One-step Phage Infection of Cyanobacteria (large-scale)

Version 3

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Abstract

Experiment purpose: measure dynamics of phage infection and gene expression in cyanobacteria. We collect samples every 1-2 hours over a 12 hour period, starting from an initial infection volume of 200ml per replicate per treatment.

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Materials

- Prepared cyanobacterial cell cultures at mid-log growth, prepared under desired media conditions by Contributed by users
- 250 ml clear polycarbonate bottles - for experiment cultures by Contributed by users
- Incubator - Light, humidity and temperature controlled by Contributed by users
- micropipettors; Sterile tips and serological pipettes by Contributed by users
- Concentrated phage lysates with known concentrations by Contributed by users
- Growth media by Contributed by users

Protocol

Before the day of the experiment

Step 1.

Prepare phage stock

Grow: On day minus-8, add phage stock to 4L of mid-log cells to start the infection (a low MOI is sufficient, e.g. 0.01-0.05), and monitor for about a week until the culture clears.

Harvest: When the culture has cleared (i.e. most cells have lysed), centrifuge the cleared culture (max speed, 10 min) to remove large cell debris and unlysed cells. Pour supernatant (containing phage) into disposable sterile vacuum filtration units containing a 0.2µm PES filter; discard the pellet containing cell debris. Connect to vacuum source and filter the lysate; save filtrate containing your phage particles.

Concentrate: Concentrate this clarified phage lysate using VivaFlow Concentration of Phage Lysate. Further concentrate the phage stock, and exchange buffers/media if necessary (e.g. to remove P, N, or other nutrients in the initial lysate) using Amicon Concentration of Viruses.

NOTES

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About 8 days prior to your one-step experiment, you will need to have sufficient cells ready, in mid-log phase, to infect for the purpose of preparing fresh lysate for the one-step experiment itself. If you need, for example, 4L of lysate, then you need 4L of mid-log cells on day minus-8, which means growing 500ml a week prior, and 25ml tubes a week before that. So growing up cyanophage lysate on a multi-liter scale requires 3-4 weeks.

Before the day of the experiment

Step 2.

Prepare cells for infection: Grow up sufficiently large volumes of cells to be infected. Plan carefully so that cells are in mid-log phase on the day of the experiment. If you are growing volumes of 1L or more, bubble your culture with an aquarium pump (ambient air) connected to a diffuser stone, to prevent CO_2 limitation.

Day of experiment: Setup

Step 3.

Before T0, use flow cytometry to measure cell concentrations of cyanobacterial cultures from Step 2. Use SYBR staining (e.g. SYBR counts of viruses) to determine concentration of your concentrated viral lysate from Step 1.

Calculate volumes of cells, phage and media to add to each experimental bottle/tube. We have an Excel template to do this calculation, based on the desired MOI, cell concentration, and final experimental culture volume. For gene expression experiments, we use an MOI of 3, final cell density of 5e7/ml, and volume of 200ml for each replicate -- this scale allows us to sample for RNA, protein, and phage & cell concentration, over a 12-14 hour time course.

Day of experiment: Setup

Step 4.

Working quickly, and with sterile technique, initialize experiment by mixing volumes (calculated in Step 3) of media, cells, and phage. First add appropriate volume of sterile medium to your experimental culture vessel (e.g. sterile 250ml polycarbonate bottles for 200ml cultures). Then add cells (prepared in Step 2). Then, to uninfected control bottles, add lysate that has been filtered to remove phage particles (e.g. the filtrate that goes through an Amicon centrifugal device). [This is important to control for small molecules that may be added with the phage particles in infected treatments; however keep in mind that other large molecules will get retained, along with phage particles, in the Amicon reservoir.] Finally, just before T0, add phage to their respective bottles. This begins the one-step infection. Mix bottles by gentle vortexing.

Experiment

Step 5.

Sample immediately for time 0. Suggested sampling: FCM for cell counts, Fluorescence, extracellular phage, RNA-protein.

Experiment

Step 6.

Place bottles in incubator until next time point. Repeat sampling in Step 5 at each time point (e.g. every 1-2 hours) until lysis is complete.