



Sep 25, 2019

The 'Three Peaks' faecal DNA extraction method for long-read sequencing [↗](#)Josh Quick<sup>1</sup><sup>1</sup>Sam Nicholls [University of Birmingham], Nicholas Loman [University of Birmingham]*In Development*[dx.doi.org/10.17504/protocols.io.584g9yw](https://doi.org/10.17504/protocols.io.584g9yw)

Long Read Club

Tech. support email: [n.j.loman@bham.ac.uk](mailto:n.j.loman@bham.ac.uk)Josh Quick   

## 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

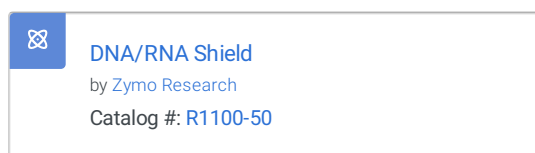
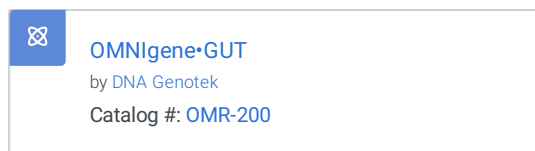
## STEPS MATERIALS








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** .








- 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** 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.

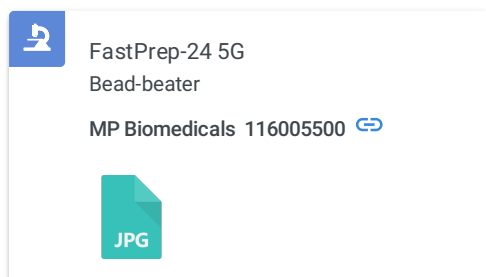




**MetaPolzyme**  
by Sigma Aldrich  
Catalog #: [MAC4L-5MG](#)


- 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**



- 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.



This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited