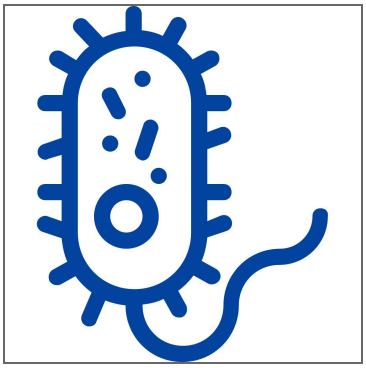

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Protocol status: Working
We use this protocol and it's working

Created: Feb 27, 2024

DNA Extraction with ZymoBIOMICS MiniPrep Kit

Carlos Goller[1]

[1]North Carolina State University

BIT Metagenomics | MICROnet

Carlos Goller

ABSTRACT

DNA extraction of bacterial DNA Using ZymoBIOMICS™ DNA Miniprep Kit (D4300) using lysis tubes with DNA/RNA Shield.

IMAGE ATTRIBUTION

Bacteria from Dmitry MIrolyubov from The Noun Project

MATERIALS

ZymoBIOMICS™ DNA Miniprep Kit

PROTOCOL MATERIALS

DNA/RNA Shield **Zymo Research Catalog #R1100-50** Step 1

Last Modified: Mar 01, 2024

PROTOCOL integer ID: 95833

Keywords: DNA, bacteria, isolate, microbe

Funders Acknowledgement:
Biotechnology Program
Grant ID: NC State University

D

14m

1 Add Sample to a ZR BashingBead™ Lysis Tubes (0.1 & 0.5 mm). Add 750 µL ZymoBIOMICS™ Lysis Solution to the tube and cap tightly. Note: For samples stored and lysed in DNA/RNA Shield **Zymo Research Catalog #R1100-50** Lysis Tubes, do not add ZymoBIOMICS™ Lysis Solution and proceed to Step 2. 10m

	A	B
	Sample Type	Maximum Input
	Feces	200 mg
	Soil	250 mg
	Liquid Samples and Swab Collections	250 ul
	Cells (isotonic buffer, el.g., PBS)	50-100 mg (wet weight) (109 bacterial and 108 yeast cells)
	Samples in DNA/RNA Shield	<1 ml

Table from ZymoBIOMICS Protocol D4300

2 Obtain DNA/RNA Shield Lysis Tubes with samples/swab heads from instructors. Secure in a bead beater fitted with a 2 ml tube holder assembly and process using optimized beat-beating conditions (speed and 40m

time) for different devices. We will use a Vortex Genie with 2ml Bashing Bead tubes for 00:40:00 .

3 Centrifuge the ZR BashingBead™ Lysis Tubes (0.1 & 0.5 mm) in a microcentrifuge at ≥ 10000 rpm, 20°C, 00:01:00 . 1m

4 Transfer up to 400 µL supernatant to the Zymo-Spin™ III-F Filter in a Collection Tube and centrifuge at 8.000 x g, 00:01:00 . Discard the Zymo-Spin™ III-F Filter. 1m

5 Add 1200 µL of ZymoBIOMICS™ DNA Binding Buffer to the filtrate in the Collection Tube from Step 4. Mix well.

6 Transfer 800 µL of the mixture from Step 5 to a Zymo-Spin™ IICR Column in a Collection Tube and centrifuge at 10.000 x g, 00:01:00 . 1m

7 Discard the flow through from the Collection Tube and repeat Step 6.

8 Add 400 µL ZymoBIOMICS™ DNA Wash Buffer 1 to the ZymoSpin™ IICR Column in a **new** Collection Tube and centrifuge at 10.000 x g, 00:01:00 . Discard the flow-through. 1m

9 Add 700 µL ZymoBIOMICS™ DNA Wash Buffer 2 to the ZymoSpin™ IICR Column in a Collection Tube and centrifuge at 10.000 x g, 00:01:00 . Discard the flow-through. 1m

10 Add 200 µL ZymoBIOMICS™ DNA Wash Buffer 2 to the ZymoSpin™ IICR Column in a Collection Tube and centrifuge at 10.000 x g, 00:01:00 . 1m

11 Transfer the Zymo-Spin™ IICR Column to a clean 1.5 ml microcentrifuge tube and add 100 µl (50 µl minimum) ZymoBIOMICS™ DNase/RNase Free Water directly to the column matrix and incubate for 00:01:00 . Centrifuge at 10.000 x g, 00:01:00 to elute the DNA5, 6. 2m

12 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, 00:03:00 . 3m

13 Transfer the eluted DNA (Step 11) to a prepared Zymo-Spin™ III-HRC Filter in a clean 1.5 ml microcentrifuge tube and centrifuge at exactly 16.000 x g, 00:03:00 . The filtered DNA is now suitable for PCR and other downstream applications. 3m