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Long-read DNA preparation for bacterial isolates.

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1 Works for me dx.doi.org/10.17504/protocols.io.64ghgtw

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ABSTRACT

General:

This is an optimized DNA isolation protocol adapted to the properties of bacteria isolated from biogas substrate and digestate, using NucleoBond® AXG Columns and NucleoBond® Buffer Set. The protocol reliably retrieves DNA of sufficient quality, length and yield. The performance has been validated on seven bacterial isolates from one biogas reactor and one biogas plant. Buffers and proteinase K can be found in NucleoBond Buffer Set III.

Observed Performance on seven isolates runs using this protocol:

N50 Ranges between 22,000 and 46,000 bp. General throughput is between 16-22 Gigabases of a single flowcell (flowcell 106 Rev D, using the LSK-109 Kit).

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MATERIALS

NAME	CATALOG #	VENDOR
Agencourt AMPure XP	A63880	Beckman Coulter
Ethanol		
Lysozyme	L6876	Sigma Aldrich
NucleoBond AXG 20 Columns	740544	Macharey Nagal

NAME	CATALOG #	VENDOR
NucleoBond Buffer Set III	740603	Macharey Nagal

Samples preparation

1 Add cation-adjusted Müller-Hinton broth in a 15 ml Falcon tube. (Müller-Hinton medium is used here for antibiotic-resistant bacteria)
5 ml CAMHB

2 Add 1/4 10 µl-loop of colony from an agar plate in the solution.

3 Vortex the mixture for 5 s at maximum speed.

4 Incubate the mixture overnight on a shaking bench at the speed of 120 rpm at 37 °C.
120 rpm **37 °C**

Cell disruption

5 Add 2 ml of the incubated mixture in a 2.5 ml Eppendorf tube.

6 Centrifuge the mixture.
5000 x g **20 °C** **00:10:00**

7 Discard the supernatant. (For anaerobes, due to low concentration of bacterial culture, it is suggested to repeat step 5 to step 7 several times in order to obtain enough amount of DNA)

8 Resuspend the bacterial pellet in Buffer G3 by vortexing.
1 ml Buffer G3

9 Add lysozyme and Proteinase K
20 µl lysozyme (100 mg/ml) **25 µl Proteinase K**

10 Gently resuspend the mixture and incubate at 37 °C for 20 min without shaking.
37 °C **00:20:00**

11 Add Buffer G4
400 µl Buffer G4

12 Gently resuspend the mixture and incubate at 50 °C for 30 min without shaking.
50 °C **00:30:00**

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13 Centrifuge the mixture.
5000 x g **20 °C** **00:05:00**

14 Transfer the supernatant to a 5 ml Eppendorf tube.

Equilibration

15 Equilibrate the column (AXG 20) with Buffer N2.
1 ml Buffer N2

Binding

16 Add Buffer N2 (room temperature) to the sample. Vortex the mixture for 15 s at maximum speed.
1 ml Buffer N2

17 Load 1 ml of the mixture on the column. Allow it to enter the resin by gravity flow.

18 Reload 1 ml of the mixture on the column. **Do not** reload if the mixture of former step has not been filtered completely in case the column gets clogged.

19 Reload the rest mixture.

Wash

20 Wash the column with Buffer N3.
1 ml Buffer N3 three times

Elution

21 Elute the genomic DNA with Buffer N5. Collect the DNA solution in a 2.5 ml Eppendorf tube.
1 ml Buffer N5

The average concentration of DNA is approximately 10 µg/ml.
Store at 4 °C if you want to stop here
4 °C Do not freeze

Cleaning & condensing

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22 Add magnetic Beads (0.35 volume, AMPure) to the DNA sample. Gently resuspend by hand.
350 µl Magnetic beads
1000 µl DNA sample

23 Incubate the mixture.
00:10:00
20 °C Room temperature

24 Spin down and let the beads bind to magnetic plate until the liquid is clear.

25 Stay on the magnet and remove supernatant.

26 Wash with Ethanol. Do not disturb pellet.
150 µl Ethanol (80%)

27 Remove supernatant.

28 **Redo the washing once**

29 Spin down and let the beads bind to magnetic plate again.
Remove the residual liquid with a 10 µl pipette.

30 Add nuclease free water and resuspend gently.
55 µl Nuclease free water

31 Incubate the DNA solution.
00:15:00
37 °C

32 Spin down and put on the magnetic plate.
Retrieve the supernatant (DNA) with a 10 µl pipette.

Determine dsDNA concentration e.g. by Qubit.
Determine DNA length by Agarose gel or Bioanalyzer.

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