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 or proceed immediately

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 10 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 (supernatant) 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 cycler
- 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 µL DNA + 40/60 µL Beads, wash 2x with 80% Ethanol, dry Beads, elute DNA in 20 µL 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/µL, 5 µL undiluted products were used for Nextera XT library preparation
- Follow Nextera XT standard protocol and sequence 151 cycles on the MiSeq

Release History

- 2.2.1
  - fix mistake, use 10 µL DNA/RNA extract for DNase treatment, not 5 µL
  - note that after extraction one could also proceed immediately
- 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