Setting up Experimental Cultures for Large-scale One-step Phage Infection of Cyanobacteria

Sarah Giuliani

Abstract

Experiment purpose is to monitor the time-course of a large-scale infection of host cyanobacteria by phage under variable media conditions and obtain samples for proteomic and transcriptomic analysis.

As a minimum, prepare 12 sample bottles for cells infected with phage and control phage lysate, under 2 different media treatments, with triplcates of each unique Phage/Treatment combination.

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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
- Treatment media by Contributed by users
- phage lysate samples for test and negative control - with known concentrations by Contributed by users
- Incubator - Light, humidity and temperature controlled by Contributed by users
- micropipettors; Sterile tips and serological pipettes by Contributed by users

Protocol

Step 1.

For a desired high MOI ratio of number of phage to number of cells (eg. 3), calculate amount of cells, phage and media to add to the experiment bottles. This will be dependent on the desired cell concentration and final experimental culture volume (eg. 200 ml).

Before time T-zero, use flow cytometry to measure cell concentrations of cyanobacterial cultures to

determine final dilutions.

Step 2.

In a clean laminar flow hood, first add media to the 250 ml polycarbonate bottles.

Step 3.

Add cyanobacteria cells.

Step 4.

Add control phage and then test phage at time T-zero. Phage volume added should be less than 5% of the total volume.

Immediately begin sampling for T-zero (see One-Step Sampling protocol).