Sampling for RNA / Protein/ DOM/ PO4: Large-scale One-step Phage Infection of Cyanobacteria

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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.

9 Hourly Timepoints: 0, 1, 2, 3, 4, 6, 8, 10, 14

Sampling is to collect supernatant fractions to determine total dissolved phosphate and soluble reactive phosphate (TDP/SRP) for analysis of inorganic phosphate concentration and phosphate in dissolved organic material (DOM); and to collect pellets to isolate RNA for transcriptomics and proteins for proteomics.

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Materials

- 15 ml conical tubes (labeled for RNA, protein, DOM) - 3 per time-point for each sample by Contributed by users
- 10 ml serological pipette - 1 per time-point for each sample by Contributed by users
- 5 ml conical tubes (labeled for PO4, Replicates 1 and 2) - 2 per time-point for each sample by Contributed by users
- 50 ml tube (numbered per bottle, reuse between time-points) by Contributed by users
- 20 ml syringe (reused between biological replicates) - 1 per time point for each unique treatment/phage experiment condition by Contributed by users
- 0.2 µm syringe filter (reused between biological replicates) - 1 per time point for each unique treatment/phage experiment cond by Contributed by users
- weighing dish (to put down filter between replicates) - 1 per time point for each unique treatment/phage experiment condition by Contributed by users
- Liquid Nitrogen by Contributed by users
- Holder for dipping samples in liquid nitrogen by Contributed by users
- Centrifuge with 50 ml and 15 ml tube adaptors by Contributed by users

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Protocol

Step 1.

Sampling method:

From each experiment bottle, pipette the scheduled sample volume (in the table below) into RNA tubes & (if needed) protein tubes.

TIMEPOINT	RNA Volume (ml)	Protein Volume (ml)
0	10	10
1	10	NO
2	10	10
3	10	NO
4	10	10
6	10	NO
8	10	10
10	10	NO
14	NO	15

Step 2.

Spin tubes for 15 minutes at 4700 rpm (4816 x g) in a swinging bucket rotor.

Step 3.

Decant both RNA & protein supernatants into designated 50 ml tubes.

Step 4.

Flash freeze cell pellets in 15 ml tubes, place in -80°C freezer.

Step 5.

Decant remaining supernatant into barrel of 20 ml syringe loaded with filter.

Step 6.

Syringe filter 10 ml of supernatant into the conical DOM tube.

Step 7.

Syringe filter 5 ml of remaining supernatant each into duplicate 5 ml PO4 tubes (for SRP/TDP assays).

Step 8.

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Remove filter from syringe, place in weighing dish, pull syringe plunger.

Step 9.

Repeat steps 6-9 for other biological replicates from same condition.

Step 10.

For T0-T3: Place DOM tubes in refrigerator and PO4 tubes in -20°C freezer.

Step 11.

For T4-T10: Place DOM tubes **and** PO4 tubes in -20°C freezer.

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