SEP 26, 2023

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.yxmvm3yybl3p/v1

Protocol Citation: Hüsna Öztoprak, Jens Bast 2023. Modified salting out method for high molecular weight gDNA extraction (oribatid mites). **protocols.io** https://dx.doi.org/10.17504/protocols.io.yxmvm3yybl3p/v1

Modified salting out method for high molecular weight gDNA extraction (oribatid mites)

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ABSTRACT

This protocol describes a low-cost, high-molecular-weight genomic DNA extraction method for a single minuscule specimen (modified from Miller et al 1988). DNA extractions from oribatid mites are typically challenging, because of the small body size of 150-1400 µm. Their chitinous exoskeleton does not dissolve during DNA extraction, impedes DNA purification, and leads to additional loss of DNA. Therefore, high-molecular DNA from oribatid mites has been thus far unattainable, especially from single individuals. We established a high-molecular-weight gDNA extraction protocol for mites that enables the generation of high-quality phased genomes for small non-model organisms. There are three options to utilize this protocol: i) for high-molecular gDNA extraction ii) for high-molecular gDNA extraction, while preserving the exoskeleton for morphological analysis, and iii) DNA extraction with Chitinase to yield more gDNA.

As specimens are collected from natural populations and are not cultured in the lab. They are cleansed prior to DNA extraction to minimize external contamination. Cleansing includes brushing the specimen in distilled water and in distilled water with detergent (fit GmbH, Zittau, Germany). Once the external residue is not visible anymore specimen is incubated in NaClO 0.05% (DonKlorix; CP GABA GmbH, Hamburg, Germany) and ethanol 70% for 30 seconds each and rinsed in distilled water again.

ATTACHMENTS

nn7eb8kcf.pdf

Protocol status: Working
We use this protocol and it's working. It enables the generation of HMW gDNA of ~20 ng, even up to 200 ng of a single oribatid mite, depending on the genus. It also works for collembolans and nematodes.

Created: Sep 25, 2023

Last Modified: Sep 26, 2023

PROTOCOL integer ID: 88360

MATERIALS

Material and Regents

Proteinase K, 2mL **Qiagen Catalog #19131**

Yeast tRNA (10 mg/mL) **Thermo Fisher Scientific Catalog #AM7119**

RNase Cocktail™ Enzyme Mix **Thermo Fisher Catalog #AM2286**

Solutions

1. TNES buffer (see Recipes)

Recipes

1. Final concentration of TNES buffer freshly made before each extraction, ddH_20 used to dilute.:

	A	B
	NaCl	400 mM
	EDTA	20 mM
	Tris pH 8.0	50 mM
	SDS	0.5%

Version i) High-molecular gDNA extraction: DNA Extraction

2h 5m 3s

1 Submerge cleansed specimen in 195 µL TNES buffer and flash freeze by holding tube in liquid nitrogen.

2 Using a sterile pestle, homogenize by applying pressure to grind the specimen between pestle head and the walls of the tube.

Note

If low DNA yield is expected. Leave pestle in tube to ensure maximum digestion of material. Consider including multiple (3x) freeze and thaw cycles and vortexing to disrupt tissue fully.

3 Add 5 µL proteinase K.

4 Vortex for 00:00:03. Centrifuge briefly. 3s

5 Incubate at 55 °C for ~ 01:00:00. 1h

Note

Completely dissolve the specimen.

5.1 If there is indigestible debris left over centrifuge 18000 rcf, 00:05:00 and transfer the supernatant to a fresh tube. 5m

6 Add 1.5 µL yeast tRNA, flick to mix briefly then spin down.

7 Add 65 µL [M] 5 Molarity (M) NaCl and 290 µL 96% EtOH, mix by inversion.

8 This should clarify the solution. Store at -20 °C for 01:00:00. 1h

Note

Solution can be stored Overnight at this step.

Version i) High-molecular gDNA extraction: DNA Purification 2h 20m

9 **Optional**: add 1 µL Pellet Paint Co-Precipitant (for colorful pellet).

10 Spin down at 18000 rcf, 4°C, 00:15:00. 15m

Note

Know the expected position of the DNA pellet, as it can be difficult to see.

11 Ghostly pellet should be visible.

12 Remove supernatant.

13 Add 0.5 mL chilled 70% EtOH (make fresh).

14 Spin at 18000 rcf, 00:05:00. 5m

15 Repeat ethanol rinse.

16 Carefully remove supernatant.

17 Leave tube open to air dry. Pellet should have a glassy appearance.

18 Using a wide-bore pipette tip, add 21 µL TE Buffer (elution buffer) and gently resuspend the DNA pellet with pipette mixing.

19 Let DNA resuspend at 4 °C Overnight . 1h

20 Add 2 µL RNase Cocktail. Incubate at 37 °C for approx. 01:00:00 . 1h

Note

Note: For femto pulse systems elution in TE Buf er, more specifically EDTA is not recommended. Alternatively use 0.1 mM EDTA or EB, Tris-HCl (pH 8-8.5).

Version ii) High-molecular gDNA extraction preserving exoske... 2h

21 To observe the specimen under a microscope, cleansed specimens are placed on a sterile slide and submerged with TNES buffer until fully covered.

22 Remove one genital plate cautiously with a sharp needle and stir tissue without destroying the exoskeleton.

23 Transfer specimen in 195 µL TNES buffer.

24 Add 5 µL proteinase K.

25 Incubate at 37 °C Overnight . 1h

26 Transfer specimen with sterile needle to a tube containing 70% EtOH and store it for morphological analysis.

27 Add 1.5 µL yeast tRNA, flick to mix briefly then spin down.

28 Add 65 µL [M] 5 Molarity (M) NaCl and 290 µL 96% EtOH, mix by inversion.

29 This should clarify the solution. Store at -20 °C for 01:00:00 . 1h

Note

DNA Purification follows the same procedure as above.

Version iii) gDNA extraction with chitinase digestion: DNA Ex...

3h 5m 10s

30 Submerge specimen in 195 µL TNES buffer and flash freeze by holding tube in liquid N.

31 Using a sterile pestle, homogenize by applying pressure to grind the specimen between pestle head and the walls of the tube.

32 Add 2 µL chitinase (1 U/ml).

33 Vortex for 00:00:05. Centrifuge briefly. 5s

34 Incubate at 55 °C for ~ 01:00:00. 1h

35 Add 5 µL proteinase K.

36 Vortex for 00:00:05. Centrifuge briefly. 5s

37 Incubate at 55 °C for ~ 01:00:00 (completely dissolve the specimen). 1h

37.1 If there is indigestible debris left over centrifuge 18000 rcf, 00:05:00 and transfer the supernatant to a fresh tube. 5m

38 Add 1.5 µL yeast tRNA, flick to mix briefly then spin down.

39 Add 65 µL [M] 5 Molarity (M) NaCl and 290 µL 96% EtOH, mix by inversion.

40 This should clarify the solution. Store at -20 °C for 01:00:00. 1h

> **Note**
>
> DNA Purification follows the same procedure as above.