# One-Step Growth Curves for Cyanophages

**Matthew Sullivan**

## Abstract

**Citation:** Matthew Sullivan One-Step Growth Curves for Cyanophages. **protocols.io**
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**Published:** 21 Jan 2016

## Guidelines

Materials:
**Part 1:**
Black 96-well microtiter plate
Pipet
25% glutaraldehyde
Liquid nitrogen

**Part II:**
None

**Part III:**
$10^8$ cell hosts & $10^7$ phages for one-step
~240 ml host for plating
70 ml SN media
950 ml of SN top agar
480 ml of SN bottom agar
Water bath
Vortex
Micropipettes and tips
Electric pipette
66 --> 1 ml serological pipettes
1 --> 1L tissue culture flask
231 --> 15 ml snap cap tubes
230 --> SN bottom agar plates (60x15mm)
33 --> 1 ml luer-lock syringes
33 --> 0.2 µm syringe filters (25mm diameter)
10 --> 50 ml conical tubes
290 --> 1.5 µl centrifuge tubes

**Phage Growth Parameters:**

| Phage(s) | Morphology | Burst Size | Latent Period | Host | Citation |
|---|---|---|---|---|---|

| | | | | | |
|---|---|---|---|---|---|
| Syn5 | podovirus | 20-30 | 1 hr | WH8109 | Raytcheva et al. (2010) |
| S-CBP1, S-CBP2, S-CBP3 | podovirus | 75-92 | | | Wang (2007) |
| P60 | podovirus | 81 | 1 hr | WH7803 | Brown et al (2006) |
| P-SSP7 | podovirus | | 8 hrs | Med4 | Lindell et al (2007) |
| S-PM2* | myovirus | 21 | 9 hrs | WH7803 | Wilson et al (1996) |
| S-CAM4 | myovirus | ~50 | | WH7803 | Kuznetsov et al (2010) |
| S-TIM5 | myovirus | ~35 | 6 hrs | WH8102 | Sabehi et al (2012) |
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| S-RIM1 | myovirus | 63 | 6 hrs | WH7803 | Stoddard et al (2007) |
| S-RIM8 | myovirus | 86 | 8 hrs | WH7803 | Stoddard et al (2007) |
| S-CBS2, S-CBS3, S-CBS3 | siphovirus | 57-175 | > podoviridae latent periods | CB0204, CB0202, CB0101 | Wang (2007) |
| S-BB1 | siphovirus | 250 | | Synechococcus strain | Mann (2003) |

*under phosphate limitation burst size is reduced by 80% and latent period is increased (Wilson et al. 1996)

NOTE: Estimates of burst sizes based on balancing estimates of viral decay and Synechococcus contacts yielded values ranging from 93 to 324 (Mann 2003)

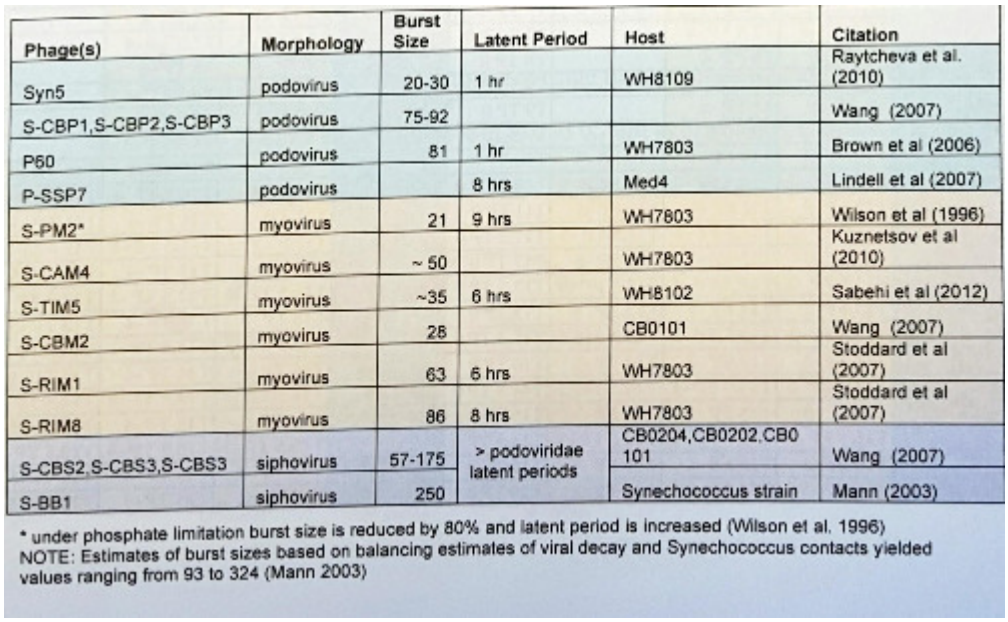


**Table 1:** Dilutions for Free Phage and Total Phage

| Free Phage Dilutions | | | Total Phage Dilutions | | |
|---|---|---|---|---|---|
| T0 FP 0 | T0 FP -2 | T0 FP -3 | T0 FP 0 | T0 FP -2 | T0 FP -3 |
| T1 FP 0 | T1 FP -2 | T1 FP -3 | T1 FP 0 | T1 FP -2 | T1 FP -3 |
| T2 FP 0 | T2 FP -2 | T2 FP -3 | T2 FP 0 | T2 FP -2 | T2 FP -3 |
| T3 FP 0 | T3 FP -2 | T3 FP -3 | T3 FP 0 | T3 FP -2 | T3 FP -3 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| T3.5 FP 0 | T3.5 FP -2 | T3.5 FP -3 | | | T3.5 FP 0 | T3.5 FP -2 | T3.5 FP -3 | | |
| T4 FP 0 | T4 FP -2 | T4 FP -3 | | | T4 FP 0 | T4 FP -2 | T4 FP -3 | | |
| T4.5 FP 0 | T4.5 FP -2 | T4.5 FP -3 | | | T4.5 FP 0 | T4.5 FP -2 | T4.5 FP -3 | | |
| T5 FP 0 | T5 FP -2 | T5 FP -3 | T5 FP -4 | | T5 FP 0 | T5 FP -2 | T5 FP -3 | T5 FP -4 | |
| T5.5 FP 0 | T5.5 FP -2 | T5.5 FP -3 | T5.5 FP -4 | | T5.5 FP 0 | T5.5 FP -2 | T5.5 FP -3 | T5.5 FP -4 | |
| T6 FP 0 | T6 FP -2 | T6 FP -3 | T6 FP -4 | | T6 FP 0 | T6 FP -2 | T6 FP -3 | T6 FP -4 | |
| T6.5 FP 0 | T6.5 FP -2 | T6.5 FP -3 | T6.5 FP -4 | | T6.5 FP 0 | T6.5 FP -2 | T6.5 FP -3 | T6.5 FP -4 | |
| T7 FP 0 | T7 FP -2 | T7 FP -3 | T7 FP -4 | | T7 FP 0 | T7 FP -2 | T7 FP -3 | T7 FP -4 | |
| T7.5 FP 0 | T7.5 FP -2 | T7.5 FP -3 | T7.5 FP -4 | | T7.5 FP 0 | T7.5 FP -2 | T7.5 FP -3 | T7.5 FP -4 | |
| T8 FP 0 | T8 FP -2 | T8 FP -3 | T8 FP -4 | | T8 FP 0 | T8 FP -2 | T8 FP -3 | T8 FP -4 | |
| T8.5 FP 0 | T8.5 FP -2 | T8.5 FP -3 | T8.5 FP -4 | | T8.5 FP 0 | T8.5 FP -2 | T8.5 FP -3 | T8.5 FP -4 | |
| T9 FP 0 | T9 FP -2 | T9 FP -3 | T9 FP -4 | | T9 FP 0 | T9 FP -2 | T9 FP -3 | T9 FP -4 | |
| T9.5 FP 0 | T9.5 FP -2 | T9.5 FP -3 | T9.5 FP -4 | | T9.5 FP 0 | T9.5 FP -2 | T9.5 FP -3 | T9.5 FP -4 | |
| T10 FP 0 | T10 FP -2 | T10 FP -3 | T10 FP -4 | T10 FP -5 | T10 FP 0 | T10 FP -2 | T10 FP -3 | T10 FP -4 | T10 FP -5 |
| T10.5 FP 0 | T10.5 FP -2 | T10.5 FP -3 | T10.5 FP -4 | T10.5 FP -5 | T10.5 FP 0 | T10.5 FP -2 | T10.5 FP -3 | T10.5 FP -4 | T10.5 FP -5 |
| T11 FP 0 | T11 FP -2 | T11 FP -3 | T11 FP -4 | T11 FP -5 | T11 FP 0 | T11 FP -2 | T11 FP -3 | T11 FP -4 | T11 FP -5 |
| T11.5 FP 0 | T11.5 FP -2 | T11.5 FP -3 | T11.5 FP -4 | T11.5 FP -5 | T11.5 FP 0 | T11.5 FP -2 | T11.5 FP -3 | T11.5 FP -4 | T11.5 FP -5 |
| T12 FP 0 | T12 FP -2 | T12 FP -3 | T12 FP -4 | T12 FP -5 | T12 FP 0 | T12 FP -2 | T12 FP -3 | T12 FP -4 | T12 FP -5 |
| T12.5 FP 0 | T12.5 FP -2 | T12.5 FP -3 | T12.5 FP -4 | T12.5 FP -5 | T12.5 FP 0 | T12.5 FP -2 | T12.5 FP -3 | T12.5 FP -4 | T12.5 FP -5 |
| T13 FP 0 | T13 FP -2 | T13 FP -3 | T13 FP -4 | T13 FP -5 | T13 FP 0 | T13 FP -2 | T13 FP -3 | T13 FP -4 | T13 FP -5 |
| T13.5 FP 0 | T13.5 FP -2 | T13.5 FP -3 | T13.5 FP -4 | T13.5 FP -5 | T13.5 FP 0 | T13.5 FP -2 | T13.5 FP -3 | T13.5 FP -4 | T13.5 FP -5 |
| T14 FP 0 | T14 FP -2 | T14 FP -3 | T14 FP -4 | T14 FP -5 | T14 FP 0 | T14 FP -2 | T14 FP -3 | T14 FP -4 | T14 FP -5 |
| T14.5 FP 0 | T14.5 FP -2 | T14.5 FP -3 | T14.5 FP -4 | T14.5 FP -5 | T14.5 FP 0 | T14.5 FP -2 | T14.5 FP -3 | T14.5 FP -4 | T14.5 FP -5 |
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| T15.5 FP 0 | T15.5 FP -2 | T15.5 FP -3 | T15.5 FP -4 | T15.5 FP -5 | T15.5 FP 0 | T15.5 FP -2 | T15.5 FP -3 | T15.5 FP -4 | T15.5 FP -5 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| T16 FP 0 | T16 FP -2 | T16 FP -3 | T16 FP -4 | T16 FP -5 | T16 FP 0 | T16 FP -2 | T16 FP -3 | T16 FP -4 | T16 FP -5 |
| T24 FP 0 | T24 FP -2 | T24 FP -3 | T24 FP -4 | T24 FP -5 | T24 FP 0 | T24 FP -2 | T24 FP -3 | T24 FP -4 | T24 FP -5 |

**Table 1.** Dilutions for Free Phage and Total Phage

| Free Phage Dilutions | | | | | Total Phage Dilutions | | | | |
|---|---|---|---|---|---|---|---|---|---|
| T0 FP 0 | T0 FP -2 | T0 FP -3 | | | T0 TP 0 | T0 TP -2 | T0 TP -3 | | |
| T1 FP 0 | T1 FP -2 | T1 FP -3 | | | T1 TP 0 | T1 TP -2 | T1 TP -3 | | |
| T2 FP 0 | T2 FP -2 | T2 FP -3 | | | T2 TP 0 | T2 TP -2 | T2 TP -3 | | |
| T3 FP 0 | T3 FP -2 | T3 FP -3 | | | T3 TP 0 | T3 TP -2 | T3 TP -3 | | |
| T3.5 FP 0 | T3.5 FP -2 | T3.5 FP -3 | | | T3.5 TP 0 | T3.5 TP -2 | T3.5 TP -3 | | |
| T4 FP 0 | T4 FP -2 | T4 FP -3 | | | T4 TP 0 | T4 TP -2 | T4 TP -3 | | |
| T4.5 FP 0 | T4.5 FP -2 | T4.5 FP -3 | | | T4.5 TP 0 | T4.5 TP -2 | T4.5 TP -3 | | |
| T5 FP 0 | T5 FP -2 | T5 FP -3 | T5 FP -4 | | T5 TP 0 | T5 TP -2 | T5 TP -3 | T5 TP -4 | |
| T5.5 FP 0 | T5.5 FP -2 | T5.5 FP -3 | T5.5 FP -4 | | T5.5 TP 0 | T5.5 TP -2 | T5.5 TP -3 | T5.5 TP -4 | |
| T6 FP 0 | T6 FP -2 | T6 FP -3 | T6 FP -4 | | T6 TP 0 | T6 TP -2 | T6 TP -3 | T6 TP -4 | |
| T6.5 FP 0 | T6.5 FP -2 | T6.5 FP -3 | T6.5 FP -4 | | T6.5 TP 0 | T6.5 TP -2 | T6.5 TP -3 | T6.5 TP -4 | |
| T7 FP 0 | T7 FP -2 | T7 FP -3 | T7 FP -4 | | T7 TP 0 | T7 TP -2 | T7 TP -3 | T7 TP -4 | |
| T7.5 FP 0 | T7.5 FP -2 | T7.5 FP -3 | T7.5 FP -4 | | T7.5 TP 0 | T7.5 TP -2 | T7.5 TP -3 | T7.5 TP -4 | |
| T8 FP 0 | T8 FP -2 | T8 FP -3 | T8 FP -4 | | T8 TP 0 | T8 TP -2 | T8 TP -3 | T8 TP -4 | |
| T8.5 FP 0 | T8.5 FP -2 | T8.5 FP -3 | T8.5 FP -4 | | T8.5 TP 0 | T8.5 TP -2 | T8.5 TP -3 | T8.5 TP -4 | |
| T9 FP 0 | T9 FP -2 | T9 FP -3 | T9 FP -4 | | T9 TP 0 | T9 TP -2 | T9 TP -3 | T9 TP -4 | |
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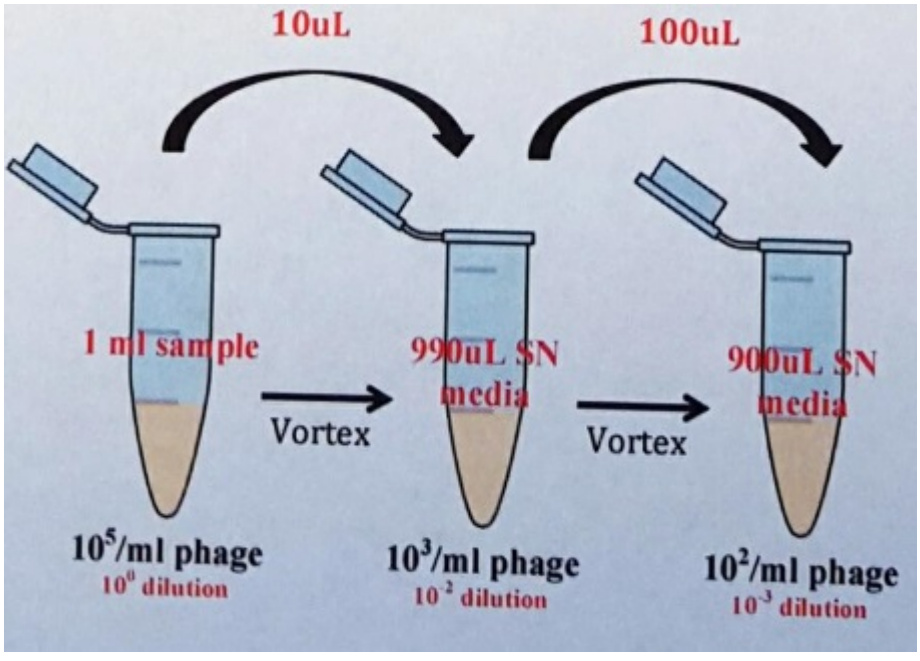
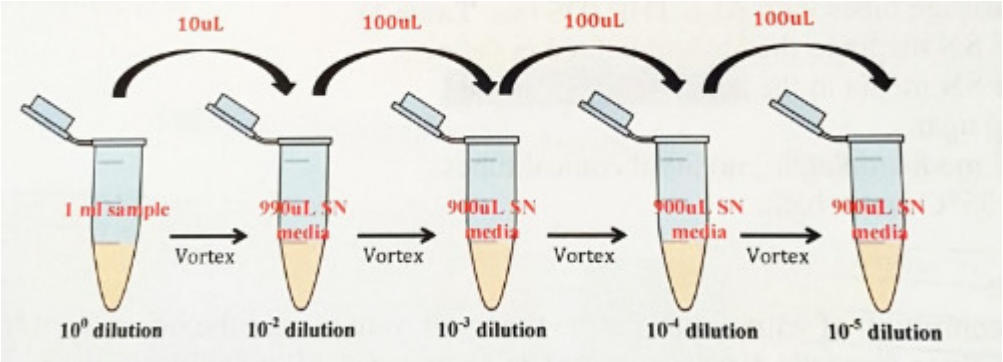
## Serial Dilution:



**Serial Dilution 2:**




## Materials

96-well Microtiter Plate (Black) 249945 by Thermo Scientific

## Protocol

Part I: Determine when the host is growing exponentially

**Step 1.**
From an already growing culture, split cells at the ratio you will be splitting for the one step experiment.

Part I: Determine when the host is growing exponentially

**Step 2.**
Immediately after the split, take a 'time 0' growth reading using a plate reader.

PROTOCOL

**. Time 0 growth reading using a plate reader**

CONTACT: VERVE Team

NOTES
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Make sure the 96-well 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 the media you are growing the cell in (eg. SN media or Pro99 media) into wells A1 and A2 of a black 96-well microtiter plate.

NOTES
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This is your blank.

**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 **without the lid.**

Part I: Determine when the host is growing exponentially

**Step 3.**

Continue taking readings in this way at approximately the same time every day for 21 days. Graph the results as you go and determine when exponential growth occurs!

Part I: Determine when the host is growing exponentially

**Step 4.**

Repeat 2 more times to ensure the host growth is consistent.

Part I: Determine when the host is growing exponentially

**Step 5.**

During your 3rd growth curve when exponential growth is occuring, fix 1 ml of cells with 5 µl 25% glutaraldehyde.

AMOUNT

5 µl Additional info:

Part I: Determine when the host is growing exponentially

**Step 6.**

Flash freeze in liquid nitrogen daily.

Part I: Determine when the host is growing exponentially

**Step 7.**

Store in -80°C freezer.

Part I: Determine when the host is growing exponentially

**Step 8.**

Determine the cell count (using FCM or DAPI) that is associated with the culture fluorescence level during exponential growth.

Part I: Determine when the host is growing exponentially

**Step 9.**

It is best to infect the host in late-exponential (log linear) phase.

Part I: Determine the titer of your phage lysate

**Step 10.**

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

Part I: Determine the titer of your phage lysate

**Step 11.**

Calculate the volume needed for $10^7$ phages.

Part II: Determine the length of time to sample during the one-step experiment

**Step 12.**

Determine the length of time to sample during the one-step experiement. Below, you will find already published phage growth parameters, which can help you determine the length to sample for your one-step experiment

| Phage(s) | Morphology | Burst Size | Latent Period | Host | Citation |
|---|---|---|---|---|---|
| Syn5 | podovirus | 20-30 | 1 hr | WH8109 | Raytcheva et al. (2010) |

| | | | | | |
|---|---|---|---|---|---|
| S-CBP1, S-CBP2, S-CBP3 | podovirus | 75-92 | | | Wang (2007) |
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| S-BB1 | siphovirus | 250 | | Synechococcus strain | Mann (2003) |

*under phosphate limitation burst size is reduced by 80% and latent period is increased (Wilson et al. 1996)

NOTES

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NOTE: Estimates of burst sizes based on balancing estimates of viral decay and *Synechococcus* contacts yielded values ranging from 93 to 324 (Mann 2003)

Part III: One-Step Growth Experiement

**Step 13.**

Prepare dilutions for free phage and total phage by following the table found in guidelines.

NOTES

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Table is in guidelines.

Part III: One Step Growth Experiment

**Step 14.**

Turn on water bath to 35°C

Part III: One Step Growth Experiment

**Step 15.**

Label a 1L flask with the phage, host, and your initials

Part III: One Step Growth Experiment

**Step 16.**

Add 396 ml SN media (or however much to q. to 400 ml) to the 1L flask

Part III: One Step Growth Experiment

**Step 17.**

Label 1 15ml snap cap tube the "Infection Tube"

Part III: One Step Growth Experiment

**Step 18.**

Label 230 15ml snap cap tubes with the red, bolded text ids

NOTES

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See Table 1 in guidelines

Part III: One Step Growth Experiment

**Step 19.**

Add 1ml host to each tube

Part III: One Step Growth Experiment

**Step 20.**

*Days in advance*, pour 240 SN bottom agar plates (2ml SN bottom agar per plate)

Part III: One Step Growth Experiment

**Step 21.**

Lay out the SN bottom agar plates

Part III: One Step Growth Experiment

**Step 22.**

Label 230 SN bottom agar plates with the **red, bolded text ids**

NOTES

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See Table 1 in guidelines

Part III: One Step Growth Experiment

**Step 23.**

Label 2 plates "Host (-)"

Part III: One Step Growth Experiment

**Step 24.**

Label 1.5µl centrifuge tubes with **ALL THE IDS** (See table 1 guidelines).

NOTES

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262 total tubes.

Part III: One Step Growth Experiment

**Step 25.**

Add 900µl SN media to the **ids highlighted in yellow**

NOTES

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See Table 1 in guidelines

Part III: One Step Growth Experiment

**Step 26.**

Add 990µl SN media to the **ids highlighted in blue**

NOTES

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See Table 1 in guidelines

Part III: One Step Growth Experiment

**Step 27.**

Microwave SN top agar

Part III: One Step Growth Experiment

**Step 28.**

Aliquot heated SN media top agar into 50ml conical tubes

Part III: One Step Growth Experiment

**Step 29.**

Keep warm in the 35°C water bath

Part III: One Step Growth Experiment

**Step 30.**

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

NOTES

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

Part III: One Step Growth Experiment

**Step 31.**

Calculate the volume of host culture needed for $10^8$ cells (q. to 2ml with SN media)

Part III: One Step Growth Experiment

**Step 32.**

Pipet this amount into the 15 ml snap cap tube labeled "Infection Tube"

Part III: One Step Growth Experiment

**Step 33.**

Add $10^7$ phages (q. to 2ml with SN media) to the tube

Part III: One Step Growth Experiment

**Step 34.**

Allow the phages to adsorb to the host cells for 1 hour

DURATION

01:00:00

Part III: One Step Growth Experiment

**Step 35.**

Put the 4ml infection into 396ml SN media in a 1L flask to dilute the infection to 1:100.

Part III: One Step Growth Experiment

**Step 36.**

Take a sample immediately after dilution - this is time 0.

0.2µl filtered (Free phage)

**Step 37.**

Remove the plunger of 1ml luer-lock syringe.

0.2µl filtered (Free phage)

**Step 38.**

Add a 0.2 µm 25mm diameter syringe filter to the syringe end

0.2µl filtered (Free phage)

**Step 39.**

Pipet 1 ml from the flask into the syringe using 1 ml serological pipet

NOTES

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NOTE: The phage concentration should be at -$10^5$ per ml.

0.2µl filtered (Free phage)

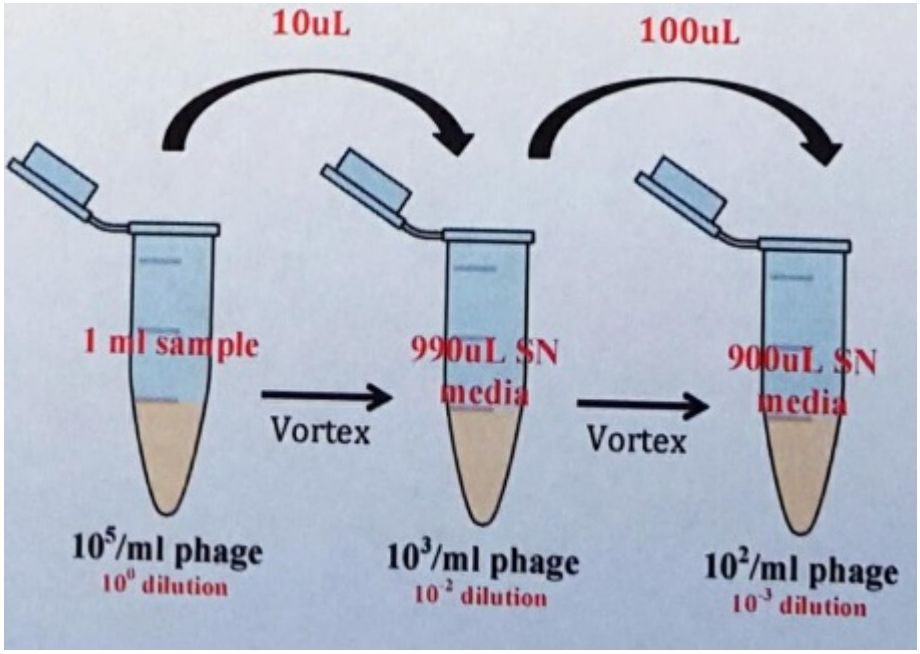
**Step 40.**

Add the plunger back to the syringe and 0.2µm syringe filter the 1 ml into a 1.5 ml centrifuge tube.

0.2µl filtered (Free phage)

**Step 41.**

Perform a serial dilution to dilute the sample to $10^2$ per ml ($10^{-3}$ dilution)




0.2µl filtered (Free phage)

**Step 42.**

Add 250µl of the $10^{-3}$ dilution and 250µl of the $10^{-2}$ dilution to the corresponding 15ml snap cap tube with 1ml host.

0.2µl filtered (Free phage)

**Step 43.**

Allow phage and host to incubate for 2 hours.

DURATION

02:00:00

0.2µl filtered (Free phage)

**Step 44.**

Plate using 4ml SN top agar per plate.

Not filtered (Total Phage)

**Step 45.**

Pipette 1ml from the flask into a 1.5ml centrifuge tube using the same 1ml serological pipette.

Not filtered (Total Phage)

**Step 46.**

Perform a serial dilution to dilute the sample to $10^2$ per ml ($10^{-3}$)

Not filtered (Total Phage)

**Step 47.**

Add 250µl of the $10^{-3}$ dilution and 250µl of the $10^{-2}$ dilution to the corresponding 15ml snap cap tube with 1ml host.

Not filtered (Total Phage)

**Step 48.**

Allow phage and host to incubate for 2 hours and then plate using 4ml SN top agar per plate.

DURATION

02:00:00

Part III: One Step Growth Experiment

**Step 49.**
Continue sampling in this way for 16 hours (and sample one more time at 24 hrs).

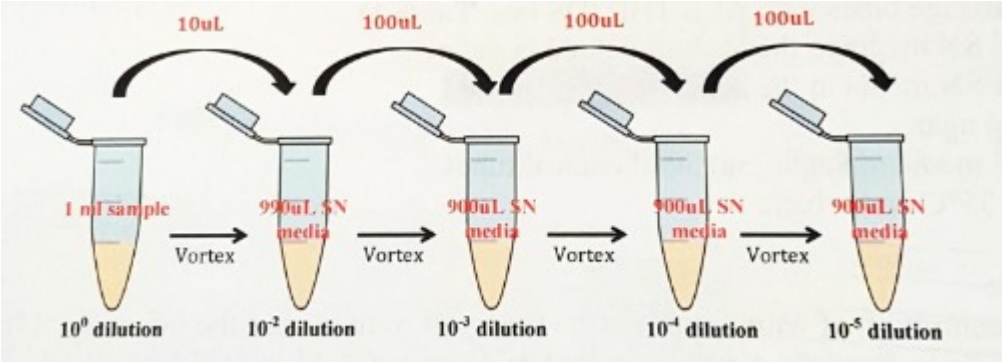
DURATION
16:00:00

Part III: One Step Growth Experiment

**Step 50.**
At later time points more dilutions will need to be plated. Be generous with what you plate (i.e., plate $10^{-2}$, $10^{-3}$, $10^{-4}$, $10^{-5}$).

Part III: One Step Growth Experiment

**Step 51.**
The serial dilutions should be performed as shown below:




Part III: One Step Growth Experiment

**Step 52.**
In seven days, count the plaques on all plates that have a countable number of them.

Part III: One Step Growth Experiment

**Step 53.**
Count again on day 14 and 21.

Part III: One Step Growth Experiment

**Step 54.**
Calculate PFU/ml at each time point for both the centrifuged (free phage only) and not centrifuged (total phage) samples. Graph the results.

Part III: One Step Growth Experiment

**Step 55.**
Calculate burst size. Take the FREE phage average of the time points on the plateau before the burst (A).

Part III: One Step Growth Experiment

**Step 56.**
Take the FREE phage average of the time points on the plateau after the burst (B).

Part III: One Step Growth Experiment

**Step 57.**
Subtract A and B. This the total burst or new phages released (C).

Part III: One Step Growth Experiment

**Step 58.**
Divide C by the number of infecting phage (TOTAL phages at T0 minus FREE at T0). This is the burst size.