NUCLEIC ACID EXTRACTION FROM VIRAL SEWAGE CONCENTRATES

Description

The aim of the protocol is to extract nucleic acids (NA) from viral sewage concentrates.

Required Instruments & Consumables

- Benchtop centrifuge (14'000 x g)
- Micropipettes and filter tips
- Sterile 1.5 mL plastic tube
- Sterile 5 mL plastic tubes
- QIAamp Viral RNA Mini Kit (QIAGEN 22906)
- RNase-free water
- Ethanol (96-100%)
- If measuring RNA with (RT)qPCR or otherwise concerned about inhibition:
    a. Zymo OneStep PCR Inhibitor Removal Kit (D6030v)

Method

A nucleic acid extraction is performed using the QIAamp Viral RNA Mini Kit (QIAGEN 22906) following the manufacturer's instructions.
The Kit is designed for 140 µL samples. We assume that our viral concentrate is 280 µL, therefore everything must be doubled up to step 8. For extraction control though, 140 µL RNase-free water is used.

A. Extraction

1. For extraction control add 140 µL RNase free water to a 5 mL tube.
2. Per 280 µL concentrate pipet 1'120 µL AVL buffer and 11.2 µL carrier RNA into another 5 mL plastic tube (or if needed a bigger plastic tube). Also add 560 µL AVL buffer and 5.6 µL carrier RNA per extraction control. Mix the tube.
3. Pipet 1'120 µL of the in step 2 prepared mix to the 5 mL tube containing the sample. Pipet 560 µL of the mix to the 5 mL tube containing the 140 µL RNase free water. Vortex the tubes.
4. Incubate at room temperature (15 - 25ºC) for 10 min.
5. Spin the tube quickly to remove drops from the inside of the lid.
6. Add 1'120 µL ethanol (96%-100%) to the sample. Add 560 µL ethanol (96%-100%) to the extraction control. Mix by vortexing.
7. Spin the tube quickly to remove drops from the inside of the lid.
8. Carefully apply 630 µL of the mixture to a QIAamp Mini spin column (placed in a 2 mL collection tube) without wetting the rim. Centrifuge at 6'000 x g (8'000 rpm) for 1 min. Place the QIAamp spin column into a clean 2 mL collection tube and discard the tube containing the filtrate.
9. Repeat step 7 until all sample has passed through the spin column. (in total 4x for sample concentrates, 2 x for extraction control)

From now on sample concentrates and extraction control are treated equally.

10. Add 500 µl of buffer AW1. Centrifuge at 6'000 x g (8'000 rpm) for 1 min. Keep the spin column and discard the collection tube containing the filtrate. Place the spin column into a new collection tube.
11. Add 500 µL of buffer AW2. Centrifuge at 20'000 x g (14'000 rpm) for 3 min. Keep the spin column and discard the collection tube containing the filtrate. Place the spin column into a new collection tube.
12. Centrifuge again at 20'000 x g (14'000 rpm) for 1 min.
13. Place the spin column into a clean 1.5 mL plastic tube.
14. Open the column and add 40 µl of AVE into the middle of the column**.** Incubate the spin Column for 2 min at room temperature and centrifuge then at 6'000 x g (8'000 rpm) for 1 min.
15. Repeat step 13 still using the same plastic tube.
16. Discard the spin column.
17. If quantifying RNA using (RT)qPCR, then purify the RNA using the Zymo spin column:
    a. Precondition the Zymo spin column by adding 600 µl of Prep-solution and centrifuging at 8'000 x g for 3 min.
    b. Discard the collection tube and place the Zymo spin column into a clean 1.5 ml Eppendorf tube.
    c. Pipet the extracted nucleic acids into the Zymo column and spin it at 16'000 x g for 3 min.
18. The sample is stored on ice at 4° C if RNA will be quantified immediately using droplet digital PCR (ddPCR) or (RT)qPCR. Otherwise, the elute is stored at -80° C for future molecular analysis. For long-term storage at -80°C, sample should be aliquoted to appropriate volumes to minimize freeze-thaw.
    a. Prepare the following aliquots:
       20 µL (sequencing)
       15 µL (ddPCR, 3x diluted)
       3 µL (qPCR, 10x diluted)

Version History

Version	Author	Date	Changes
1.0.0	Xavier Fernandez-Cassi, Carola Bänziger	2020-07-01	Protocol Development, Testing, and First Draft
1.0.1	Anina Kull	2020-10-05	Formalization of Protocol for Publishing
2.0	All	2020-10-09	Added Zymo Column for (RT)-qPCR
2.1	Anina Kull	2021-02-11	Minor changes, added aliquots
2.2	Tim Julian	2021-06-02	Authorship and Minor Editing