
# User-Developed Protocol:

# Purification of DNA from fungi using the EZ1 DNA Tissue Kit

This procedure has been adapted by customers and is for automated purification of total DNA from yeast or filamentous fungi using the EZ1 DNA Tissue Kit and the BioRobot® EZ1 workstation. **The procedure has not been thoroughly tested and optimized by QIAGEN.**

**IMPORTANT**: Please read the Safety Information and Important Notes sections in the *EZ1 DNA Tissue Handbook* before beginning this procedure. For safety information on the additional chemicals mentioned in this protocol, please consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

## Equipment and reagents to be supplied by the user

- BioRobot EZ1 workstation (cat. no. 9000705)
- EZ1 DNA Bacteria Card (cat. no. 9016362)
- EZ1 DNA Tissue Kit (cat. no. 953034)
- Water bath or heating block capable of incubation at 30°C
- Centrifuge and centrifuge tubes capable of attaining 5000 x *g*
- Lyticase, 25 units/µl

## Important point before starting

- Proteinase K is not required for this protocol.

## Things to do before starting

- Heat a water bath or heating block to 30°C.

## Pretreatment of fungi

1. **Centrifuge a fungal culture or primary sample for 5 min at 5000 x *g* and remove the supernatant. Alternatively, use several fungal colonies. Resuspend the pellet or colonies in 190 µl Buffer G2.**

   **Note**: It is often possible to isolate DNA directly from 200 µl of culture without centrifugation.

2. **Add 10 µl lyticase (25 units/µl) and incubate at 30°C for 30 min.**

   **Note**: The amount of lyticase can be increased for difficult-to-lyse cells.

3. **Proceed to the DNA purification procedure.**

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## DNA purification

1. **Insert the EZ1 DNA Bacteria Card completely into the EZ1 Card slot of the BioRobot EZ1.**
2. **Switch on the BioRobot EZ1.**
3. **Press "START" to display the "Protocols" menu.**
4. **Press "1" to select the "Bact_200ul" protocol.**
5. **Choose an elution volume of 100 µl or 200 µl.**
6. **Press any key to proceed through the text displayed in the LCD.**

   The text summarizes the following steps, which describe the loading of the worktable.
7. **Open the workstation door.**
8. **Invert 1–6 reagent cartridges twice to mix the magnetic particles. Then tap the cartridges to deposit the reagents at the bottom of their wells.**
9. **Load the reagent cartridges into the cartridge rack. Load the cartridge rack into the BioRobot EZ1.**

   **Note**: After sliding a reagent cartridge into the cartridge rack, ensure that you press down on the cartridge until it clicks into place.

   If there are fewer than 6 reagent cartridges, you can load them in any order on the rack. However, when loading the other labware in steps 10–12, ensure that they also follow the same order.
10. **Load 1–6 opened elution tubes into the first row.**
11. **Load 1–6 tip holders containing filter-tips into the second row.**
12. **Load 1–6 opened sample tubes containing the samples into the fourth row.**

    The third row remains empty.
13. **Close the workstation door.**
14. **Press "START" to start the purification procedure.**

    The automated purification procedure takes 17 min.
15. **When the protocol ends, the LCD displays "Protocol finished". Open the workstation door.**
16. **Retrieve the elution tubes containing the purified DNA. The DNA is ready to use, or can be stored at 2–8°C for 24 h or at –20°C for longer periods. Discard the sample-preparation waste.**

    If the purified DNA is to be analyzed by real-time PCR, tubes containing eluate should first be applied to a suitable magnetic separator and the eluate transferred to a clean tube (see the *EZ1 DNA Tissue Handbook*) in order to minimize the risk of magnetic-particle carryover.
17. **To run another protocol, press "ESC", prepare samples, and follow the procedure from step 4 onward. Otherwise, press "STOP" twice to return to the first screen of the LCD, close the workstation door, and switch off the BioRobot EZ1.**
18. **Clean the BioRobot EZ1.**

    Follow the maintenance instructions in the *BioRobot EZ1 User Manual*.


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