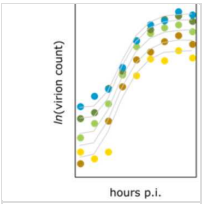

Version 2

Oct 09, 2022

Modified one-step growth (mOSG) assay V.2

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ABSTRACT

This protocol describes a modified one-step growth assay, as presented in the manuscript "*Life history diversity and signals of trade-offs in a large group of chloroviruses*" (Lievens et al., bioRxiv). It is designed to quantify the adsorption constant, depolarization probability, lysis time, and burst size of chloroviruses (large, lytic dsDNA viruses). The protocol should also be applicable to other lytic viruses that are large enough to be counted by flow cytometry.

Compared to a classic one-step growth assay, this protocol uses multiple MOPs (virion:host ratios), flow cytometry, and non-linear statistical modeling to increase efficiency. Briefly, viruses and host cells (algae) are mixed at a high concentration and allowed to adsorb, infections are synchronized by 1:1000 dilution, diluted solutions are aliquoted, and independent aliquots are sampled every 2h. The samples are then measured with flow cytometry, and statistical models used to estimate the adsorption constant, lysis time, burst size, and depolarization probability. See the associated manuscript for a full description and schematic figure, as well as the statistical model.

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MATERIALS TEXT

For this protocol, we use:

- to store virus samples: 15ml PP centrifuge tubes from Sarstedt (ref. 62.554.502, Sarstedt, DE) - *don't interfere with viruses**
- culture plates: 96-well polystyrene tissue culture plates from Techno Plastic Products (ref. TPP92696, Techno Plastic Products, CH) - *don't interfere with viruses*, can withstand centrifugation*
- deep well plates: 96-well 2.2ml PP deep well plates from VWR (ref. 732-0585, VWR International, USA) or Abgene (ref. AB0932, Thermo Scientific, DE) - *don't interfere with viruses*, can withstand centrifugation*
- transparent seals: PCR plate seals from VWR (ref. 732-3212, VWR International, USA) - *very adhesive, which is necessary to deal with the inversion steps*

**We find that in many storage containers, the virion concentration decreases over time (EJPL, unpublished data). This includes many types of PCR plates, eppis, and centrifuge tubes. The effect may be because virions adsorb to certain plastics. Any new storage container should be tested before use!*

Also necessary:

- up to 36 virus samples
- an algal culture in a growth phase that can be infected by the viruses
- centrifuge with rotors for plates and 15/50ml tubes
- vortex
- orbital shaker
- multichannel pipettes
- haemocytometer and microscope, or some other way of counting algae
- flow cytometry materials (see https://www.protocols.io/view/virion-quantification-by-flow-cytometry-without-fi-6qpvr6q93vmk/v1)

BEFORE STARTING

This version is for two adsorption plates, which corresponds to max. 36 different viruses when using 5 MOPs/virus sample. It is assumed that the virion concentration of the virus samples is known (see dx.doi.org/10.17504/protocols.io.6qpvr6q93vmk/v1).

Use sterile techniques throughout this protocol. Using materials that don't bind viruses is important; see *Materials* for more information.

Definitions:

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- MOP = Multiplicity of Particles, the ratio of virions to host cells
- BBM = modified Bold's Basal Medium, see the associated publication
- growth conditions = the conditions under which the one-step growth assay is being run (e.g. a specific temperature)
- culture plates = 96-well flat-bottomed tissue culture plates (see *Materials*)
- deep well plates = 96-well 2.2ml PP deep well plates (see *Materials*)
- transparent seal = transparent PCR plate seal (see *Materials*)
- if the type of seal is not specified, it can be any adhesive plate seal that does not breathe (e.g. aluminum, thermo, ...)

Preparation (can be done the day beforehand)

1 Prepare the **1:1000 dilution plates.**

1.1 Prepare 34 deep well plates with 1998µl BBM in wells 1A to 12D (i.e. wells 12E, 12F, 12G, 12H free). Label half of these plates "1", and the other half "2".

If following the alternative 1:1000 dilution option (see step 9.1):
Prepare 34 deep well plates with 1980µl BBM in wells 1A to 12D (i.e. wells 12E, 12F, 12G, 12H free). Label half of these plates "1", and the other half "2".

1.2 Seal the 1:1000 dilution plates and store at room temperature.

2 Prepare the **1:10000 dilution plates.**

2.1 Prepare two deep well plates with 1800µl BBM in wells 1A to 12D (i.e. wells 12E, 12F, 12G, 12H free). Label one plate "1*" and the other "2*".

If following the alternative 1:1000 dilution option (see step 9.1):
Prepare two deep well plates with 1998µl BBM in wells 1A to 12D (i.e. wells 12E, 12F, 12G, 12H free). Label one plate "1" and the other "2*".*

2.2 Seal the 1:10000 dilution plates and store at room temperature.

3 *If following the alternative 1:1000 dilution option (see step 9.1):*
*Prepare the **1:10 intermediate dilution plates**.*

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3.1 *Prepare two culture plates with 180µl in wells 1A to 12 D (i.e. wells 12E, 12F, 12G, 12H free). Label one plate "1" and the other "2".*

3.2 *Seal the 1:10 intermediate dilution plates and store at room temperature.*

4 Prepare the **adsorption plates**.

4.1 In two culture plates, mix viral solutions so they have a volume of 50µl and varing virion concentrations:

- final MOP 0.5: concentration $1.5*10^7$ virions/ml at this stage; will be $0.75*10^7$ virions/ml when the volume is 100µl
- final MOP 1: concentration $3*10^7$ virions/ml at this stage; will be $1.5*10^7$ virions/ml when the volume is 100µl
- final MOP 2: concentration $6*10^7$ virions/ml at this stage; will be $3*10^7$ virions/ml when the volume is 100µl
- final MOP 5: concentration $15*10^7$ virions/ml at this stage; will be $7.5*10^7$ virions/ml when the volume is 100µl
- final MOP 10: concentration $30*10^7$ virions/ml at this stage; will be $15*10^7$ virions/ml when the volume is 100µl

Avoid reusing tips for virus pipetting; reusing tips leads to lower accuracy (possibly because viruses bind to the plastic) (EJPL, unpublished data).

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A	B	C	D	E	F	G
plate	column	row	virus	final MOP	virus to add (µl)	BBM to add (µl)
1	1	A	NE-JV4	0.5	1.03	49.0
1	1	B	NE-JV4	1	2.07	47.9
1	1	C	NE-JV4	2	4.14	45.9
1	1	D	NE-JV4	5	10.4	39.7
1	1	E	NE-JV4	10	20.7	29.3
1	1	F	MA-1E	0.5	1.04	49.0
1	1	G	MA-1E	1	2.07	47.9
1	1	H	MA-1E	2	4.15	45.9
1	2	A	MA-1E	5	10.4	39.6
1	2	B	MA-1E	10	20.7	29.3
1	2	C	PBCV-1	0.5	0.72	49.3
1	2	D	PBCV-1	1	1.44	48.6
1	2	E	PBCV-1	2	2.89	47.1
1	2	F	PBCV-1	5	7.21	42.8
1	2	G	PBCV-1	10	14.4	35.6
...	...	...	...	...	...	...
1	12	C	neg. ctrl.			50
1	12	D	neg. ctrl.			50
1	12	E	flow cytometry neg. ctrl.			
1	12	F	flow cytometry neg. ctrl.			
1	12	G	flow cytometry pos. ctrl.			
1	12	H	flow cytometry pos. ctrl.			

Example pipetting scheme for one adsorption plate.

4.2 Seal the adsorption plates (with the lids on top of the seal), vortex, and store at 4°C.

5 Prepare 8ml of "flow cytometry positive control" sample in a 15ml tube (use any sample with a known virion concentration). Store at 4°C.

mOSG

6 Get the adsorption plates out of 4°C storage, vortex & spin down. They will warm to room

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temperature during the next step.

7 Prepare an intermediate algal solution.

7.1 Centrifuge the algae for 5min at 4500rpm.

7.2 Gently pour off the supernatant and resuspend the pellet.

7.3 Count the concentration of algal cells in the pellet using a haemocytometer.

7.4 Using the algal pellet and BBM, prepare 10ml of an intermediate algal solution at $3*10^7$ cells/ml.

8 Adsorption.

8.1 Using a 200µl multichannel pipette, aliquot 50µl of the intermediate algal solution into wells 1A-12D of the adsorption plates. Make sure the algal solution is added into the virus solution (don't leave drops on the side of the wells), and use new pipette tips for every column to avoid contamination.

8.2 Close the adsorption plates with their lid.

8.3 Shake the adsorption plates at high speed: 15min at 1200rpm on a microplate shaker with orbital diameter 1mm, or 15min at 500rpm on a shaker with orbital diameter 10mm.
Background info: These conditions led to ≥ 80% adsorbed virions in preliminary tests with PBCV-1 (EJPL, unpublished data).

Note the time at the end of the 15min adsorption period. This is time point 0.

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8.4

9 Do a 1:1000 dilution to synchronize infections.
Notes: Do this step as quickly as possible: the time of dilution should be as close as possible to time point 0.
Background info: 1:100 dilutions were insufficient to prevent secondary infection (EJPL, unpublished data).

9.1 Using a 10µl multichannel pipette, transfer 2µl from adsorption plate "1" into each of the 1:1000 dilution plates "1". Do the same for adsorption plate "2" and the 1:1000 dilution plates "2".

Alternative 1:1000 dilution option, using a 200µl multichannel pipette:

- *Transfer 20 µl from adsorption plate "1" into 1:10 intermediate dilution plate "1". Do the same for adsorption plate "2" and 1:10 intermediate dilution plate "2".*
- *Mix the 1:10 intermediate dilution plates by pipetting each column up and down 3x.*
- *Transfer 20 µl from 1:10 intermediate dilution plate "1" into each of the 1:1000 dilution plates "1". Do the same for intermediate dilution plate "2" and the 1:1000 dilution plates "2".*

9.2 Seal all but one of the 1:1000 dilution plates "1" and all but one of the 1:1000 dilution plates "2". Use a transparent seal, and make sure that the seal sticks very well. Invert the sealed plates to mix, and leave under the growth conditions.

10 Do a 1:10000 dilution, which will be used to look for signals of secondary infection.

10.1
- Mix the remaining 1:1000 dilution plate "1" by using a 1000µl multichannel pipette to pipette each column up and down 3x. Do the same for the remaining 1:1000 dilution plate "2".
- Transfer 200µl from the remaining 1:1000 dilution plate "1" into the 1:10000 dilution plate "1*". Do the same for the remaining 1:1000 dilution plate "2" and the 1:10000 dilution plate "2*".

If following the alternative 1:1000 dilution option (see step 9.1):

- *Using a 10µl multichannel pipette, transfer 2 µl from the 1:10 intermediate dilution plate "1" into the 1:10000 dilution plate "1*". Do the same for the 1:10 intermediate dilution plate "2" and the 1:10000 dilution plate "2*".*

10.2 Seal the remaining 1:1000 dilution plates.

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10.3 Seal the 1:10000 dilution plates. Use a transparent seal, and make sure that the seal sticks very well. Invert the sealed plates to mix, and leave under the growth conditions.

11 Time point 0 (0h) sampling.
Notes: Due to the time taken by steps 9 and 10, the "time point 0" samples are typically collected ~30min after the actual time point 0 (which is the end of the adsorption period, i.e. the idealized dilution time). As long as the infected cells don't start lysing within ~30min, this doesn't affect the results.

11.1 Centrifuge the remaining 1:1000 dilution plates "1" and "2" for 15min at 2000g, to separate free virions from algae.

11.2 Transfer 100µl of the supernatants from the centrifuged 1:1000 dilution plates "1" and "2" into culture plates. Label these "**supernatant plate** 1 0h" and "**supernatant plate** 2 0h".

11.3 Add 100µl BBM to wells 12E and 12F of the supernatant plates, and 100µl of the "flow cytometry positive control" to wells 12G and 12H of the supernatant plates.

11.4 Seal the supernatant plates and store at 4°C.

11.5 Discard the 1:1000 dilution plates.

12 Every hour after time point 0, invert the 1:1000 dilution plates to mix the contents.
Background info: Several mixing methods were tested; inverting was similar to mild shaking (EJPL, unpublished data).

13 Every two hours after time point 0, sample one "1" and one "2" 1:1000 dilution plate as in step 11. After 16h, sample the "1*" and "2*" plates as well.

Flow cytometry

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14 Between 1-7 days after the mOSG (ideally 1-4 days), measure the virion concentration in the supernatant plates using protocol dx.doi.org/10.17504/protocols.io.6qpvr6q93vmk/v1 (for low-concentration virion samples).
Tip: To avoid conflating flow cytometer errors with mOSG results, don't measure the supernatant plates in chronological order.

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