Apr 01, 2025

Shearing of Nematode HMW DNA for HiFi Sequencing

DOI
https://dx.doi.org/10.17504/protocols.io.kxygx7ep4l8j/v1

Manuela R. Kieninger[1]

[1]Blaxter Faculty, Wellcome Sanger Institute, Hinxton, Cambridgeshire, CB10 1SA

Manuela R. Kieninger
Blaxter Faculty, Wellcome Sanger

DOI: https://dx.doi.org/10.17504/protocols.io.kxygx7ep4l8j/v1

Protocol Citation: Manuela R. Kieninger 2025. Shearing of Nematode HMW DNA for HiFi Sequencing . **protocols.io** https://dx.doi.org/10.17504/protocols.io.kxygx7ep4l8j/v1

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

Created: March 28, 2025

Last Modified: April 01, 2025

Protocol Integer ID: 125656

Keywords: HMW DNA, Shearing, HiFi sequencing, Nematodes, C. elegans, Megaruptor, DNA, Genomics, PacBio, long read sequencing, reference genome, nematode hmw dna for hifi, nematode hmw dna, shearing of hmw dna, sheared dna, hmw dna, nematode, diagenode, input hifi library preparation, dna, shearing, sequencing

Funders Acknowledgements:
Wellcome Trust
Grant ID: 220540/Z/20/A

Abstract

This protocol describes the shearing of HMW DNA extracted from nematodes using the Megaruptor 3 (Diagenode). We expect an average fragment size of 20 kb after shearing. The sheared DNA is then size-selected and SPRI cleaned before the PacBio low-input HiFi library preparation (not included here).

Guidelines

Use a volume of at least 100 µl for shearing.
The shearing volume can be up to 500 µl. Calculate the concentration of the solution you want to shear or measure it directly after dilution.
Multiple samples can be run with the **same concentration and same volume**. Top-up the sample to the necessary volume with nuclease free water.

Materials

- Eppendorf DNA LoBind Tubes 2.0 ml Cat. No. 022431048
- Megaruptor 3 Shearing Kit (x 16 samples) Cat. No. E07010003
- Megaruptor 3 (Diagenode) Cat. No. B06010003
- Bore tips low-bind (200 µl, 1000 µl) ← dependent on volume used
- Nuclease-free water

additionally:
- NanoDrop One Cat. No. ND-ONE-W

or
- Qubit™ 4 Fluorometer Cat. No. Q33238
- Qubit Assay tubes Catalog number Q32856
- Qubit reagents for DNA (for example Qubit 1X dsDNA HS Assay Kit Cat. No. Q33231)

Safety warnings

It is highly recommended to wear a lab coat and nitrile gloves when performing this laboratory procedure. Make sure to comply to all Health and Safety rules in your laboratory.

Before start

We start with HMW DNA eluted in Elution Buffer.
This protocol is prepared to shear 2 µg DNA in total, which is later used for the PacBio low-input HiFi library preparation. But shearing more or less DNA is of course possible.
Check your HMW DNA fragment profile on the FemtoPulse (Agilent) or other fragment analysers before the shearing.
Initialise the Megaruptor.

Shearing with the Megaruptor 3 (Diagenode)

1h 3m

1 Spin down the DNA solution before adding it to the shearing tube. 5m
I spin 8.000 rcf for 3 min.
This is important because sometimes the DNA is not fully eluted or the solution contains indissoluble particles. This can block the capillary of the syringe and the DNA cannot get sheared!

1.1 If you want to run multiple samples in the same Megaruptor run, you have to bring the DNA solution to the same volume with approx. the same concentration. I add nuclease-free water for dilution and mix well with a bore tip. 10m
If the sample is meant for the PacBio low-input HiFi sequencing, I would recommend to make a shearing sample with 2 µg DNA in total.

2 Prepare the syringe: 5m

2.1 Check the syringe for loose elements (tighten if necessary).

2.2 Label syringe and tube.
You might want to re-shear the sample if the sheared DNA still include too long fragments.

2.3 Make sure that the Megaruptor is initialised.

3 For the Megaruptor: 3m
Go to "Protocols", then go to "Go + shear" and add the concentration, volume, and speed (usually a speed of 29 is good to get 18kb, but samples can differ and might get too short or fragments are still too long).

3.1 I shear the sample twice at the setting 28 for the first shearing and 29 for the second shearing. I get an average fragment size of 18 to 22 kb. But the shearing result can vary and might be species-dependent.

4 Add the hydro tube containing your DNA with the syringe on top in the cassette, load from outside to the inside and make sure the Megaruptor is balanced (use a balancing empty syringe with tube if you have an uneven number of samples to shear).

4.1 Make sure the cassette is in its right position after the initialisation step and make sure the samples are completely clicked in.

5 Start the shearing run.
One run should take about 30 to 40 min.

40m

5.1 Check after 10 min if the solution is going up and down, if the syringe is blocked the sample cannot be sheared.

6 After shearing is finished check the shearing result with the FemtoPulse.
Note: I usually shear twice.
First with the setting **28** and a second time with the setting **29**. However, the final fragment size you get is species dependent and can be adjusted if needed.

6.1 If the peak size in the FemtoPulse is between 10 to 20 kb (a bit higher than 20 kb is acceptable) and the peak width is tight, the DNA solution can now be taken to the size-selective SPRI clean-up.