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Whole genome amplification and long read sequencing using ONT V.2

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We use this protocol and it's working

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Abstract

A genome reference is a prerequisite for a complete understanding of the biology and evolution of a species. However, the major challenge remains to obtain high-quality DNA and RNA from the majority of organisms. Therefore, there is a need for having a protocol to bypassed the challenging stage of obtaining axenic cultures and limited the amount of DNA material from a limit individual. This protocol build on whole genome sequencing single nematode. With multiple displacement amplification (MDA) allows the genome from a single nematode to be amplified and can sequence with both long- and short-read sequencing. This protocol can be completed within two week including genome amplification and sequencing.Also, combines MDA and Oxford Nanopore sequencing and provides a cost- and labor-effective solution to generate complete assemblies in organisms with as little as 50 picograms of starting material and assemble a draft genome assembly.

Guidelines

Version 2 correct the steps in T7 endonuclease I digestion.

Materials

REAGENTS
22 gauge needle
0.2ml PCR tube
DNA LoBind Tubes, Eppendorf, #EP0030108078

- 1X PBS (Phosphate-buffered saline)
- REPLI-g Single Cell Kit **Qiagen** **Catalog #**150345
- AMPure XP **Bechman Coulter** **Catalog #**A63882
- T7 Endonuclease I - 1,250 units **New England Biolabs** **Catalog #**M0302L
- Qubit dsDNA HS Assay Kit **Thermo Fisher Scientific** **Catalog #**Q32854
- Ligation Sequencing Kit **Oxford Nanopore Technologies** **Catalog #**SQK-LSK109
- Flow Cell Priming Kit **Oxford Nanopore Technologies** **Catalog #**EXP-FLP002
- NEBNext Quick Ligation Module - 100 rxns **New England Biolabs** **Catalog #**E6056L
- NEBNext FFPE DNA Repair Mix - 96 rxns **New England Biolabs** **Catalog #**M6630L
- NEBNext Ultra II End Repair/dA-Tailing Module - 96 rxns **New England Biolabs** **Catalog #**E7546L

EQUIPMENT
Magnetic separator
Vortex mixer
Microfuge
Thermal cycler
Rotator mixer
Magnetic separator
Qubit
Gridion

[Optional] extraction and denature of genomic DNA

1 Prepare DLB Lysis buffer:
33 µL REPLI-g DLB buffer
3 µL 1M DTT

2 Add 4 µL PBS buffer in 0.2ml PCR tube.

3 Transfer worm to the 0.2ml PCR tube with 4 µL PBS buffer.

Note

[Optional] You may cut the worm with 22 gauge needle. This may release the cells from the cuticles

4 Add 3 µL DLB Lysis buffer. Incubate sample on thermocycler at 65 °C for 00:10:00 10m

5 Add 3 µL of REPLI-g Stop Solution. Store the sample on ice.

Whole genome amplification 8h 38m 30s

6 Prepare REPLI-g polymerase master mix
9 µL Nuclease-free water
29 µL REPLI-g Reaction Buffer
2 µL REPLI-g DNA Polymerase

Note

The difference of these kits is the final amount of expected amplified templates from the polymerase master mix (SC 40 µg, midi 40 µg, and mini 10 µg with 10ng DNA). We initially started with SC, but were only able to obtain midi and mini kits during the COVID pandemic. We have inserted this explanation and option in the publicised protocol.

7 Add 40 µL REPLI-g polymerase master mix to each reaction. 8h 3m

Note

set heated lid temperature to 70 °C)

Incubate sample at 30 °C for 08:00:00 .
Inactivate reaction at 65 °C for 00:03:00 .
Store amplified DNA at 4 °C .

8 Dilute 1 µL amplified DNA to 100X in dH2O.
Take 2 µL diluted amplified DNA for quantification with Qubit dsDNA High sensitivity assay.

9 Warm AMPure XP beads to Room temperature .
Resuspend the AMPure XP beads by vortexing.

10 Transfer the sample to a 1.5 ml Microtubes.
Add 90 µL of resuspended AMPure XP beads to the amplification reaction and thoroughly mixed.

11 Incubate the sample on a Tube Revolver for 00:10:00 at Room temperature . 10m

12 Keep the tube on the magnet until eluate is clear and colorless, and pipette off the supernatant. 30s
Wash the beads with 200 µL of freshly prepared [M] 70 % (v/v) ethanol for 00:00:30 and remove the ethanol using a pipette and discard.

Spin down and place the tube back on the magnet. Pipette off any residual ethanol.

13 Allow to dry for 00:05:00 5m

> **Note**
>
> Do not dry the pellet to the point of cracking.

14 Remove the tube from the magnetic rack and resuspend pellet in 30 µL Nuclease-free water. Incubate for 00:20:00 at Room temperature . 20m

15 Spin down and place the tube back on the magnet until the eluate is clear and colorless. Remove and retain 30 µL of eluate in a clean 1.5 ml Microtube.

16 Dilute 1 µL purified amplified DNA to 100X in dH2O.
Take 2 µL diluted purified amplified DNA for quantification with Qubit dsDNA High sensitivity assay.

T7 endo I digestion 1h 15m 30s

17 For each reaction, mix the reagents in the following order in a clean 0.2 ml PCR tube. Add nuclease-free water until final volume of 25 µL . Mix gently by flicking the tube, and spin down.

	A	B
	1.5 µg (Xµl)	amplified DNA
	3µl	NEBuffer 2
	1.5µl	T7 Endonuclease I
	25-Xµl	Nuclease-free water

18 Incubate at 37 °C for 00:30:00 in thermal cycler. 30m

19 Make up the amplified DNA sample to a total volume of 50 µL with Nuclease-free water.

20 Transfer the sample to a clean 1.5 ml Microtubes.

21 Resuspend the AMPure XP beads by vortexing. 10m
Add 30 µL of resuspended AMPure XP beads to the amplification reaction and thoroughly mixed. Incubate the sample on a Tube Revolver for 00:10:00 at Room temperature.

22 Keep the tube on the magnet until eluate is clear and colorless, and pipette off the supernatant.

23 Wash the beads with 200 µL of freshly prepared [M] 70 % (v/v) ethanol for 30s
00:00:30 and remove the ethanol using a pipette and discard. Spin down and place the tube back on the magnet. Pipette off any residual ethanol.

24 Repeat step 21 again.

25 Allow to dry for 00:05:00, but do not dry the pellet to the point of cracking. 5m

26 Remove the tube from the magnetic rack and resuspend pellet in 49 µL Nuclease-free water. Incubate for 20 minutes at RT. Spin down and place the tube back on the magnet until the eluate is clear and colorless.

27 Remove and retain 49 µl of eluate in a clean 1.5 ml microtubes.

28 Take 1 µl purified DNA for quantification with Qubit dsDNA High sensitivity assay.

ONT library prep and ONT sequencing

29 ONT library prep were followed ONT Ligation sequencing gDNA (SQK-LSK109) protocol.