Sampling for RNA / Protein/ dissolved nutrients from phage infection experiments Version 2

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Abstract

This sampling protocol is designed to collect two fractions: cell pellets and cell-free filtered supernatants. The cell pellets are used for gene expression analyses by RNA-Seq and proteomics. The cell-free fraction is used for analysis of dissolved analytes (e.g. N, P, DOC).

Volumes are based on our experience with cyanobacterial cultures at cell densities ~ 5E7/ml. For more/less concentrated cultures, adjust volumes accordingly.

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Materials

- 10 ml serological pipette - 1 per time-point for each sample 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
- conical tubes, 15ml by Contributed by users
- conical tubes, 50ml by Contributed by users
- syringes, 20ml by Contributed by users
- syringe filters, 0.2µm by Contributed by users
- eppendorf tubes, 5ml by Contributed by users

Protocol

Step 1.

At each time point, collect 10ml from each experimental bottle/tube into a 15ml conical tube, using a serological pipet. Collect one 10ml sample for RNA and one 10ml sample for protein.

Step 2.

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

Step 3.

If you wish to collect the cell-free fraction (e.g. for analyzing dissolved inorganic or organic nutrients or phage particles): decant supernatants into labeled sterile 50 ml conical tubes. We pool the RNA and protein supernatants together resulting in a 20ml combined supernatant.

If you do not wish to collect the cell-free fraction: decant supernatant into waste container.

Step 4.

Flash-freeze cell pellets in 15 ml tubes using liquid nitrogen; store pellets in -80°C freezer until later analysis.

If you are not collecting the cell-free fraction, stop here.

If you are collecting the cell-free fraction, go to Step 5.

Step 5.

Remove the plunger from a 20ml clean syringe. Attach a new 0.2µm syringe filter to the syringe. Pour your 20ml combined supernatant into the barrel of the syringe. Replace plunger on the syringe and filter your 20ml supernatant into appropriate tubes for your downstream nutrient/phage analysis (we use a 15ml conical tube plus a 5ml snap-cap Eppendorf tube).

Step 6.

Repeat step 5 for each sample. You can reuse the same syringe for replicates of the same condition/treatment.

Step 7.

Store 0.2µm filtered samples in -20ºC until analysis. (if analyzing phage particles in the filtrate, store at 4ºC instead.)