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## DNA Extraction: Zymo Research Quick-DNA Fecal/Soil Microbe Midiprep Kit (Cat #: D6110)

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**Protocol status:** Working

**We use this protocol and it's working**

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## Abstract

DNA Extraction: Zymo Research Quick-DNA Fecal/Soil Microbe Midiprep Kit (Cat #: D6110); kit and protocol used for DNA extraction of soil samples in the Bik Lab.



## DNA Extraction: Zymo Research Quick-DNA Fecal/Soil Microbe Midiprep Kit (Cat #: D6110)

















15m

- 1 Add **beta-mercaptoethanol** (not included in kit) to the **Genomic Lysis Buffer (Cat #: D3004-1-100)** to a final dilution of 1M 0.5 % (v/v) (v/v) *i.e., 2.5 ml per 500 ml* for optimal performance.
- 2 Add 5 g of soil sample to the bead/filter of a **ZR Bashing Bead Lysis/Filtration Tube (50 mL w/ 0.5 mm Beads; Cat #: S6010)**.
- 3 Add 6 mL of **BashingBead Buffer (Cat #: D6001-3-150)** to the sample. Cap tube tightly and secure with parafilm.
  - 3.1 Place tubes in a bead beater (Bullet Blender) and process the samples for 10 minutes at speed 8.  
*Tip: Store tubes sideways until they go into the bead beater to prevent liquid from prematurely going through filter.*  
*Tip: Select "Zymo MidiPrep" pre-loaded protocol on Bullet Blender in Bik Lab.*
  - 3.2 Centrifuge the **ZR BashingBead Lysis/Filtration Tube** in a centrifuge at 5000 rpm, 00:05:00.
  - 3.3 Remove bead/filter chamber from the top of the **ZR BashingBead Lysis/Filtration Tube** and transfer supernatant from the bottom of the tube (using a 10 mL pipette) to a new 50 mL tube.
- 4 Add 18 mL of **Genomic Lysis Buffer** to the supernatant. Mix well.  
*Tip: Centrifuge for a few seconds if there is too much foam.*
  - 4.1 Filter the entire mixture using a **Zymo-Spin V-E Column/Zymo-Midi Filter (Cat #: C1021-25)** mounted on a vacuum manifold, at  $\geq 600$  mm Hg.
  - 4.2 Disconnect the **Zymo-Spin V-E Column/Zymo-Midi Filter** and transfer the **Zymo-Spin V-E Column** to a **Collection Tube (Cat #: C1001-50)**. Spin the column at 10000 rpm, 00:01:00 in a microcentrifuge.

5m

1m



- 5 Add  300  $\mu$ L of **DNA Pre-Wash Buffer (Cat #: D3004-5-15)** to the column and spin at  10000 rpm, 00:01:00 . Discard the flow through.   
*Tip: Add reagent by inserting the pipette tip at an angle while pressing it against the wall of the column and without touching the filter to avoid overflow.*
- 6 Add  400  $\mu$ L of **g-DNA Wash Buffer (Cat #: D3004-2-50)** to the column and centrifuge at  10000 rpm, 00:01:00 . Discard the flow through. 
- 7 **REPEAT WASH STEP:** Add  400  $\mu$ L of **g-DNA Wash Buffer** to the column and centrifuge at  10000 rpm, 00:01:00 . Discard the flow through. 
- 8 Transfer the **Zymo-Spin V-E Column** to a 1.5 mL microcentrifuge tube. Add  150  $\mu$ L of **DNA Elution Buffer (Cat #: D3004-4-16)** directly to the column matrix. Wait for 1 minute and then centrifuge at  10000 rpm, 00:01:00 to elute the DNA. 
- 9 Place the **Zymo-Spin III-HCR Filter** in a clean **Collection Tube**. Add  600  $\mu$ L of **Prep Solution (Cat #: D6035-1-30)**. Centrifuge at  8000 rpm, 00:03:00 . 
- 9.1 Place the **Zymo-Spin III-HCR Filter** in a clean 1.5 mL microcentrifuge tube. Transfer the eluted DNA to the prepared **Zymo-Spin III-HCR Filter** and centrifuge at exactly  16000 rpm, 00:03:00 . The filtered DNA is now suitable for PCR and other downstream applications. 