Establishing non-axenic monoclonal cultures of Skeletonema marinoi

1. Make micropipettes by heating the capillary tubes over the flame and pull the ends of the tubes to obtain a pointed end. Slide the silicon tube over the unprocessed part of the capillary tube.
2. Put a drop from the plankton net tow on a microscope slide and place it in the inverted microscope. Prepare another slide with several drops of clean f/2 medium.
3. Isolate a single chain of *Skeletonema* by sucking it into the micropipette. Release it in a drop of f/2 medium and repeat to new drops until only one chain of *Skeletonema* remains. Use a new micropipette for every transfer to a new drop.
4. Transfer the cell chain to a Petri dish, half filled with f/2 medium.
5. Keep the Petri dishes with isolated cell chains in a transparent plastic box with a wet filter paper in the bottom. The filter paper will keep the moisture in the box and prevent evaporation from the Petri dishes.
6. The box with the Petri dishes should be kept in a culturing chamber. We use 10°C, 50 µE m^{-2} s^{-1}, and L:D 12:12.
7. Monitor the Petri dish daily at 10x magnification in the inverted microscope. When several chains are recorded, transfer to a 40 ml NUNC flask and fill up to 10 ml with f/2 medium.
8. When growth is confirmed in the NUNC flask fill up to 40 ml with f/2 medium.
9. Re-inoculate culture every 3-4 weeks by adding 1-5 ml of culture to 40 ml fresh f/2 medium.

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Plankton sample: Fresh (maximum 1 day old) collected net sample

Apparatus: capillary tubes, silicon tube, Bunsen burner and inverted microscope.

Cell culture medium: f/2 medium (Guillard 1975)

Plasticware: Petri dishes (ø 5 cm), 50 ml NUNC flasks.

Additional information:

***Guillard RRL** (1975) Culture of phytoplankton for feeding marine invertebrates. In: Culture of Marine Invertebrate Animals (eds. Smith W, Chanley M), pp. 29-60. Plenum Press, New York.*

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