Bi1x, Spring 2010

Virus Epifluorescence Protocol

In this experiment your group will be imaging viruses from pond samples using SYBR Gold. The samples are stained and then run through a 0.02 micron filter which is small enough to catch viruses. The filters will then be loaded onto a slide and imaged under the fluorescence microscope. Your group will prepare all four of the conditions described below. Each person should complete the entire process at least once.

Conditions

1. Unmodified pond sample
2. Pond sample prefiltered
    a. Get a syringe and a .2 micron filter
    b. Remove the plunger, screw on the filter
    c. Add 2ml of sample and force it slowly and gently through the filter into a new labeled tube
3. Control viruses
    a. Get an aliquot of lambda phage at known concentration from the TAs
4. Milli-Q water filtered through a 0.02 micron filter

Staining Protocol

1. Add 2ml of your sample into a 15ml falcon tube
2. We will have diluted the stock 10,000X SYBR Gold solution to 100X (into 100 uL of water that was prefiltered with a 0.02 micron filter – supplied by the TAs)
3. Add 20ul of 100X SYBR Gold to your sample to make it 1X
   SYBR Gold stains DNA and is toxic. Don't get it on you.
   SYBR Gold is also bleached by light. Cover it with aluminum foil at all times.
4. Cover with aluminum foil to protect SYBR Gold from light and incubate at room temperature for 10 minutes (longer is fine)

Filtering Protocol

1. Watch demonstration of how to prepare the filter apparatus
   Wear safety goggles when handling the 0.02 micron filters, they are brittle and can shatter.
2. Using tweezers load the 0.02 micron filters onto the filter apparatus
3. Place the top on and secure it using the clamp
4. Attach the vacuum then begin adding your sample
   Be sure to get the end of the pipette very close to the filter so you don't lose any sample on the edges. But also be sure not to stab the filter.
5. Once the sample is all the way through remove the vacuum and clamp
6. Before each time you run a sample on this device you need to rinse the pedestal and tower for about 1 minute with Milli-Q water (prefiltered water source) - ask the TAs where to find this device. Then flush a few mL of milli-Q water through

pedestal using the vacuum. This minimizes carry over contamination from filter to filter.

Slide Preparation

1. Add 15ul of mountant to the center of a glass slide
2. Remove your filter from the apparatus and place it top up on the mountant
3. Add 15 more ul of mountant to the top of the filter
4. Place on cover slip
5. Seal edges with nail polish
6. Cover loosely with aluminum foil and let it dry for a few minutes
7. Once dried the slide can be looked at under the microscope, cover slip side down.
8. SYBR Gold fluoresces in the FITC channel