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Long Read Viromics Amplification Library Preparation (VirION 2)

In 1 collection

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

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Guidelines

Use DNA from "preparation of extracted DNA for long read library prep" as starting material for this protocol.

Use DNA LoBind tubes throughout this entire protocol (except for PCR tubes).

DNA LoBind Tubes **Eppendorf** **Catalog #**#022431021

Protocol materials

DNA LoBind Tubes **Eppendorf** **Catalog #**#022431021

NEBNext FFPE DNA Repair Mix - 24 rxns **New England Biolabs** **Catalog #**M6630S

NEBNext Ultra II End Repair/dA-Tailing Module - 24 rxns **New England Biolabs** **Catalog #**E7546S

Ampure XP beads **Beckman Coulter** **Catalog #**A63881

NEB Blunt/TA Ligase Master Mix **Catalog #**M0367

PCR Barcoding Expansion 1-12 **Catalog #**EXP-PBC001

LA TaKaRa Hot Start **Takara Bio Inc.** **Catalog #**RR042A

1D Genomic DNA by Ligation **Catalog #**SQK-LSK109

Troubleshooting

Sample Concentration

1 Prepare 48 ul of sample to use as input for the next step. Sample should have between 1ng - 100 ng of DNA for reliable library success. See "Preparation of extracted DNA for long-read library prep" concerning preparation of DNA for this protocol.

DNA repair, End repair and dA tailing

2 Prepare reaction mix using the following, making enough master mix for the total number of samples you are working with.

- 3.5 ul NEB Ultra II End Repair/dA Tailing reaction buffer
- 3 ul NEB Ultra II End Repair/dA Tailing enzyme mix
- 3.5 ul NEB FFPE DNA repair buffer
- 2 ul NEB FFPE DNA enzyme mix

NEBNext Ultra II End Repair/dA-Tailing Module - 24 rxns **New England Biolabs Catalog #**E7546S

NEBNext FFPE DNA Repair Mix - 24 rxns **New England Biolabs Catalog #**M6630S

Note

We recommend making 0.2 reactions worth of extra master mix to account for pipetting error.

2.1 In a PCR tube, add 12 ul master mix to 48 ul DNA from step 1.

2.2 Using a thermal cycler, incubate at 25 C for 5 minutes and 65 C for 5 minutes.

25 °C 00:05:00

65 °C 00:05:00

2.3 Clean up using AMPure XP beads (1:1 sample:beads) and elute in 31ul nuclease-free water into a 1.5mL tube.
- See "Ampure Bead Clean up For HMW DNA" protocol.
- For final elution step, incubate at 55 °C 00:02:00

Ampure XP beads **Beckman Coulter** **Catalog #**A63881

2.4 Use 1ul to assess DNA concentration with Qubit.

Adapter ligation

3 Add the following to 30 ul DNA from previous step:
- 50 ul NEB Blunt/TA ligase
- 20 ul BCA (from Oxford Nanopore PCR Barcoding Expansion 1-12 kit)

NEB Blunt/TA Ligase Master Mix **Catalog #**M0367

PCR Barcoding Expansion 1-12 **Catalog #**EXP-PBC001

3.1 Incubate at room termperature for 10 minutes.
Room temperature 00:10:00

3.2 Clean up using AMPure XP beads (1:0.4 sample:beads) and elute in 15ul Nuclease-free water into a PCR tube.
- See "Ampure Bead Clean up For HMW DNA" protocol.
- For final elution step, incubate at 55 °C 00:02:00

3.3 Use 1ul to assess DNA concentration with Qubit.

PCR Amplification

4 Prepare reaction mix using the following from the LA TaKaRa Hot Start kit, making enough master mix for the total number of sample you are working with.

- 16 ul dNTPs
- 10 ul 10x reaction buffer
- 1 ul LA TaKaRa enzyme
- 66 ul water

LA TaKaRa Hot Start **Takara Bio Inc. Catalog #**RR042A

Note

We recommend making 0.2 reactions worth of extra master mix to account for pipetting error.

4.1 Add 2 ul of your chosen barcode from Oxford Nanopore PCR Barcoding Expansion 1-12 kit to 5 ul of DNA from previous step.

Note

Be sure to use different barcodes for any samples that will be sequenced together.

4.2 Add 93 ul of master mix to the barcode - DNA mix

4.3 Use the following thermocycler conditions to amplify the library using minimum of 15 cycles.

- repeat steps 2-4 of this sub-step 15-25 times

1.) 94 °C 00:01:00

2.) 94 °C 00:00:30
3.) 62 °C 00:00:30
4.) 68 °C 00:16:00 for 20kb

5.) 72 °C 00:16:00 for 20kb

4.4 Clean up using AMPure XP beads (1:0.5 sample:beads) and elute in 20ul Nuclease-free water.

- See "Ampure Bead Clean up For HMW DNA" protocol.
- For final elution step, incubate at 55 °C 00:02:00

4.5 Check the concentration (we recommend Qubit) and purity (NanoDrop) of your DNA, and run on a Genomic DNA TapeStation to assess library size.

5 The amplified libraries should be used as input for the Oxford Nanopore 1D Genomic DNA by Ligation (SQK-LSK109) protocol, beginning with the "DNA repair and end-prep" step. Libraries with different barcodes can be pooled at your desired ratio before beginning. We recommend loading 10 - 100 fmol of DNA into the flow cell.

1D Genomic DNA by Ligation **Catalog #**SQK-LSK109