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

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1 Works for me dx.doi.org/10.17504/protocols.io.7rshm6e

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EXTERNAL LINK

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

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

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

MATERIALS

NAME	CATALOG #	VENDOR
MetaPolyzyme	MAC4L-5MG	Sigma Aldrich

STEPS MATERIALS

NAME	CATALOG #	VENDOR
OMNIgene•GUT	OMR-200	DNA Genotek
DNA/RNA Shield	R1100-50	Zymo Research
MetaPolyzyme	MAC4L-5MG	Sigma Aldrich

MATERIALS TEXT

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).

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
by DNA Genotek
Catalog #: OMR-200

DNA/RNA Shield
by 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 MetaPolyzyme, 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.

MetaPolyzyme
by 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 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**

FastPrep-24 5G
Bead-beater
MP Biomedicals 116005500

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.