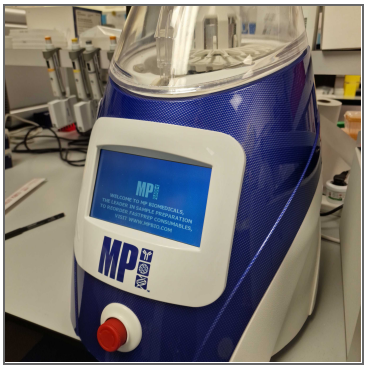

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Protocol status: Working
We use this protocol and it's working

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

Lee Murphy[1], Alan Maclean[1], Katarzyna Hafezi[1]

[1]Genetics Core, Edinburgh Clinical Research Facility, University of Edinburgh

ECRF Genetics Core

Lee Murphy
Genetics Core, Edinburgh Clinical Research Facility, Univers...

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^{o}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 Flourometer
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

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PROTOCOL integer ID: 90847

Keywords: DNA extraction, rectal mucosa, biopsies, faecal, stool, microbiome

MATERIALS

Samples:

Patient samples (rectal mucosa biopsies & faecal samples) collected during surgery and quickly frozen at -80^{o}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

JPG

BEFORE START INSTRUCTIONS

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 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 supernatent 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 °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 °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 t 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 th 7m binding matrix at the bottom of the 15ml tube.

31 Remove and discard 500 µL of supernatent 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)**

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 t 5m resuspend the Matrix. **(Heating the DES buffer on the block for 00:05:00 at 55 °C before using may make this easier).**

39 Centrifuge 14k for 00:01:00 to collect eluted sample. Remove the spin column and store the DN 1m sample tube for QC and microbial DNA enrichment.