VERSION 1

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<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

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

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High molecular weight DNA extraction from all kingdoms

Long Read Club

Josh Quick

MATERIALS

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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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research, metagenomics,
long-read sequencing,
nanopore sequencing,
genomics, DNA extraction,
sample preparation

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

OMNlgene•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 μ L PBS and 5 μ L MetaPolzyme, 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.

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

6 Add 100 μ L DNA/RNA Shield, 10 μ L 10 % (w/v) SDS and 10 μ L 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 μ L supernatant depending on size of pellet.

- 8 Resuspend pellet in **750 μ L** 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 μ L** 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.