Archiving Plaques: saving virus purification for later

Kathryn Kauffman[1], Martin Polz[1]

[1]Massachusetts Institute of Technology

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Polz Lab

Kathryn Kauffman
Massachusetts Institute of Tec...

ABSTRACT

In plaque-assay based viral discovery many more plaques are often generated than can be examined at any one time, it is therefore of value to be able to archive plaques for future investigation. To address the potential for differences among viruses in tolerance to storage, this protocol provides large-scale archival approaches that include storage of picked plaques at both 4°C and -20°C or -80°C. We note however, that viruses differ in their sensitivity to storage conditions (Clark 1962 [1]; Fortier and Moineau 2009 [2]) and, though we describe methods that include multiple conditions, we highlight also that including additional approaches, such as storage in infected cells (Golec et al. 2011 [3]), is likely to increase total proportion of recovered viruses in large collections.

[1] Clark, W.A. (1962). Comparison of Several Methods for Preserving Bacteriophages. Appl. Microbiol. 10, 466–471.
[2] Fortier, L.-C., and Moineau, S. (2009). Phage Production and Maintenance of Stocks, Including Expected Stock Lifetimes. In Bacteriophages, M.R.J. Clokie, and A.M. Kropinski, eds. (Humana Press), pp. 203–219.
[3] Golec, P., Dąbrowski, K., Hejnowicz, M.S., Gozdek, A., Łoś, J.M., Węgrzyn, G., Łobocka, M.B., and Łoś, M. (2011). A reliable method for storage of tailed phages. Journal of Microbiological Methods 84, 486–489.

EXTERNAL LINK

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THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Kauffman, K. M. & Polz, M. F. Streamlining standard bacteriophage methods for higher throughput. MethodsX 5, 159–172 (2018). https://doi.org/10.1016/j.mex.2018.01.007 - This protocols.io protocol has been adapted from the MethodsX article, which is published under the Creative Commons Attribution License (CC BY).

Kauffman and Polz - 2018 - Streamlining standard bacteriophage methods for hi.pdf

Supplementary_File_1.Archiving_Template.xlsx

PROTOCOL STATUS

Working

GUIDELINES

The described procedures are of greatest utility when the number of plaques to be archived is on the order of several hundreds - and it is then optimal to divide the tasks of cataloging plaques and physically archiving them over three consecutive days, this is how the protocol is presented here.

MATERIALS TEXT

See "Guidelines & Warnings" section for materials to have ready before start.

BEFORE STARTING

Materials for plaque archival procedures

Virus material: Agar overlay plates with plaques to be archived Archival and filtration materials:

- Sterile host growth media (250uL per plaque)
- Sterile 50% glycerol solution (50:50 glycerol:host growth media; 125uL per plaque)

When opting for centrifugation-based filtration:

- 96-well polypropylene microplates for collection and as filtration receivers (Greiner Bio-One #651261); requires two 96-well plates per every 48 plaques.
- 96-well filter-bottom plates, 0.22um (EMD Millipore MSGVS2210) or 0.45um (EMD Millipore MSHVS4510); requires one 96-well plate per every 48 plaques.
- Centrifuge that can accommodate 96-well plates
- Sterile adhesive 96-well plate aluminum foil covers

When opting instead for vacuum-based filtration:

- Users will need to ensure compatibility of selected manifold, filter-bottom plates, and receiver plates. The below items are an example of compatible components.
- 96-well polypropylene microplates for collection and as filtration receivers (Greiner Bio-One #651261); requires two 96-well plates per every 48 plaques.
- 96-well filter-bottom plates, 0.22um (EMD Millipore MSGVS2210) or 0.45um (EMD Millipore MSHVS4510); requires one 96-well plate per every 48 plaques.
- MultiScreenHTS Vacuum Manifold for 96-well plate format (EMD Millipore MSVMHTS00)
- Vacuum pump
- Sterile adhesive 96-well plate aluminum foil covers

Cataloging spreadsheet: see template example document provided with this protocol - "Supplementary File 1" from the MethodsX publication.

Cataloging Plaques (Day 1)

1 **Mark the plaques:** Mark all plaques to be archived by writing a number adjacent to the plaque on the bottom of the petri dish.

NOTE

Note: In cases where there are hundreds of plaques of interest it is optimal to divide the tasks of cataloging plaques and physically archiving them over three consecutive days, this is how the protocol is presented here.

2 **Log the plaques:** Log all plaque properties of interest into an archival spreadsheet, such as the example provided in example dataset. Properties of interest might include: day plaque was first detected, size, and appearance.

Collecting Plaque Plugs (Day 2)

3 **Prepare 96-well storage plates:** Prepare 96-well plates for receiving plaque plugs by aliquoting 250uL of host growth media (or desired buffer) into 48 alternating wells. Note that alternating wells are used to minimize potential for cross-contamination between stocks.

4 **For each plaque, collect a plaque plug as follows:**
Hold a 1mL pipette-tip by hand and use it to bore through the plaque to the bottom of the petri dish
Twist the tip at the bottom of the dish to completely sever the connection between the plug and the rest of the agar
Pull the pipette tip free from the agar and insert the delivery end into the designated well in the 96-well plate, as defined in the cataloging spreadsheet
Press down on the opening at the top of the pipette tip with a finger to create sufficient air pressure to expel the agar plug containing the plaque into the media in the well. This may require several presses. Confirm that the plug is dislodged and in the media.
Once all plaque plugs have been harvested seal the 96-well plate with sterile adhesive aluminum covers and store at 4C overnight to allow the virions to elute from the plugs.

Processing Plaque Plugs (Day 3)

Prepare filter-bottom plates: Prepare a filter-bottom plate by removing it from its package and placing it on top of a receiver plate

5 snugly. For centrifugation-based approach use lab tape to seal the filter plate snugly to the receiver plate.

6 **Transfer plaque eluates for filtration:** Using a multichannel pipette, transfer 125uL from each well of the storage plate to the filter-bottom plate. Though it is preferable to avoid collecting the plaque-plug in the pipette tip during transfer it ultimately does not matter either way as the virions are expected to have been eluted into the media overnight.

7 **Filter the plug eluate through the filter plate, either:**
- By vacuum filtration using a vacuum manifold, or
- By centrifugal filtration using a centrifuge that can accommodate filter plates, spin up to 30 minutes at max speed ≤5,000rcf to collect maximum filtrate

8 **Store 4°C stocks:** Remove and discard the filter-bottom plate from the receiver plate and cover the receiver plate with a sterile adhesive aluminum foil cover. This virus stock material is cell-free and can be stored at 4°C.

9 **Store frozen stocks:** Add 125uL of 50% glycerol solution to the 125uL remaining in the original plaque-eluate plate. This virus stock material contains cells and should be stored at -20°C or -80°C to prevent cell growth.

Recovering viruses from archives (future)

10 Use the Tube-free Agar Overlay or Molten Streaking for Singles protocols to prepare fresh plates of plaques from the archived plaque eluates.