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

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Abstract

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 MPN (most probable number) assay to determine % phage infectivity.

For qEXT and MPN sampling, **250 µL of sample in duplicates** were collected and filtered through a 0.2 µm, 96-well filter plate, for each time point. (Flow through filtrate containing phage is the sample to analyze).

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Materials

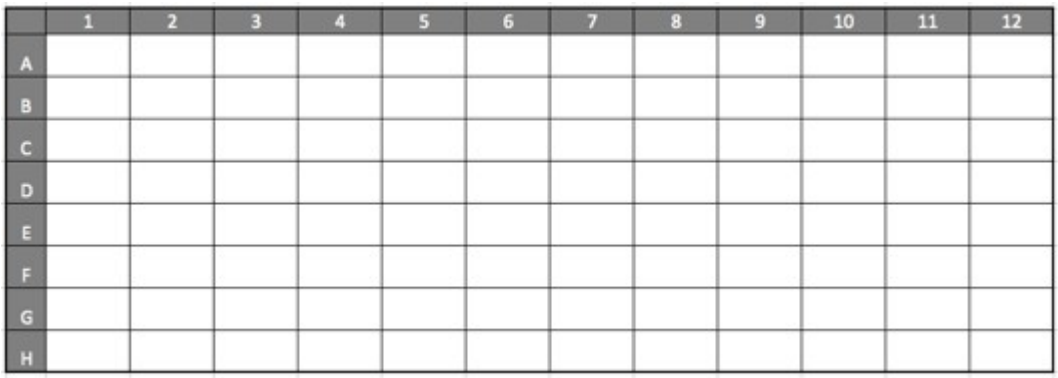
- 96-well filter plates (0.2um) Millipore #MSGVN2210 by Contributed by users
- 96 well plates for filtrate Fisher #087722C by Contributed by users
- Eppendorf centrifuge with plate adapters and a balance plate by Contributed by users
- P1000 micropipet and Filter Tips by Contributed by users
- Parafilm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



Step 2.

Transfer 250 uL of sample from each experiment bottle into a filter plate in duplicates. Only use sample bottles with phage and not 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.