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WORKS FOR ME 1

Oral microbiome DNA extraction using Zymobiomics

COMMENTS 0

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DISCLAIMER

Modified Zymobiomics

ABSTRACT

A method of DNA extraction of oral mouthwash samples for use in microbiome studies that utilize next-generation sequencing (NGS) and/or third-generation sequencing.

PROTOCOL CITATION

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MATERIALS TEXT

- Mouthwash sample
- ZymoBIOMICS™ DNA Miniprep Kit (D4300) – can be stored at RT
- Disruptor Genieâ or Vortex (Zymo Research: S6001-2-120)
- 1.5 mL microfuge tube (Eppendorf™ 022431021) and rack
- 15 ml centrifuge
- 5 ml or 15 ml tube
- Filter tip 1000
- Filter tip 200
- Filter tip 10
- Pipette set (1000, 200, 100, 10)
- Magnetic bead (Beckman Coulter: A63881) – store at 4°C (DO NOT FREEZE)
- Magnetic separation rack (NEB: S1506S)
- 100% Ethanol (prepare from 200 proof Ethanol 100%, RNase-free; Fisher Cat. BP2818-500)
- 75% Ethanol solution (prepare from 200 proof Ethanol 100%, RNase-free; Fisher Cat. BP2818-500)
- Nuclease-free water (NFW) (NEB: B1500S)
- Biohazard bag
- Gloves
- DNA quality check

- Qubit™ dsDNA BR Assay Kit (Catalog number:Q32850)
- Qubit™ Fluorometer
- Agarose gel electrophoresis system
- DNA ladder (1 kb and high molecular weight DNA marker)
- TAE buffer (Thermo Fisher: FERB49)
- Sybersafe (Invitrogen: S33102)
- NanoDrop™ Spectrophotometers
- Microwave
- 1 kb DNA ladder (Thermo Fisher: 10787018)
- High Molecular weight DNA ladder (GeneRuler™ High Range DNA Ladder) (Thermo Fisher: SM1351) or Quick-Load®1 kb Extend DNA Ladder (NEB: N3239S)

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1 **ZymoBIOMICS™ DNA Extraction (following the manufacturer's instruction except for beat beating time)** (reference no. 1)

1.1. Transfer 5-10 ml collected saliva/ mouthwash sample to 15 ml sterile conical centrifuge tube

1.2. Centrifuge the tube at 4000 rpm for 20 minutes at 4°C

1.3. Discard supernatant as much supernatant as possible. Save the pellet

1.4. Add 750 µl ZymoBIOMICS™ Lysis Solution to the tube that contain pellet and mix the lysis buffer with the pellet.

1.5. Move all buffer to ZR BashingBead™ Lysis Tubes and cap tightly.

1.6. Secure in a bead beater or vortex with a 2 ml tube and process at maximum speed for 2-3 minutes (modified from original protocol which suggests to beat for 20 minutes).
1.7. Centrifuge the ZR BashingBead™ Lysis Tubes in a microcentrifuge at ≥ 10,000 x g for 1 minute.
1.8. Transfer up to 400 µl supernatant to the Zymo-Spin™ III-F Filter in a Collection Tube and centrifuge at 8,000 x g for 1 minute. **Discard the Zymo-Spin™ III-F Filter.**
1.9. Add 1,200 µl of ZymoBIOMICS™ DNA Binding Buffer to the filtrate in the Collection Tube (from **Step 1.8**). Mix well.
1.10. Transfer 800 µl of the mixture from Step 5 to a Zymo-Spin™ IIC-Z Column in a Collection Tube and centrifuge at 10,000 x g for 1 minute.
1.11. Discard the flow through from the Collection Tube and **repeat Step 1.10**.
1.12. Add 400 µl ZymoBIOMICS™ DNA Wash Buffer 1 to the Zymo-Spin™ IIC-Z Column in a new collection Tube and centrifuge at 10,000 x g for 1 minute. Discard the flow-through.
1.13. Add 700 µl ZymoBIOMICS™ DNA Wash Buffer 2 to the Zymo-Spin™ IIC-Z Column in a Collection Tube and centrifuge at 10,000 x g for 1 minute. Discard the flow-through.
1.14. Add 200 µl ZymoBIOMICS™ DNA Wash Buffer 2 to the Zymo-Spin™ IIC-Z Column in a Collection Tube and centrifuge at 10,000 x g for 1 minute.
1.15. Transfer the Zymo-Spin™ IIC-Z Column to a clean 1.5 ml microcentrifuge tube and add 100 µl (50 µl minimum) NFW directly to the column matrix and incubate for 1 minute. Centrifuge at 10,000 x g for 1 minute to elute the DNA.
1.16. Place a Zymo-Spin™ III-HRC Filter in a new Collection Tube and add 600 µl ZymoBIOMICS™ HRC Prep Solution. Centrifuge at 8,000 x g for 3 minutes.
1.17. Transfer the eluted DNA (**Step 1.15**) to a prepared Zymo-Spin™ III-HRC Filter in a clean 1.5 ml microcentrifuge tube and centrifuge at 16,000 x g for 3 minutes. The filtered DNA is now ready for the cleanup step.

2 **(Optional) DNA** clean-up **using magnetic beads** (modified from reference no. 2)
2.1. Allow magnetic bead reagent to come to room temperature prior to use.
2.2. Mix the reagent well before use. It should appear homogenous and consistent in color (called mixture).
2.3. Add 1 volume of the mixture to 1 volume of sample. For example, adding 80 µl bead to 80 µl extracted DNA.
2.4. Gently pipette up and down 10 times and gently flick the tube for 5 times then spin down (1-2 second).
2.5. Incubate the mixture at room temperature for 5-10 minutes.
2.6. Place the mixture onto magnetic separation rack for 3 minutes to separate beads from solution
2.7. Prepare fresh 80% ethanol (400 µl ethanol and 100 µl NFW).
2.8. Aspirate the cleared solution from the reaction tube and discard. This step must be performed while the reaction tube is situated on the magnetic separation rack. Do not disturb the area of magnetic beads.
2.9. Dispense 200 µl of 80% ethanol to reaction tube and incubate for 1 minute at room temperature. Aspirate out the ethanol and discard. Repeat for a total of two washes.
2.10. Remove the tube from the magnetic separation rack and spin down the tube (1 second).Then, place the tube onto magnetic separation rack and use 10 µl pipette to aspirate out the remained ethanol and discard.
2.11. Place the reaction tube on bench top to air-dry for 3-10 minutes. Be sure to allow the dry (not

shiny).
2.12. Add 40 µl of nuclease free water to each tube of the reaction and flicking 5 times or until no clump and perform spin down (1-2 second). Incubate for 2 minutes.
2.13. Place the tube onto magnetic separation rack and use 10 µl pipette to aspirate out the DNA in the NFW and put into the new tube.
2.14. Label and aliquot purified DNA into 2 tubes (for Illumina and Nanopore). Store aliquoted DNA at -20°C, unless proceeding to the downstream application.

3 Determining DNA quality

3.1. *Purity:* Apply 1 µl of DNA. Use the NanoDrop™ to measure absorbance and record sample purity. The OD 260/280 ratio should be 1.8 and OD 260/230 should be 2.0-2.2.
3.2. *Concentration:* Apply 1 µl of DNA. Use the Qubit™ to measure concentration (reference no. 3).
3.3. *Integrity:* Apply 1-5 µl of DNA. Use the 0.8% Agarose gel to check the integrity (reference no. 4). Load 1 kb DNA ladder on the first lane and high molecular weight DNA ladder on the last lane. After running the gel electrophoresis, high integrity of DNA will locate above 10 kb in the area of high molecular weight DNA ladder.

4 References

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