Sampling for Flow Cytomtery (FCM) Cell Quantification: 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.

8 Hourly Timepoints: 0, 2, 4, 6, 8, 10, 12, 14

Sampling is to determine total cell concentration by flow cytometry.

- For Flow cytometry, **100 µl of sample in duplicate** were taken from each experiment bottle at every other time-point and diluted 10 X in ASW salts, followed by fixing the sample by adding 5 µl of 25% glutaraldehyde, incubating in dark for about 10 minutes, and then flash freezing in liquid nitrogen. Samples were stored at -80°C for further processing.

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Materials

- P1000 micropipets and 1 ml filter tips by Contributed by users
- P200 micropipets and 200 µl filter tips by Contributed by users
- Labeled 1.2 ml cryo tubes by Contributed by users
- Repeator pipet and tips (that hold total volume of 100 µl and can dispense 2 µl) by Contributed by users
- Racks to hold cryo tubes by Contributed by users
- Cryo tube canes for dipping into liquid nitrogen by Contributed by users
- Labeled cryo boxes for -80°C storage by Contributed by users
- ASW salts by Contributed by users
- 25% Glutaraldehyde by Contributed by users
- Liquid Nitrogen: 1-3 L by Contributed by users

Protocol

Step 1.

Before the experiment, add 900 µl of ASW salts into the labeled sample 1.2 ml cryo tubes.

Step 2.

Transfer 100 µl of each sample, in duplicate, from experiment bottles into the tubes containing 900 µl of ASW salts.

Step 3.

In the chemical fume hood, add 5 µl of glutaraldehyde to each tube, using the repeator pipet. Cap the tubes and mix well by inverting them a few times, then incubate for about 10 minutes in the dark.

Step 4.

Flash-freeze tubes in liquid nitrogen, using the cryo vial canes.

Step 5.

Store the samples in labeled cryo boxes at -80°C for further processing.