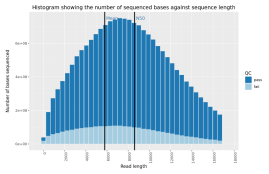
Nov 19, 2019 Version 2

Long-read DNA preparation for metagenomic samples V.2



DOI
dx.doi.org/10.17504/protocols.io.5feg3je

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OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.5feg3je

Protocol Citation: Christian Brandt 2019. Long-read DNA preparation for metagenomic samples. **protocols.io** **https://dx.doi.org/10.17504/protocols.io.5feg3je**

Manuscript citation:
https://www.researchsquare.com/article/d9fdc53c-a0a7-44a1-bce4-4d1fafa60f9e

Protocol status: Working
We use this protocol and it's working

Created: July 12, 2019

Last Modified: November 19, 2019

Protocol Integer ID: 25798

Keywords: sludge, biogas, substrate, digestate, manure, metagenome, DNA isolation, ONT

Abstract

General:
This is an DNA isolation protocol adapted to the properties of sludge samples from biogas and wastewater treatment plants. The protocol reliably retrieves metagenomic DNA of sufficient quality, length and yield. The performance has been validated on 20 different reactor plants.

Observed Performance on 20 ONT runs using this protocol:
It does not negatively impact the overall throughput for Nanopore Sequencing. Sequencing runs were active over 2.5 days, with one refuel step after 18 h.
N50 Ranges between 4000 and 8000 bp, depending on the sample type. E.g. frozen samples have a lower N50, fresh samples a higher N50. General throughput is between 18-28 Gigabases of a single flowcell (flowcell 106 Rev D, using the LSK-109 Kit).

Funding:
This work was funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) - BR 5692/1-1 and BR 5692/1-2

Materials

MATERIALS

- RNase A **Qiagen** **Catalog #**19101
- Ethanol
- Guanidine thiocyanate **Bio Basic Inc.** **Catalog #**GB0244.SIZE.1Kg
- Agencourt Ampure XP **Beckman Coulter** **Catalog #**A63880
- FastDNA Spin Kit for Soil **MP Biomedicals**

Before start

Prepare HA-wash solution (12 uses)

1.) Add and mix the following from the FastDNA Kit:

4.564 mL sodium phosphate buffer

0.568 mL MT buffer

1.166 mL PPS

2.) Vortex at full speed for 2 min

3.) Add 6.3 mL 5.5 M guanidine thiocyanate

4.) Vortex again.

Vortex **HA-wash solution** for 2 min before use

Get a ice bucket

Sample pre preparation

1 Add 1 ml of homogenic sludge/substrate via 10 ml syringe (without needle) to a 2 ml tube

1 mL sludge/substrate

2 Centrifuge for 5 min at 20,000g and remove supernatant

00:05:00

3 Resuspend in 400 µl nuclease free water

400 µL nuclease free water

Cell Lysis

4 Transfer sample to lysing matrix E tube and add 778 µl sodium phosphate buffer

778 µL sodium phosphate buffer

5 Add 122 µl MT buffer, homogenize tube by hand

122 µL MT buffer

6 Homogenize via FastPrep®-24 Classic I. for 20 s at 6 m/s and **put directly on ice** after lysis.

00:00:20

Equipment

MP Biomedicals™ FastPrep -24™ Classic Instrument	NAME
Benchtop homogenizer	TYPE
Fisher Scientific	BRAND
12079310	SKU
https://www.fishersci.co.uk/shop/products/mp-biomedicals-fastprep-24-instrument/12079310	LINK

Note

The device may strongly influence your expected total yield and read lengths. Try different times and strengths first if you are using another device and analyse your DNA length distribution at the end, e.g. by agarose gel or Agilent Bioanalyzer, and determine the best settings for your device.
6 m/s corresponds to 3700 rpm, according to the manufacturer

Precipitation and Binding

7 Centrifuge for 15 min at 4 °C with 14,000g

00:15:00

4 °C

8 Transfer supernatant to a clean 2 ml tube

9 Add 1 µl RNase A to each sample and incubate for 5 min

1 µL RNase A

00:05:00

10 Add 250 µl PPS (protein precipitation solution) and mix carefully but efficiently by inverting at least 10 times

250 µL PPS

11 Centrifuge at 14,000 g for 10 min at 4 °C

00:10:00

4 °C

12 Transfer supernatant to clean 5 ml tube

13 Resuspend binding matrix suspension and add 1 ml binding matrix suspension to the 5 ml tube

1 mL binding matrix suspension

14 Invert by hand for 2 min (avoid "matrix pellet" here)
Place on a rack for 0.5 to 1 h

00:02:00

00:30:00 or up to 1 hour

15 Discard 650 µl supernatant without disturbing the binding matrix

Washing

16
- Transfer approx. 600 µl of the mixture to a spin filter and centrifuge at 14,000g for 1 min

00:01:00
- Empty the catch tube and repeat the steps until all the binding matrix is in the spin filter
- Empty the catch tube.

17
- Add 500 µl HA-wash solution and gently resuspend the matrix by stirring using a pipette tip
- Centrifuge at 14,000 g for 1:00min, empty catch tube

500 µL HA-wash solution

00:01:00

Note

Slightly stir with the pipette tip, do not overdo resuspending, as well for the next step

18
- Add 500 µl SEWS-M and gently resuspend the pellet using a pipette tip
- Centrifuge at 14,000 g for 1:00 min, replace the catch tube

500 µL SEWS-M

00:01:00

19
- Centrifuge a second time at 14,000 g for 2 min to dry the membrane
- Discard the catch tube and add a new clean 1.5 ml tube

00:02:00

20 Air-dry the spin-filter for 5 min at room temperature

00:05:00

20 °C (room temperature)

DNA retrival

21 Add 90 µl 55 °C warm nuclease-free water to the spin filter and gently stir the binding matrix slightly with a pipette, then incubate for at least 5 min

90 µL 55 °C warm nuc. free water

00:05:00 at least

22 Centrifuge at 14,000 g for 1 min to get the eluate

00:01:00

Note

Store at 4 °C if you want to stop here

4 °C don't freeze

Pre Cleaning

23 Add 31.5 µl magnetic beads (0.35 volume, e.g. AMPure or HighPrep) to 90 µl of DNA sample,
shake gently by hand
90 µL DNA sample
31.5 µL magnetic beads

Note

Around 500 ng/µl DNA is reduced to around 250 ng/µl after cleaning.
Alternatively, take less DNA volume and add water if the concentration is over 500 ng/µl

24 Incubate for 10 min at room temperature
00:10:00
20 °C room temperature

25 Spin down and **bind to magnet** until the liquid is clear,

26 Stay on the magnet and remove supernatant

27 **(wash 1/2)** Add 150 µl Ethanol, don't disturb pellet
150 µL Ethanol (80 %)

28 **(wash 2/2)** Remove supernatant

29 go to step #27 and redo the washing once

30 Spin down, bind to magnet again.
Remove the residual liquid **with a 10 µl pipette**

31 Add 30 µl nuclease free water and resuspend gently
30 µL nuclease free water

32 Incubate for 10 min at room temperature

00:10:00

20 °C room temperature

33 Spin down and put on magnetic

34 Retrieve the supernatant with your DNA

Note

Determine dsDNA concentration e.g. via Qubit.
Determine DNA length via agarose gel or Bioanalyzer.

Expected result

You should usually lose around half of your total DNA during this process.