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The 'Three Peaks' faecal DNA extraction method for long-read sequencing V.1

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High molecular weight D... Long Read Club

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External link: https://www.slideshare.net/scalene/the-three-peak-challenge-for-longread-ultradeep-stool-metagenomics-on-the-promethion

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Protocol status: In development
We are still developing and optimizing this protocol

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Keywords: Microbiome research, metagenomics, long-read sequencing, nanopore sequencing, genomics, DNA extraction, sample preparation, faecal dna extraction method, dna

Materials

MATERIALS

MetaPolyzyme **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**MAC4L-5MG

STEP MATERIALS

500 µL PBS pH 7.5

DNA/RNA Shield **Zymo Research Catalog #**R1100-50

MetaPolyzyme **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**MAC4L-5MG

Resuspend the contents of the bottle in 500 µL PBS pH 7.5 and aliquot for optimal activity

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OMNIgene•GUT **DNA Genotek Catalog #**OMR-200

DNA/RNA Shield **Zymo Research Catalog #**R1100-50

MetaPolyzyme **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**MAC4L-5MG

Troubleshooting

1 Add 100 mg fresh stool or 50 µL OMNIgene GUT to 200 µL DNA/RNA Shield, vortex briefly and place on a tube rotator at 20 rpm 00:10:00 .

OMNIgene•GUT **DNA Genotek Catalog #**OMR-200

DNA/RNA Shield **Zymo Research Catalog #**R1100-50

2 Centrifuge at 5000 x g 00:05:00 and retain up to 200 µL supernatant depending on size of pellet.

3 Add 100 µL PBS and resuspend material by pipetting up and down, centrifuge 5000 x g 00:05:00 and retain up to 100 µL supernatant depending on size of pellet.

4 Add 1 mL PBS and resuspend material by pipetting up and down, centrifuge 5000 x g 00:05:00 and discard supernatant.

5 Add 100 µL PBS and 5 µL MetaPolyzyme, mix by pipetting and incubate at 35 °C 02:00:00 . Gently mix by pipetting up and down to ensure solution is homogeneous before proceeding.

MetaPolyzyme **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**MAC4L-5MG

6 Add 100 µL DNA/RNA Shield, 10 µL [M] 10 % (w/v) SDS and 10 µL [M] 20 mg/mL Proteinase K and mix by pipetting. Incubate at 55 °C 00:30:00 with 300 rpm mixing. Gently mix by pipetting up and down to ensure solution is homogeneous before proceeding.

7 Centrifuge 5000 x g 00:05:00 and retain up to 200 µL supernatant depending on size of pellet.

8 Resuspend pellet in 750 µL Lysis Solution and transfer to a ZR BashingBead Lysis Tube. Bead-beat on a FastPrep instrument for 1 cycle of 00:00:40 at 6 m/s

Equipment	
FastPrep-24 5G	NAME
Bead-beater	TYPE
MP Biomedicals	BRAND
116005500	SKU
https://uk.mpbio.com/fastprep-24-5g-instrument	LINK

9 Centrifuge tube at 10000 x g for 00:01:00 and retain 400 µL supernatant.

10 Pool the supernatants retained at each of the steps in a 2 ml Eppendorf tube and measure the volume using a P1000 pipette. Add 2 volumes of Genomic Lysis Buffer and 2 mL MagBinding beads.