

virome-protocols

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Filtration

Material

- Filter (0.45 µm), TPP, [99745](#)
- Syringe
- Container, 8 mL or 25 mL

Procedure

- Centrifuge sample and use supernatant to remove cells (e.g. WBC and RBC in case of blood) or other solids
- With syringe aspirate the sample and press through filter in a new container
- Replace filter when clogged

Nucleic acid extraction

Material

- 1 mL sample
- Pipettes and tips
- 1.5 mL tubes
- [NucliSENS easyMag](#) (bioMérieux)

Procedure

- from 1 mL sample extract total nucleic acid and elute in 25 µL using the NucliSENS easyMag

- store extract at -20°C

DNase treatment

Reagents

- TURBO DNA-free Kit, Invitrogen, [AM1907](#)

Material

- DNA/RNA extract from nucleic acid extraction
- Pipettes and tips
- 1.5 mL tubes
- Thermomixer
- Centrifuge

Procedure

- Combine 5 µL DNA/RNA extract, 1.2 µL 10× TURBO DNase Buffer and 1 µL TURBO DNase (2 U)
- Incubate at 37°C for 20 min
- Add 2 µL resuspended DNase Inactivation Reagent
- Incubate 5 min at room temperature, mixing occasionally
- Centrifuge at 10'000 g for 1.5 min and transfer the RNA to a fresh tube

SSIV split protocol (RT and 2nd strand synthesis)

Reagents

- UltraPure DNase/RNase-Free Distilled Water, Invitrogen, [10977035](#)
- Primer 6N, 100 µM (mh413_6N, 5'-NNNNNN-3')
- dNTP, Thermo Scientific, [R0192](#)
- SuperScript IV Reverse Transcriptase, Invitrogen, [18090200](#)
- RNaseOUT Recombinant Ribonuclease Inhibitor, Invitrogen, [10777019](#)
- RNase H, New England Biolabs, [M0297S](#)
- DNA Polymerase I, Large (Klenow) Fragment, New England Biolabs, [M0210L](#)
- Agencourt AMPure XP Beads, Beckman Coulter, [A63881](#)
- Ethanol absolute
- QuantiFluor ONE dsDNA System, Promega, [E4870](#)

- Nextera XT DNA Library Preparation Kit (96 samples), Illumina, [FC-131-1096](#)
- Nextera XT Index Kit (96 indexes, 384 samples), Illumina, [FC-131-1002](#)
- MiSeq Reagent Kit v3 (150-cycle), Illumina, [MS-102-3001](#)

Material

- DNA/RNA extract from nucleic acid extraction (for DNA workflow)
- DNase treated extract (for RNA workflow)
- Pipettes and tips
- 1.5 mL tubes
- PCR strips
- PCR cyclers
- Magnetic Separation Rack
- Quantus Fluorometer, Promega, [E6150](#)
- Thermomixer
- [MiSeq](#), Illumina

Procedure

- Fill in and follow instructions in [SSIV_standard_split.xltx](#)
- Purify total volume of 2nd strand synthesis products with 2x AMPure beads (20/30 μ L DNA + 40/60 μ L Beads, wash 2x with 80% Ethanol, dry Beads, elute DNA in 20 μ L Water)
- Quantify DNA in 2nd strand synthesis products (Note: if unpurified, quantification might be too high)
- If purified 2nd strand synthesis products were below 0.2 ng/ μ L, 5 μ L undiluted products were used for Nextera XT library preparation
- Follow Nextera XT standard protocol and sequence 151 cycles on the MiSeq

Release History

- [2.2.0](#)
 - reduce ratio of AMPure beads from 3x to 2x for purification of 2nd strand synthesis products
- [2.1.1](#)
 - describe centrifugation step before filtration
- [2.1.0](#)
 - DNA/RNA split workflow including DNase treatment in RNA workflow
 - always purify 2nd strand synthesis products
- [2.0.0](#)
 - no nuclease-treatment
 - switch RT enzyme from SSIII to SSIV

- combined DNA/RNA workflow
 - exclude anker PCR
- 1.0.0
 - published virome-protocol, including DNA/RNA split workflow and anker PCR