QIAGEN Supplementary Protocol:

Purification of total DNA from yeast using the DNeasy® Blood & Tissue Kit

This protocol is designed for purification of DNA from up to 5 x 10^7 yeast cells.

Introduction

In this protocol, the cell wall of yeast cells is lysed enzymatically with lyticase. Sphereoplasts are then collected by centrifugation and processed according to the standard DNeasy tissue protocol.

IMPORTANT: Please read the *DNeasy Blood & Tissue Handbook*, paying careful attention to the "Safety Information" and "Important Notes" sections, before beginning this procedure. DNeasy Blood & Tissue Kits are intended for research use. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

Sample collection and storage

For certain yeast cultures that accumulate large amounts of metabolites and/or form very dense cell walls, it is preferable to harvest cells in the early log phase of growth.

Fresh or frozen cell pellets can be used in the procedure. Best results are obtained with fresh material or material that has been immediately frozen and stored at –20°C or –70°C. Repeated freezing and thawing of stored samples should be avoided, since this leads to reduced DNA size. Use of poor-quality starting material will also lead to reduced length and yield of purified DNA.

Quantification of starting material

Yeast growth is usually measured using a spectrophotometer. However, it is very difficult to give specific and reliable recommendations for the correlation between OD values and cell numbers in yeast cultures. Cell density is influenced by a variety of factors (e.g., species, media, and shaker speed) and OD readings of cultures measure light scattering rather than absorption. Measurements of light scattering are highly dependent on the distance between the sample and the detector and therefore readings vary between different types of spectrophotometer. In addition, different species show different OD values at defined wavelengths (e.g., 600 or 436 nm).

We therefore recommend calibrating the spectrophotometer used by comparing OD measurements at appropriate wavelengths with viable cell densities determined by plating experiments (e.g., see Ausubel, F.M. et al., eds. [1991] *Current Protocols in Molecular Biology*, New York: John Wiley & Sons, Inc.). OD readings should be between 0.05 and 0.3 to ensure significance. Samples with readings above 0.3 should be diluted so that the readings fall within this range and the dilution factor used in calculating the number of cells per milliliter.

The following calculation can be considered as a rough guide, which may be helpful. A *Saccharomyces cerevisiae* culture of 1–2 x 10^7 cells per milliliter, diluted 1 in 4, gives OD_{600} values of approximately

0.25 measured using a Beckman DU®-7400 or 0.125 using a Beckman DU-40 spectrophotometer. These correspond to calculated OD values of 1.0 or 0.5 respectively for 1–2 x 10^7 cells per milliliter.

Equipment and reagents required

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

- DNeasy Blood & Tissue Kit (cat. no. 69504 or 69506)
- Pipets and pipet tips
- Vortexer
- Microcentrifuge tubes (1.5 ml or 2 ml)
- Microcentrifuge with rotor for 1.5 ml and 2 ml tubes
- Centrifuge capable of attaining 300 x *g* and 5000 x *g* with centrifuge tubes for harvesting yeast
- Thermomixer, shaking water bath, or rocking platform for heating at 30°C and 56°C
- Ethanol (96–100%)*
- Sorbitol buffer (1 M sorbitol; 100 mM sodium EDTA; 14 mM β-mercaptoethanol)
- Lyticase (yeast-lysing enzyme)

Important points before starting

- If using the DNeasy Blood & Tissue Kit for the first time, read "Important Notes" in the *DNeasy Blood & Tissue Handbook*.
- All centrifugation steps are carried out at room temperature (15–25°C).
- Vortexing should be performed by pulse-vortexing for 5–10 s.

Things to do before starting

- Buffer ATL and Buffer AL may form precipitates upon storage. If necessary, warm to 56°C until the precipitates have fully dissolved.
- Buffer AW1 and Buffer AW2 are supplied as concentrates. Before using for the first time, add the appropriate amount of ethanol (96–100%) as indicated on the bottle to obtain a working solution.
- Preheat a thermomixer, shaking water bath, or rocking platform to 30°C for use in step 2.
- If using frozen cell pellets, equilibrate the sample to room temperature. Avoid repeated thawing and freezing of samples since this will lead to reduced DNA size.

* Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.

Procedure

1. **Harvest cells (maximum 5 x 10^7) by centrifuging for 10 min at 5000 x *g* (approximately 7500 rpm). Discard supernatant.**
2. **Resuspend the pellet in 600 µl sorbitol buffer. Add 200 units lyticase and incubate at 30°C for 30 min.**

   Note: Lysis time and yield will vary from sample to sample, depending on the cell number and species processed. Please refer to the enzyme supplier for further guidelines.

   After incubation, heat the thermomixer, shaking water bath, or rocking platform to 56°C if it is to be used for the incubation in step 5.
3. **Pellet the spheroblasts by centrifuging for 10 min at 300 x *g*.**
4. **Resuspend the spheroplasts in 180 µl Buffer ATL.**
5. **Add 20 µl proteinase K. Mix thoroughly by vortexing, and incubate at 56°C until the spheroplasts are completely lysed. Vortex occasionally during incubation to disperse the sample, or place in a thermomixer, shaking water bath, or on a rocking platform.**

   Lysis time varies depending on the species and amount of yeast processed. Lysis is usually complete in 15 min. If it is more convenient, samples can be lysed overnight; this will not affect them adversely.
6. **Vortex for 15 s. Add 200 µl Buffer AL to the sample, and mix thoroughly by vortexing. Then add 200 µl ethanol (96–100%), and mix again thoroughly by vortexing.**

   It is essential that the sample, Buffer AL, and ethanol are mixed immediately and thoroughly by vortexing or pipetting to yield a homogeneous solution. Buffer AL and ethanol can be premixed and added together in one step to save time when processing multiple samples.

   A white precipitate may form on addition of Buffer AL and ethanol. This precipitate does not interfere with the DNeasy procedure.
7. **Pipet the mixture from step 6 (including any precipitate) into the DNeasy Mini spin column placed in a 2 ml collection tube (provided). Centrifuge at ≥6000 x g (8000 rpm) for 1 min. Discard flow-through and collection tube.***
8. **Place the DNeasy Mini spin column in a new 2 ml collection tube (provided), add 500 µl Buffer AW1, and centrifuge for 1 min at ≥6000 x *g* (8000 rpm). Discard flow-through and collection tube.***

* Flow-through contains Buffer AL or Buffer AW1 and is therefore not compatible with bleach. See *DNeasy Blood & Tissue Handbook* for safety information.

9. **Place the DNeasy Mini spin column in a new 2 ml collection tube (provided), add 500 µl Buffer AW2, and centrifuge for 3 min at 20,000 x *g* (14,000 rpm) to dry the DNeasy membrane. Discard flow-through and collection tube.**

   It is important to dry the membrane of the DNeasy Mini spin column, since residual ethanol may interfere with subsequent reactions. This centrifugation step ensures that no residual ethanol will be carried over during the following elution.

   Following the centrifugation step, remove the DNeasy Mini spin column carefully so that the column does not come into contact with the flow-through, since this will result in carryover of ethanol. If carryover of ethanol occurs, empty the collection tube, then reuse it in another centrifugation for 1 min at 20,000 x *g* (14,000 rpm).

10. **Place the DNeasy Mini spin column in a clean 1.5 ml or 2 ml microcentrifuge tube (not provided), and pipet 200 µl Buffer AE directly onto the DNeasy membrane. Incubate at room temperature for 1 min, and then centrifuge for 1 min at ≥6000 x *g* (8000 rpm) to elute.**

    Elution with 100 µl (instead of 200 µl) increases the final DNA concentration in the eluate, but also decreases the overall DNA yield (see *DNeasy Blood & Tissue Handbook*).

11. **Recommended: For maximum DNA yield, repeat elution once as described in step 10.**

    This step leads to increased overall DNA yield.

    A new microcentrifuge tube can be used for the second elution step to prevent dilution of the first eluate. Alternatively, to combine the eluates, the microcentrifuge tube from step 10 can be reused for the second elution step.

    Note: Do not elute more than 200 µl into a 1.5 ml microcentrifuge tube because the DNeasy Mini spin column will come into contact with the eluate.

Troubleshooting

For general troubleshooting, please consult the Troubleshooting Guide in the *DNeasy Blood & Tissue Handbook*.

	Comments and suggestions
Low yield	
Insufficient lysis	In future preparations, extend incubation with cell-wall–lysing enzyme and/or increase the amount of lysing enzyme. Harvest yeast during early log phase of growth (see "Sample collection and storage", page 1).

QIAGEN kit handbooks can be requested from QIAGEN Technical Services or your local QIAGEN distributor.

Selected kit handbooks can be downloaded from www.qiagen.com/literature/handbooks/default.aspx .
Material safety data sheets (MSDS) for any QIAGEN product can be downloaded from www.qiagen.com/ts/msds.asp .