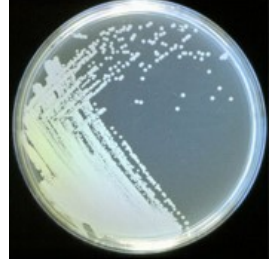


Jun 12, 2020

🌐 Long-read DNA preparation for bacterial isolates.

DOI

dx.doi.org/10.17504/protocols.io.64ghgtw



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DOI: dx.doi.org/10.17504/protocols.io.64ghgtw

Protocol Citation: He Sun, Christian Brandt, Anna Schnürer 2020. Long-read DNA preparation for bacterial isolates.. [protocols.io https://dx.doi.org/10.17504/protocols.io.64ghgtw](https://dx.doi.org/10.17504/protocols.io.64ghgtw)

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Protocol status: Working

We use this protocol and it's working

Created: September 04, 2019

Last Modified: June 12, 2020

Protocol Integer ID: 27496

Keywords: bacterial isolate, Long-read DNA,

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).

Funding:

This work was funded by the China Scholarship Council (CSC) [Grant No. 201606350190] and SLU.

Materials

MATERIALS

 Agencourt AMPure XP **Beckman Coulter Catalog #A63880**

 Ethanol

 Lysozyme **Merck MilliporeSigma (Sigma-Aldrich) Catalog #L6876**

 NucleoBond AXG 20 Columns **Macharey Nagal Catalog #740544**

 NucleoBond Buffer Set III **Macharey Nagal Catalog #740603**



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

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

Note

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


- 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  [go to step #26](#) Redo the washing once

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

30 Add nuclease free water and resuspend gently.

 55 μ L 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 μ l pipette.

Note

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