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FindingNemo Extraction 4: Circulomics 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 the **Circulomics Nanobind CBB Big DNA Kit**.
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 **Circulomics Nanobind CBB Big DNA Kit** was initially the recommended extraction protocol for UL sequencing using SQK-ULK001.

We tested this sub-protocol in **human cell line**, with standard input cells of 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:
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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

- ☒ Nanobind CBB Big DNA Kit **Circulomics Catalog #NB-900-001-01** (optional)

Disposables

- ☒ DNA LoBind Tubes, 1.5 mL **Eppendorf Catalog #0030108051**
- ☒ DNA LoBind 2.0ml PCR Clean Eppendorf Tubes **Eppendorf Catalog #0030 108.078**
- ☒ Protein LoBind Tubes 1.5 mL **Eppendorf Catalog #0030108442**
- 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 the **Nanobind CBB Big DNA Kit** (see Materials).

2 Follow the manufacturer's instructions as described here.

3 After overnight incubation at room temperature, quantify the DNA as per **"UHMW DNA QC"** and check homogeneity by calculating %CV values.

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

4 °C for storage

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