Sampling to Monitor Fluorescence during Cell Lysis: Large-scale One-step Phage Infection of Cyanobacteria

Sarah Giuliani

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 (unfiltered)

8 Even-hour Timepoints: 0, 2, 4, 6, 8, 10, 12, 14 (filtered)

Sampling is for monitoring the fluorescence vs. time and tracking the real-time progression of the phage infection.

For Fluorescence measurement, **20 µl of sample** was taken from each experiment bottle and diluted 10X in ASW in a 96-well plate for both filtered and unfiltered samples. The plate was read with the program “chla + phyco_onestep.prt” on the Tecan plate reader, which reads fluorescence at 680 nm (excitation 440 nm) for chlorophyll a and reads fluorescence at 577 nm (excitation at 544 nm) for phycoerythrin.

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Materials

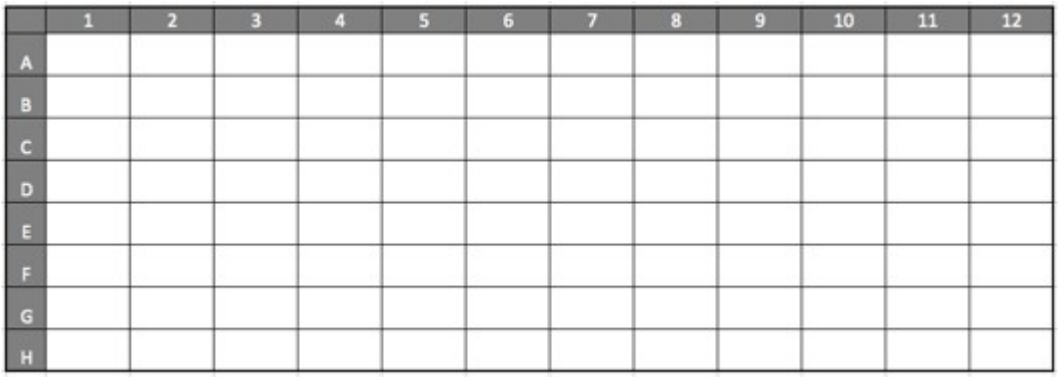
- 96 well plates with lids FISHER SCIENTIFIC #087722C by Contributed by users
- P20 micropipet and filter tips by Contributed by users
- Tecan fluorescence plate reader by Contributed by users
- ASW media by Contributed by users
- 96-well filter plates, 0.2um size Millipore #MSGVN2210 by Contributed by users
- Centrifuge with plate adapters by Contributed by users

Protocol

Step 1.

Before sampling, add 180 ul ASW into the wells of a 96-well plate labeled as a fluorescence plate.

Plate-Setup:



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

Step 2.

Add 20 ul of unfiltered sample into the ASW for a 10X dilution. Mix well with a pipette.

Step 3.

For filtered samples, transfer 280 ul from each experiment bottle or sample tube into a 96-well filter plate stacked onto a bottom 96-well filtrate plate, and centrifuge the plates at 1000 X g for 3 minutes.

Step 4.

From the filtrate, add 20 ul of sample into the ASW for a 10X dilution. Mix well with a pipette.

Step 5.

Take fluorescence readings using the “chla + phyco_onestep.prt” program on the Tecan plate reader, which reads fluorescence at 680 nm (excitation 440 nm) for chlorophyll a and reads fluorescence at 577 nm (excitation at 544 nm) for phycoerythrin.

Step 6.

Plot the fluorescence vs. time and use this to track the real-time progression of the infection.