Transcriptomics During One-Step Growth Curves for Cellulophaga Phages

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Abstract

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Guidelines

Note: one-step growth experiment instructions are for MOI=3.

Before start

You must determine the titer of your phage lysate before performing the experiment.

Protocol

Titer Determination

Step 1.
Do a plaque assay to determine the PFU/ml of the lysate you plan to use

Preparing the Host

Step 2.
Inoculate a new culture; i.e., pick a colony into a 125 ml flask containing MLB media

NOTES
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Be sure to consider how many 500 ml cultures you will need (controls, replicates) so you grow enough, up to 50 ml. *Cellulophaga* should grow at room temperature on the benchtop. Do this late in the workday (3-6 PM).

Preparing the Host

Step 3.
The next day, transfer 10 ml of this culture to 500 ml of new media in a Fernbach flask (short and fat 2800 ml)

Preparing the Host

Step 4.
Inoculate enough 500 ml cultures for your experiment

DURATION
20:00:00

NOTES

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Grow for 16-20 hours (based on your host's growth curve) before you want to start the one-step.

Preparing the Host

Step 5.

Immediately after the transfer, take a 'time 0' growth reading

PROTOCOL

. Cellulophaga growth reading

CONTACT: VERVE Team

Step 5.1.

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

NOTES

VERVE Team 24 Aug 2015

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.

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

Step 5.2.

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

NOTES

VERVE Team 24 Aug 2015

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

Step 5.3.

Read the plate on the plate reader

ANNOTATIONS

Bonnie Poulos 15 Mar 2016

Take absorbance reading at 595nm.

Preparing the Host

Step 6.

Continue taking readings in this way periodically

NOTES

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Don't forget to inoculate a culture to use for the plaque assays!

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Make sure you take a few readings shortly before you start the one-step to ensure the host is still growing.

Preparing the Host

Step 7.

Graph the results as you go!

NOTES

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It is best to infect the host in mid exponential (log linear) phase, when OD ≅ 0.02.

One-Step Growth Experiment

Step 8.

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

NOTES

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

One-Step Growth Experiment

Step 9.

Calculate the total number of cells in each of your 500 ml cultures

One-Step Growth Experiment

Step 10.

Calculate how many phages you should add for MOI 3

NOTES

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Multiply the total cell number by 3 and calculate the volume of lysate containing this number of phages.

One-Step Growth Experiment

Step 11.

Add phages to the experimental flask and an equal volume of MSM to the control flask and start your timer

One-Step Growth Experiment

Step 12.

Immediately, dilute the infection 1:1 in MLB media; i.e., add 500 ml fresh media to each flask and swirl to mix

One-Step Growth Experiment

Step 13.

Take a sample immediately after dilution

NOTES

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This is 'time 0', but check your timer and make a note of how much time has actually passed since phage addition. Each sampling should be completed as quickly as possible, so set up your plates, tubes containing buffer for dilutions, top agar, etc. before you start.

One-Step Growth Experiment

Step 14.

Steps for centrifuged plaque assay:

PROTOCOL

. Centrifuged Plaque Assay Sample Steps

CONTACT: VERVE Team

Step 14.1.

Pipet 10 µl from the flask into 990 µl of MSM in a 1.5 ml tube

NOTES

VERVE Team 15 Jul 2015

You are diluting your sample 100x: 10^{-2}.

Step 14.2.

Vortex briefly

Step 14.3.

Centrifuge for 5 min. at 1000 rpm

DURATION

00:05:00

Step 14.4.

Very carefully remove the tube

NOTES

VERVE Team 15 Jul 2015

Do not disturb the pellet!

Step 14.5.

Do serial dilutions to make 10^{-4} and 10^{-5} dilutions

Step 14.6.

Plate 100 µl each from the 10^{-4} and 10^{-5} dilutions

One-Step Growth Experiment

Step 15.

Steps for non-centrifuged plaque assay:

PROTOCOL

. Non-Centrifuged Plaque Assay Sample Steps

CONTACT: VERVE Team

Step 15.1.

Pipet 10 µl from the flask into 990 µl of MSM in a 1.5 ml tube

NOTES

VERVE Team 15 Jul 2015

You are diluting your sample 100x: 10^{-2}.

Step 15.2.

Vortex briefly

Step 15.3.

Do serial dilutions to make 10^{-4} and 10^{-5} dilutions

Step 15.4.

Plate 100 µl each from the 10^{-4} and 10^{-5} dilutions

One-Step Growth Experiment

Step 16.

Steps for RNA samples:

PROTOCOL

. RNA Samples Steps

CONTACT: VERVE Team

Step 16.1.
Use a serological pipet to sample 30 ml from each flask into a 50 ml falcon tube

Step 16.2.
Centrifuge for 5 min. at 4000 rpm, 4°C

DURATION
00:05:00

Step 16.3.
Remove the supernatant

Step 16.4.
Resuspend the pellet in 1 ml RNALater and transfer to a 1.5 or 2 ml tube

Step 16.5.
Store samples at 4°C for up to 4 weeks

One-Step Growth Experiment

Step 17.
The next day, count the plaques on all plates that have a countable number of them

NOTES

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At later time points, more dilutions will need to be plated. Based on trial one-steps, decide when and which dilutions should be plated. Be generous, as we don't want to have to repeat the whole experiment!

Bonnie Poulos 23 Feb 2016
Sample once an hour for a total of 9 time points.

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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; add these to your original count

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 19.
Count again on the third day

One-Step Growth Experiment

Step 20.
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. Based on trial one-steps, decide when and which dilutions should be plated. Be generous, as we don't want to have to repeat the whole experiment!

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Sample once an hour for a total of 9 time points.

One-Step Growth Experiment

Step 21.

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 22.

Graph the results

One-Step Growth Experiment

Step 23.

Calculate burst size

PROTOCOL

. Calculating burst size

CONTACT: VERVE Team

Step 23.1.

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

Step 23.2.

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

Step 23.3.

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

Step 23.4.

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