

VERSION 2

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WORKS FOR ME

Extraction and ONT MinION Library Preparation of uHMW gDNA V.2

DOI

dx.doi.org/10.17504/protocols.io.j8nlkww11l5r/v2

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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 chunk of tissue from an *Ascaris* sp. adult).

Protocols on which this workflow is based:

- Zymo® Quick-DNATM Magbead Plus Kit protcol
- Oxford Nanopore Technologies® SQK-LSK-109 gDNA Ligation Sequencing protocol
- Zymo® DNA Clean & ConcentratorTM Magbead Kit protocol (best-testing phase only)

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PROTOCOL CITATION

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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.

Part 1: Ultra-HWM gDNA extraction | Zymo Quick-DNA HWM MagBea

1 Set dry bath to \$\ 55 \cdot \cdot \)

2 For each sample, add the following to a clean 1.5 mL microcentrifuge tube to create a master mix:

Δ 95 μL
 Δ 2ymo DNA Elution Buffer Zymo Research Catalog #D3004-4-1
 Δ 95 μL
 Δ 2ymo Biofluid & Solid Tissue Buffer Zymo Research Catalog #D4081-3-25
 Δ 10 μL
 Δ 2ymo 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



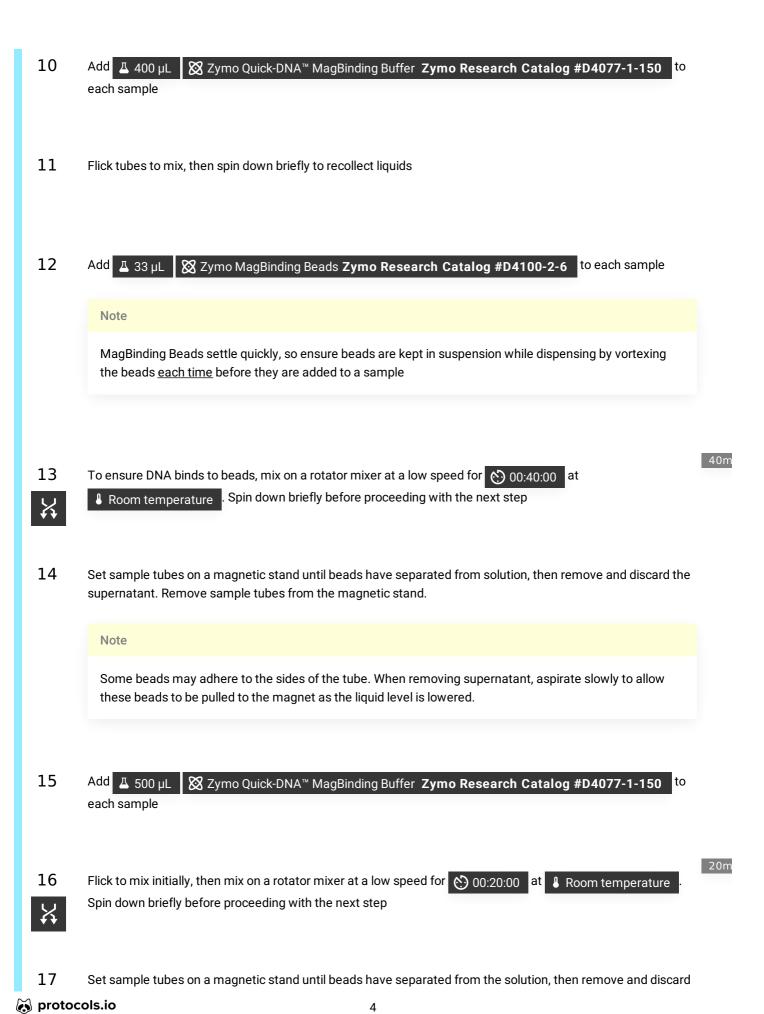
- 4 Use a new NEB Monarch Pestle NEB Catalog #T3002-1 to grind and crush the tissue in the tube. Keep the pestle in the tube
- Add \perp 200 μ L master mix (prepared in Part 1 Step 2) to each tube containing tissue and pestle
- 6 Continue using the pestle to grind the tissue within the master mix until 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

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 new 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 retained supernatant for Part 2.

Part 2: Ultra-HWM gDNA purification | Zymo Quick-DNA HWM MagBea 9 Set dry bath to 37°C

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Note



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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.

- 21 Add Δ 900 μL 🛭 Zymo g-DNA Wash Buffer **Zymo Research Catalog #D3004-2-200** to each sample
- 22 Flick to mix, then spin down briefly
- Transfer the entire sample (all liquid and beads) to a new clean 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

- 25 Add Δ 900 μL 🛭 Zymo g-DNA Wash Buffer **Zymo Research Catalog #D3004-2-200** to each sample
- Flick to mix, then spin down briefly
- 27 Transfer the entire sample (all liquid and beads) to a new clean 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. Leave sample tubes on the magnetic stand

Note

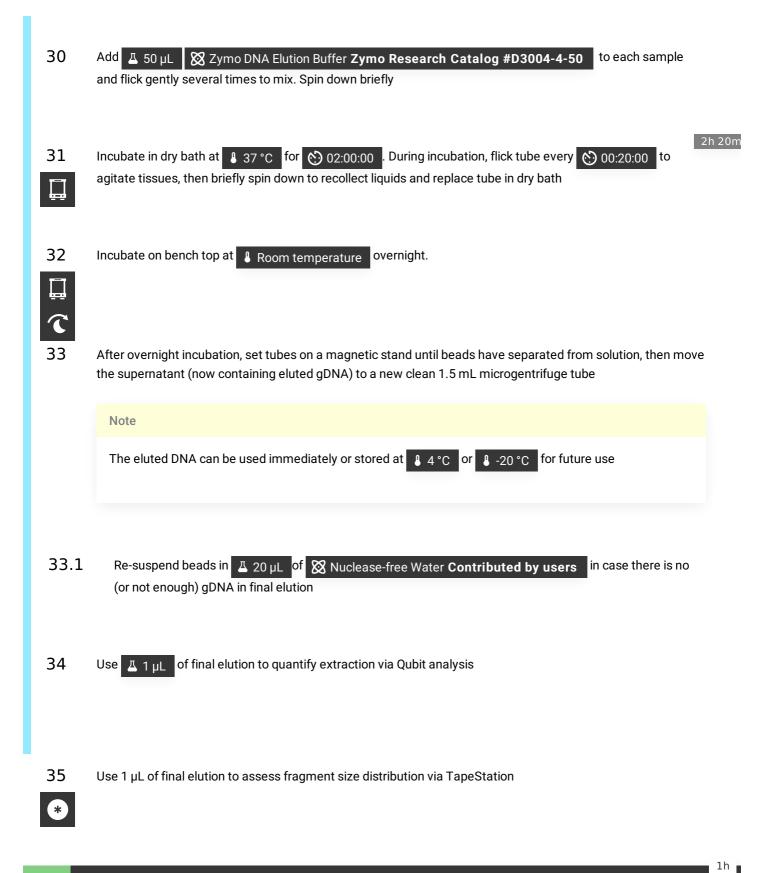
Some beads may adhere to the sides of the tube. When removing supernatant, aspirate slowly to allow these beads to be pulled to the magnet as the liquid level is lowered

- 28.1 Use a P10 pipette to remove any residual liquid from the bottom of the tube
- Air dry the beads for up to 00:20:00 and proceed to next step once beads are dry, but not over-dry

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Note

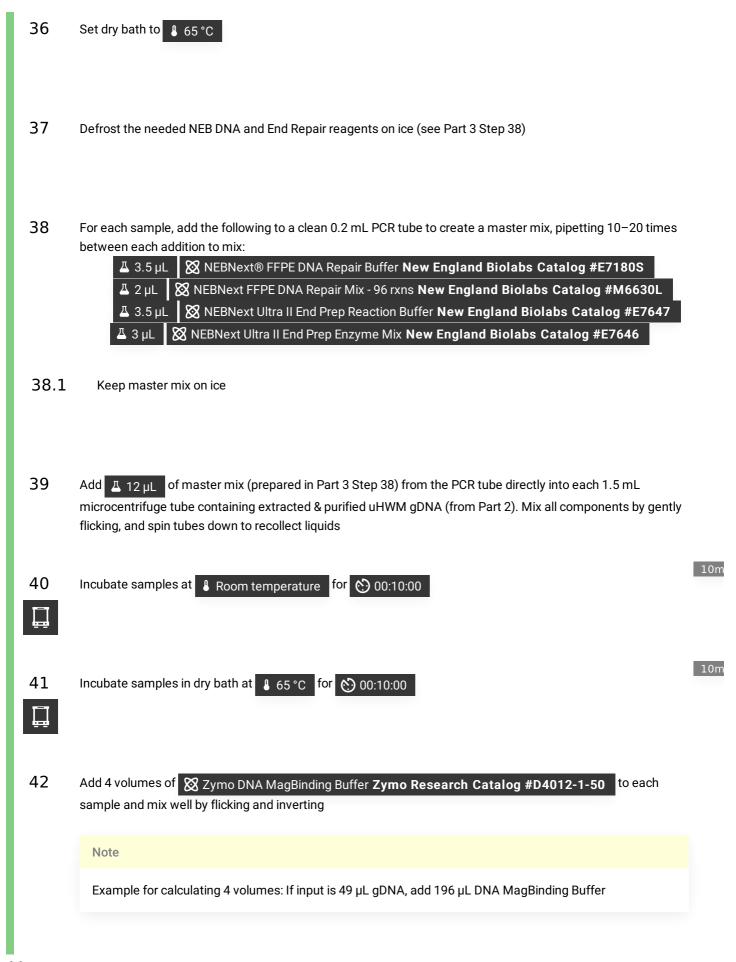
It may take less time for the beads to dry, so check them often during this process. Beads will change in appearance from glossy black when still wet to a matte black/brown when fully dry. Over drying the beads may result in lower gDNA recovery.



Part 3: DNA repair and end-prep | Zymo Clean & Concentrator, ONT L



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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 beads <u>each time</u> before they are added to a sample	
44	Mix samples on rotating mixer at a low speed at Room temperature for 00:20:00	201
45	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	
46	Add A 500 µL Symo DNA Wash Buffer Zymo Research Catalog #D4003-2-24 and then remove from magnetic stand, and mix well by flicking and inverting	
47	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	
48	Add A 500 µL S Zymo DNA Wash Buffer Zymo Research Catalog #D4003-2-24 and then remove from magnetic stand, and mix well by flicking and inverting	
49	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	
50	Air dry the beads for 00:10:00	10

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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 completely removed/evaporated from the beads

51 Add 🚨 50 µL 🛛 🔀 Zymo DNA Elution Buffer **Zymo Research Catalog #D3004-4-10**



Manually agitate samples for 00:10:00 by gently flicking/inverting (and occasionally spinning dow to recollect liquids)

Note

This volume is too small to be able to use most rotator mixers effectively, so manually agitation is necessary

- 53 Briefly spin samples down and pellet the beads on a magnet until the eluate is clear and colorless (1-2 min)
- 54 Remove and retain the 🔼 50 µL of eluate (containing repaired & end-prepped DNA) to a new clean 1.5 mL microcentrifuge tube
- 55 Use I 1 uL of final elution to quantify via Qubit assay

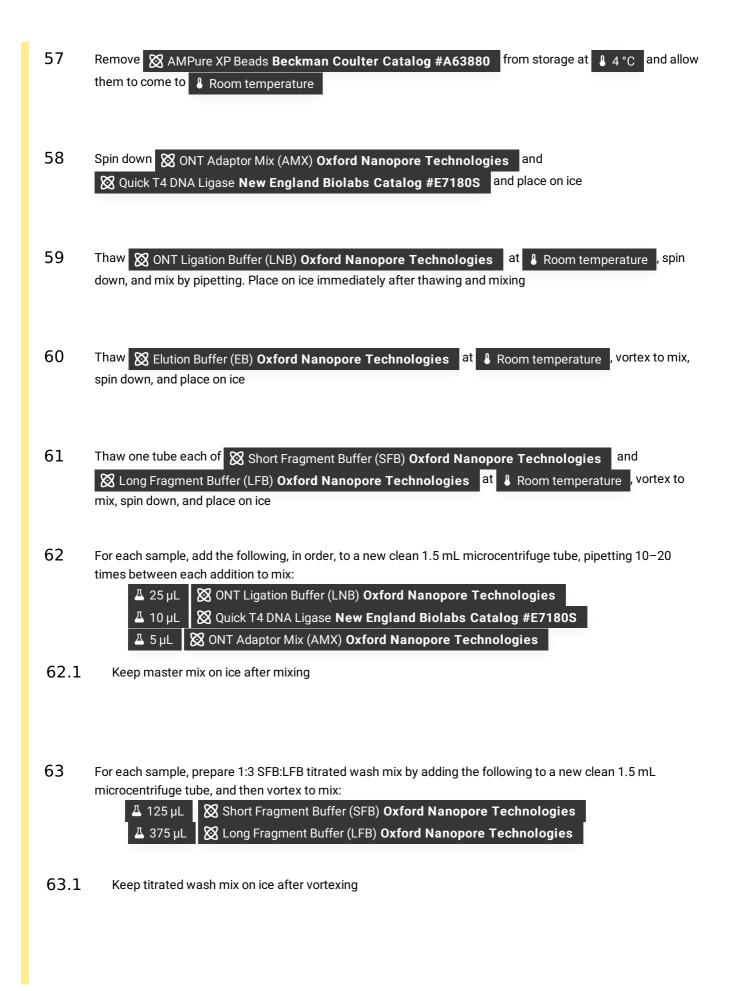
Part 4: Adaptor ligation and clean up | ONT Ligation Sequencing & NE

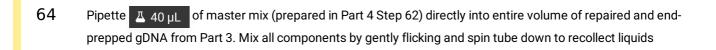
56 Set dry bath to 37 °C



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Note

If you have omitted the bead-based purification steps from the second half of Part 3, <u>do not</u> incubate the reaction for longer than 00:10:00

Resuspend AMPure XP Beads Beckman Coulter Catalog #A63880 by vortexing and add 0.4X volume resuspended beads to each sample, then flick to mix

Note

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

Note

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

- Mix on a rotator mixer at a low speed for 01:00:00 at 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 Z 250 µL 1:3 SFB:LFB titrated wash mix (prepared in Part 4 Step 63). 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 \pm 250 μ L 1:3 SFB:LFB titrated wash mix (prepared in Part 4 Step 63). 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

2m 30s

Note

Do not allow the pellet of beads 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 beads in Δ 15 μL
- Elution Buffer (EB) Oxford Nanopore Technologies
- Briefly spin down and incubate in dry bath at 37 °C for 02:00:00 During incubation, flick tube every 00:20:00 to agitate tissues, then briefly spin down to recollect liquids and replace tube in dry bath

Note

For HMW & uHMW gDNA, incubation at \$\ 37 \circ for longer times can improve the recovery of long fragments

74 Incubate on the bench top at 8 Room temperature overnight



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1. After overnight incubation, pellet the beads on a magnet until the eluate is clear and colorless (at least 1 min)

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- Remove and retain the $_{15\,\mu L}$ of eluate (containing the prepared library) to a new clean 1.5 mL microcentrifuge tube
- 77 Use $\underline{\text{A}}_{1 \, \mu L}$ 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 to be loaded onto a flow cell. Otherwise, store libraries at -20 °C until ready