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Nanobind extraction protocol for DNA that fragments easily

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Giada Ferrari[1]

[1]Norwegian Sequencing Centre, Department of Biosciences, University of Oslo

Biodiversity Genomics E...

Tuuli Lundbäck-Larva
Department of Immunology, Genetics and Pathology, SciLifeLab...

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We use this protocol and it's working

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Disclaimer

The duration times presented are estimations only.

Abstract

This protocol describes a modified PacBio NanoBind procedure for the Extraction of HMW DNA from samples that are challenging because of DNA that fragments excessively following standard protocols. To reduce this, the protocol includes the following modifications:
- The frozen tissue is allowed to thaw in L buffer. The high concentration of EDTA protects DNA integrity. (From **dx.doi.org/10.17504/protocols.io.n92ldzj4ov5b/v1**)
- No mechanical disruption of the tissue is performed, except for mincing with a scalpel blade.
- Reduced time and rpms during tissue digestion.

Attachments

DOCX

Nanobind_DNA_extract..

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17KB

Materials

- PacBio NanoBind Kit
- 1.5 ml Protein LoBind tubes
- 2 ml Protein LoBind tubes

- 10 mM Tris pH 7.6
- 100 mM EDTA
- 20 mM NaCl
- Isopropanol

- Petri dish or weighing boat
- Scalpel blades
- Centrifuge (cooling; up to 10,000 x g
- ThermoMixer
- Mini-centrifuge
- Pipettes, pipette tips + wide-bore pipette tips
- Vortexer
- Platform rocker
- Magnetic separation rack

Safety warnings

The operator must wear a lab coat, powder-free nitrile gloves and safety specs to perform the laboratory procedures in this protocol.

Waste needs to be collected in a suitable container and disposed of in accordance with local regulations.

Liquid waste needs to be collected in a suitable container and disposed of in accordance with local regulations.

See best practices, thawing and handling instructions, and safety instructions for all the kit reagents, as well as instructions and safety data sheets for other reagents used in this protocol **before starting**.

Ethics statement

The protocols.io team notes that research involving animals and humans must be conducted according to internationally-accepted standards and should always have prior approval from an Institutional Ethics Committee or Board.

Before start

Prepare 1-5 ml / sample Buffer L according to the recipe in Step 2.

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1h 55m

1 Cut a suitable piece of frozen tissue with a scalpel blade in a pre-cooled petri dish or weighing boat. Work on ice or dry ice. 2m

2 Remove the sample from the ice and immediately cover with 1-5 mL of L buffer (from Sambrook and Russel, Molecular Cloning: A laboratory Manual, 2001). 1m

	Buffer L
	10 mM Tris pH 7.6
	100 mM EDTA
	20 mM NaCl

Recipe for Buffer L from Sambrook and Russel, Molecular Cloning: A laboratory Manual, 2001

3 Allow the sample to thaw in the L buffer. 2m

4 Mince the tissue into small pieces with a scalpel blade. 3m

5 Collect the buffer and tissue pieces into microcentrifuge tubes. 2m

6 If necessary, rinse the petri dish with L buffer and collect into the same tubes. 1m

7 Centrifuge at 3,000 x g for 00:05:00 and discard the supernatant. 5m

8 Use a total of 1 mL of cold Buffer CT to resuspend the pellets by pipette mixing 10X with a wide bore P200 pipette. 1m

9 Transfer all the resuspended pellets into one 2 mL Protein LoBind microcentrifuge tube. 1m

10 Pellet homogenate by centrifuging at 3,000 x g and 4°C for 00:05:00 . Discard supernatant. 5m

11 Pulse vortex for 1s x 2 times (max setting) to dislodge pellet.

12 Add 20 µL of Proteinase K to the previous pellet. 1m

13 Add 150 µL of Buffer CLE3 and pipette mix 10X with a wide bore P200 pipette. 1m

14 Incubate on a ThermoMixer at 55°C and 600 rpm for 00:20:00 . 20m

15 Spin the tube on a mini-centrifuge for 2 s to remove liquid from the cap.

16 Add 20 µL of RNase A. 1m

17 Incubate on a ThermoMixer at 55°C and 600 rpm for 00:20:00 . 20m

18 Spin the tube on a mini-centrifuge for 2 s to remove liquid from the cap.

19 Add 60 µL of Buffer SB and pulse vortex for 1s x 5 times (max setting) to mix. 1m

20 Centrifuge at 10,000 x g and RT (15–30°C) for 00:05:00 . 5m

21 Transfer up to 300 µL of supernatant to a new 1.5 mL Protein LoBind microcentrifuge tube using a wide bore P200 pipette. (Discard the 2 mL Protein LoBind microcentrifuge tube containing the precipitated pellet.) 1m

22 Add 50 µL of Buffer BL3 to the previous supernatant and inversion mix 10X. 1m

23 Spin the tube on a mini-centrifuge for 2 s to remove liquid from the cap.

24 Add Nanobind disk to lysate and add 350 µL of isopropanol. Inversion mix 10X. 1m

25 Mix on a platform rocker at 20 rpm for 00:15:00 at RT. 15m

26 Place tube rack on the magnetic base using the method described in the Nanobind tissue kit Guide & overview “Magnetic rack handling procedure” section (PacBio).

27 Discard supernatant with a pipette, taking care to avoid pipetting the DNA or contacting the Nanobind disk. 1m

28 Add 500 µL of Buffer CW1, remove tube rack from magnetic base, inversion mix 4X, replace the tube rack on the magnetic base, and discard the supernatant. 1m

29 Repeat CW1 wash. 2m

30 Add 500 µL of Buffer CW2, remove tube rack from magnetic base, inversion mix 4X, replace the tube rack on the magnetic base and discard the supernatant. 2m

31 Repeat CW2 wash. 2m

32 Spin the tube on a mini-centrifuge for 2 s. With the tube rack already on the magnetic base and right-side-up, place tube on tube rack and remove residual liquid. 1m

33 Repeat previous step. 1m

34 Remove tube from tube rack.

35 Add 100 µL of Buffer LTE directly onto the Nanobind disk and incubate at RT for 00:10:00. 11m

36 Collect DNA by transferring eluate to a new 1.5 mL microcentrifuge tube. 1m

37 Spin the tube containing the Nanobind disk on a mini-centrifuge for 5 s. Use a standard P200 pipette to combine any additional liquid that comes off the disk with the previous eluate. Repeat if necessary. 2m

38 Pipette mix the sample 5X with a wide-bore 200 µL pipette tip to homogenize and disrupt any unsolubilized “jellies” that may be present. 1m

39 Let sample rest at RT overnight to allow DNA to solubilize.

Protocol references

- L buffer, Sambrook and Russel, Molecular Cloning: A laboratory Manual, 2001.
- "Squeeze” enrichment of intact cells (eukaryotic and prokaryotic) from marine sponge tissues prior to routine DNA extraction" dx.doi.org/10.17504/protocols.io.n92ldzj4ov5b/v1