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HMW DNA extraction from frozen nematodes (bulk)

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We use this protocol and it's working

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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

Start with at least 40 mg of pellet. I usually use 60 mg.
It may be necessary to use more, dependent on the nematode species.
I would not recommend to 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
- Wheel or rocker (for example: Stuart rotator SB3)
- 1000 µl bore tips low-bind
- 200 µl bore tips low-bind
- 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 (Catalog number Q32856)
- Qubit reagents (for example Qubit 1X dsDNA HS Assay Kit Cat. No. Q33231)
- NanoDrop One (Cat. No. ND-ONE-W)
- Femto Pulse System (Agilent) (Cat. No. M5330AA)
- FemtoPulse reagents (Agilent): Genomic DNA 165 kb Kit (Cat. No. FP-1002-0275)

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 I would recommend to keep them on dry ice. If you process only one or two samples, you can put them in wet ice.

Lysis

5h 17m

1 Use a worm pellet of about 40 to 80 mg which was previously flash frozen in liquid nitrogen.
More than 100 mg is not recommended as DNA purity gets lower.

2 If necessary: transfer the frozen nematode pellet to a Monarch pestle tube;
The pellet must be in a 1.5 ml microtube in which the pestle fits.
2m

3 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

3.1 Mix well with pipette or vortex.
1m

4 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

5 Perform 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 is possible.
1m

6 Add 550 µL LysisMix buffer.

6.1 Pipette mix well with 1000 µL wide-bore tip.
1m

7 Transfer all of (~630 µL) the sample-LysisMix into a 2 mL LoBind tube and digest at 56°C in the ThermoMixer which 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

7.1 Lysis time: 2 h
Can be increased to 4 h if needed. Time for digestion is sample-dependent but 2 h is fine for most nematode species I have processed.
2h

8 Check if worms have fully digested (longer lysis and more proteinase K can be added if worms are still visible).
1m

8.1 Possible stopping point: Lysate can be kept in the fridge overnight after this step.

RNase A treatment

5h 17m

9 Add 10 µL RNase A and mix well (invert/spin or pipette mix with bore tip). 1m

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

Extraction

5h 17m

11 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

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

12 Put sample on ice for 5 min. 5m

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

13.1 Invert at 20 rpm for 1 to 3 min (wheel or rocker). 3m

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

14 Spin at room temperature 16.000 rcf for 25 min or longer if necessary. 25m

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

15 Take all the upper phase to a new 2 mL LoBind tube.

Very slow pipetting is necessary!

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

17 Add 550 µL Isopropanol and **invert right away after adding several times.**

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

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

19.1 **Keep the supernatant and add it to a new microtube in case the DNA did not bind to beads!**

19.2 **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. I usually add 10 µL of 3 M NaAc pH 5.2 and 200 µL additional Isopropanol and mix well (40x invert). Use a 4 degrees centrifuge here.

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

21 Carefully invert 2 to 3 times.

22 Remove wash buffer with a tip without disturbing the beads.
Add again 500 µL Monarch gDNA Wash Buffer and invert.

23 Remove the wash buffer with a tip.

23.1 **Check** if there is any **wool-like structure** in the wash buffer that has been taken away; that 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.

24 Short spin the tube for 1 s in a small centrifuge.

25 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.

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

5m

27 Put bead retainer in new 1.5 mL DNA LoBind tube.

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

29 Spin in centrifuge for 30 sec at 12.000 rcf.

30s

30 Pipette mix 20 times up and down with bore tip.

1m

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

30m

32 Pipette mix well with bore tip and leave the DNA at room temperature overnight for elution.

2m

33 The next day:
Pipette mix with bore tip at least 20 times.
Then do QC: NanoDrop measurement, Qubit measurement, and FemtoPulse run.

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