Sampling for qEXT and MPN assays: Large-scale One-step Phage Infection of Cyanobacteria Version 3

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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.

15 Hourly Timepoints: 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14

Sampling is for qPCR assay to determine total extracellular phage (qEXT), and for MPN (most probable number) assay to determine % phage infectivity.

For qEXT and MPN sampling, **250 µL of each sample in duplicates** were collected and filtered through a 0.2 µm, 96-well filter plate, for each time point. The flow through filtrate containing phage was stored at 4°C as the sample to analyze.

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Materials

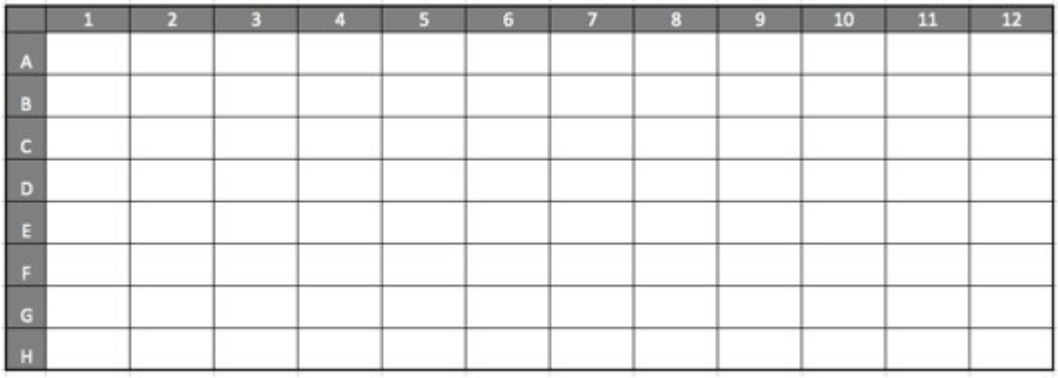
- 96-well filter plates (0.2um) Millipore #MSGVN2210 by Contributed by users
- 96 well plates for filtrate Fisher #087722C by Contributed by users
- Eppendorf centrifuge with plate adapters and a balance plate by Contributed by users
- P1000 micropipet and Filter Tips by Contributed by users
- Parafilm by Contributed by users

Protocol

Step 1.

Stack filter plate onto bottom 96-well plate without touching the bottom of the sterile filter plate.

Plate-Setup Template:



	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Step 2.

Transfer 250 uL of sample from each experiment bottle into the filter plate in duplicates. Only sample from the bottles containing test phage and not the controls.

Step 3.

Centrifuge at 1000 x g for 3 min.

Step 4.

Between sampling, store stacked filter/filtrate plates at 4°C, covered with a plate lid.

Step 5.

After filling the plate, remove the filter plate and store the filtrate plate at 4°C, covered with a plate lid and wrapped well with parafilm.