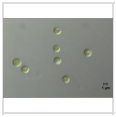

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Basic protocol for elimination of bacteria from microalgal culture using antibiotics

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ABSTRACT

Method used to eliminate bacterial contamination of a marine micro-algal culture. Note, this method is not guaranteed to be 100% successful either due to the fact that the antibiotic combination is not lethal to the bacteria present or it is possible that the strain may not be capable of prolonged growth without the presence of bacteria. This method is based on that of Droop, adapted according to Andersen but using a modern antibiotic mix suggested by S. Slocombe (SAMS - Scottish Association for Marine Science). The method described here have been successfully used by Christian Jeanthon (ECOMAP) to produce axenic cultures of diatoms and E. huxleyi.

1. Droop, M. R. A procedure for routine purification of algal cultures with antibiotics. Br. Phycol. Bull. 3, 295–297 (1967).
2. Andersen, R. Algal Cultering Techniques. (Academic Press, 2005).

1 Using appropriate protective clothing, prepare 20ml of an antibiotic solution mixture 10X in pure water as follows:
- Cefotaxime (5g/L)
- Carbenicillin (5g/L)
- Kanamycin (2g/L)
- Augmentin (2g/L)

2 Sterilize through a 0.1 or 0.22 µm filter into a sterile bottle. Keep refrigerate for up to weeks, or store at -20 °C in small aliquots.

3 Centrifuge 20ml of a healthy micro-algal culture in log phase of growth. Resuspend the pellet in fresh media sterilize through a 0.1 or 0.22 µm filter. If it is possible, check the media a priori by flow cytometer (FC) for bacteria contaminant.

The speed and time of the centrifugation will vary among the different groups of algae. For E.hux, Christian Jeanthon used 1600g during 2'.

4 Repeat steps 3 and 4 up for 5 – 6x.

The wash step (3) needs to be optimized depending on the algae. A good balance between how many times the cells are washed, how many algae cells are remained and the efficiency of bacteria removal needs to be check for each algal group and can be done by FC.

5 Resuspend the cells in 600 µl of fresh sterile media.

The inoculum should be dense therefore the volume to resuspend the cells and to add in the next step can be changed accordingly.

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6 Mix the cells with antibiotic as describe below and ensure the contents are well mixed - gently vortex if necessary.

Tube ANT concentration	1 10%	2 15%	3 20%	4 25%	5 35%	6 50%
Antibiotic Mix 10X (µl)	40	60	80	100	140	200
Algae cells (µl)	100	100	100	100	100	100
Algae Media (µl)	1860	1840	1820	1800	1760	1700
Enriched Media (µl) (LB, SOC)	2	2	2	2	2	2

7 Incubate the tubes at the optimum growth temperature in an illuminated incubator.

8 After 16/24/48/72/80 hours of incubation take ~ 10 µl from each tube and inoculate into 1 ml of fresh growth medium in a 24 well plate.

9 Incubate the plate at the optimum growth temperature in an illuminated incubator.

10 Follow the algae growth and bacteria contaminant on the plate samples during a week by using the flow cytometer.

11 Transfer the multiples wells that contain the axenic algae to 15 ml of sterilize fresh media even if they are from the same strain

12 Cryopreserve immediately the axenic strain if it is possible. For genome sequencing purpose, once the axenic strain is stablished, prepare 1 – 2L of dense culture, collect the cells by centrifugation and freeze at -80 the pellet for future extraction.

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