



Oct 07, 2019

The 'Three Peaks' faecal DNA extraction method for long-read sequencing V.2 [↗](#)Josh Quick¹¹Sam Nicholls [University of Birmingham], Nicholas Loman [University of Birmingham]

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Works for me

[dx.doi.org/10.17504/protocols.io.7rshm6e](https://doi.org/10.17504/protocols.io.7rshm6e)

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

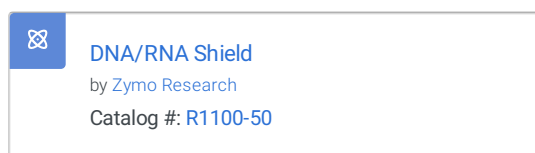
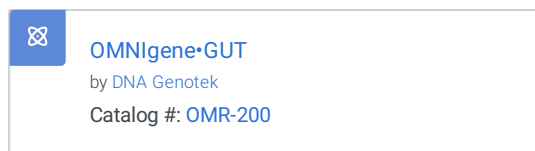
NAME ▾	CATALOG # ▾	VENDOR ▾
OMNigene-GUT	OMR-200	DNA Genotek
DNA/RNA Shield	R1100-50	Zymo Research
MetaPolyzyme	MAC4L-5MG	Sigma Aldrich











MATERIALS TEXT





Resuspend the contents of the bottle in **500 µl PBS pH 7.5** and aliquot for optimal activity.


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**.







- 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.

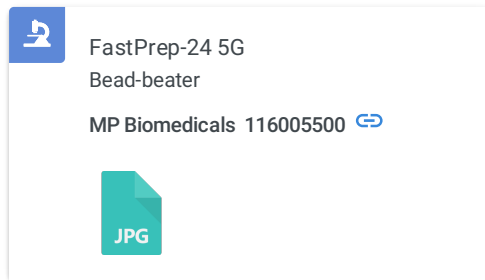
- 5 Add  **100 µl** PBS and  **5 µl** MetaPolzyme, mix by pipetting and incubate at  **35 °C** for  **02:00:00** . Gently mix by pipetting up and down to ensure solution is homogeneous before proceeding.





























MetaPolzyme
by [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**



- 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.



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