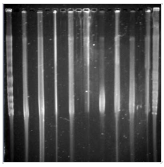

Version 2

Sep 03, 2021

FindingNemo Extraction 2: Phenol-free Method V.2

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dx.doi.org/10.17504/protocols.io.bxx2ppqe

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ABSTRACT

This is a sub-protocol designed to extract/isolate ultra-high molecular weight (UHMW) DNA to obtain ultra-long (UL) reads on Nanopore sequencers using a **phenol-free extraction** method.
A DNA extraction protocol that yields clean and homogeneous UHMW DNA is important for a good UL sequencing output. The choice of protocol should be based on achieving these parameters.

Kit-free, phenol-free protocol is a modification of NEB's Monarch HMW DNA Extraction Kit for Cells & Blood, with the option to use SDS or CTAB in the lysis buffer. This protocol also uses glass beads for DNA precipitation matrix.

We tested this sub-protocol in **human cell line**, with input cells of 3 millions but can be varied from 1-5 millions. As a rule of thumb, a million cells will suffice for one load on a MinION.

DOI

dx.doi.org/10.17504/protocols.io.bxx2ppqe

PROTOCOL CITATION

Inswasti Cahyani, John Tyson, Nadine Holmes, Josh Quick, Nicholas Loman, Matthew Loose 2021. FindingNemo Extraction 2: Phenol-free Method. **protocols.io**
https://dx.doi.org/10.17504/protocols.io.bxx2ppqe
Version created by Inswasti Cahyani

WHAT'S NEW

This is the correct phenol-free protocol, with corrected steps at Cell Lysis and DNA Precipitation sections. The first version was copying steps from the phenol-based protocol (FindingNemo Extraction 1)

KEYWORDS

ultra-long sequencing, cohex, glass bead, nanopore, MinION, UHMW DNA, Monarch, Circulomics, phenol, SDS, CTAB, GM12878, Whatman, PromethION, Nanobind

CREATED

Sep 03, 2021

LAST MODIFIED

Sep 03, 2021

PROTOCOL INTEGER ID

52954

GUIDELINES

Acknowledgements

The FindingNemo protocol series was developed by Inswasti Cahyani for the Long Read Club with significant contributions from John Tyson and Nadine Holmes, also Josh Quick, and continuous discussion and support of Matt Loose and Nick Loman. We would also like to thank Giron Koetsier (NEB) and Kelvin Liu (Circulomics) for lending their expertise and advance product trials.

Please follow on Twitter for latest updates and results:
@NininUoN
@mattloose

MATERIALS TEXT

Chemicals/Compounds

- 5M Ammonium Acetate **Sigma –**
- **Aldrich Catalog #A-7330**
- Tris-HCl pH 8.0 **Thermo**
- **Scientific Catalog #J22638-AE**
- Ethanol
- Absolute **Honeywell Catalog #32221-2.5L**
- 1X Phosphate Buffer Saline **Fisher**
- **Scientific Catalog #15453819**
- Proteinase K,
- 2mL **Qiagen Catalog #19131**
- RNase
- A **Qiagen Catalog #19101**
- Nuclease-free
- Water **Thermofisher Catalog #AM9920**
- NaCl (5 M) RNase-free **Thermo Fisher**
- **Scientific Catalog #AM9759**
- EDTA (0.5 M, pH 8.0, nuclease-free) **Thermo Fisher**
- **Scientific Catalog #AM9260G**
- 20% Sodium dodecyl sulfate (SDS) **Contributed by users**
- Cetyltrimethylammonium Bromide (CTAB) **MP**
- **Biologicals Catalog #02194004-CF**

Made-up Buffer

Tris Lysis Buffer (TLB-SDS or TLB-CTAB)

- 100 mM NaCl
- 10 mM Tris-HCl, pH 8.0
- 25 mM EDTA, pH 8.0
- **TLB-SDS** = 0.5% (w/v) SDS ***or***
- **TLB-CTAB** = 2% (w/v) CTAB

Disposables

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DNA LoBind Tubes, 1.5

- mL **Eppendorf Catalog #0030108051**

DNA LoBind 2.0ml PCR Clean Eppendorf

- Tubes **Eppendorf Catalog #0030 108.078**

Glass Beads 3 mm **Scientific Laboratory Supplies**

- **Ltd Catalog #DD68501** **OR**

Monarch DNA Capture Beads **New England**

Biolabs Catalog #T3005L

Thin-wall PCR Tubes 0.5 ml **Fisher**

- **Scientific Catalog #12194142**

cut tube 2-3 mm from the bottom to make a bead retainer

Monarch Bead Retainers **New England**

OR Biolabs Catalog #T3004L

Monarch Collection Tubes II - 100 tubes **New England**

- **Biolabs Catalog #T2018L** (optional)

or use any 1.5 ml centrifuge tube as collection tube

- Wide-bore (or cut off) P1000 and P200 tips

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SAFETY WARNINGS

When handling phenol always wear PPE, keep a solution of 50% (w/v) PEG-400 nearby to treat the burn in the case of accidental splashes.

BEFORE STARTING

Things to observe at all times:

- Excessive and vigorous pipetting and vortexing should be avoided as these may shear the DNA.
- Make up buffers with nuclease-free water to avoid introducing nucleases to solutions.
- Avoid unnecessary heating and freezing; isolated DNA should be stable for storage in the fridge for months.

UHMW DNA Extraction

1 This protocol is adapted from Monarch® HMW DNA Extraction Kit for Cells & Blood.

Either SDS (anionic surfactant) or CTAB (cationic surfactant) can be used in the lysis buffer.
Providing alternative surfactants in the lysis buffer may help with different cell systems that require different biochemistry.

Cell Lysis 5m

2 Pellet 3 million cells in 1.5 ml tube by centrifuging at 1000 x g for 1 min at 4°C. 1m

1000 x g, 4°C, 00:01:00

3 Wash with PBS (make sure all media and serum are rinsed off), spin at 1000 x g for 1 min at 4°C. 1m

1000 x g, 4°C, 00:01:00

4 Add 149 µl of TLB-SDS **or** TLB-CTAB which has been added with 100 µg RNaseA (1 µl) and vortex at full speed for 3 seconds.

Make master mix of the TLB and RNaseA if handling more than one sample.

5 Incubate at 37°C for 10 minutes. 10m

37 °C **00:10:00**

6 Add 140 µl TLB and 200 µg Proteinase K (10 µl). Mix by slow inversion 5 times or with a P1000 wide-bore pipette tip.

7 Incubate at 55°C for 20 minutes. 20m

55 °C **00:20:00**

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Shaking in a thermomixer at 300-700 rpm can help lysis and homogenization, especially when more than 3 million cells are used.

DNA Precipitation

8 Add 75 µl of 5M Ammonium acetate, mix well with wide-bore P1000 pipette tip.

9 Add 3 clean glass beads to the cell lysate.

10 Add 275 µl of Isopropanol

11 Rotate the tube with a vertical rotator at 9 rpm for 5 minutes. 5m

00:05:00 vertical rotator

If a rotator is not available, hand inversion for 30-40 times can be used. Invert the tube slowly by hand so that a full inversion cycle takes 4-5 seconds.

12 Remove and discard liquid by pipetting.

13 Wash bound DNA with 1 ml of 70% ethanol, invert tube 3 times, remove and discard ethanol.

14 Repeat step 13 once with 500 µl. Discard the ethanol, taking care not to disturb the DNA precipitate.

DNA Elution 31m

15 Insert a bead retainer to a collection tube.
Pour the beads into the bead retainer and spin for 1 s in a mini centrifuge (or the shortest time possible) to remove residual wash buffer. Keep the bead retainer.

16 ⚠

Quickly pour the beads into a new 2 ml low-bind tube and immediately add 250 µl of elution buffer.

Do not let the beads with DNA dry out. (As an alternative, 250 µl of elution buffer can be aliquoted into a 2 ml tube

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prior to this step.)

17 30m
Incubate at 37°C for 30 min. Gently aspirate and dispense the eluate over the glass beads at regular intervals with a wide-bore P1000 tip to aid elution.
37 °C **00:30:00 mix per 10 min**

18 1m
Insert the bead retainer from step 15 into a clean 2 ml DNA low-bind tube.
Pour the beads from step 17 and centrifuge at 12,000 x g for 1 minute.
12000 rpm, Room temperature , 00:01:00

19
Quantify DNA as per "**UHMW DNA QC**" and check homogeneity by calculating %CV values. If the DNA is not sufficiently homegeneous, incubate the DNA for longer.

20
Store at 4°C or continue to **UL Library Preparation** as per "**Modified ULK001**".
If only SQK-RAD004 is available, follow library preparation in "**Modified RAD004**" or "**KrazyStarFish (KSF)**".
4 °C for storage

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