

Feb 14, 2022

MDA for virome analysis

DOI

dx.doi.org/10.17504/protocols.io.b42uqyew

Frej Larsen¹

¹Copenhagen University

FOOD Micro UCPH



Frej Larsen

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.b42uqyew>

Protocol Citation: Frej Larsen 2022. MDA for virome analysis. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.b42uqyew>

License: This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working



Created: February 14, 2022

Last Modified: February 14, 2022

Protocol Integer ID: 58164

Keywords: mda for virome analysis, virome analysis, ssdna virus, stranded dna, amplification of dna, phi29 dna polymerase enzyme, dna, dsdna, ssdna

Abstract






This protocol is not intended for amplification of DNA. Rather, it uses the Phi29 DNA polymerase enzyme to turn single stranded DNA (ssDNA) into double stranded DNA (dsDNA). This is required for ssDNA viruses to be sequenced.

The procedure should be performed in a fume hood with a UV-light. Prior to starting, the UV-light should be turned on to disinfect the workspace and UV-tolerant materials such as empty PCR-plates, lids, and pipette tips.








Whenever working with the samples, keep them on ice.

Troubleshooting



- 1 Place materials that tolerate UV-treatment in fume hood and turn on the UV light and recirculation for 30 minutes before use.
- 2 Pick up a bucket of ice and thaw samples on ice
- 3 Add  10 µL denaturation buffer to a PCR tube
- 4 Add  10 ng DNA in  10 µL MQ water to the PCR tube and mix by pipetting.
If sample has a concentration below 1 ng/ul, add  10 µL of undiluted sample to the tube and mix by pipetting
- 5 Briefly centrifuge the plate
- 6 Denature template DNA by 95 degrees for 3 minutes in a ThermoCycler. Directly after, put the samples back on ice
- 7 Add all  20 µL sample content to the MDA cake tube. Keep both sample and cake on ice while transferring the sample
- 8 Seal tube with provided lid and briefly centrifuge the sample
- 9 Run sample on a ThermoCycler with the following program:

40m

 -  30 °C for  00:30:00
 -  65 °C for  00:10:00
 -  4 °C
- 10 Purify samples with bead purification or store sample at  -20 °C or  -80 °C