viruses

Supplementary Material

Supplementary Table S1. List of anelloviruses species and accession numbers included in the ICTV9 reference set. Asterisks (*) denote viruses that are listed as a putative member of a genus but have not been approved as a species.

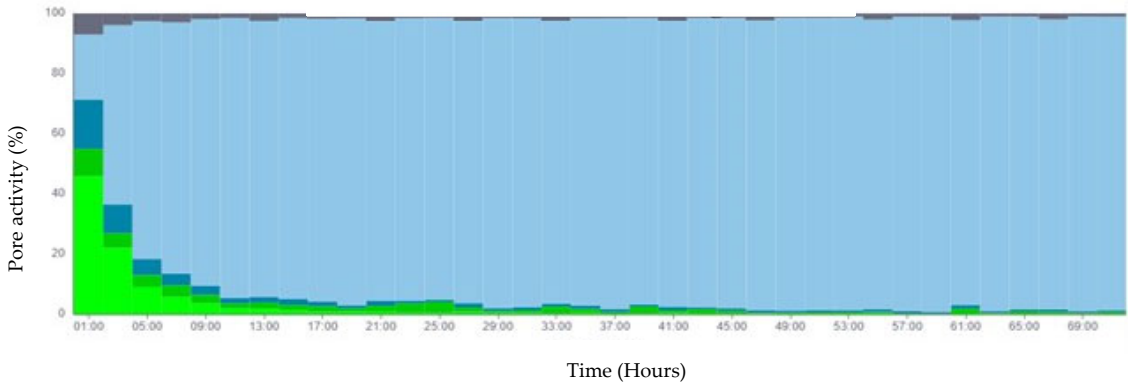
Accession Number	Species	Isolate
AB076002	Torque teno canis virus	Torque teno canis virus-Cf-TTV10
AB041961	Torque teno douroucouli virus	Torque teno douroucouli virus-At-TTV3
AB076003	Torque teno felis virus	Torque teno felis virus-Fc-TTV4
EF538877	Torque teno felis virus*	Torque teno felis virus-PRA1
AB290918	Torque teno midi virus 1	Torque teno midi virus 1-MD1-073
AB290919	Torque teno midi virus 2	Torque teno midi virus 2-MD2-013
EF538875	Torque teno midi virus*	Torque teno midi virus-2PoSMA
EF538876	Torque teno midi virus*	Torque teno midi virus-6PoSMA
AB303552	Torque teno midi virus*	Torque teno midi virus-MDJHem2
AB303553	Torque teno midi virus*	Torque teno midi virus-MDJHem3-1
AB303554	Torque teno midi virus*	Torque teno midi virus-MDJHem3-2
AB303555	Torque teno midi virus*	Torque teno midi virus-MDJHem5
AB303560	Torque teno midi virus*	Torque teno midi virus-MDJN14
AB303559	Torque teno midi virus*	Torque teno midi virus-MDJN2
AB303561	Torque teno midi virus*	Torque teno midi virus-MDJN47
AB303562	Torque teno midi virus*	Torque teno midi virus-MDJN51
AB303564	Torque teno midi virus*	Torque teno midi virus-MDJN69
AB303566	Torque teno midi virus*	Torque teno midi virus-MDJN97
AB449062	Torque teno midi virus*	Torque teno midi virus-Pt-TTMDV210
AB026931	Torque teno mini virus 1	Torque teno mini virus 1-CBD279
AB038629	Torque teno mini virus 2	Torque teno mini virus 2-NLC023
AB038630	Torque teno mini virus 3	Torque teno mini virus 3-NLC026
AB041963	Torque teno mini virus 4	Torque teno mini virus 4-Pt-TTV8-II
AB041962	Torque teno mini virus 5	Torque teno mini virus 5-TGP96

AB026929	Torque teno mini virus 6	Torque teno mini virus 6-CBD203
AB038627	Torque teno mini virus 7	Torque teno mini virus 7-CLC156
AF291073	Torque teno mini virus 8	Torque teno mini virus 8-PB4TL
AB038631	Torque teno mini virus 9	Torque teno mini virus 9-NLC030
EF538880	Torque teno mini virus*	Torque teno mini virus-LIL-y1
EF538881	Torque teno mini virus*	Torque teno mini virus-LIL-y2
EF538882	Torque teno mini virus*	Torque teno mini virus-LIL-y3
AB076001	Torque teno sus virus 1	Torque teno sus virus 1-Sd-TTV31
AY823990	Torque teno sus virus 2	Torque teno sus virus 2-1p
AY823991	Torque teno sus virus*	Torque teno sus virus-2p
AB041960	Torque teno tamarin virus	Torque teno tamarin virus-So-TTV2
AB057358	Torque teno tupaia virus	Torque teno tupaia virus-Tbc-TTV14
AB008394	Torque teno virus 1	Torque teno virus 1-TA278
AB064607	Torque teno virus 10	Torque teno virus 10-JT34F
AF345524	Torque teno virus 11	Torque teno virus 11-TCHN-D1
AB064605	Torque teno virus 12	Torque teno virus 12-CT44F
AF345526	Torque teno virus 13	Torque teno virus 13-TCHN-A
AB037926	Torque teno virus 14	Torque teno virus 14-CH65-1
AB028668	Torque teno virus 15	Torque teno virus 15-TJN01
AB017613	Torque teno virus 16	Torque teno virus 16-TUS01
AX025830	Torque teno virus 17	Torque teno virus 17-SENV-G
AX025718	Torque teno virus 18	Torque teno virus 18-SENV-C
AB025946	Torque teno virus 19	Torque teno virus 19-SANBAN
AB049608	Torque teno virus 2	Torque teno virus 2-CH71
AB060594	Torque teno virus 20	Torque teno virus 20-SAa-10
AF348409	Torque teno virus 21	Torque teno virus 21-TCHN-B
AX174942	Torque teno virus 22	Torque teno virus 22-svi-1
AB049607	Torque teno virus 23	Torque teno virus 23-CH65-2
AB060597	Torque teno virus 24	Torque teno virus 24-SAa-01

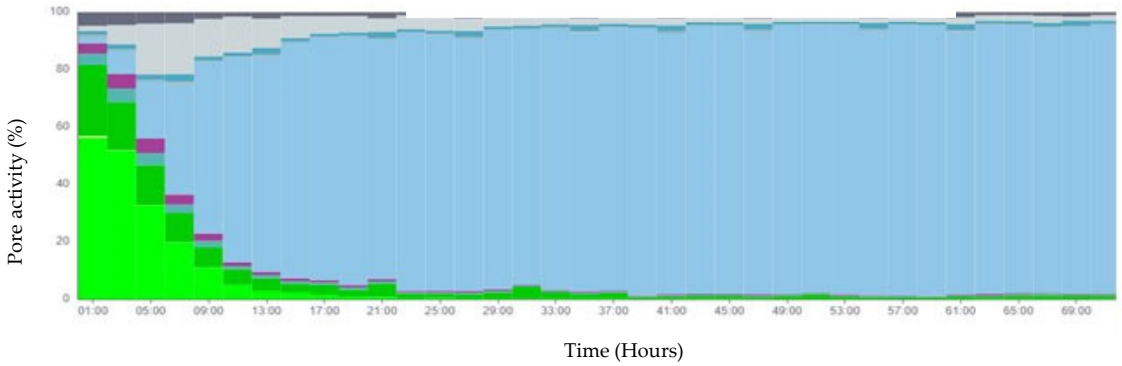
AB041959	Torque teno virus 25	Torque teno virus 25-Mf-TTV9
AB041958	Torque teno virus 26	Torque teno virus 26-Mf-TTV3
AB064595	Torque teno virus 27	Torque teno virus 27-CT23F
AB064598	Torque teno virus 28	Torque teno virus 28-CT43F
AB038621	Torque teno virus 29	Torque teno virus 29-yonKC009
AY666122	Torque teno virus 3	Torque teno virus 3-HEL32
AB041957	Torque teno virus 4	Torque teno virus 4-Pt-TTV6
AF345523	Torque teno virus 5	Torque teno virus 5-TCHN-C1
AF435014	Torque teno virus 6	Torque teno virus 6-KAV
AF261761	Torque teno virus 7	Torque teno virus 7-PMV
AB054647	Torque teno virus 8	Torque teno virus 8-Kt-08F
DQ187006	Torque teno virus 9	Torque teno virus 9-BM1C-18
FJ459582	Torque teno zalophus virus*	Torque teno zalophus virus – ZcAV

Supplementary Figure S1: Effect of DNA shearing on GridION pore activity over time.
GridION pore activity over time in the a) absence of DNA shearing, b) DNA shearing using g-TUBE, and c) mechanical shearing of DNA using Bioruptor™. The y-axis represents pore activity, which is shown as a percentage.

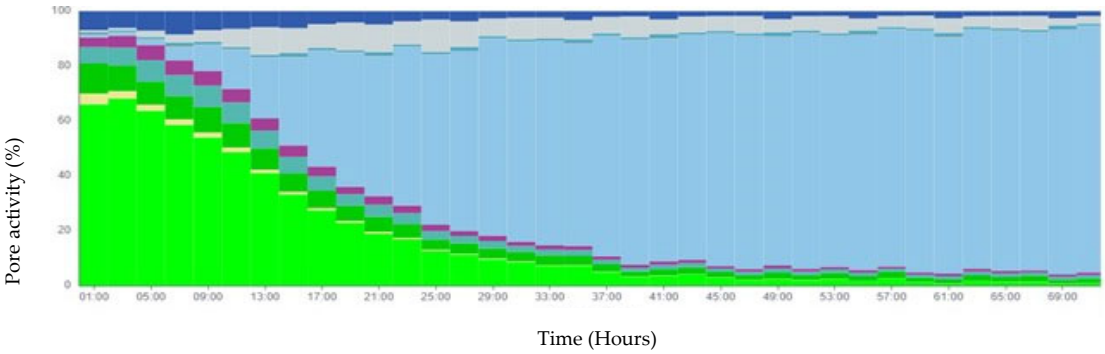
a. No shearing

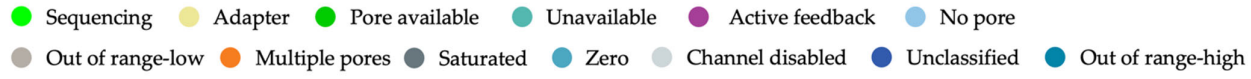


b. g-TUBE based shearing



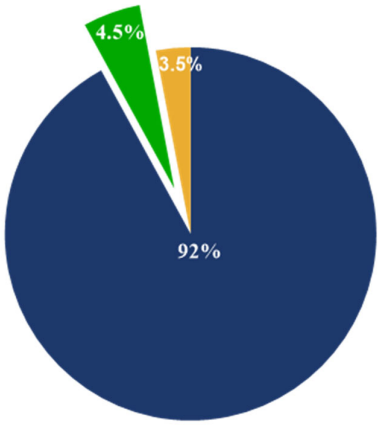
c. Bioruptor™ based shearing



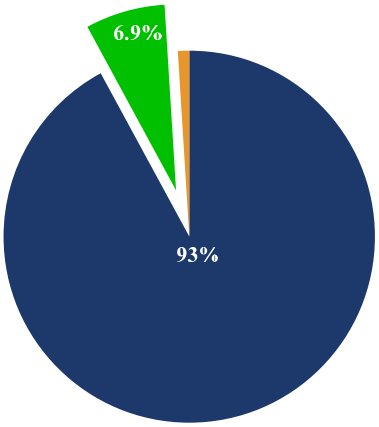


Supplementary Figure S2: Percentage of total reads classified as *Anelloviridae* family when using different library preparation protocols.

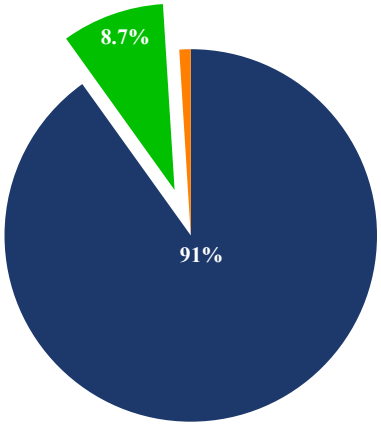
a. Concatemer debranching + native barcoding



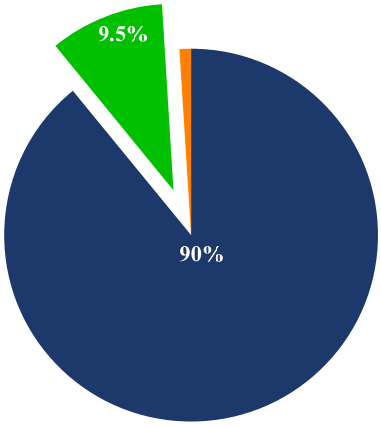
b. Concatemer debranching + g-TUBE + non-PCR barcoding



c. Concatemer debranching + Bioruptor™ + native barcoding



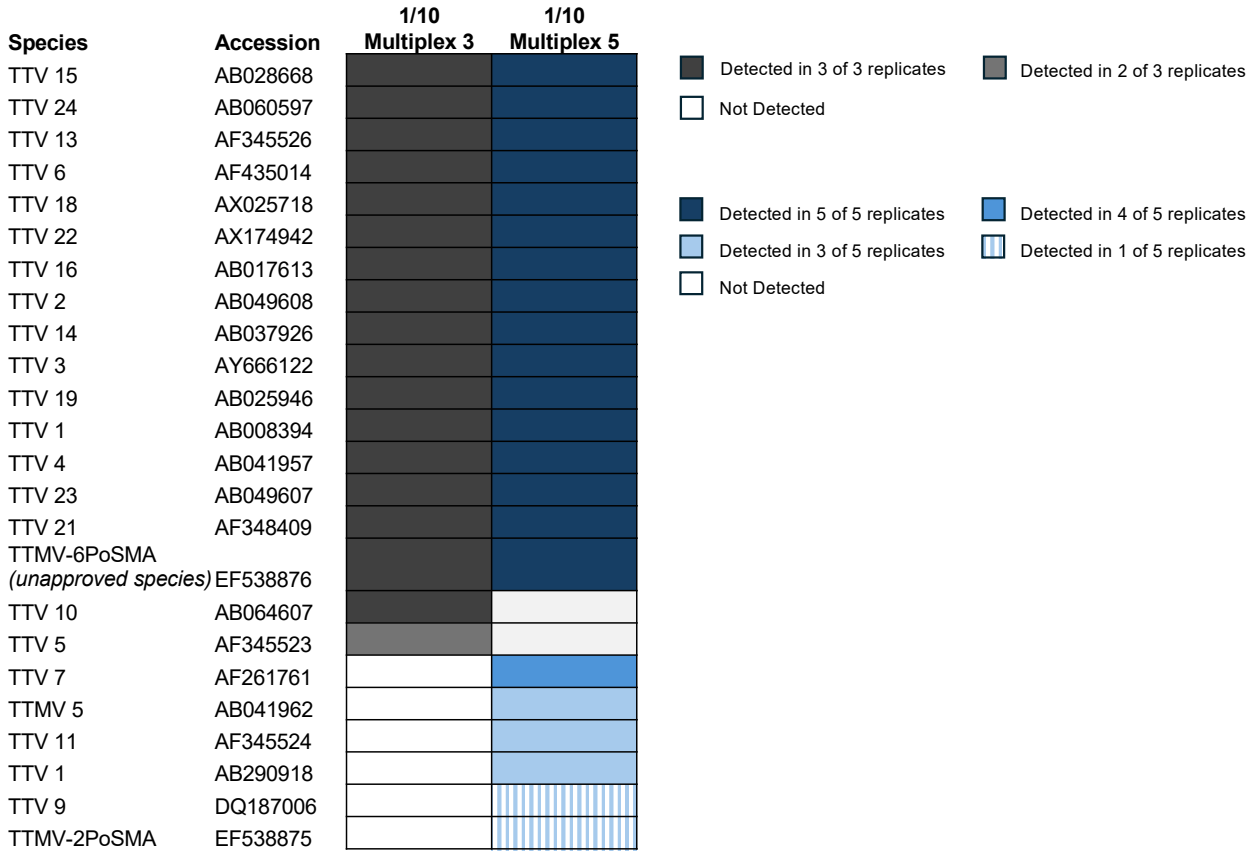
d. Concatemer debranching + Bioruptor™ + PCR barcoding



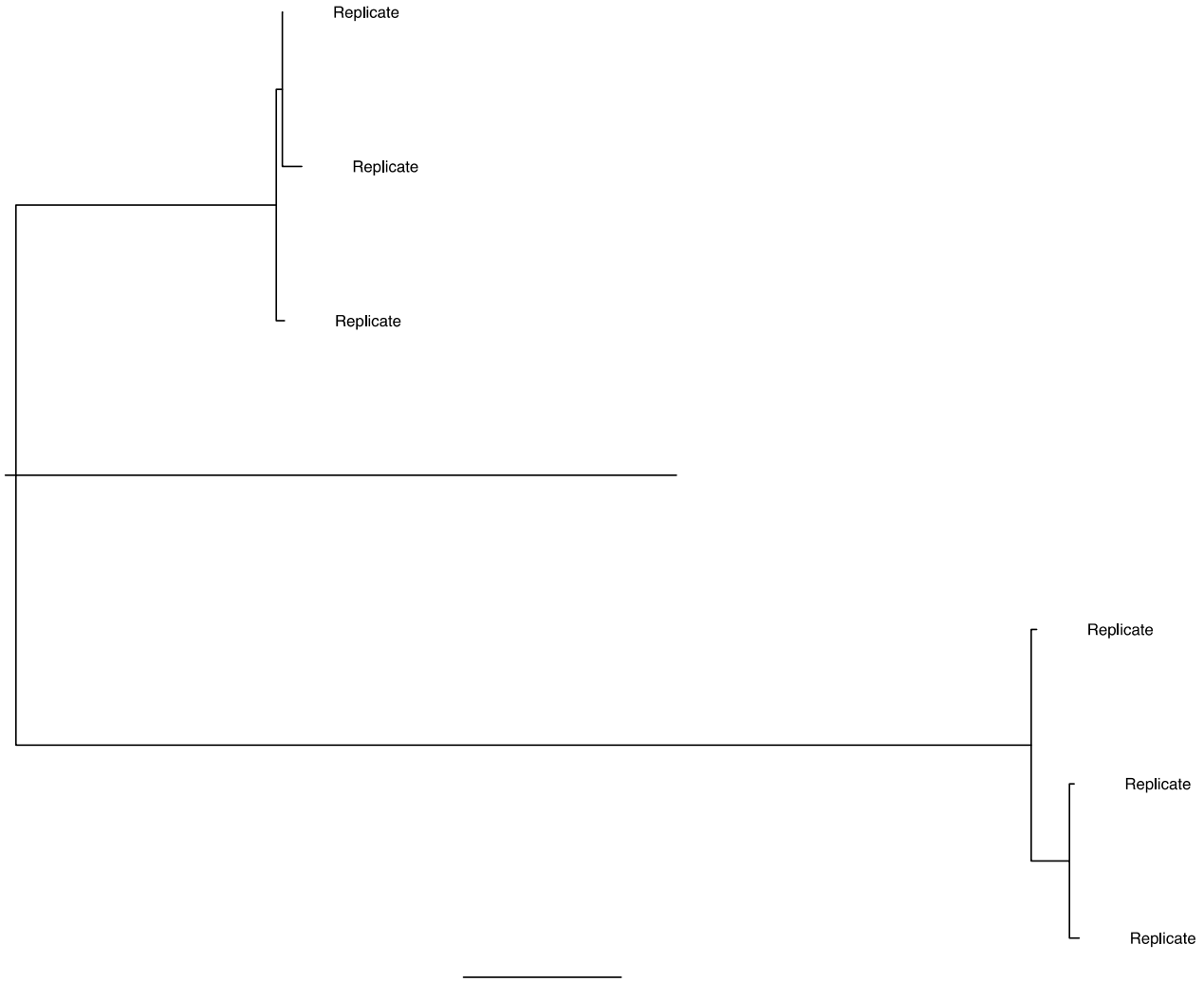
Legend: *Homo sapiens* *Anelloviridae* Others

The percentage of anellovirus reads is based on the Epi2Me workflow analysis. In figures b-d, "Others" represent < 1% of total reads.

Supplementary Figure S3: Comparison of anellovirus species identified when multiplexing 3 and 5 samples. This figure illustrates the detection of anellovirus species in a 1/10 dilution series of PS-2 plasma DNA, using PS-1 plasma as the diluent, with multiplexing at two different scales: triplex (3 samples) and pentaplex (5 samples).



Supplementary Figure S4: Maximum likelihood phylogenetic tree of TTV 13 ORF1 consensus sequences from PS-1 and PS-2 across three replicates along with the TTV 13 reference sequence (AF345526). The tree was inferred under a GTR+Γ_4+I model with 1000 bootstrap replications using RAxML. Positions containing gaps and missing data were eliminated, resulting in a total of 1096 positions being retained for tree inference. The tree with the highest log likelihood is shown. Node labels depict bootstrap values, and tip labels denote the sample name and replicate number.



Supplementary Methods

A. Enrichment of circular DNA:

For enzymatic digestion of linear DNA, the reaction was prepared as shown below.

Master mix	Volume (µL)	Final concentration
NEB buffer	5	1X
Exo-I	2	0.4 U/µL
Exo-III	2	2 U/µL
Lambda	1	0.1 U/µL
Water	10	
DNA	30	
Total	50	

The mixture was incubated in a thermal cycler at 37 °C for 2 hours, followed by heat inactivation at 80 °C for 20 minutes.

B. CIDER-Seq protocol: The following protocol was adapted from Mehta *et al.* [1].

i) Rolling circle amplification

Master mix	Volume (µL)	Final concentration
10xPhi29 buffer	2	1X
dATP	0.5	2.5 mM
dGTP	0.5	2.5 mM
dCTP	0.5	2.5 mM
dTTP	0.5	2.5 mM
20x Exo-resistant random primer	1	50 µM
Phi29 DNA polymerase	1	0.5 U/µL
Input DNA	~10 – 14	10 – 40 ng
Nuclease Free Water	To make up to 20	
Total	20	

Amplification was carried out in a thermal cycler at 30 °C for 18 hours and stopped by heating at 65 °C for 10 minutes. Amplified DNA was purified using 3M sodium acetate and 100% ice-cold ethanol as described [1].

ii) Debranching step:

Master mix	Volume (µL)	Final concentration
10xPhi29 buffer	3	1X
Each dNTP	0.3	1 mM
Phi29 DNA polymerase	1.5	0.5 U/µL
Input DNA	18.0	
Nuclease Free Water	6.3	
Total	30	

The mixture was incubated in a thermal cycler at 30 °C for 2 hours, followed by 65 °C for 10 minutes. Post-debranching, DNA was used using 3M sodium acetate and 100% ice-cold ethanol as described [1].

iii) DNA branch release step:

Master mix	Volume (µL)	Final concentration
5x S1 Nuclease Buffer	4	1X
S1 nuclease	0.5	2.5 U/ µL
DNA	15.5	
Total	20	

The mixture was incubated in a thermal cycler at 37 °C for 30 minutes. DNA was purified using 3M sodium acetate and 100% ice-cold ethanol as described [1].

iv) DNA repair:

Master mix	Volume (µL)	Final concentration
10x NEB Buffer 2	5	
dNTPs	1	0.2 mM
DNA polymerase 1	1	0.2 U/µL
T4 DNA polymerase	1	0.06 U/µL
DNA	20	10 ng/ µL
Water	22	
Total	50	

DNA repair was carried out at room temperature for 30 minutes, followed by clean-up using the NEB Monarch DNA clean-up assay as per the manufacturer's protocol. The final DNA concentration was measured using Qubit dsDNA High sensitivity kit (Thermo Fisher scientific, Cat#Q32854).

C. DNA shearing using Bioruptor™:

Prior to shearing, 100 µL of DNA (2 – 100 ng/µL) in TE buffer solution was incubated on ice for 10 minutes. Sonication was done using 15 sec on/30 sec off for 2 cycles with the using Bioruptor™ set at 4 °C. Sheared DNA should be ~800 – 1000 bp in size.

D. Library preparation:

The library preparation was carried out using ligation sequencing kit V14 (Oxford Nanopore Technologies, Cat#SQK-LSK114) and PCR barcoding expansion kit (EXP-PBC001). Protocol modifications included using an annealing temperature of 54 °C and 27 cycles of amplification in the PCR barcoding step. The final library at 10 – 20 fmol concentration was loaded on the GridION for sequencing.

References

1. Mehta D, Cornet L, Hirsch-Hoffmann M, Zaidi SS, Vanderschuren H. Full-length sequencing of circular DNA viruses and extrachromosomal circular DNA using CIDER-Seq. Nat Protoc. 2020;15(5):1673-89. Epub 2020/04/05. doi: 10.1038/s41596-020-0301-0. PubMed PMID: 32246135.