Fluorescent in situ Hybridization

Protocol adapted from Pernthaler et al. 2001

Equipment and supplies:

· Zeiss AX10 LSC microscope equipped with filter set for DAPI, FITC and CY3

· Dry-type incubator or hybridization oven

· Water bath

· Freezer

· Fridge fixation of plankton samples: Use PFA or Glutaraldehyde fixed samples

· 100 ml glass bottles · plastic petri dishes (diameter, 5 cm)

· white polycarbonate membrane filters (diameter, 47 mm; pore size, 0.2 µm)

· cellulose nitrate support filters (diameter, 47 mm; pore size, ³ 0.45 µm)

· filter towers for 47 mm membrane filters

· vacuum pump

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| **Stock Reagent** | **Volume** | **Final Concentration in Hybridization buffer** |
| **5M NaCl** | 360 µl | 900 mM |
| **1M Tris/HCl** | 40 µl | 20 mM |
| **Formamide** | % Depending on probe/stringency |  |
| **MilliQ H2O** | Add to 2 ml |  |
| **10% SDS (added last to avoid precipitation)** | 2 µl | 0.01% |

1. A hand-operated vacuum pump and several autoclavable plastic filter towers that can be linked together for parallel sample processing make an inexpensive filtration apparatus for field work. Place the moistened support filter and polycarbonate filter into the filtration tower, and filter an appropriate volume of the fixed sample by applying gentle vacuum. Support filters may be utilized for several samples. For cell numbers of around 106 ml-1 , 10 ml of sample are sufficient.

2. After complete sample filtration, wash with 10-20 ml of sterile H2O; remove H2O by filtration.

3. After sample filtration, wash filter with 1x PBSs (pH 7.4) then dehydrate with ethanol as follows:

2mL 50% EtOH for 3 min

2mL 80% EtOH for 3 min

2mL 100% EtOH for 3 min

(3% H2O2 for 5 min to dull autofluorescence – optional, chloroplasts don’t overwhelm image, but not as sharply defined)

2x with MilliQ water

1x 100% EtOH

If not treated immediately, the filters can be dried and stored at -20C.

4. Each filter is cut in four sections, which are placed on glass slides.

5. Filter sections are overlayed with 20 µI of hybridization solution containing 0.9 M NaCl, 20 mM Tris-HCI (pH 7.4), 35% formamide, 0.01% SDS, and 50 ng of fluorescently labeled oligonucleotide [add 38 uL of hybridization buffer and 2 uL diluted probe (1 ul probe to 11 uL water) to each filter] and incubated for 3 hrs at at 37˚C in the dark.

6. The filters are washed twice at 37C with wash buffer for 20 min without shaking in the dark. Then they are washed with TNT buffer (For 50mL TNT buffer: 1.5 mL NaCl 5M, 5 mL Tris 1M, 37 uL Tween 20%, 43.46 mL water) for 15 min at room temperature in the dark.

7. Filters are then mounted on a glass slide with Prolong Gold DAPI (Invitrogen) immersion oil. The filters can also be stained with 2.5 ug/mL DAPI for 3 min then washed with water and then mounted on a glass slide.)

8. Filters may be stored wrapped in foil at -20C.