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ONT Library Prep for Whole Genome Sequencing

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We use this protocol and it's working

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Abstract

This protocol outlines the preparation genomic DNA for whole genome sequencing using Oxford Nanopore platform.

Guidelines

Longer incubation with AMPure beads leads to higher yield.

Materials

REAGENTS

	Item	Supplier	Part Number	Notes
	Ligation Sequencing Kit V14	Oxford Nanopore Technologies	SQK-LSK114	The Short Fragment Buffer (SFB) will NOT be used because it selects for fragments that are 3kb and longer.
	NEBNext® Companion Module for Oxford Nanopore Technologies® Ligation Sequencing	New England BioLabs	E7180	
	AMPure® XP Reagent	Beckman Coulter	A63880 (5 mL) A63881 (60 mL)	
	Nuclease-Free Water	Sigma-Aldrich	W4502	Or equivalent nuclease-free water.
	Ethyl Alcohol, Pure	Sigma-Aldrich	459844	Or equivalent 100% non-denatured ethanol.

EQUIPMENT

	A	B	C	D
	Thermomixer	Various Suppliers	Varies	Compatible with 1.5 mL tubes.
	Microcentrifuge	Various Suppliers	Varies	Compatible with 1.5 mL tubes.
	Single Channel Pipettes: P2, P20, P200, P1000	Various Suppliers	Varies	
	Vortex-Genie 2	Scientific Industries	SI-0236	Or an equivalent vortex mixer.
	6-Tube Magnetic Separation Rack	New England Biolabs	S1506S	Or an equivalent magnetic rack for 1.5 mL tubes.
	Qubit Flex Fluorometer	Thermo Fisher Scientific	Q33327	Or an equivalent fluorometer.

	A	B	C	D
	HulaMixer™ Sample Mixer	Thermo Fisher Scientific	15920D	

SUPPLIES

	A	B	C	D
	Pipette Tips TR LTS 10 µL, 20 µL, 200 µL, 1,000 µL	Rainin	17014961 17014963 17014967	Or appropriate sterile, DNA low-binding, and filtered pipette tips. We do not recommend using wide bore tips.
	DNA LoBind® Tubes, 1.5 mL, Snap Cap	Eppendorf	022431021	Or equivalent DNA low-binding, nuclease-free 1.5 mL tubes.
	Qubit dsDNA HS (High Sensitivity) Assay Kit	Thermo Fisher Scientific	Q33230 (100 assays) Q33231 (500 assays)	Or equivalent fluorescent DNA dye based quantification kit.
	Ice bucket	Various Suppliers	Varies	

Preparation

1 **Prepare the following:**

1.1 For each sample: Adjust 1.4 µg of DNA volume to 47 µL with **Nuclease-free water** in a 1.5 mL tube and mix using a wide-bore tip.

1.2 Prepare fresh **80% ethanol** for the wash steps. Each sample will need 400 µL.

1.3 Set two heat blocks to 65 °C and 37 °C .

End-prep

2 **End-prep Reaction**

2.1 In a 1.5 mL tube, mix the following for each sample:

	Item	Volume (µL)
	DNA CS (optional)	1
	DNA sample	47
	Ultra II End-prep Reaction Buffer	3.5
	Ultra II End-prep Enzyme Mix	3
	NEBNext FFPE DNA Repair Buffer	3.5
	NEBNext FFPE DNA Repair Mix	2

If you do not use DNA CS, add 1 µL of Nuclease-free water instead.

2.2 Thoroughly mix by pipetting using a wide-bore tip and avoid creating bubbles.

2.3 Incubate at Room temperature for 00:05:00 on bench top and 65 °C for 00:05:00 on thermomixer. 10m

3 **Clean-Up**

3.1 Vortex AMPure XP Beads (AXP).

3.2 Add 60 µL of the resuspended **AMPure XP Beads (AXP)** to the end-prep reaction(s).

3.3 Incubate tube(s) on the Hula mixer 10 rpm, Room temperature , 00:05:00 .

3.4 Spin down the tube(s) and pellet on a magnet.

3.5 Keep the tube(s) on the magnet and pipette off the supernatant.

3.6 Keep the tube(s) on the magnet and wash the beads with 200 µL of freshly prepared **80% ethanol** without disturbing the pellet.

3.7 Remove the ethanol using a pipette and discard without disturbing the pellet.

3.8 Keep the tube(s) on the magnet and wash the beads with 200 µL of freshly prepared **80% ethanol** without disturbing the pellet (again).

3.9 Remove the ethanol using a pipette and discard without disturbing the pellet (again).

3.10 Allow to dry for ~30 seconds, but do NOT dry the pellet to the point of cracking.

3.11 Remove tube(s) from magnet and resuspend the pellet in 61 µL **Nuclease-free water**.

3.12 Incubate tube(s) for minimum 00:10:00 at 37 °C . 10m

3.13 Pellet the beads on a magnet.

3.14 Remove and retain 60 µL of eluate into a new 1.5 mL tube(s).

Adapter Ligation 20m

4 **Adapter ligation reaction**

4.1 In the same 1.5 mL tube, mix in the following order for each sample:

	Item	Volume (µL)
	DNA sample (from the previous step)	60
	Ligation Adapter (LA)	5
	Ligation Buffer (LNB)	25
	NEBNext Quick T4 DNA Ligase	10

4.2 Thoroughly mix by gently pipetting using a wide-bore tip and avoid creating bubbles.

4.3 Incubate tube(s) at Room temperature for 00:10:00 . 10m

5 **Clean-up**

5.1 Vortex AMPure XP Beads (AXP).

5.2 Add 40 µL of the resuspended **AMPure XP Beads (AXP)** to the reaction(s).

5.3 Incubate tube(s) on the Hula mixer at 10 rpm, Room temperature , 00:05:00 .

5.4 Spin down the tube(s) and pellet on a magnet.

5.5 Keep the tube(s) on the magnet and pipette off the supernatant.

5.6 Remove tube(s) from magnet and wash the beads by adding 250 µL **Short Fragment Buffer (SFB)**.

5.7 Resuspend the beads by pipetting with wide-bore tip.

5.8 Pellet the beads on a magnet and pipette off the supernatant.

5.9 Remove tube(s) from magnet and wash the beads by adding 250 µL **Short Fragment Buffer (SFB)** (again).

5.10 Resuspend the beads by pipetting with wide-bore tip (again).

5.11 Pellet the beads on a magnet and pipette off the supernatant (again).

5.12 Allow to dry for ~30 seconds, but do NOT dry the pellet to the point of cracking.

5.13 Remove tube(s) from magnet and resuspend the pellet in 32 µL **Elution Buffer (EB)**.

5.14 Incubate for minimum 00:10:00 at 37 °C 10m

5.15 Pellet the beads on a magnet.

5.16 Remove and retain 32 µL of eluate into a new 1.5 mL tube(s).

5.17 Qubit each library and keep on ice until loading or store in -20°C.