Cryopreservation of marine microalgae employing a controlled rate cooler

PREAMBLE. For all cryopreservation procedures it is optimal to employ a healthy, vigorous, relatively dense, late log-phase, or early stationary phase, culture. Where practicable axenic strains, or those with low levels of bacterial contaminants should be employed and those contaminated with eukaryotes avoided.

1. Prepare 10-ml of 10% (v/v) DMSO in the appropriate culture medium and filter-sterilize the cryoprotectant solution into a sterile Universal tube.
2. Aseptically transfer 10-ml of dense culture into a Universal tube, add 10-ml of the sterilized cryoprotectant solution. Seal the tube and invert twice to ensure thorough mixing.
3. Aseptically decant 1-ml aliquots into cryovials and incubate for 10 min. at room temperature.

   At SAMS 15 vials are normally filled and processed (see below).
4. Programme the controlled cooler: Start temperature 20°C; ramp, cool at -1°C $min.^{-1}$ to -40°C; dwell, hold at -40°C for 10 min.

   For some taxa optimal cooling rates need to be empirically ascertained. In general slower cooling rates (<-1°C $min.^{-1}$) are optimal for larger cells (>25µm diameter). In general mechanical seeding of ice is not used for algal cryopreservation, but if an option in the system employed empirical experimentation may be used to ascertain if it results in higher post-thaw viability.
5. Start the programme to purge the system with nitrogen vapour and to allow the system to stabilise at the start temperature.
6. On reaching the start temperature, (most systems have an audible alarm) transfer the cryovials to the cooling chamber of the programmable cooler and initiate the cooling ramp
7. After the end of the programme an alarm will sound, rapidly transfer the cryovials to a small dewar containing liquid nitrogen using long forceps.

   It is essential that the cryovials are not allowed to warm up prior to plunging into liquid nitrogen.
8. Samples for storage should be transferred to the cryostat (ultra-cold freezer) in the liquid nitrogen containing dewar. Transfer of cryovials to the storage system should be done rapidly using long forceps.

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Algae: Method has been successfully employed for a variety of unicellular algae including: unicellular greens, prasinophytes, heterokonts and diatoms.

Apparatus: Cell incubators; a class I biological safety cabinet; a programmable, controlled rate cooler; a small (1-l) dewar; a heated water-bath; storage cryostat/refrigerator; long forceps.

Cell culture medium: f/2 or other appropriate medium.

Solutions: cryoprotectant solution 10% (v/v) dimethyl sulphoxide (DMSO) in appropriate culture medium; 70% (v/v) ethanol; liquid nitrogen.

Plasticware: membrane filters (0.5µm pore size); Universal tubes (20ml); disposable pipettes; 2-ml cryogenic tubes.

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

Additional information:

Day JG *(2007) Cryopreservation of Microalgae and Cyanobacteria. In: Day JG & Stacey GN (eds) Cryopreservation and Freeze-drying Protocols. Humana Press. pp 139-149.*

Day JG and Brand JJ *(2005) Cryopreservation Methods for Maintaining Cultures. In: Algal Culturing Techniques. Andersen RA (ed) Academic Press, New York. pp 165-187.*

culturecollection of algae and protozoa

Storage temperature is critical and should be <-130°C. It is advisable that replicate samples are stored in at least two separate "refrigerators". At SAMS 10 cryovials are normally stored in a working bank, 2 vials are stored in a master/ back-up bank and 3 are used to check viability/ efficacy of the protocol.

9. To recover cultures, vials are thawed by placing in a pre-heated water-bath (40°C) and agitate until the last ice crystal has just melted.

   For most marine taxa it is important not to prolong their incubation at 40°C. Alternative, slower warming e.g. in a 25°C water-bath may be beneficial for some strains, but in general rapid warming is optimal as it avoids/ minimises ice crystal regrowth.

10. On thawing rapidly transfer to a laminar flow/ biological safety cabinet and wipe the outside of the vial with 70% (v/v) ethanol.

    Note: there may be high levels of viable bacterial and fungal spores in liquid nitrogen that may contaminate recovered cultures.

11. Using a disposable plastic pipette transfer the 1-ml of thawed culture into a suitable, labelled, culture vessel containing >20-ml of an appropriate medium. Then cover in aluminium foil and re-label with strain designation and date.

    It is optimal to prevent further biochemical-based injuries to incubate for a period in the dark.

12. Incubate at standard culturing temperature for the cryopreserved organism, after 24h partially remove the foil and after a further 24-96 hours remove all the foil covering.

    It is important to ensure that cells are not subjected to light levels likely to induce photo-oxidative stress during the recovery phase. It is worth noting that cell numbers are generally very low and particularly for strains where recovery levels may be low that there is little self-shading.

13. After an appropriate period (2-8 weeks, depending of the strain) a normal culture should be obtained. This may be maintained by routine serial transfer, or employed for experimental use.