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DNA extraction from rectal mucosa biopsies and matched faecal samples taken from surgery for microbiome analysis

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Protocol status: Working

We use this protocol and it's working

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Keywords: DNA extraction, rectal mucosa, biopsies, faecal, stool, microbiome, microbiome dna from rectal mucosa biopsy tissue, dna extraction from rectal mucosa biopsy tissue, dna extraction from rectal mucosa biopsy, dna extraction from faecal sample, using neb nebnex microbiome dna enrichment kit, neb nebnex microbiome dna enrichment kit, surgery for microbiome analysis, procedure for manual dna extraction, manual dna extraction, microbiome dna, dna extraction, extracted dna, rectal mucosa biopsy tissue, microbiome analysis, rectal mucosa biopsy, library preparation for microbiome wg, using mp biomedical fastdna spin kit, microbial dna enrichment, mp biomedical fastdna spin kit, modification of mp biomedical fastdna spin kit, microbiome wg, matched faecal sample, ultra ii fs dna library prep kit, faecal sample, extracted sample, extraction, dna quantitation, dna, using neb

Abstract

This protocol outlines the procedure for manual DNA extraction of microbiome DNA from rectal mucosa biopsy tissue and matched faecal samples taken during surgery and frozen at -80°C.

The protocol covers

1. DNA extraction from faecal samples using MP Biomedicals FastDNA Spin Kit and FastPrep-24 for bead-beating
2. DNA extraction from rectal mucosa biopsy tissue using a modification of MP Biomedicals FastDNA Spin Kit and FastPrep-24 for bead-beating

Other steps not covered in this protocol are

3. Microbial DNA enrichment of extracted samples using NEB NEBNext Microbiome DNA Enrichment kit
4. DNA Quantitation of extracted DNA using the Qubit Fluorometer
5. Library Preparation for microbiome WGS using NEB NEBNext Ultra II FS DNA Library Prep Kit for Illumina
6. DNA sequencing on the Illumina NextSeq2000

Materials

Samples:

Patient samples (rectal mucosa biopsies & faecal samples) collected during surgery and quickly frozen at -80°C

Reagents:

 FastDNA Spin Kit for Soil **MP Biomedicals** #6560200

Lysozyme 5 GM; Thermo; #89833

Mutanolysin from Streptomyces globisporu; SLS; #SAE0092- 10KU

Equipment:

MP Biomedicals FastPrep-24 5G

Centrifuge (Eppendorf 5425 or similar)

Heating block

Vortex

Pipettes and tips

Equipment

FastPrep-24 5G

NAME

Bead-beater

TYPE

MP Biomedicals

BRAND

116005500

SKU

<https://uk.mpbio.com/fastprep-24-5g-instrument>

LINK



Troubleshooting

Before start

Ensure SEWSM buffer has 100% Ethanol added according to the kit instructions before use



DNA extraction from faecal samples

- 1 Label and weigh a  2 mL Matrix E tube (MPBio). Transfer stool (approximately  0.2 g) and weigh to record weight of stool sample to be extracted.
- 2 Add  928 μL Sodium Phosphate buffer;  122 μL MT buffer (both from MPBio Kit) to the sample in the Matrix E tube.
- 3 Perform Bead Beating with the MP Biomedicals FastPrep-24 for  00:01:00 at 5m/s.
- 4 Incubate at  85 °C for  00:15:00 on the heating block.
- 5 Repeat steps **3 and 4**.
- 6 Centrifuge at  14000 rpm for  00:05:00 and transfer supernatant to a fresh 2ml Eppendorf tube.
- 7 Add  250 μL PPS Solution (MPBio), mix by inverting tube 12 times.
- 8 Centrifuge at  14000 rpm for  00:05:00 and transfer supernatant to a 15ml tube.
- 9 Vortex the Binding Matrix (MPBio) before use to ensure matrix is fully resuspended and add  1 mL to the 15ml tube.
- 10 Mix by inverting the 15ml tubes for  00:02:00 then leave standing for  00:05:00 to collect the binding matrix at the bottom of the 15ml tube.
- 11 Remove and discard  500 μL of supernatant without disturbing the Binding Matrix at the bottom of the tube.



- 12 Gently resuspend the Matrix in the remaining solution by pipetting up and down and transfer  600 μL to a spin filter column (MPBio).
- 13 Centrifuge at  14000 rpm for  00:01:00 then empty the flow through from the tube.
- 14 Repeat steps **12 and 13** if necessary until all the Binding Matrix solution is applied to the spin filter column.
- 15 Add  500 μL SEWSM wash buffer (MPBio) to the spin column, pipette gently until the Matrix is fully resuspended. **(Ensure SEWSM buffer has 100% Ethanol added according to the kit instructions before use)**
- 16 Centrifuge at  14000 rpm for  00:01:00 . Discard the flow through and centrifuge at  14000 rpm for  00:02:00 to dry.
- 17 Air dry for  00:05:00 then transfer the spin column to a fresh, labelled catch tube (MPBio).
- 18 Apply  80 μL DES elution buffer to the Matrix in the spin column and pipette gently up and down to resuspend the Matrix. **(Heating the DES buffer on the block for  00:05:00 at  55 °C before using may make this easier).**
- 19 Centrifuge 14k for  00:01:00 to collect eluted sample. Remove the spin column and store the DNA sample tube for QC.



DNA extraction from rectal mucosa tissue samples

1h 48m

- 20 Label and weigh a 2ml Eppendorf tube. Transfer rectal mucosa biopsy tissue in to the 2ml Eppendorf tube. Re-weigh to calculate weight of biopsy tissue and record. (Aim for  5-60 mg of starting tissue).



- 21 Add  828 μL Sodium Phosphate buffer;  122 μL MT buffer (both from MPBio Kit),  50 μL Lysozyme (100mg/ml) and  50 μL Mutanolysin (5U/ μL) to the tube, briefly vortex to mix and incubate on a heat block at  37 $^{\circ}\text{C}$ for  01:00:00 1h
- 22 Transfer entire contents of the Eppendorf tube to a Matrix E tube (MPBio Kit).
- 23 Perform Bead Beating with the MP Biomedicals FastPrep-24 for  00:01:00 at 5m/s. 1m
- 24 Incubate at  85 $^{\circ}\text{C}$ for  00:15:00 on the heating block. 15m
- 25 Repeat steps **23 and 24**.
- 26 Centrifuge at  14000 rpm for  00:05:00 and transfer supernatant to a fresh 2ml Eppendorf tube. 5m
- 27 Add  250 μL PPS Solution (MPBio), mix by inverting tube 12 times.
- 28 Centrifuge at  14000 rpm for  00:05:00 and transfer supernatant to a 15ml tube. 5m
- 29 Vortex the Binding Matrix (MPBio) before use to ensure matrix is fully resuspended and add  1 mL to the 15ml tube.
- 30 Mix by inverting the 15ml tubes for  00:02:00 then leave standing for  00:05:00 to collect the binding matrix at the bottom of the 15ml tube. 7m
- 31 Remove and discard  500 μL of supernatant without disturbing the Binding Matrix at the bottom of the tube.
- 32 Gently resuspend the Matrix in the remaining solution by pipetting up and down and transfer  600 μL to a spin filter column (MPBio).



- 33 Centrifuge at  14000 rpm for  00:01:00 then empty the flow through from the tube. 1m
- 34 Repeat steps **32 and 33** if necessary until all the Binding Matrix solution is applied to the spin filter column.
- 35 Add  500 μL SEWSM wash buffer (MPBio) to the spin column, pipette gently until the Matrix is fully resuspended. **(Ensure SEWSM buffer has 100% Ethanol added according to the kit instructions before use)**  5m
- 36 Centrifuge at  14000 rpm for  00:01:00 . Discard the flow through and centrifuge at  14000 rpm for  00:02:00 to dry. 3m
- 37 Air dry for  00:05:00 then transfer the spin column to a fresh, labelled catch tube (MPBio). 5m
- 38 Apply  80 μL DES elution buffer to the Matrix in the spin column and pipette gently up and down to resuspend the Matrix. **(Heating the DES buffer on the block for  00:05:00 at  55 °C before using may make this easier).** 5m
- 39 Centrifuge 14k for  00:01:00 to collect eluted sample. Remove the spin column and store the DNA sample tube for QC and microbial DNA enrichment. 1m

Protocol references

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