

**VERSION 1** 

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WORKS FOR ME

Extraction and ONT MinLibrary Preparation of uHMW gDNA V.1

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COMMENTS 0

## **ABSTRACT**

This custom protocol optimizes extraction, purification, and Oxford Nanopore Technologies (ONT) MinION library preparation for ultra-high molecular weight genomic DNA (uHMW gDNA) from parasitic nematodes. It can be used effectively with both low-input samples (e.g., a single adult hookworm) and high-input samples (e.g., a large chunk of tissue from an Ascaris sp. adult).

## Protocols on which this workflow is based:

- Zymo® Quick-DNA<sup>TM</sup> Magbead Plus Kit protcol
- Oxford Nanopore Technologies® SQK-LSK-109 gDNA Ligation Sequencing protocol
- Zymo® DNA Clean & Concentrator<sup>TM</sup> Magbead Kit protocol (best-testing phase only)

PROTOCOL CITATION

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https://protocols.io/view/extraction-and-ont-minlibrary-preparation-of-uhmw-ciyhuft6

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# BEFORE STARTING

- Add 1,040 μL Zymo Proteinase K Storage Buffer to each tube of Zymo Proteinase K (20 mg) prior to use. The final concentration of Proteinase K is ~20 mg/ml. Store resuspended Proteinase K at -20°C after mixing.
- For best results, allow AMPure XP beads (stored at 4°C) to come to RT prior to use.

DNA HWM MagBea

- 1 Set dry bath to 8 55 °C
- 2 For each sample, add the following to a clean 1.5 mL microcentrifuge tube to create a master mix:

型 95 μL 🐰 Zymo DNA Elution Buffer **Zymo Research Catalog #D3004-4-1** 

Δ 95 μL 🐰 Zymo Biofluid & Solid Tissue Buffer **Zymo Research Catalog #D4081-3-25** 

△ 10 μL 🛮 🔀 Zymo Proteinase K **Zymo Research Catalog #D3001-2-20** 

- 2.1 Vortex the master mix gently to mix, then spin down and keep on ice
- 3 Using a new pipette tip or sterilized forceps, add one whole worm (or a piece of tissue) directly from tissue preservative to the bottom of a clean 1.5 mL microcentrifuge tube,

Note

Transfer as little tissue preservative liquid as possible to the new tube during this process

4 Use a new NEB Monarch Pestle **NEB Catalog #T3002-1** to grind and crush the tissue in the tube. Keep the pestle in the tube

- 5 Add 200 µL master mix (prepared in Part 1 Step 1) to each tube containing tissue and pestle
- 6 Continue using the pestle to grind the tissue within the master mix until fully homogenized. Remove the pestle, being careful to keep any tissue in the tube by wiping the pestle on the tube edges as it is removed
- 7 Close the tube and mix by inverting and flicking gently, then spin down briefly to recollect tissue and liquids
- Incubate sample in dry bath at 55 °C for 02:30:00 or until tissue solubilizes. During incubation, flick tube every 00:20:00 to agitate tissues, then briefly spin down to recollect liquids and replace tube in dry bath

If a very large amount of input tissue was used: It is likely there will still be visible tissue even after hours of lysis. If so, centrifuge the sample for 00:01:00 at 10000 x g or greater to pellet debris, then pipette all liquids into a clean 1.5 μL microcentrifuge tube. (The majority of gDNA will be contained in the layer of liquid just above the pellet, so pipette carefully to get as much liquid as possible without disturbing the debris.) Discard the tube contain the pelleted debris and use the supernatant to move forward for Part 2.

# Part 2: Ultra-HWM gDNA purification | Zymo Quick-DNA HWM MagBe 9 Set dry bath to \$\ 37 \cdot \cdot \) 10 Add \$\ \triangle 400 \ \mu L \ \triangle \cdot \

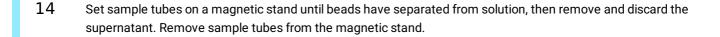
Note

12 Add Δ 33 μL 🛭 Zymo MagBinding Beads **Zymo Research Catalog #D4100-2-6** to each sample

### Note

MagBinding Beads settle quickly, so ensure beads are kept in suspension while dispensing by vortexing <u>each time</u> before beads are added to a sample

To ensure DNA binds to beads, mix on a rotator mixer at a low speed for 40 min at room temperature. Spin down briefly before proceeding with the next step



## Note

Some beads may adhere to the sides of the tube. When removing supernatant, aspirate slowly to allow these beads to be pulled to the magnet as the liquid level is lowered.

- Flick to mix initially, then mix on a rotator mixer at a low speed for 20 min. Spin down briefly before proceeding with the next step
- Set sample tubes on a magnetic stand until beads have separated from the solution, then remove and discard the supernatant. Remove sample tubes from the magnetic stand

## Note

Some beads may adhere to the sides of the tube. When removing supernatant, aspirate slowly to allow these beads to be pulled to the magnet as the liquid level is lowered.

- Add Δ 500 μL 🛭 Zymo DNA Pre-Wash Buffer **Zymo Research Catalog #D3004-5-250** to each sample
- 19 Flick to mix, then spin down briefly
- Set sample tubes on a magnetic stand until beads have separated from solution, then remove and discard the supernatant. Remove sample tubes from the magnetic stand

## Note

Some beads may adhere to the sides of the tube. When removing supernatant, aspirate slowly to allow these beads to be pulled to the magnet as the liquid level is lowered.

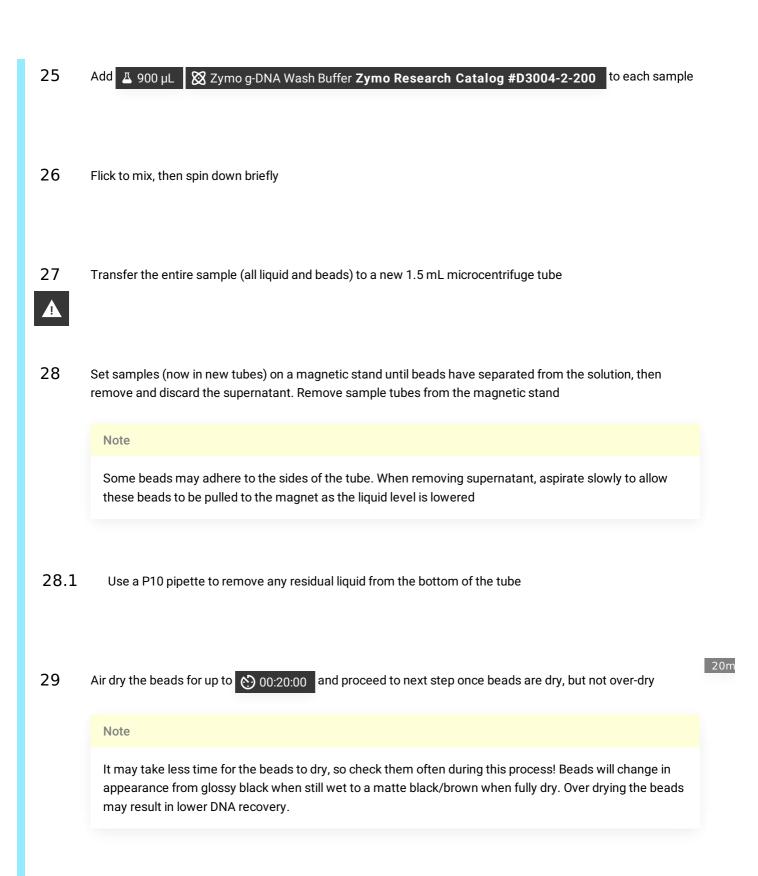
- Flick to mix, then spin down briefly
- Transfer the entire sample (all liquid and beads) to a new 1.5 mL microcentrifuge tube



Set samples (now in new tubes) on a magnetic stand until beads have separated from the solution, then remove and discard the supernatant. Remove sample tubes from the magnetic stand

## Note

Some beads may adhere to the sides of the tube. When removing supernatant, aspirate slowly to allow these beads to be pulled to the magnet as the liquid level is lowered

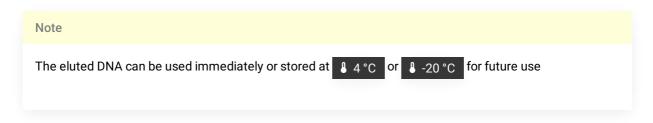


Add Δ 50 μL Symo DNA Elution Buffer **Zymo Research Catalog #D3004-4-50** to each sample and flick gently several times to mix. Spin down briefly





- 32 Incubate on bench top at § Room temperature overnight.
- After overnight incubation, set tubes on a magnetic stand until beads have separated from solution, then move the supernatant (now containing eluted DNA) to a new tube



- Use  $\underline{\mathsf{L}}_{1\,\mu\mathsf{L}}$  of final elution to quantify extraction via Qubit analysis
- 35 Use 1  $\mu$ L of final elution to assess fragment size distribution via TapeStation



# Part 3: DNA repair and end-prep | Zymo Clean & Concentrator, ONT L 36 Set dry bath to 65 °C Defrost the needed NEB DNA and End Repair reagents on ice (see Part 3 Step 38)

38 For each sample, add the following to a clean 0.2 mL PCR tube to create a master mix, pipetting 10-20 times between each addition to mix: X NEBNext® FFPE DNA Repair Buffer New England Biolabs Catalog #E7180S X NEBNext FFPE DNA Repair Mix - 96 rxns New England Biolabs Catalog #M6630L 🔀 NEBNext Ultra II End Prep Reaction Buffer New England Biolabs Catalog #E7647 X NEBNext Ultra II End Prep Enzyme Mix New England Biolabs Catalog #E7646 38.1 Keep master mix on ice 39 Add A 12 uL of master mix (prepared in Part 3 Step 38) from the PCR tube directly into each 1.5 mL microcentrifuge tube containing extracted & purified uHWM gDNA (from Part 2). Mix all components by gently flicking, and spin tubes down to recollect liquids 40 Incubate samples at | Room temperature | for **(?)** 00:10:00 10m 41 Incubate samples at \$\\ 65 \cdot \cdot \text{for } \cdot \cdot \cdot 00:10:00 42 Add 4 volumes of Zymo DNA MagBinding Buffer Zymo Research Catalog #D4012-1-50 sample and mix well by flicking and inverting Note Example for calculating 4 volumes: If input is 49 µL gDNA, add 196 µL DNA MagBinding Buffer 43 Spin samples down briefly and add 🗸 20 µL 🔯 Zymo MagBinding Beads **Zymo Research Catalog #D4100-5-2** Note MagBinding Beads settle quickly, so ensure beads are kept in suspension while dispensing by vortexing each time before beads are added to a sample

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20n

Mix samples on rotating mixer at a low speed at Room temperature for 00:20:00



- Briefly spin down samples and pellet on a magnetic stand (1–2 min) until the supernatant is clear and colorless. With the tubes still on the magnet, pipette off and discard the supernatant
- Add <u>Add</u> 500 µL Zymo DNA Wash Buffer **Zymo Research Catalog #D4003-2-24** and then remove from magnetic stand, and mix well by flicking and inverting
- Briefly spin samples down briefly and transfer to magnetic stand to allow beads to pellet until solution is clear (1–2 min). With the tubes still on the magnet, pipette off and discard the supernatant
- Air dry the beads for 00:10:00

10m

## Note

MagBinding Beads utilize a different chemistry than SPRI beads (e.g., AMPure XP beads) so there is not the same risk of over-drying. It is important for optimal elution that the residual buffer is <u>completely removed/evaporated</u> from the beads

- 49 Add Δ 50 μL 🛭 Zymo DNA Elution Buffer **Zymo Research Catalog #D3004-4-10**
- Manually agitate samples by gently flicking (and occasionally spinning down) for 00:10:00

10r



## Note

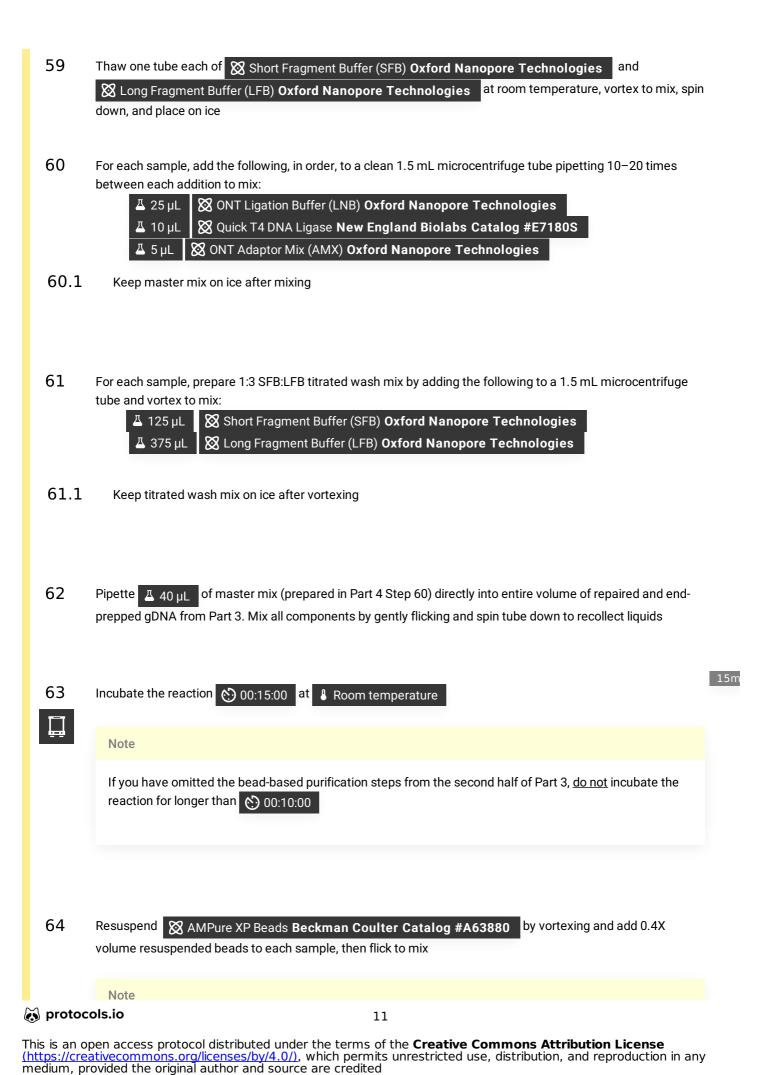
This volume is too small to be able to a the rotator mixer effectively at this step. Manually agitation is necessary



- Briefly spin samples down and pellet the beads on a magnet until the eluate is clear and colorless (1–2 min)
- Remove and retain the 50  $\mu$ L of eluate (containing repaired & end-prepped DNA) to a new clean 1.5 mL microcentrifuge tube
- Use A 1 uL of final elution to quantify via Qubit assay



- Set dry bath to 37 °C
- Remove AMPure XP Beads Beckman Coulter Catalog #A63880 from storage at them to come to Room temperature
- Spin down ONT Adaptor Mix (AMX) Oxford Nanopore Technologies and Quick T4 DNA Ligase New England Biolabs Catalog #E7180S and place on ice
- Thaw ONT Ligation Buffer (LNB) Oxford Nanopore Technologies at Room temperature, spin down, and mix by pipetting. Place on ice immediately after thawing and mixing
- Thaw Elution Buffer (EB) Oxford Nanopore Technologies at spin down, and place on ice



AMPure XP Beads settle quickly, so ensure beads are kept in suspension while dispensing by vortexing <u>each time</u> before beads are added to a sample

## Note

Example for calculating 0.4X volume: If input is 89  $\mu$ L (after adding master mix), add 35.6  $\mu$ L AMPure XP Beads

- Mix on a rotator mixer at a low speed for 01:00:00 at 8 Room temperature
- Spin down the sample and pellet on a magnetic stand. Keeping the tube on the stand, pipette off and discard the supernatant
- Wash the beads by adding 250 µL 1:3 SFB:LFB titrated wash mix (prepared in Part 4 Step 61). Flick the beads to resuspend, spin down, then return to the magnetic rack and allow the beads to pellet. Remove the supernatant using a pipette and discard
- Wash the beads by adding 250 µL 1:3 SFB:LFB titrated wash mix (prepared in Part 4 Step 61). Flick the beads to resuspend, spin down, then return to the magnetic rack and allow the beads to pellet. Remove the supernatant using a pipette and discard
- Spin down the beads and place them back on the magnetic rack. Use a P10 pipette to pipette of any residual liquid and allow beads to air-dry for 00:00:30 to 00:02:00

## Note

Do not allow the pellet to dry to the point of cracking! Over-drying beads will result in reduced yields

Remove the tube from the magnetic stand and resuspend the pellet in  $\frac{15 \, \mu L}{1}$ 

2m 30s

# 

Briefly spin down and incubate in dry bath at \$\ 37 \circ for \ 02:00:00 with occasional gentle flicking and spinning down throughout incubation

## Note

For HMW & uHMW gDNA, incubation at 37°C for longer times can improve the recovery of long fragments

2h

72 Incubate on the bench top at 8 Room temperature overnight



- 1. After overnight incubation, pellet the beads on a magnet until the eluate is clear and colorless (at least 00:01:00)
- 75 Use  $\underline{\mathsf{L}}_{1\,\mu\mathsf{L}}$  of final elution to quantify library via Qubit analysis

## Note

Note: For same-day or near-future sequencing, store the prepared library on ice (or at 4 °C) until ready to be loaded onto a flow cell. Otherwise, store libraries at -20°C