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MDA for virome analysis

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Protocol status: Working
We use this protocol and it's working

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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 transfering 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