

# Tips for physical isolation of bacteria-free, clonal microalgae from marine environmental samples

Ensure all equipment is sterile prior to initiation of the procedure and maintain the highest possible standard of aseptic technique.

1. Reduce the number of bacteria in a culture by:
  - a. Centrifuging samples at low speed (~100 x g) for 5 minutes. Decant and discard the supernatant, then resuspend the pellet in sterile seawater, or dilute culture medium.
  - b. Wash the sample with sterile medium through a filter of a suitable mesh size (e.g. use CellTrics 20 micron mesh to wash algal cells > 25 microns diameter). Place sterile filter in the neck of a test tube and pipette medium over the cells. Finally, invert the filter and wash the cells into fresh sterile medium.
2. Dilute the sample in filtered seawater so that it is easy to pick up individual cells using a micro-pipette. (See Tool-kit method of Gohde, Egardt & Appelgren)
3. Transfer individual cells through several drops of sterile medium on a slide or Petri dish then inoculate into sterile medium in the well of the 24-well plate. Repeat the process with as many individual cells as you can pick out, then seal the plate with Parafilm™ and incubate in a moist illuminated incubator for around 1 week before checking for growth of pure cultures.
4. To remove bacteria sticking to cell surface mucilage, place the cell in the centre of an agar plate and, using the knob-like tip of a melted micro-pipette, drag or push the cell over and/or through the agar. This is best carried out viewing the plate through a stereomicroscope, wiped clean with 70% alcohol, located in a laminar flow cabinet. Incubate the plate for a few days under standard conditions, then examine it to see if the cell(s)/culture(s) is/are growing free of contaminants. If the procedure has been successful, then carefully pick off cells and transfer them to a clean agar plate, remote from any other contaminants growing on the original plate. (Pers. communication M Melkonian & B Melkonian).
5. Ultra-sonication can be used to break up cells in clumps, or surrounded in extracellular mucilage, before attempting to isolate algae using the methods described.
6. For strains that are capable of growth on solid media, spray cells directly onto an agar plate. Undertake all manipulations in a laminar flow cabinet, or sterilisable work area.

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*Field Sample:* From tow of phytoplankton net mesh size 20microns or water sample from rock pool with dense bloom.

*Apparatus:* Inverted microscope with low magnification objective (x5, x10) or stereomicroscope; glass capillary tube attached to 0.5m silicon tubing, sterilised by boiling.

*Culture medium:* f/10 (ie f/2 diluted x5), filtered to sterilise and remove detritus (see [www.ccap.ac.uk/media](http://www.ccap.ac.uk/media))

*Micropipettes:* Blaubrand 20 microlitre and 50 microlitre capacity, 125 mm long.

*Plasticware:* 24-well plates and 35 mm Petri dishes filled with culture medium.

Celltrics® sterile filters, mesh size 10,20,30,50,100 and 150 microns, available from Partec ([www.partec.com](http://www.partec.com))

All chemicals were purchased from Sigma-Aldrich, unless otherwise stated.

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*Additional information:*

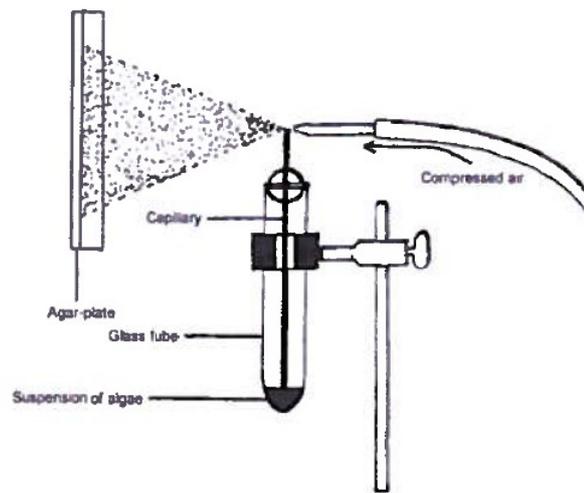
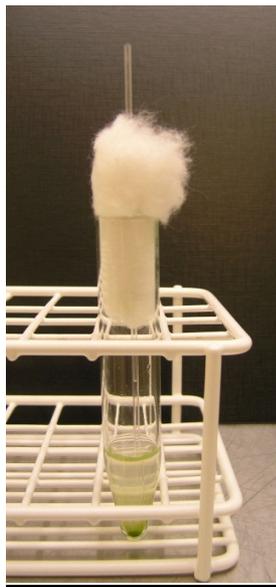
**Andersen RA and Kawachi (2005)**  
*Traditional Microalgae Isolation Techniques. In Algal Culturing Techniques ed Andersen RA. Academic Press.*

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- a. Prepare in advance glass ~15 ml centrifuge/glass tubes, sealed with a micropipette wrapped in non-absorbent cotton-wool and then autoclaved (see Figure).
- b. Aseptically transfer the algal cell suspension into the tube and then reclose the tube by placing the cotton bung/micropipette into the tube.
- c. Hold a tube with a flow of compressed air in front of the top of the micropipette and the air current should induce the capillary flow of a fine mist of liquid and algal cells onto the agar plate (see Figure). The plate should be sealed and incubated for a few days. Any discrete algal colonies observed can be picked off to initiate potentially bacteria free clonal algal cultures. (Pers. communication M Melkonian & B Melkonian)



7. 'Sterility' tests on cultures to check if the resultant strains are bacteria free: a small inoculum of the culture should be added to an enriched liquid medium such as E27 or PPY ([www.ccap.ac.uk/media/pdfrecipes/](http://www.ccap.ac.uk/media/pdfrecipes/)), or streaked onto plates of nutrient agar made up in ½ strength seawater. The test cultures are incubated for up to 6 weeks then checked for turbidity of the liquid cultures, or presence of bacterial colonies on agar. If there is no evidence of bacteria, then the culture is likely to be 'axenic'. Further evidence can be obtained by microscopic examination after staining with a DNA fluorochrome such as DAPI (4',6-diamidino-2-phenylindole), which should visualise any bacteria present in the culture.