SYBR Green or Gold Staining

Forest Rohwer

Abstract

This protocol is based on RT Noble and JA Fuhrman (1998) Aquatic Microbial Ecology 12:113-118: Use of SYBR Green I for rapid epifluorescence counts of marine viruses and bacteria.

Citation: Forest Rohwer SYBR Green or Gold Staining. **protocols.io**
dx.doi.org/10.17504/protocols.io.c7sznd
Published: 07 Jan 2016

Guidelines

Old Procedure

1.) Make dilution of virus prep in 0.02µm filtered seawater.

2.) Prepare staining droplet using 2.5-4µl SYBR green or gold (10,000x stock diluted to 10% with 0.02µm filtered Q-water) mixed with 97µl 0.02µm filtered Q-water and place cover over droplets to keep dark.

3.) Prepare 25 mm SYBR towers: 0.02µm Anodiscs, careful not to break membrane (can use 0.2µm backing filter underneath if desired). Filter sample onto membrane using vacuum. Rinse tower in 1L Q-water in between samples and blot onto paper towel to dry.

4.) Remove membrane carefully and set down on the edge of the droplet of diluted SYBR stain, letting the SYBR float under the filter. Incubate RT, dark for 15 min.

5.) Pick up filter off drop of stain and blot by passing over kimwipe two times. Place membrane, cell side up, onto microscope slide. Pipet 32µl 0.1% phenylenediamine (made in PBS/50% glycerol) on 22mm square coverslip. Invert over filter on microscope slide and press down to spread mounting medium.
6.) Place slide at –20°C to enhance fluorescence. Read slides using 100x oil immersion objective and inverted fluorescent microscope.

Protocol

Step 1.
Make dilution of virus prep in 0.02µm filtered seawater (100 kDa permeate if available).

Step 2.
Add diluted virus to 1 ml 0.02 µm filtered seawater and add 1 µl SYBR green or gold (10,000x stock) to solution.

Step 3.
Incubate RT, in the dark for 15 min.

DURATION
00:15:00

Step 4.
Prepare 25mm SYBR towers: use 0.02µm Anodiscs, careful not to break membrane.

Step 5.
Filter sample onto membrane (use vacuum).

Step 6.
Rinse tower in 1L Q-water in between samples and blot onto paper towel to dry.

Step 7.
Remove membrane and place cell-side up on microscope slide.

Step 8.
Pipet 32µl 0.1% phenylenediamine (made in PBS/50% glycerol) on 22mm square coverslip. Invert over filter on microscope slide and press down to spread mounting medium.

Step 9.
Place slide at –20°C to enhance fluorescence.

Step 10.
Read slides using 100x oil immersion objective and inverted fluorescent microscope.