One-step growth curves for Cellulophaga phages

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Abstract

Citation: Matthew Sullivan One-step growth curves for Cellulophaga phages. **protocols.io**
dx.doi.org/10.17504/protocols.io.ddh235
Published: 02 Feb 2016

Guidelines

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

Before start

Before performing a one-step experiment, you determine when the host is growing exponentially.

Protocol

Host Growth Curve

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

NOTES
VERVE Team 24 Aug 2015
Cellulophaga should grow at room temperature on the benchtop.

Host Growth Curve

Step 2.
Immediately after the transfer, take a ‘time 0’ growth reading

PROTOCOL

. Cellulophaga growth reading

CONTACT: VERVE Team

NOTES
VERVE Team 13 Jul 2015
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.

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

NOTES

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This is your ‘blank’.

ANNOTATIONS

Bonnie Poulos 15 Mar 2016

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.

Bonnie Poulos 15 Mar 2016

For a determination of growth, an absorbance reading at 595nm will be taken of the culture.

Step 2.2.

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

NOTES

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

Step 2.3.

Read the plate on the plate reader

ANNOTATIONS

Bonnie Poulos 15 Mar 2016

Take absorbance reading at 595nm.

Host Growth Curve

Step 3.

Continue taking readings as performed in step 2 periodically

NOTES

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

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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

Step 4.

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

Phage Lysate Titer

Step 5.

Calculate the volume needed for 10^7 phages

NOTES

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If this is less than 1 µl, you will need to dilute your lysate first.

One-Step Growth Experiment

Step 6.

Determine the concentration of your culture at the time you want to start the infection

NOTES

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

One-Step Growth Experiment

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

One-Step Growth Experiment

Step 8.
Pipet this amount into a 1.5 ml tube

One-Step Growth Experiment

Step 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

DURATION
00:15:00

One-Step Growth Experiment

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

One-Step Growth Experiment

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

One-Step Growth Experiment

Step 12.
Steps for centrifuged sample:

PROTOCOL

. Centrifuged Sample Steps

CONTACT: VERVE Team

Step 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})

NOTES

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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.

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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.

ANNOTATIONS

Bonnie Poulos 15 Mar 2016
Refer to the protocol "One-step growth curves for Cellulophaga phages" for more details about

preparing and diluting the phage samples.

Step 12.2.
Vortex briefly

Step 12.3.
Centrifuge at 5 min at 1000 rpm

DURATION
00:05:00

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

One-Step Growth Experiment

Step 13.
Steps for samples that are not centrifuged:

PROTOCOL

. Non-Centrifuged Sample Steps

CONTACT: VERVE Team

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

ANNOTATIONS
Bonnie Poulos 15 Mar 2016
Refer to the protocol "One-step growth curves for Cellulophaga phages" for details regarding the preparation and dilution of the phage preparation.

Step 13.2.
Vortex briefly

Step 13.3.
Plate 100 µl

One-Step Growth Experiment

Step 14.
Continue sampling in this way for 8 hours

DURATION
08:00:00

NOTES
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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 next time.
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T1 and T2 can be at 1 and 2 hours, respectively, but then switch to every 30 minutes.

One-Step Growth Experiment

Step 15.
Store the filtered samples at 4°C

One-Step Growth Experiment

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

One-Step Growth Experiment

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

NOTES

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

One-Step Growth Experiment

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

One-Step Growth Experiment

Step 19.
Count again on the third day

One-Step Growth Experiment

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

One-Step Growth Experiment

Step 21.
Graph the results

One-Step Growth Experiment

Step 22.
Calculate burst size

PROTOCOL

. Calculating burst size

CONTACT: VERVE Team

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

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

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

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