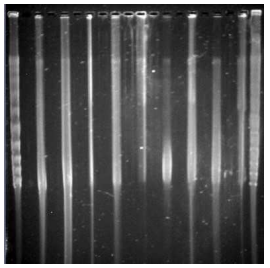
Sep 03, 2021 Version 2

FindingNemo Extraction 2: Phenol-free Method V.2



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We use this protocol and it's working

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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.

Guidelines

Acknowledgements

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

Materials

Chemicals/Compounds

- 5M Ammonium Acetate **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**A-7330
- Tris-HCl pH 8.0 **Thermo Scientific** **Catalog #**J22638-AE
- Ethanol Absolute **Honeywell Fluka** **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)
- Cetyltrimethylammonium Bromide (CTAB) **MP Biomedicals** **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

- 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

Note

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

OR Monarch Bead Retainers **New England Biolabs Catalog #**T3004L

- Monarch Collection Tubes II - 100 tubes **New England Biolabs Catalog #**T2018L (optional)

Note

or use any 1.5 ml centrifuge tube as collection tube

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

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 start

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.

Note

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.

Note

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

Note

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

Note

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.

Note

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 prior to this step.)

17 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. 30m

37 °C 00:30:00 mix per 10 min

18 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. 1m

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