VERSION 2

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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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protocols.io

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<https://doi.org/10.1093/gigascience/giz043>

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Protocol status: Working

We use this protocol and it's working

The 'Three Peaks' faecal DNA extraction method for long-read sequencing V.2

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Long Read Club

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Josh Quick

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.

This protocol is adapted and uses reagents from the Quick-DNA HMW MagBead Kit (Zymo, D4081).

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28178

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

- 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 for 00:10:00 at 20 rpm .

OMNIgene•GUT DNA Genotek Catalog #OMR-200

DNA/RNA Shield Zymo Research Catalog #R1100-50

- 2 Centrifuge at 5000 x g for 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 at

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 at 5000 x g for 00:05:00 and discard supernatant.

5 Add 100 µL PBS and 5 µL MetaPolzyme, mix by pipetting and incubate at 35 °C for 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 µL DNA/RNA Shield, 10 µL 10 % (w/v) SDS and 10 µL 20 mg/mL Proteinase K and mix by pipetting. Incubate at 55 °C for 00:30:00 with 300 rpm mixing. Gently mix by pipetting up and down to ensure solution is homogeneous before proceeding.

7 Centrifuge at 5000 x g for 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.
- 11 Add 2 volumes of Genomic Lysis Buffer and 50 µL MagBinding beads and place on a tube rotator for 00:10:00 at 20 rpm .

- 12 Put tube on a magnetic rack and incubate for 00:02:00 or until clear. Discard supernatant taking care not to disturb bead pellet.
- 13 Remove from the magnetic rack and using a P1000 pipette add 100 μL DNA elution buffer. Mix by pipetting up and down x10.
- 14 Add 500 μL Quick-DNA MagBinding Buffer and place on a tube rotator for 00:10:00 at 20 rpm .
- 15 Put tube on a magnetic rack and incubate for 00:02:00 or until clear. Discard supernatant taking care not to disturb bead pellet.
- 16 Remove from the magnetic rack and using a P1000 pipette add 900 μL DNA Pre-Wash Buffer. Mix by pipetting up and down x10 and transfer to a new 1.5 mL Eppendorf tube.
- 17 Put tube on a magnetic rack and incubate for 00:02:00 or until clear. Discard supernatant taking care not to disturb bead pellet.
- 18 Remove from the magnetic rack and using a P1000 pipette add 900 μL g-DNA Wash Buffer. Mix by pipetting up and down x10 and transfer to a new 1.5 mL Eppendorf tube.
- 19 Put tube on a magnetic rack and incubate for 00:02:00 or until clear. Discard supernatant taking care not to disturb bead pellet.

- 20 go to step #18 and repeat wash with g-DNA Wash Buffer for a second time.
- 21 Using a P1000 wide-bore pipette tip to reduce turbulence add 900 μL DNA elution buffer to the front of the tube and immediately remove it again. Try to do this in a fast, smooth motion disturbing the beads as little as possible.
- 22 Remove the residual buffer from the bottom of the tube and discard.
- 23 With a P1000 pipette add 50 μL DNA elution buffer to the tube and mix by pipetting up and down x10.
- 24 Place on a tube rotator for 00:05:00 at 20 rpm .
- 25 Put tube on a magnetic rack and incubate for 00:02:00 or until clear. Transfer DNA to a new 1.5 mL Eppendorf tube. Store DNA at 5 °C for up to a month or use immediately.