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FindingNemo Extraction 3: NEB Monarch Kit

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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 **New England Biolabs (NEB) Monarch HMW DNA Extraction Kit for Cells & Blood**.
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.

The **New England Biolabs (NEB) Monarch HMW DNA Extraction Kit for Cells & Blood** is a quick, tweakable extraction protocol fitting for one-day library prep.

We tested this sub-protocol in **human cell line**, with input cells varied between 1-6 millions. As a rule of thumb, a million cells will suffice for one load on a MinION.

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PROTOCOL CITATION

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KEYWORDS

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

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GUIDELINES

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

Chemicals/Compounds

- Tris-HCl pH 8.0 **Thermo Scientific Catalog #J22638-AE**
- Ethanol Absolute **Honeywell Catalog #32221-2.5L**
- Isopropanol Absolute **Fisher Scientific Catalog #P/7500/15**
- 1X Phosphate Buffer Saline **Fisher Scientific Catalog #15453819**
- Nuclease-free Water **Thermofisher Catalog #AM9920**

Kits

- Monarch HMW DNA Extraction Kit for Cells & Blood **New England Biolabs Catalog #T3050S** Step 1

Disposables

- DNA LoBind Tubes, 1.5 mL **Eppendorf Catalog #0030108051**
- 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 using **Monarch® HMW DNA Extraction Kit for Cells & Blood**.

Monarch HMW DNA Extraction Kit for Cells & Blood **New England Biolabs Catalog #T3050S**

2 We have obtained optimal extractions using the NEB Monarch kit. This combines speed with high quality UHMW DNA. Follow the manufacturer's instructions as described here, BUT incorporate the following changes as described below.

Our most homogeneous extracted DNA samples were obtained by lysis at 600-700 rpm speed using the Monarch kit. This is one area that can be optimised depending on the input sample.

One-day Protocol 4h

3 To complete DNA extraction from cell to library prep and sequencing in one day, start early!

One million human cells are sufficient for a single library load on the MinION. At least two million human cells are required for a single load on the PromethION. Other samples can be scaled according to total amount of DNA recovered. So for a 1 gigabase genome you would require 3 million cells etc.

4 Dilute the eluted DNA with 150 µl of NEB Elution Buffer II.

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- This is following the NEB UHMW Monarch kit protocol where DNA is first eluted with 100 µl buffer. After this step, sample volume will be 250 µl total.
- Quantification of a very viscous UHMW DNA is problematic and will not produce accurate results, hence the dilution.
- Gradual dilution is recommended to achieve homogeneous concentration of 50-100 ng/µl.

5 Incubate the eluted DNA at 37°C for about 2-3 hours with regular pipette mixing. 3h

37 °C **03:00:00 max (2-3 hours)**

During mixing, observe by eye that the viscous DNA 'blob' has been more or less dissolved to the different parts of the tube (*i.e.*, less heterogeneous). This is usually observed after 2 hours of incubation. Otherwise, continue the incubation to 3 hours and proceed to the next step.

6 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.

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

4 °C for storage

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