Mar 15, 2016

One-step growth curves for Cellulophaga phages

Forked from One-step growth curves for Cellulophaga phages

DOI
dx.doi.org/10.17504/protocols.io.eqfbdtn

VERVE Net | Sullivan Lab

Bonnie Poulos
Matthew Sullivan Lab, University of Arizona, The Ohio State ...

DOI: dx.doi.org/10.17504/protocols.io.eqfbdtn

Protocol Citation: Bonnie Poulos: One-step growth curves for Cellulophaga phages. **protocols.io** **https://dx.doi.org/10.17504/protocols.io.eqfbdtn**

Protocol status: Working

Created: March 15, 2016

Last Modified: March 28, 2018

Protocol Integer ID: 2535

Abstract

One-step growth curves are used to make determinations about the life cycle of a virus on a particular host. By following a virus infection during one life cycle phase of host a growth curve can be constructed and the burst size can be calculated.

Guidelines

Note: One-step growth experiment instructions are for MOI=0.1.

Before start

Before performing a one-step experiment, you need to determine when the host is growing exponentially. You must also determine the titer of the phage lysate that will be used for the experiment. These are described in the protocol.

Host Growth Curve

1 Inoculate a new culture; ie, pick a colony into a new flask containing 10 ml of MLB media

Note

Cellulophaga should grow at room temperature on the benchtop.

2 Immediately after the transfer, take a ‘time 0’ growth reading (T0)

Note

Take the T0 growth reading at 595nm using 200ul of culture, in duplicate. Make sure the microtiter plate you are using is clean inside and out, with no scratches or spots on its surface, as it will interfere with the light reading. Subtract the media blank reading from the culture reading to arrive at the OD595 for the culture.

Protocol

NAME

Cellulophaga growth reading

CREATED BY

Verve Team

PREVIEW

2.1 Pipet 200 µl of MLB media into wells A1 and A2 of a white microtiter plate

Note

This is your ‘blank’.

2.2 Pipet 200 µl of sample (the new culture you just inoculated) into wells B1 and B2 of the same plate

Note

Ensure that there are no bubbles in the wells, as they will affect your readings. Pipet away any bubbles.

2.3 Read the plate on the plate reader

3 Continue taking readings as performed in step 2 periodically

Note

Graph the results as you go! It is best to infect the host in med-exponential (log linear) phase, when OD ≅ 0.02.

Note

You can start with longer intervals (1–2 hours) until you start to see growth, then shorter intervals (15–30 minutes) until the growth starts to level off. If it's taking a while, you can go back to reading at longer intervals.

Phage Lysate Titer

4 Do a plaque assay to determine the PFU/ml of the lysate you plan to use

5 Calculate the volume needed for 10^7 phages

Note

If 10^7 phages are contained in less than 1µl, you will need to dilute the lysate prior to performing the growth curve experiment.

One-Step Growth Experiment

6 Determine the concentration of your culture at the time you start the infection

Note

Use a correlation of readings from the plate reader and cell counts (CFU, DAPI, or FCM counts) to estimate this.

7 Calculate the volume of host culture needed for 10^8 cells

8 Pipet this amount into a 1.5 ml tube

9 Add 10^7 phages to the tube and start your timer for 15 minutes to allow the phages to adsorb to the host cells

00:15:00

10 After 15 minutes, dilute the infection 1:1000 in MLB media in a 250 ml flask.

Note

If you have 50mL of MLB in the flask, add 50ul of host for a 1:1000 dilution.

11 Take a sample immediately after dilution – this is 'time 0'

12 Steps for centrifuged sample:

Protocol

NAME

Centrifuged Sample Steps

CREATED BY

Verve Team

PREVIEW

12.1 Pipet 100 µl from the flask into 900 µl of MSM in a 15 ml tube (you are diluting your sample 10x: 10^{-1})

Note

Once you know how many phages to expect, you know what dilutions of your early samples to plate to get good counts For example, if the T0 expected concentration is 10^4, there should be 100 plaques if you plate 100 µl of a 10^{-1} dilution.

Note

Note that if you are using a different MOI, you will need to calculate the expected number of phage at T0 to guide you in what dilution to plate This will depend on the total volume of the initial infection (ie, the volume of cells plus phages) So the concentration at T0 should be total phage added/volume of infection, divided by 1000 (for the 1:1000 dilution) Convert this to phages per ml.

12.2 Vortex briefly

12.3 Centrifuge at 5 min at 1000 rpm

00:05:00

12.4 Very carefully remove the tube (do not disturb the pellet!) and plate 100 µl

13 Steps for samples that are not centrifuged:

Protocol

NAME

Non-Centrifuged Sample Steps

CREATED BY

Verve Team

PREVIEW

13.1 Pipet 100 µl from the flask into 900 µl of MSM in a 1.5 ml tube

13.2 Vortex briefly

13.3 Plate 100 µl

14 Continue sampling in this way for 8 hours

08:00:00

Note

At later time points, more dilutions will need to be plated. On the first trial, be generous with what you plate (ie, plate 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}) and use the results as a guide for what you should plate in repeat experiments.

Note

T1 and T2 can be at 1 and 2 hours, respectively, but then switch to every 30 minutes for the duration of the experiment.

15 Store the filtered samples at 4°C

16 The next day, count the plaques on all plates that have a countable number of them

17 Decide which dilution gives the best count at each time point for the next time you do this same phage-host pair

Note

Depending on the size of the plaques, a "good" count will be somewhere between 10 and a few hundred.

18 The next day, count any new plaques that have appeared and add these to your original count

19 Count again on the third day

20 Calculate PFU/ml at each time point for both the centrifuged (free phage only) and not centrifuged (total phage) samples

21 Graph the results

22 Calculate burst size

Protocol

NAME

Calculating burst size

CREATED BY

Verve Team

PREVIEW

22.1 Take the FREE phage average of the time points on the plateau before the burst (A)

22.2 Take the FREE phage average of the time points on the plateau after the burst (B)

22.3 Subtract A from B; This is the total burst or new phages released (C)

22.4 Divide C by the number of infecting phage (TOTAL phages at T0 minus FREE at T0); This is the burst size