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DNA Extraction with Zymo Quick-DNA™ Fungal/Bacterial Miniprep Kit

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We use this protocol and it's working

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Abstract

This protocol is used to extract genomic DNA from bacterial and fungal isolates using the Quick-DNA™ Fungal/Bacterial Miniprep Kit (Zymo Research). The kit enables rapid DNA extraction in as little as 15 minutes. The resulting ultra-pure DNA is suitable for PCR, next-generation sequencing (NGS), and other molecular applications.

This protocol includes three key modifications to the manufacturer's instructions:

- A sample preparation step has been added.
- Guidance is provided for addressing insufficient bead beating.
- The lysis mixture is loaded onto the column once, rather than twice, to streamline the workflow.

Materials

Kit

- Quick-DNA™ Fungal/Bacterial Miniprep Kit (Zymo, D6005)

Equipment:

- Microcentrifuge
- Vortex mixer with horizontal tube holder

Supplies

- Microcentrifuge tubes
- Inoculation loops

Chemical

- β-mercaptoethanol

Troubleshooting

Before start

For a new kit, add β-mercaptoethanol (not provided) to the Genomic Lysis Buffer to a final concentration of 0.5% (v/v), i.e., 500 µL per 100 mL.

1 Both bacterial/fungal colonies on agar plates and broth cultures can be used for DNA extraction.

1.1 Colonies

- Colonies from fresh culture plates can be used directly. Plates can be stored at 2°C – 8°C for up to two weeks before extraction.
- Add 750 µL of BashingBead Buffer to a ZR BashingBead Lysis Tube.
- Use a sterile inoculation loop to transfer one or several pure colonies (depending on colony size) into the tube.

Note: *If colonies are difficult to pick, cut a small piece of agar containing colonies and transfer the agar into the lysis tube.*

1.2 Broth Culture

- Centrifuge 1 mL of culture at 6,000 × g for 10 minutes in a microcentrifuge tube. Discard the supernatant and retain the pellet. The pellet can be stored at -25°C to -15°C until DNA extraction.
- Resuspend the pellet in 750 µL of BashingBead Buffer and transfer the suspension to a ZR BashingBead Lysis Tube.

2 Secure the lysis tubes in a bead beater (e.g., a vortex mixer with a horizontal tube holder) and homogenize at maximum speed for 5 minutes.

Note: *Do not discard the tube at this stage. If DNA extraction fails due to insufficient bead beating, you may re-process the tube by extending bead beating to 20 minutes and* repeating *the downstream steps.*

3 Centrifuge the lysis tubes at 10,000 × g for 1 minute.

4 Transfer 200 µL of the supernatant into a Zymo-Spin™ III-F Filter in a collection tube. Centrifuge at 10,000 × g for 1 minute.

5 Discard the filter. To the flow-through, add 600 µL of Genomic Lysis Buffer and mix thoroughly by pipetting.

6 Load the entire 800 µL mixture into a Zymo-Spin™ IICR Column in a new collection tube. Centrifuge at 10,000 × g for 1 minute.

7 Place the Zymo-Spin™ IICR Column into a new collection tube. Discard the old tube with the flow-through.

8 Add 200 µL of DNA Pre-Wash Buffer to the column. Centrifuge at 10,000 × g for 1 minute.

9 Add 500 µL of g-DNA Wash Buffer to the column. Centrifuge at 10,000 × g for 1 minute.

10 Transfer the column to a clean 1.5 mL microcentrifuge tube (not provided). Add 35-100 µL of DNA Elution Buffer directly onto the column matrix. Centrifuge at 10,000 × g for 1 minute to elute the DNA.

11 Store the eluted DNA at -20°C.

Protocol references

Zymo: Manufacturer’s protocol for the Quick-DNA Fungal/Bacterial Miniprep Kit