Viral BONCAT

Alexis Pasulka,Victoria Orphan

Abstract

Experimental set up for BONCAT coupled with fluorophore addition via copper (I)- catalysed azide-alkyne cycloaddition (i.e., click chemistry) to detect the the transfer of HPG, a methionine analogue, from newly synthesized proteins in host cells to their viruses during lytic infection.

Citation: Alexis Pasulka,Victoria Orphan Viral BONCAT. **protocols.io**
dx.doi.org/10.17504/protocols.io.p9ydr7w
Published: 22 May 2018

Materials

- SYBR Gold Nucleic Acid Gel Stain S-11494 by Contributed by users
- Tris[(1-hydroxypropyl-1H-1,2,3-triazol-4-yl)methyl]amine (THPTA) by Click Chemistry Tools
- 25% Glutaraldehyde by Contributed by users
- L-Homopropargylglycine 1067-100 by Click Chemistry Tools
- PTFE Printed Slides by Electron Microscopy Sciences
- Amicon Ultra Centrifugal Filter (100K) UFC810096 by Emd Millipore

Protocol

Sample Collection

Step 1.

Collect seawater - important to collect gently to avoide damaging cells (e.g., via Niskin bottles with polycarbonate tubing). It is important to take into account light and temperature conditons both while prepping the sample prior to incubation and for the duration of the incubation. This is of course ecosystem dependent.

Experimental Set Up

Step 2.

Fill bottles gently with sewater to equal volumes. Make sure to include replicate treatment bottles as well as control bottles (e.g., no HPG addition). Bottle choice can vary - possible options include glass erlenmyer flasks with cheesecloth cotton stoppers, glass incubation bottles with screw tops, or clear polycarbonate bottles with screw tops. If you plan on running a time course and taking a variety of samples from your bottles, consider making replicate bottles to be taken down each time point such that too much volume is not removed from your incubation bottles at any given sampling point.

Experimental Set Up

Step 3.

HPG additions:

HPG Stock Concentration = 100 mM*

Final concentration of HPG in Incubation Bottles = 100 µM[†]

**Note you may want to alter your HPG stock concentration depending on the volume of your incubation such that you add a small volume (µL range) of HPG.*

[†]Final concentration of HPG can vary as well. See Hatzepichler et al. 2014 (doi:10.1111/1462-2920.1243) for discussion on different concentrations of modified amino acid.

Add appropropriate volume of HPG to incubation bottles. For example, to 250 mL bottles, add 250 µL of 100 mM HPG.

Experimental Set Up

Step 4.

Incubate bottles under appropriate light and temperature conditions. This of course will vary depending on where your samples were collected.

Experimental Set Up

Step 5.

Sampling time points may vary. It is important to consider the rate of viral mortality in your system. If you believe this to be happening quickly, you may want to sample on the order of 6 and 12 hours, up to 24 hours. If this is happening at a slower rate, then perhaps 24 and 48 hour samples will be sufficient. This method only works if the viral pool does not get saturated with HPG. If that happens, and all the viruses get labeled, you can no longer detect changes in the viral pool. We are continuing to develop the 'best practices' for this method and will update our protocol regularly.

See Pasulka et al 2018 for other considerations about the sensitivity of fluoresence detection for Viral BONCAT.

Sampling Viruses for BONCAT

Step 6.

Samples for determining HPG incorporation into viruses are prepared differently than typical viral microscopy (e.g., anodisc filters) to concentrate viruses from a larger volume of seawater and reduce background fluorescence for image analysis.

The volume collected for concentrating viruses may vary depending on the environment. For samples collected from a coastal pier site 8 mLs (in duplicate) was pre-filtered through a 0.2 µm filter, and then concentrated using an Amicon Ultra Centrifugal filter (Millipore, 100 K, UFC810096) to approximately 30 µL following the manufacturer's recommendations (i.e., 4000 rcf for 15 min). Samples were then fixed with glutaraldehyde (0.5% final concentration), flash frozen and stored at -80deg until further analysis.

Click Reactions

Step 7.

Prior to the copper (I)-catalysed cycloaddition, or click reaction, spot samples (2–5 µl) directly onto Teflon printed glass slides (Electron Microscopy Sciences, PTFE Printed Slides), air-dry. Do NOT use poly-l-lysine coated slides as this results in a bad reaction between the glutaraldehyde and the poly-l-lysine and creates a strong background signal.

Gently rinse with 0.02 µm filtered water (this can be done by gently pipetting over the well, careful to pipette in the direction off the slide and not onto another well). Air-dry sample again. We have not had trouble with the viruses washing off in this or the subsequent steps.

Click Reactions

Step 8.

Put samples through an ethanol dehydration series (50:50, 80:20, 96:4 v/v EtOH:H2O) prior to click reaction. This can be done by placing the slide into 50 mL falcon tubes with ethanol solutions. Make up fresh ethanol solutions.

Click Reactions

Step 9.

Make fresh click solution immediately prior to reaction.

Buffer solution:

12.5 µL of 100 mM sodium ascorbate (made in 1X PBS)

12.5 µL of 100 mM aminoguanidine hydrochloride (made in 1X PBS)

220 µL 1X PBS

Dye pre-mix:

1.25 µl - 20 mM CuSO4

2.50 µl - 50 mM THPTA (Tris[(1-hydroxypropyl-1H-1,2,3-triazol-4-yl)methyl]amin)

0.5 µl - 1 mM azide dye (TAMRA-azide fluorophore is best if you do a SYBR Gold counter stain)

Let dye pre-mix incubate in the dark for 3 minutes. Then add dye-premix to buffer solution to make final click solution and invert tube (do not vortex).

Click Reactions

Step 10.

Place 20 µL of click solution over each well. Transfer slide to a humid chamber (50-ml falcon tube with water on tissue paper in the bottom) and incubate in the dark at RT for 30 minutes.

Click Reactions

Step 11.

Following incubation, wash the sample with 1X PBS (to remove excess dye), followed by 0.02 µm filtered water (to remove salts). This is then followed with a wash in 50:50 v/v EtOH:H2O. Let the sample dry in the dark.

Counter Stain Viruses

Step 12.

Counterstain for 15 min with SYBR Gold (0.25% final concentration), wash with water and air dry in the dark.

Imaging Viruses

Step 13.

Capture digital images with two filter sets: a FITC filter set (excitation 480/40 nm; emission 535/50 nm) and a Cy3 filter set (excitation 545/30 nm; emission 620/60 nm).

Quantification of HPG incorporation can then be determined from these digital images by measuring the fluorescence signal of the azide-containing TAMRA fluorophore (i.e., click signal in Cy3) relative to the fluorescence signal from the SYBR Gold (i.e., DNA signal in FITC) within individual viral particles using a custom Matlab analysis pipeline. Example images and code are available on our Github Repository (https://github.com/apasulka/Viral-BONCAT-nanoSIMS).