SYBR Green I Counts of Viruses and Bacteria
Citation: Noble and Fuhrman (Aquatic Microb. Ecol.)
Updated March 2017 – Rachel Parsons

Need:

• 0.02 μm pore size, 25 mm Anodisc filters (from Whatman; made of aluminum oxide
• 0.8 μm GN4 metricel filters or equivalent.
• Sample (typically with 1% 0.02μm filtered formalin), plastic Petri dish, pipettes and tips.
• SYBR I solution from Molecular Probes•: make a working solution by diluting a subsample 1:10 of the original cncentration with 0.02 μm filtered deionized water. Make enough for the days use. From this stock solution, make a 2.5% working solution (final 2.5 X 10-3 dilution of stock) just before use- preferably on the dish as described below.
• Antifade mountin solution: Prolong antifade available from Molecular Probes.

PROCEDURE (BEST DONE IN SUBDUED LIGHT)

Place a 0.02μM Anodisc filter backed by a 0.8μm GN4 metricel filter on a glass filter tower in the hood in lab 303.

Sample volumes to use: For BATS oceanic seawater volume 3ml for 0m to 160m and 5mls for 200m to 400m, Bermuda coastal waters filter 2mls though sometimes be better with 0.5-1ml with volumes made up to 2mls by adding 0.02 μm filtered isotonic seawater.

Filter fixed seawater sample through the Anodisc filter at approx. 7 in Hg. While the samples are filtering, place 2.5μ1 of SYBR Green working solution on the bottom of a small plastic petri dish and mix with 97.5 μ1 0.02 μm filtered Q-water using a pipet.

Samples are filtered through the Anodisc membranes, and the filter removed just after the last liquid passes through, with the vacuum left on while removing the filter. Be careful, the filters are very fragile! The Anodisc filters is dried on whatman filter paper or a kimwipe and then placed sample side up on the drops of the staining solution for 15 min in a dark drawer or box.

After staining period, any remaining moisture is then carefully wicked away from the back side of the membrane (or on the top plastic rim of the petri dish) and the filter dried on a Kimwipe. If there is a film of liquid on top of the filter, it was not prepared properly and could be an issue. Note this on the slide.

Once dry, place the filter on a glass slide, then put a 30μl drop of Prolong solution on a 25mm cover slip and invert it over the filter. Push the cover slip to be sure the mounting solution fills the square space under the cover slip. You may need some mounting solution under the filter to ensure that is sticks to the slide.

View with narrow blue excitation. Image viruses before they fade usually in a day or two of preparing. Store slide FROZEN and dark.