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

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Sep 25, 2019

In Development dx.doi.org/10.17504/protocols.io.584g9yw

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

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

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 **00:10:00** .

OMNIgene•GUT
by DNA Genotek
Catalog #: OMR-200

DNA/RNA Shield
by Zymo Research
Catalog #: R1100-50

2 Centrifuge at **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 **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 **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
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 00:30:00 with mixing. Gently mix by pipetting up and down to ensure solution is homogeneous before proceeding.

7 Centrifuge 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 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.