Analysis of Viral Morphological Characteristics

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Abstract

Purpose: This protocol describes how to analyze a natural aquatic virus sample using transmission electron microscopy (TEM). The purpose is to obtain the capsid diameter distributions of the viral assemblage, tail length distributions of the viral assemblage, and percentages of each viral morphotype in a sample.

Note: Prior to using this protocol, deposit viruses from your sample onto a TEM grid using the protocol "Quantitatively Depositing Viruses onto TEM Grids" then stain the viruses using the protocol "Positive and Negative Staining of Viruses on TEM Grids".

Citation: Jennifer Brum Analysis of Viral Morphological Characteristics. **protocols.io** dx.doi.org/10.17504/protocols.io.dde23d
Published: 21 Jan 2016

Guidelines

Steps:

1) Bring a thumb drive to the TEM so you can save all of your digital micrographs

2) When using the TEM, take digital micrographs (pictures) of the first 100 viruses that you see
a) no skipping viruses because they are ugly (no bias!)
b) the pictures do not have to be works of art (this is "quick and dirty") so take them as fast as you can and just make sure that you will be able to get accurate measurements of them later (e.g. they should be in focus, but don't take 5 minutes to obtain the perfect focus and lighting)
c) take all pictures at the same magnification (110,000X generally works) because this will make it much easier to analyze them later
d) if you are unsure if something is a virus, take a picture of it anyway, but do not count it as part of the 100 viruses (ask knowledgeable colleagues for help later)
e) take pictures of anything else that is cool or interesting (it's a digital camera – no limits)

3) Make sure that each micrograph has a scale bar

4) Save each micrograph as follows: "Box-Grid-Virus number", e.g. 1-2b-37

5) Once you have the micrographs, analyze them with ImageJ analysis software
a) see the protocol "Using ImageJ to Measure Viral Dimensions in Micrographs"
b) for each virus:
- record the sample information (e.g. location, depth, date sample collected, data grid prepared, date grid analyzed), plus the grid box, grid number, and virus number
- measure the capsid diameter (Figure 1)
- if the virus has a tail, measure the tail length (Figure 1)

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- indicate the morphotype of the virus (myovirus, podovirus, siphovirus, non-tailed) (Figure 2)

Figure 1:

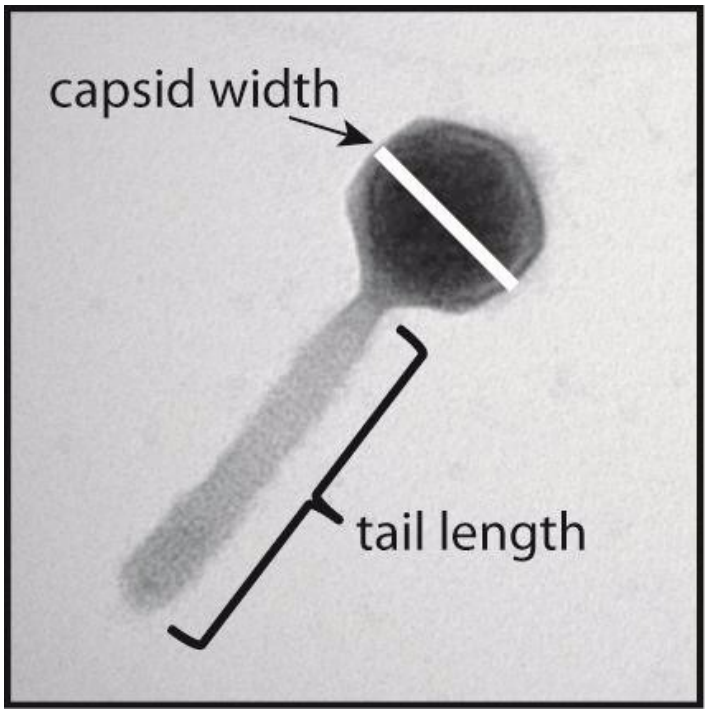

Figure 1. Measuring the capsid width and tail length of a virus.

Figure 2:

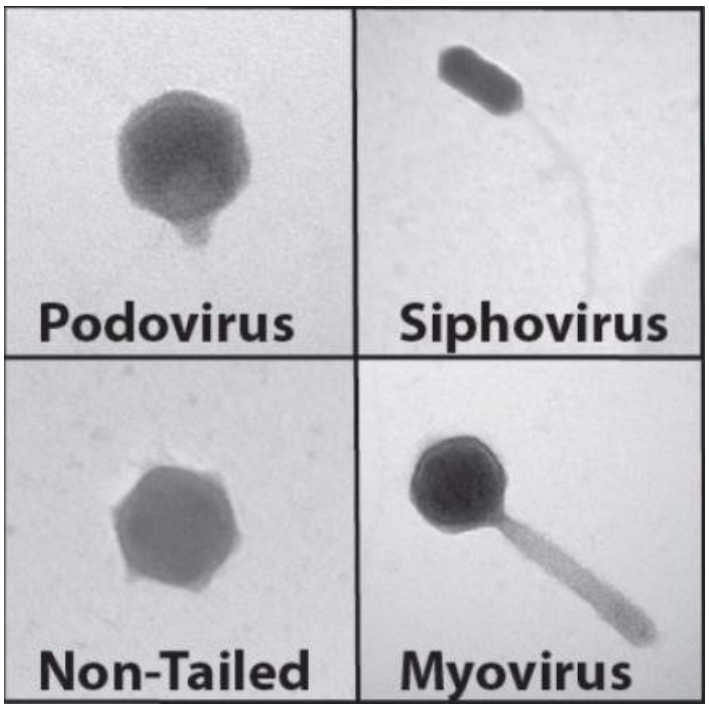

Figure 2. Examples of different viral morphotypes.

6) See Brum and Steward (2010) and Brum et al. (2013) for ideas regarding analysis of your results
- e.g. compare relative percentage of morphotypes between samples

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- e.g. compare capsid width histograms among samples using Morisita's index
- e.g. compare capsid width histograms among samples using correspondence analysis, including analysis of the influence of environmental parameters on the differences between samples

Applicable References:

Brum and Steward, 2010 – A reference that uses this protocol to analyze morphological characteristics of aquatic viral assemblages.

Brum et al., 2013 – A reference that fully describes and evaluates the accuracy of this protocol, plus uses it to characterize global marine viral assemblages.

Brum, J.R., and Steward, G.F. (2010) Morphological characterization of viruses in the stratified water column of alkaline, hypersaline Mono Lake. Microbial Ecology 60: 636-643.

Brum, J.R., Schenck, R.O., and Sullivan, M.B. (2013) Global morphological analysis of marine viruses shows minimal regional variation and dominance of non-tailed viruses. The ISME Journal doi:10.1038/ismej.2013.67

Protocol

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