ZYMO RESEARCH

DNA Purification Made Simple

Quick-DNA™ Fungal/Bacterial 96 Kit

DNA from tough-to-lyse fungi and bacteria samples.

Highlights

- Simple, high-throughput (96-well) isolation of DNA from all types of tough-to-lyse fungi (*e.g.*, yeast) and bacteria in as little as 40 minutes.
- State-of-the-art, ultra-high density **BashingBeads™** are fracture resistant and chemically inert.
- Omits the use of organic denaturants as well as proteinases.

Catalog Numbers:
D6006

Scan with your smart-phone camera to view the online protocol/video.

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Product Contents

Quick-DNA™ Fungal/Bacterial 96 Kit	D6006 (2 x 96 Preps.)	Storage Temperature
ZR BashingBead™ Lysis Rack (0.1 & 0.5 mm)	2	Room Temp.
BashingBead™ Buffer	40 ml (2x)	Room Temp.
Genomic Lysis Buffer[1]	150 ml	Room Temp.
DNA Pre-Wash Buffer[2]	50 ml	Room Temp.
g-DNA Wash Buffer	100 ml	Room Temp.
DNA Elution Buffer[3]	10 ml (2x)	Room Temp.
96-Well Block	2	Room Temp.
Silicon-A™ Plate	2	Room Temp.
Collection Plate	2	Room Temp.
Cover Foil	4	Room Temp.
Instruction Manual	1	-

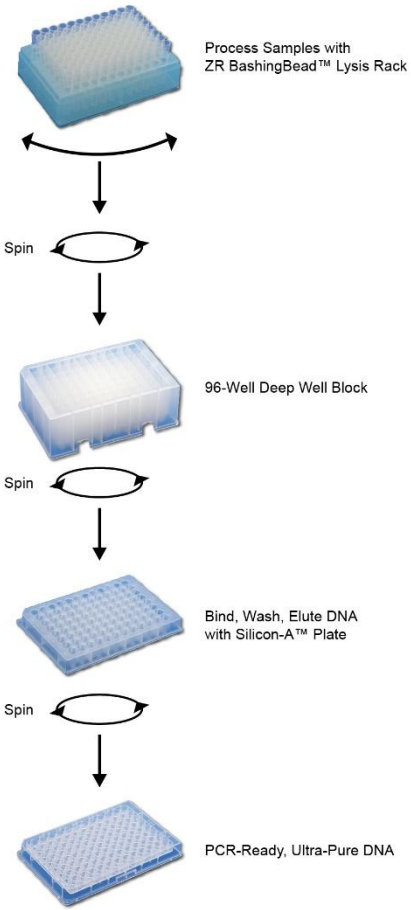
[1] For optimal performance, add beta-mercaptoethanol to 0.5%(v/v) *i.e.*, 750 µl per 150 ml.
[2] A precipitate may have formed in the **DNA Pre-Wash Buffer** during shipping. To completely resuspend the buffer, incubate the bottle at 30-37ºC for 30 minutes and mix by inversion. DO NOT MICROWAVE.
[3] The **DNA Elution Buffer** contains 10 mM Tris-HCl, pH 8.5, 0.1 mM EDTA. If required, pure water can also be used to elute the DNA.

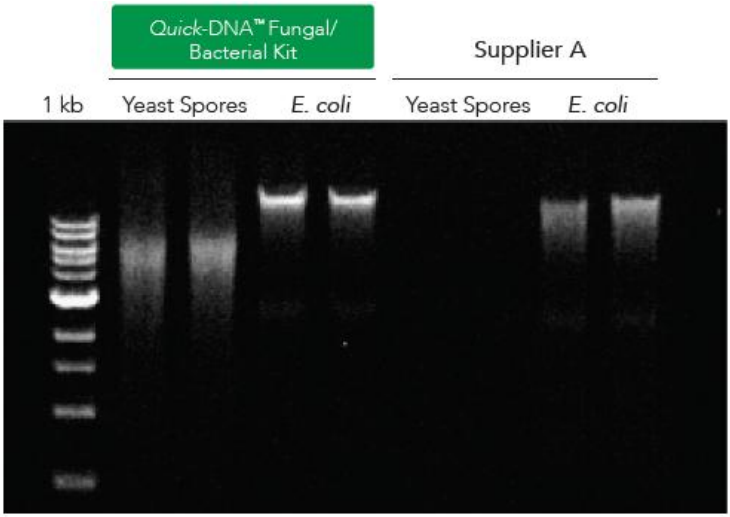
Specifications

- **Format –** Bead Beating, 96-Well Plate Purification.
- **Sample Sources –** 10 – 20 mg (wet weight) fungi or bacteria; this equates to approximately $2x10^8$ bacterial cells and $2x10^7$ yeast. Spores, pollen, nematodes, as well as other microorganisms can also be sampled.
- **DNA Purity –** High quality DNA is eluted with **DNA Elution Buffer** making it perfect for PCR (A_{260}/A_{280} >1.8).
- **DNA Size Limits –** Capable of recovering genomic DNA up to and above 40 kb. In most instances, mitochondrial DNA and viral DNA (if present) will also be recovered.
- **DNA Recovery –** Typically, up to 5 µg total DNA is eluted into 100 µl (25 µl minimum) **DNA Elution Buffer** per sample.
- **Equipment –** Centrifuge w/ microplate carriers, 96-well plate/block disruptor or pulverizer

Product Description

The ***Quick*-DNA™ Fungal/Bacterial 96 Kit** is designed for the simple, rapid, and high-throughput (96-well) isolation of DNA from tough-to-lyse fungi including: *A. fumigatus, C. albicans, N. crassa, S. cerevisiae, S. pombe*, as well as from mycelium and Gram-positive and Gram-negative bacteria. The procedure is easy and can be completed in as little as 40 minutes. Fungal and/or bacterial samples are added directly to a **ZR BashingBead™ Lysis Rack (0.1 & 0.5 mm)**[1] and rapidly and efficiently lysed by bead beating without using organic denaturants or proteinases. The DNA is then isolated and purified using our Zymo-Spin™ Technology and is ideal for downstream molecular-based applications including PCR, array, etc. A schematic of the ***Quick*-DNA™ Fungal/Bacterial Miniprep Kit** procedure is shown below.





DNA isolated from *Saccharomyces cerevisiae* (spores) and *E. coli* using the ***Quick*-DNA™ Fungal/Bacterial Kit** was high-quality and structurally intact. Equivalent amounts of yeast and bacteria were processed using the ***Quick*-DNA™ Fungal/Bacterial Kit** or the Supplier A kit. Equal volumes of eluted DNA were analyzed on a 0.8% (w/v) agarose gel stained with EtBr.

DNA/RNA Shield™ (R1100-50, R1100-250) can be used to stabilize nucleic acids and inactivate infectious agents in a variety of samples, without the need for reagent removal.

For rapid, robust, and simple purification of high quality, inhibitor-free DNA from any sample including feces, soil, water, biofilms, swabs, saliva, body fluids, etc. use the **ZymoBIOMICS® DNA Miniprep Kit (D4300).**

Protocol

This protocol consists of: (I) Buffer Preparation and (II) DNA Purification

(I) Buffer Preparation

- ✓ For optimal performance, add ~100% beta-mercaptoethanol (user supplied) to the **Genomic Lysis Buffer** to a final dilution of 0.5% (v/v) *i.e.*, 750 µl per 150 ml.

(II) DNA Purification

1. Add 10 – 20 mg (wet weight) bacterial/fungal cells[1] that have been resuspended in up to 50 µl of water or isotonic buffer (*e.g.*, PBS) to a **ZR BashingBead™ Lysis Rack (0.1 mm & 0.5 mm)**. Add 400 µl **BashingBead™ Buffer** to each tube and cap tightly.

   *For samples stored in **DNA/RNA Shield™**, see **Samples in DNA/RNA Shield™** (**pg. 7**).*

2. Secure ZR BashingBead™ Lysis Rack (0.1 mm & 0.5 mm) in a bead beater fitted with the appropriate holder assembly for your bead beating module and process using optimized beat beating conditions (speed and time) for your device. See Optimized Lysis Protocols for Bead-Beating (**pg.8**) for recommended lysis protocols.

   ***Optional Stopping Point:** Samples can be stored after Step 2 at -80°C.*

3. Centrifuge the ZR BashingBead™ Lysis Rack (0.1 & 0.5 mm) at ≥ 3,000 x *g* (5,000 x *g* max.) for 5 minutes.

4. Transfer up to 250 µl supernatant to each well of a **96-Well Block.**

5. Add 750 µl of **Genomic Lysis Buffer** to the supernatant in the 96-Well Block from Step 4. Cover completely with **Cover Foil** and mix thoroughly by vortexing block for 2 minutes. Centrifuge the 96-Well Block at ≥ 3,000 x *g* (5,000 x *g* max.) for 5 minutes.

6. Remove or pierce foil and transfer 500 µl from the wells of Step 5 to the wells[2] of a **Silicon-A™ Plate,** mounted on a **Collection Plate**. Centrifuge the assembly at ≥ 3,000 x *g* (5,000 x *g* max.) for 5 minutes.

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

8. Add 200 µl **DNA Pre-Wash Buffer** to the wells of the Silicon-A™ Plate, mounted on the emptied Collection Plate, and centrifuge the assembly at ≥ 3,000 x *g* for 5 minutes.

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[1] This equates to approximately $2x10^8$ bacterial cells and $2x10^7$ yeast cells.
[2] Be careful to avoid pipetting debris that can clog the wells of the Silicon-A™ Plate.

9. Add 500 µl **g-DNA Wash Buffer** to the wells of the Silicon-A™ Plate on the Collection Plate and centrifuge the assembly at ≥ 3,000 x *g* for 5 minutes.

10. Transfer the Silicon-A™ Plate to an **Elution Plate** and add 100 µl (25 µl minimum) **DNA Elution Buffer** directly to the matrices in the plate. Centrifuge the assembly at ≥ 3,000 x *g* for 5 minutes.

    Eluted, ultra-pure DNA is now ready for use in your experiments, or the **Elution Plate** can be covered with **Cover Foil** for storage of the DNA.

Appendix

Samples in DNA/RNA Shield™

DNA/RNA Shield™ *ensures nucleic acid stability during sample storage and transport at ambient temperatures. There is no need for refrigeration or specialized equipment. DNA/RNA Shield™ effectively lyses cells and inactivates nucleases and infectious agents (virus), and it is compatible with various collection and storage devices (vacutainers, swabs, nasal, buccal, fecal, etc.).*

DNA/RNA Shield™ *purchased separately (R1100 or R1200).*

1. For samples collected in DNA/RNA Shield™, transfer up to 450 µl of sample into the **ZR BashingBead™ Lysis Rack (0.1 & 0.5 mm)**.

   Note: If using < 450 µl sample, increase the volume to 450 µl using **BashingBead™ Buffer** or **DNA/RNA Shield™** before continuing.

2. Continue from Step 2 of the main protocol (**pg. 5**).

Optimized Lysis Protocols for Bead-Beating

The following conditions with different mechanical lysis machines were validated with minimum bias using the **ZymoBIOMICS® Microbial Community.**

1 Vortex Genie with 2ml BashingBead™ Tubes

Recommended for ease of use and accessibility

Use Microtube Adaptor (Scientific Industries, Inc. Cat. No. S5001-7)

1. 40 minutes of continuous bead beating (max of 18 tubes per adaptor)

2 Bertin Precellys Evolution with 2 ml BashingBead™ Tubes

Recommended for ease of use and ultra-high speed.

1. 1 minute on at 9,000 RPM
2. 2 minutes rest
3. Repeat cycle 4 times for a total of 4 minutes of bead beating

3 MP Fastprep-24™ (Classic & 5G) with 2 ml BashingBead™ Tubes

Maximum of 20 tubes. The weight of > 20 tubes may cause a system error.

1. 1 minute on at 6.5 m/s
2. 5 minutes rest
3. Repeat cycle 5 times for a total of 5 minutes of bead beating

4 Omni Bead Ruptor Elite with 2 ml BashingBead™ Tubes

1. 1 minute on at 6 m/s
2. 5 minutes rest
3. Repeat cycle 3 times for a total of 3 minutes of bead beating

5 Biospec Mini-BeadBeater-16 with 2 ml BashingBead™ Tubes

1. 1 minute at maximum speed
2. 5 minutes rest
3. Repeat cycle 5 times for a total of 5 minutes of bead beating

6 Biospec Mini-BeadBeater-96 with 2 ml BashingBead™ Tubes

1. 5 minutes on at Max RPM
2. 5 minutes rest
3. Repeat cycle 4 times for a total of 20 minutes of bead beating

7 Biospec Mini-BeadBeater-96 with 96 well lysis rack

1. 5 minutes on at Max RPM
2. 5 minutes rest
3. Repeat cycle 8 times for a total of 40 minutes of bead beating

✕ TissueLyser II

No tested conditions yielded accurate profiles. This device is not validated by Zymo Research for microbiome research.

✕ TissueLyser LT

No tested conditions yielded accurate profiles. This device is not validated by Zymo Research for microbiome research.

✕ Retsch Mixer Mill MM 400

No tested conditions yielded accurate profiles. This device is not validated by Zymo Research for microbiome research.

Troubleshooting

For **Technical Assistance**, please contact 1-888-882-9682 or E-mail tech@zymoresearch.com

Problem	Possible Causes and Suggested Solutions
Background Contamination	**Contaminated workspace or equipment**. Clean workspace, centrifuge, and pipettes with 10% bleach routinely to avoid contamination. Make sure bags of columns and buffer bottles are properly sealed for storage. Use of these outside a clean room or hood can result in contamination.
DNA Degradation	**DNase contamination:** Check pipettes, pipette tips, microcentrifuge tubes, etc. for DNase contamination and exercise the appropriate precautions during the DNA purification procedure. If water is used to elute the DNA, ensure that DNase-free water is used.
Low DNA Yield	**Incomplete sample lysis:** Bead beating devices that oscillate in a single dimension (only vertically or only horizontally) have been observed to inefficiently lyse very recalcitrant species. Devices that oscillate three-dimensionally or in a figure-8 motion often lyse efficiently. See **Optimized Lysis Protocols for Bead-Beating** (**pg. 8**) for recommended protocols. **Incomplete debris removal:** For high density samples, ensure lysate is centrifuged properly to pellet insoluble debris following bead beating. Centrifugation duration can be increased to create a tighter pellet. Ensure that none of the debris is transferred to the **Silicon-A™ Plate** in the next step. **Too much input material used.** If the lysate does not pass through the column or is extremely viscous, use less input material. Too much sample input can cause cellular debris to overload the column and insufficient flow. Consult the Sample Sources under Specifications for information on your input limit based on sample.

Problem	Possible Causes and Suggested Solutions
Low DNA Yield (cont.)	**Incomplete elution:** Ensure the DNA Elution Buffer hydrates the matrix for 5 minutes at room temperature before centrifugation. To increase yields, heat the DNA Elution Buffer to 60ºC before use. You can also load the eluate a second time, incubate at room temperature for 3 minutes, and centrifuge again.
Low DNA Purity	**Improper handling:** The column tip can be contaminated with wash buffer flow through. Ensure the tip does not touch the flow through. Empty the collection tube or use a new collection tube when instructed. **Insufficient centrifugation:** Ensure the indicated centrifugation times and speeds are used. Increase the centrifugation time of the final wash step by one minute to ensure complete wash buffer removal. When applicable, centrifuge at maximum recommended speeds.

Ordering Information

Product Description	Catalog No.	Size
***Quick*-DNA™ Fungal/Bacterial Microprep Kit**	D6007	50 Preps.
***Quick*-DNA™ Fungal/Bacterial Miniprep Kit**	D6005	50 Preps.
***Quick*-DNA™ Fungal/Bacterial Midiprep Kit**	D6105	25 Preps.
***Quick*-DNA™ Fungal/Bacterial 96 Kit**	D6006	2 x 96 Preps.

Individual Kit Components	Catalog No.	Amount
ZR BashingBead™ Lysis Rack (0.1 & 0.5 mm)	S6002-96-3	1 Rack
BashingBead™ Buffer	D6001-3-40 D6001-3-150	40 ml 150 ml
Genomic Lysis Buffer	D3004-1-50 D3004-1-100 D3004-1-150 D3004-1-200 D3004-1-250	50 ml 100 ml 150 ml 200 ml 250 ml
DNA Pre-Wash Buffer	D3004-5-15 D3004-5-30 D3004-5-50 D3004-5-250	15 ml 30 ml 50 ml 250 ml
g-DNA Wash Buffer	D3004-2-50 D3004-2-100 D3004-2-200 D3004-2-250 D3004-2-400	50 ml 100 ml 200 ml 250 ml 400 ml
DNA Elution Buffer	D3004-4-1 D3004-4-4 D3004-4-10 D3004-4-16 D3004-4-50	1 ml 4 ml 10 ml 16 ml 50 ml
96-Well Block	P1001-2	2 Blocks
Silicon-A™ Plate	C2001	2 Plates
Collection Plate	C2002	2 Plates
Elution Plate	C2003	2 Plates
Cover Foil	C2007-4	4 Foils

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ZymoTaq DNA Polymerase	Size	Catalog No.
ZymoTaq™ PreMix	50 Rxns. 200 Rxns.	E2003 E2004
ZymoTaq™ DNA Polymerase	50 Rxns. 200 Rxns.	E2001 E2002
ZymoTaq™ qPCR PreMix	50 Rxns. 200 Rxns.	E2054 E2055

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NGS Library Prep Kits	Size	Catalog No.
Zymo-Seq™ SPLAT DNA Library Kit	12 Preps.	D5464

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