Jun 19, 2025

High Molecular Weight DNA extraction from frozen nematodes (bulk) - Andersen

Forked from HMW DNA extraction from frozen nematodes (bulk)

DOI
https://dx.doi.org/10.17504/protocols.io.dm6gpq67jlzp/v1

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DOI: https://dx.doi.org/10.17504/protocols.io.dm6gpq67jlzp/v1

Protocol Citation: Manuela Kieninger 2025. High Molecular Weight DNA extraction from frozen nematodes (bulk) - Andersen. **protocols.io** https://dx.doi.org/10.17504/protocols.io.dm6gpq67jlzp/v1

Protocol status: Working
This protocol has been used to make ~200 HiFi genomes.

Created: May 14, 2025

Last Modified: June 19, 2025

Protocol Integer ID: 218261

Keywords: HMW DNA, DNA extraction, HiFi sequencing, nematodes, DNA, C. elegans, monarch hmw dna extraction kit for tissue, dna extraction, frozen nematode, monarch hmw dna extraction kit, nematode, weight dna, quantity of dna, extraction, eluted dna, dna, frozen pellet, high molecular weight

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

Abstract

This protocol describes the High Molecular Weight (HMW) DNA extraction from a frozen pellet of nematodes. We use the Monarch HMW DNA Extraction Kit for Tissue from NEB with some modification to their protocol. The eluted DNA is usually about 150 kb in length and without low molecular-weight DNA. The quantity of DNA extracted is highly species dependent.

Guidelines

We prepare pellets that are between 40 mg and 80 mg.
It may be necessary to use more, dependent on the nematode species.
Do not use more than 100 mg. This leads to less pure DNA.
All temperatures are in Celsius.

Materials

For Extraction:

- Monarch® HMW DNA Extraction Kit for Tissue Kit #T3060L. **Note:** every item in this kit can also be ordered as a single item.
- Eppendorf DNA LoBind Tubes 1.5 ml Cat. No. 022431021
- Eppendorf DNA LoBind Tubes 2.0 ml Cat. No. 022431048
- Eppendorf™ ThermoMixer™ C Cat. No. 15158953
- Eppendorf Smart Block 2.0 ml Cat. No. 5362000035
- Eppendorf Smart Block 1.5 ml Cat. No. 5360000038
- ThermoFisher Hula Mixer Cat No. 15920D
- VWR 1000 µl wide-bore tips Cat. No. 76635-652
- VWR 1000 µl wide-bore tips Cat. No. 76635-646
- Ice
- Dry ice (if you work with multiple samples)
- Fridge
- Centrifuge (must go up to 16.000 rcf)

eventually:

- Na Acetate pH 5.2 3M
- Ethanol 75%

For QC:

- Qubit™ 4 Fluorometer Cat. No. Q33238
- Qubit Assay tubes Cat. No. Q32856
- Qubit reagents: HS Assay Kit Cat. No. Q32854 or BR Assay Kit Cat. No Q32853
- NanoDrop One Cat. No. ND-ONE-W
- Agilent Femto Pulse System Cat. No. M5330AA)
- Agilent FemtoPulse reagents Genomic DNA 165 kb Kit Cat. No. FP-1002-0275
- Agilent TapeStation Cat No. G2992AA
- Agilent Genomic DNA Screen Tape Cat. No 5067-5365
- Agilent Genomic DNA Reagents Cat. No 5067-5366

Safety warnings

- Make sure to keep the supernatant after the Isopropanol step.
- Sometimes the DNA will not bind to the glass beads but you will be able to do a Isopropanol precipitation with the saved supernatant. The DNA is usually less clean and has more low molecular weight DNA. However, it can be size-selected and cleaned with magnetic beads.
- This protocol requires you to wear lab coat, nitrile gloves. Cotton glove liners are strongly recommended when handling the samples on dry ice and samples from the ULT freezer.
- Please collect the waste in a suitable container and dispose of the waste in accordance with local regulations.

Before start

Preheat ThermoMixer to 56°C.
Get samples out of -80°C freezer.
If you processes more than two samples, keep them on dry ice. If you process only one or two samples, you can put them in wet ice.

Worm Pellet

1 For each pellet that you wish to prep, grow animals on four NGMA 10 cm plates seeded with OP50.
- We aim to make pellets that are 40 mg - 80 mg.
- We recommend making multiple pellets at a time, if possible. This way, if the first prep does not pass QC, you already have more samples to process. For example, if you want to make three pellets, grow animals on 12 10 cm plates.
- No matter how many pellets we collect, we wash off all of the worms into one conical so that all animals are washed with the same conditions. The individual pellets are aliquoted after the last wash step.

2 When the plates have just starved, wash off worms from all of your plates with cold M9 into a 15 or 50 ml conical, depending on the number of plates you are using.
- Use 10 ml of cold M9 for every 2 plates.

2.1
- Add 5 ml of M9 to plate 1
- Gently swirl to release the worms
- Pour the M9 from plate 1 to plate 2
- Gently swirl plate 2
- Pour the M9 into the 15/50 ml conical
- Repeat with another 5 ml of M9

2.2 If you have many plates:
- Set the plates in two rows
- Add 5 ml of M9 to each plate in the top row
- Swirl each plate and then pour onto the corresponding plate in the second row.
- Add another 5 ml of M9 to each plate in the top row.
- Swirl the bottom row plates and then decant the M9 into a 50 ml conical.
- Swirl the M9 in the top row plates and then pour the M9 onto the bottom row plates.
- Swirl the bottom row plates and decant into the 50 ml conical.

3 Centrifuge at 2000 rpm for 8 minutes with a break speed of 3.

4 Allow the sample to settle on ice for 2 minutes.

5 Aspirate the supernatant. Be careful because the pellet is quite loose. For the first wash, you can leave behind a considerable amount of supernatant (5-10 ml) to ensure you do not lose animals.
If you are processing multiple strains, be sure to change tips between samples to avoid cross contamination.

6 Add 20 mL of cold M9 + 0.01% Tween. Centrifuge at 2000 rpm for 8 minutes with a break speed of 3.

7 Allow the sample to settle on ice for 2 minutes.

8 Aspirate the supernatant. Be careful because the pellet is quite loose.
Be sure to change tips between samples to avoid cross contamination.

9 Repeat steps 6-8 two more times for a total of three washes.

10 Add 20 ml of cold M9. Centrifuge at 2000 rpm for 8 minutes with a break speed of 3.

11 Aspirate the supernatant, but leave approximately 1 mL of M9.
Be sure to change tips between samples to avoid cross contamination.

12 Transfer the pellet to labeled 1.7 ml microfuge tubes with a Pasteur pipette.
- When choosing a microfuge tube, make sure that the pestles provided with the NEB Monarch HMW DNA kit fit in the tube. If the pestle fits in your microfuge tube, you do not need to transfer your pellet to a new pestle tube when you start the DNA extraction.

12.1 If you are generating multiple pellets, transfer the material a drop at a time to each tube to split the pellet equally between all tubes.

13 Spin in a microcentrifuge at 2000 rpm for 5 minutes.
If the supernatant is not clear and the pellet does not look sharp, spin again.

14 Remove as much supernatant as possible.

15 Weigh each pellet. You should have between 40 mg and 80 mg of worms.

15.1
- Use a fine-measure scale.
- Tare the scale using an empty microfuge from the same batch in which you collected worms.
- One at a time, place a tube with a pellet on the scale.
- Record the weight and write it on the side of the tube.

16 Flash freeze in liquid nitrogen.

Lysis

5h 17m

17 If necessary, transfer the frozen nematode pellet to a Monarch pestle tube.
The pellet must be in a 1.5 ml microfuge tube in which the pestle fits.
2m

18 Per sample: take 600 µL of HMW gDNA Tissue Lysis Buffer and add 20 µL Proteinase K to make the LysisMix buffer. You can prepare a master mix when processing multiple samples. The mix can be prepared at room temperature and then put on ice.
2m

18.1 Mix well with a pipette.
1m

19 Add 50 µL of cold LysisMix buffer to the 1.5 mL tube with the frozen nematode pellet. Keep the sample on ice especially during the pestle step.
1m

20 Perform the pestle step (at least 30 times up-down and turn the pestle in one direction; not both)
It can be necessary to let the pellet thaw a bit in the lysis mix on ice before disruption with the pestle if possible.
1m

21 Add 550 µL LysisMix buffer.

21.1 Pipette to mix well with a 1000 µL wide-bore tip.
1m

22 Transfer all of (~630 µL) the sample-LysisMix into a 2 mL LoBind tube (provided with the kit) and digest at 56°C in the ThermoMixer that is set to 750 rpm for the first 15 min. After 15 min, reduce the rpm to 550 rpm for the rest of the lysis time.
2h

22.1 After 1.5 hrs of total lysis, check for the level of digestion. If the sample is fully digested, no visible worms should remain.
1m

- If there are still worms, continue digesting at 56°C and 550 rpm. Check every 30 minutes for digestion.
- two hours of lysis time is generally fine, but you may need to increase up to four hours.

22.2 Possible stopping point: Lysate can be kept in at 4°C overnight after this step.

RNase A treatment

5h 17m

23 Add 10 µL RNase A and mix well with a wide-bore pipette tip. 1m

24 Add to ThermoMixer for 10 min at 56°C and 550 rpm. 10m

Extraction

5h 17m

25 Spin the sample for 5 min at 2500 rcf. Take the supernatant leaving 30 µL behind. Add the supernatant to a new 1.5 mL LoBind tube. The old tube can be discarded. 6m
- The 30 µl is left behind to avoid pipetting debris.

25.1 Add 30 µL of HMW gDNA Tissue Lysis Buffer before next step, so the total volume is the same as before the centrifugation. 1m

26 Put the sample on ice for 5 min. 5m

27 Add 300 µL cold Monarch Protein Separation Solution and invert right away.

27.1 Invert at 20 rpm for 1 to 3 min. 3m
- We use ThermoFisher's HulaMixer (cat# 15920D) set only for orbital rotation.

27.2 Leave the sample on ice for 10 min. 10m

28 Spin at room temperature 16,000 rcf for 25 min or longer if necessary. 25m

28.1 Check if the sample has two stable phases (lower phase can be very small).
If the phases are not stable enough for pipetting, centrifuge longer.

29 Take all the upper phase to a new 2 mL LoBind tube.
Very slow pipetting is necessary!

30 Add **3** Monarch DNA Capture Beads to the DNA solution.
Make sure to add the beads now! Before you add Isopropanol!

31 Add 550 µL Isopropanol and **invert 20 times right away.**

31.1 Invert on wheel or rocker at 7 rpm for 5 to 7 min. 7m

- We use ThermoFisher's HulaMixer (cat# 15920D) set only for orbital rotation.

32 Remove liquid without disturbing the beads (HMW DNA should have attached to the glass beads).

32.1 **Keep the supernatant and add it to a new microfuge tube in case the DNA did not bind to beads!**

32.2 **Note:** If the DNA extraction should fail, use the kept supernatant and perform an Isopropanol precipitation
to get the DNA out.
The supernatant can be saved, and an Isopropanol Precipitation can be done if the DNA extraction fails. Add 10 µL of 3 M NaAc pH 5.2 and 200 µL additional Isopropanol and mix well (40x invert). Centrifuge at 4 degrees.

33 Add 500 µL Monarch gDNA Wash Buffer right away to the tube with the beads.

34 Carefully invert 2 to 3 times.

35 Remove wash buffer with a tip without disturbing the beads.

36 Add again 500 µL Monarch gDNA Wash Buffer and invert.

37 Remove the wash buffer with a tip without disturbing the beads.

37.1 **Check** if there is any **wool-like structure** in the wash buffer that has been taken away. This precipitate happens if the DNA did not stay attached to the beads. You can save this structure (spin and remove liquid) and elute in a separate tube! However, the DNA eluted this way will not be as pure as if it was bound to the beads.

38 Put a labeled bead retainer into a collection tube. Pour beads into the retainer.

39 Short spin the tube for 1 s in a small centrifuge to remove residual liquid.

40 Pour the glass beads to 2 mL tube and add 200 µL Monarch gDNA Elution Buffer II.
Do not let the glass beads dry for too long.

40.1 Put the bead retainer in new 1.5 mL DNA LoBind tube.

41 Put the tube in the ThermoMixer at 56°C and 300 rpm for 5 min.

5m

42 Pour liquid with beads into the bead retainer and add 50 µL Monarch gDNA Elution Buffer II on top to wash the beads.

43 Spin in centrifuge for 30 sec at 12,000 rcf.

30s

44 Pipette mix 20 times up and down with a wide-bore tip.

1m

45 Let the DNA elute further in ThermoMixer for 30 min at 37°C and 300 rpm.

30m

46 Pipette mix well with a wide-bore tip and leave the DNA at room temperature overnight for elution.

2m

47 The next day, pipette mix with a wide-bore tip at least 20 times.

Quality Control

48 Use 2 µl of your sample to perform a Qubit reading. A Qubit Broad Range (BR) reaction should be fine.

48.1 Follow the manufacturer's instructions to complete the Qubit.

48.2 There can be a wide-range of results. Ultimately, you need to ensure that there is enough material for your downstream needs.

49 Use 1 µl of your sample for NanoDrop or equivalent.

49.1 You want the amount of DNA in the NanoDrop reading to be no more than 2X greater than the Qubit reading.

49.2 The A260/280 should be between 1.8 - 2.0 and the A260/A230 should be 2.0 - 2.2.

50 Use the required amount of sample for a TapeStation Run using the Genomic DNA Reagents

50.1
- The resulting band/peak should be sharp and HMW.
- The DIN should be greater than 7.5.
- More than 50% of the fragments should be >15 kb