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Genomic DNA extraction from anaerobic digester samples

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1 *Works for me* This protocol may be deleted by the owner

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GUIDELINES

Genomic DNA extraction from anaerobic digester samples

MATERIALS

NAME	CATALOG #	VENDOR
FastDNA Spin Kit for Soil		MP Biomedicals
FastPrep-24 Homogenizer	116004500	MP Biomedicals
Guanidine Thiocyanate (MW - 118.16 g/mol)	G9277	Sigma Aldrich

Kit

1 Genomic DNA extraction from anaerobic digester samples using FastDNA

FastDNA™ SPIN Kit for Soil from MP Biomedicals (https://www.mpbio.com/116560000-fastdna-spin-kit-for-soil-samp-cf)

Cell lysis

2

In Lysing matrix E, weigh **250 mg** solid/semi-solid sample **OR** add **200 µl** of liquid sample

Lysing matrix E used for the cell lysis

*** Label** the lysing matrix tube **on the side** of the tube and not on the top of the cap (chances of label being disappear during the process)

3 **Make lysis master Mix**

Select carefully Sodium phosphate buffer and MT buffer from the kit and vortex them.

A. Sodium phosphate buffer (SPB)

B. Sodium phosphate buffer (SPB)

Calculation for lysis master mix

X = number of sample, Y = extras/reserve samples
A = Volume of SPB = 978 µl, B = Volume of MTB = 122 µl

Master mix

Final volume of SPB = A * (X + Y)
Final volume of MTB = B * (X + Y)

In a tube (according to requirement) add Final volume of SPB and Final volume of MTB and mix well by gentle vortexing.

4 Add 1 ml of lysis master mix to the Lysing matrix containing samples. Recap the lysing tube cap tightly.

5 Lyse the cell by bead beating process for 40 seconds at the speed of 6.0 in FastPrep-24™ homogenizer.

FastPrep-24™ homogenizer

Centrifugation

6 Centrifuge at 14,000 g for 10-15 min

7 Meanwhile the samples are in centrifuge, prepare for the next step, label the 2 ml centrifuge tube and add **250 µl** of **PPS buffer** in each empty tubes.

PPS buffer

8 Carefully take out the lysing tube from the homogenizer and collect the supernatant without disturbing the pellet/beads. Transfer the supernatant to the labelled tubes containing 250 µl PPS buffer and mix well by pipetting. Alternatively mixing can be done by gently inverting the tubes several 20 times or for 20 seconds.

Centrifuge the tubes for 5-10 min at 14,000 g.

9 Meanwhile the samples are in centrifuge, prepare for the next step. Use of sterile 5 ml centrifuge tube can be very helpful.

Rigorously vortex the binding matrix to resuspend the particles

Pipette 1 ml of Binding Matrix into the labelled 5 ml centrifuge tubes.

Binding Matrix

5 ml centrifuge tube

After centrifuge, transfer the supernatant into the 5 ml centrifuge tube containing 1 ml binding matrix.

Mix well binding matrix and supernatant by inverting the tube at least for 2 min (**DO NOT VORTEX**)

Incubate the tube for 5-10 min at room temperature. Allow binding matrix to precipitate and liquid becomes clear.

It is possible to take break at this step and incubation can be done in a fridge (4 °C).

10 Meanwhile incubation prepare the spin filter and label them.

Quick-Clean Spin Filters

Discard 500 - 700 µl of clear liquid without disturbing the setteled binding matrix

Gently mix the binding matrix with remaining amount of liquid and transfer 600 µl to the labelled spin filters.

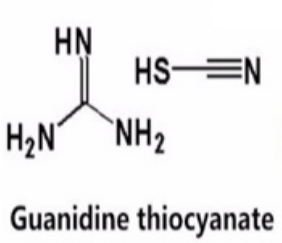
Centrifuge for 1 min at 14,000 g and discard the filtrate.

Repeat the process for the remaing amount the suspension.

Humic Acid Removal

11 Prepare **5.5 M** Guanidine thiocyanate solution by mixing 64.988 grams of molecular grade Guanidine thiocyanate into 100 ml of sterile deionized water. Completely dissole Guanidine thiocyanate with the help of magnetic stirrer and heating.

5.5 M Guanidine thiocyanate solution can be prepared before the DNA extraction process and can be stored for several weeks.



Guanidine thiocyanate chemical structure

Directly add **500 µl** of 5.5 M Guanidine thiocyanate to the quick-spin filter and resuspend the binding matrix. Care should be taken not the damage the filter.

Centrifuge at 14, 000g for 1 min or till the complete liquid is removed.

Clean-up

12 Prepare SEWS-M buffer with adding 100 ml of 100 % (>95 %) ethanol to the new SEWS-M buffer bottle. The prepared bottle can be used for several weeks.

SEWS-M buffer

Add 500 µl of ethanol added SEWS-M buffer directly to the spin filter and mix well by flickering with a finger.

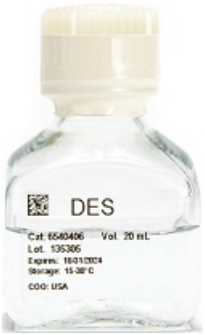
Centrifuge at 14,000 g for 1 min or until the complete liquid is removed. Discard the filtrate and replace the spin-filter into the tube.

Centrifuge the empty filter for 2 min to dry the binding matrix.

After the centrifuge, air dry the binding matrix for 3-5 min at room temperature.

Elution

13 Replace the spin filter into the catch tubes of 1.5 ml centrifuge tubes (labelled).



DES-water

Carefully add 70 µl of DES-water directly to the binding matrix inside the spin filter. Mix well by flickering with a finger.

Incubate the tube for 1-2 min at room temperature.

Centrifuge the tube at 14,000 g for 2 min.

Quality control

14 Quality of the extracted DNA can be accessed by the agarose gel electrophoresis.

The concentration of eluted DNA can be measured with nanopore or preferably with Qubit.

Storage

15 The DNA tubes must be labelled and sealed well and can be stored at -20 °C.