Oct 07, 2019 Version 2

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

DOI
https://dx.doi.org/10.17504/protocols.io.7rshm6e

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DOI: https://dx.doi.org/10.17504/protocols.io.7rshm6e

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

Protocol Citation: Josh Quick 2019. The 'Three Peaks' faecal DNA extraction method for long-read sequencing. **protocols.io** **https://dx.doi.org/10.17504/protocols.io.7rshm6e**

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

Protocol status: Working
We use this protocol and it's working

Created: September 27, 2019

Last Modified: October 07, 2019

Protocol Integer ID: 28178

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

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).

Protocol materials

MetaPolyzyme **Merck MilliporeSigma (Sigma-Aldrich) Catalog** #MAC4L-5MG

DNA/RNA Shield **Zymo Research Catalog** #R1100-50

OMNIgene•GUT **DNA Genotek Catalog** #OMR-200

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 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 **Merck MilliporeSigma (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

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.