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Preparation of extracted DNA for long-read library prep

In 1 collection

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

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Abstract

This protocol details steps to prepare DNA for long-read library prep, including clean-up and shearing.

Guidelines

Use DNA from "DNA extraction for HMW DNA" or other high molecular weight DNA as starting material for this protocol.

Pipet carefully to avoid shearing DNA.

Protocol materials

- g-TUBE **Covaris** **Catalog #**520079
- Ampure XP beads **Beckman Coulter** **Catalog #**A63881
- DNeasy PowerClean Pro **Qiagen** **Catalog #**12997-50

DNA clean-up

1 Begin with high molecular weight DNA (see "DNA Extraction for HMW DNA"). Assess the purity of your DNA using NanoDrop. If you have already done this and have the results available, you do not need to repeat it.

> Note
>
> When assessing low concentration samples (<5ng/ul), do not rely on 260/280 and 260/230 values. Look at the graph to find contaminants, as indicated by peaks that are not at 260nm.

2 If your DNA has a visible peak at 260nm and your NanoDrop values are outside the following ranges -

260/280: 1.7 - 2.1
260/230: 1.8 - 2.2

then your DNA needs clean-up!

If your DNA does not have an easily visible peak at 260nm but has peaks elsewehere, then your DNA needs clean-up!

2.1 If your DNA is contaminated with phenol or other extraction reagents, perform an Ampure Bead clean-up (see "Ampure Bead Clean-up for High Molecular Weight DNA"). Elute in 100ul.

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

2.2 If your DNA is from an iron chloride precipitate resuspended with citrate buffer, clean up your DNA using the Qiagen DNeasy PowerClean Pro kit. Be sure to always mix gently by inversion or flicking, NOT vortexing or pipetting. Elute in 100ul.

DNeasy PowerClean Pro **Qiagen** **Catalog #**12997-50

3 Assess the conencentration of your DNA using Qubit, the purity using NanoDrop, and the size using Genomic DNA TapeStation.

Shear DNA

4 Load at least 90ul of high molecular weight DNA into the top of a Covaris g-TUBE. Loading less than 90ul will result in poorly sheared DNA.

g-TUBE **Covaris Catalog #**520079

4.1 To shear DNA to 15kb, centrifuge at 4,700 rpm for 60 sec in an Eppendorf 5424 centrifuge with a 24 position rotor. Flip the g-TUBE upside-down and repeat this centrifugation.

4700 rpm , 60 sec

4700 rpm , 60 sec

Note

You may also use Eppendorf model 5415 with a 24 position rotor. Using any other model or rotor size will result in DNA that is not sheared to the target size.

4.2 To shear DNA to 20kb, centrifuge at 4,200 rpm for 60 sec in an Eppendorf 5424 centrifuge with a 24 position rotor. Flip the g-TUBE upside-down and repeat this centrifugation.

4200 rpm , 60 sec

4200 rpm , 60 sec

Note

You may also use Eppendorf model 5415 with a 24 position rotor. Using any other model or rotor size will result in DNA that is not sheared to the target size.

4.3 Recover DNA from the lid of the g-TUBE.

4.4 Dilute a small aliquot to 2 - 50ng/ul and assess DNA size using Genomic DNA TapeStation.

Note

If your DNA is less than 2ng/ul, proceed without checking DNA size.

5 Proceed to "Long Read Viromics Amplification Library Preparation (VirION 2)".