Apr 13, 2021

Detection of bacteria in antibiotic-treated diatom cultures and cell harvesting

Francesco Manfellotto[1], Monia Teresa Russo[1], Pina Marotta[1], Antonella Ruggiero[1], Mariella Ferrante[1]

[1]Stazione Zoologica Anton Dohrn

1 Works for me dx.doi.org/10.17504/protocols.io.btt5nnq6

Francesco Manfellotto
Stazione Zoologica Anton Dohrn

ABSTRACT

We illustrate a simple and rapid method for detecting the presence/absence of bacteria in diatom cultures using DAPI and fluorescence microscopy.
We also describe how we harvest cells for genomic DNA extraction once we have assessed that bacteria are absent from the culture.

DOI

dx.doi.org/10.17504/protocols.io.btt5nnq6

PROTOCOL CITATION

Francesco Manfellotto, Monia Teresa Russo, Pina Marotta, Antonella Ruggiero, Mariella Ferrante 2021. Detection of bacteria in antibiotic-treated diatom cultures and cell harvesting. **protocols.io** https://dx.doi.org/10.17504/protocols.io.btt5nnq6

KEYWORDS

DAPI, AXENIC, DIATOMS, MICROSCOPY

CREATED

Mar 31, 2021

LAST MODIFIED

Apr 13, 2021

PROTOCOL INTEGER ID

48733

information contained in or linked to this protocol or any of our Sites/Apps and Services.

1 For the antibiotic treatment to obtain axenic cultures, see the published protocol:

Axenic Diatoms cultures protocol
dx.doi.org/10.17504/protocols.io.bgudjws6

DMI6000 b
Fully Automated Inverted Research Microscope
LEICA
https://www.leica-microsystems.com/products/light-

Transfer 1 mL of Culture in a new 2 mL tube.

2 

Add 1,6% of neutralized formaldehyde to fix cells and mix gently by inversion

3 Add 1uL of DAPI 1 mg/mL and mix gently by inversion.

Keep away from direct light

☒4,6-Diamidino-2-Phenylindole, Dihydrochloride (DAPI) **Thermo Fisher Scientific Catalog #D1306**

4 Transfer cells in a 35mm glass bottom dish.

µ-Dish 35 mm, high Glass Bottom
A 35 mm imaging dish with a glass bottom for use in TIRF, single molecule and super-resolution microscopy applications
ibidi
https://ibidi.com/dishes/176--dish-35-mm-high-glas

Citation: Francesco Manfellotto, Monia Teresa Russo, Pina Marotta, Antonella Ruggiero, Mariella Ferrante (04/13/2021). Detection of bacteria in antibiotic-treated diatom cultures and cell harvesting. https://dx.doi.org/10.17504/protocols.io.btt5nnq6

Incubate for 10 min away from direct light to perimt cells sedimentation

5

Use an inverted microscope with fluorescence excitation.

* We use a Leica DMI 6000 B microscope.

Set light sources:

- **bright-field**
- **Fluorescence for chlorophyll (580 / 604 nm)**
- **Fluorescence for DAPI (364 / 454 nm)**

6 Assess bacteria absence-presence in the cultures.

Move across the plate, focusing on the cells and on the bottom of the dish.

7 Capture different photos

Citation: Francesco Manfellotto, Monia Teresa Russo, Pina Marotta, Antonella Ruggiero, Mariella Ferrante (04/13/2021). Detection of bacteria in antibiotic-treated diatom cultures and cell harvesting. https://dx.doi.org/10.17504/protocols.io.btt5nnq6

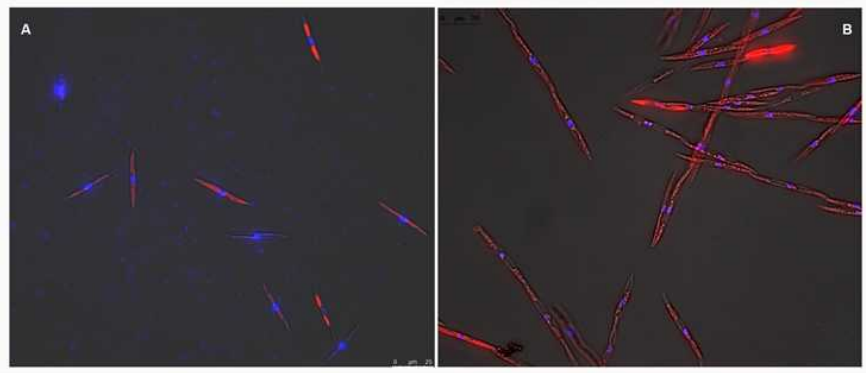


Fig.3A. Normal culture with bacteria (blue spots)
Fig.3B. Axenic culture without bacteria.

Cells filtration

8 Filter cells on MF-Millipore™ Membrane Filter, 1.2 µm pore size, gridded 47 mm.

Fig.4

9 Move the filter in a new 50 mL tube using tweezers

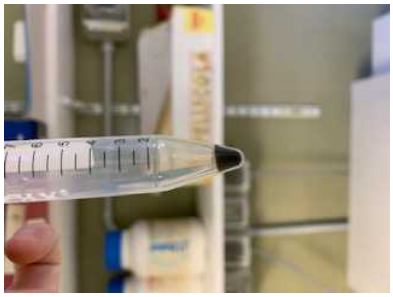
Citation: Francesco Manfellotto, Monia Teresa Russo, Pina Marotta, Antonella Ruggiero, Mariella Ferrante (04/13/2021). Detection of bacteria in antibiotic-treated diatom cultures and cell harvesting. https://dx.doi.org/10.17504/protocols.io.btt5nnq6

10 Rinse the filter with 1 mL of clean medium using a pasteur pipette

pipet gently, to avoid cell lysis

11 Collect the resuspended cells in a new 15 mL tube

12 Pellet cells by centrifugation
15 min. 2000 g 18°C and discard supernatant



13 Freeze pellet immediately in liquid nitrogen

Citation: Francesco Manfellotto, Monia Teresa Russo, Pina Marotta, Antonella Ruggiero, Mariella Ferrante (04/13/2021). Detection of bacteria in antibiotic-treated diatom cultures and cell harvesting. https://dx.doi.org/10.17504/protocols.io.btt5nnq6

14 **Store at -80°C**

Citation: Francesco Manfellotto, Monia Teresa Russo, Pina Marotta, Antonella Ruggiero, Mariella Ferrante (04/13/2021). Detection of bacteria in antibiotic-treated diatom cultures and cell harvesting. https://dx.doi.org/10.17504/protocols.io.btt5nnq6