arrival, please notify us immediately with details (e.g. picture of the flask, microscopy images). Not that none of cultures are axenic, so you will always see some bacteria in the cultures. Under our term and conditions, you have two weeks to notify us and we will try to send you a new culture free of charge except for shipping.

Special instruction for viruses

Upon delivery of a virus – host systems, store the viral flask at 4°C in darkness. As described above, the host culture needs to be transferred in your culture incubator upon delivery. To propagate efficiently a virus isolate, the host must be growing in exponential phase. The host culture can be diluted 1/10 – 1/50 (vol/vol) into fresh culture medium and incubated for 3 to 4 days under appropriate conditions. The viral suspension can be added using a virus:host ratio of 1:10 – 1:50 (vol/vol) and incubated under host growing conditions. You can use an aliquot of non-infected host culture as a control to monitor cell lysis. Cell lysis is detected by complete clearing of the host culture (usually 3 – 7 days after viral inoculation depending on the viral strain, the initial inoculation ratio, and host growing conditions). The resulting lysate can either be stored at 4°C or need to be transferred as soon as possible. Do not keep the lysate for extended period of time under host growing conditions as it may induce the development of resistant hosts. It is possible to filter the lysate through 0.2 µm filters (use PES, PC, GF but avoid cellulose acetate membranes) to remove remaining host cells and debris. Viral suspension can be stored several months at 4°C (usually 3 – 4 months). Be aware that viruses are sensitive to intense light irradiance, UV, and heat.

If you need any further advice, please contact us at rcc@sb-roscoff.fr