

Isolation of Nucleic Acids

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Summary

Saccharomyces cerevisiae is an excellent model organism for the study of eukarotic genetics. Easy manipulation of yeast DNA is essential to its role in research, and studies of gene expression or regulation require analysis of RNA. This chapter presents quick and straightforward methods to isolate genomic DNA, plasmid DNA, or RNA from yeast. The isolation protocols presented here, which utilize a glass bead method to break through the cell wall, will yield plasmid DNA of sufficient quality to transform into *Escherichia coli*, genomic DNA that can be digested with restriction enzymes for Southern blotting, or RNA for use in applications such as Northern blots.

Key Words: Yeast; DNA isolation; plasmid isolation; RNA isolation; method; gene expression; gene regulation; glass bead method.

1. Introduction

The budding yeast *Saccharomyces cerevisiae* is often studied as a model eukaryotic organism. It is an excellent tool for genetic studies, both because of the ease of manipulation of the organism and the wealth of reference information available; Web sites such as the *Saccharomyces* Genome Database (SGD; <http://www.yeastgenome.org>) curated by Stanford University contain sequence information, database analysis tools, links to papers, and even links to researchers working in the area. An essential component of using yeast as a model for study is the ability to manipulate the genetic material. RNA is isolated for studies such as gene expression and regulation, for reverse transcriptase polymerase chain reaction, or for ribonuclease protection assays. DNA is isolated to confirm genotypes by Southern blotting or by sequencing, and plasmid DNA may be isolated to confirm that an observed phenotype is a result of a plasmid construct, to recover a plasmid for sequencing, or to transform into *Escherichia coli* for amplification.

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One of the challenges of isolating nucleic acids from yeast cells is the cell wall. The two main methods of overcoming this barrier are first to create spheroplasts and isolate from them (1), or to use vortexing and glass beads to break through the cell wall (2). The methods presented here use the glass bead method; it is quick and straightforward, and eliminates the expense of using zymolyase. The DNA isolated by this method is suitable for restriction digest and Southern blots, or for transformation into *E. coli*.

The method used to isolate RNA is straightforward; the difficulty usually encountered in working with RNA is contamination by exogenous ribonucleases (RNases). Great care has to be taken to avoid this. Gloves must be worn at all times because RNases are present on the skin. Equipment or surfaces touched with bare hands are likely to be contaminated, and because RNases are quite stable this can be a persistent problem. Work areas, reagents, and tools must be specially prepared and protected. It is best to set aside a work area specifically for RNA work if possible so that it can be maintained as an RNase-free area. Separate bags of disposables such as tips and tubes can be kept in this area so that they are only opened with gloved hands. Disposables guaranteed to be RNase-free can be purchased. It is also best to have a separate set of chemicals set aside for RNA work. Even if not specified, unopened chemicals can be assumed to be RNase-free, but if chemicals have been used they may have been measured out with spatulas that previously have been handled without gloves, and thus the chemicals are likely to be contaminated.

2. Materials

2.1. DNA Extraction

1. DNA lysis buffer: 2% Triton X-100, 1% sodium dodecyl sulfate (SDS), 0.1 M NaCl, 1 mM ethylenediaminetetraacetic acid (EDTA), pH 8.0, 10 mM Tris-HCl, pH 8.0.
2. Acid-washed glass beads: 0.4–0.5 mm glass beads, washed in hydrochloric acid then rinsed in copious amounts of water repeatedly until pH reaches 7.0. Beads are baked dry before use.
3. Phenol:chloroform:isoamyl alcohol (25:24:1): Phenol is prepared as per **ref. 3**. Phenol is corrosive, so gloves should be worn during its preparation and handling. Phenol is first melted at 68°C, then hydroxyquinilone is added to a final concentration of 0.1% (see **Note 1**). One volume of 0.5 M Tris-HCl, pH 8.0, is added and the solution is mixed vigorously. Once the two phases have separated, the upper phase is removed and discarded. One volume of 0.1 M Tris-HCl is added and mixed, and again the phases are allowed to separate and the upper phase is removed and discarded. This step is repeated until the phenol reaches a pH of 7.8 or higher (see **Note 2**). The phenol is then stored under 0.1 M Tris-HCl, pH 8.0, at 4°C.
4. 10 – 1 TE: 10 mM Tris-HCl, pH 8.0, 1 mM EDTA, pH 8.0.

5. RNase free of DNase: RNase stock is prepared as per **ref. 1**. RNase A is dissolved in 0.01 M sodium acetate, pH 5.2, at a concentration of 10 mg/mL. It is placed in a boiling water bath for 15 min to inactivate DNase, and allowed to cool to room temperature. One-tenth volume of 1 M Tris-HCl, pH 7.4, is added to adjust the pH (*see Note 3*). Aliquot and store at -20°C .

2.2. RNA Extraction

1. RNA lysis buffer: 0.5 M NaCl, 10 mM EDTA, 1% SDS, 0.2 M Tris-HCl, pH 7.6.
2. Acid-washed glass beads: prepared as in **Subheading 2.1**.
3. Phenol:chloroform:isoamyl alcohol (25:24:1): the phenol is prepared as in **Subheading 2.1**.
4. Diethylpyrocabonate (DEPC)-water: 0.1% DEPC dissolved in distilled and deionized water, then autoclaved (*see Note 4*).

Wear gloves during the preparation of any reagents for use with RNA. Solutions must be prepared RNase-free: any glassware used should be baked overnight at 200°C ; any reusable plastics should be soaked in DEPC water for at least 1 h and autoclaved; and DEPC water should be used in the making of solutions.

3. Methods

3.1. DNA Extraction (*see Fig. 1*)

1. Yeast cells may be cultured on plates or in liquid. It is best to use freshly grown cells. If the DNA to be isolated is genomic, cells may be grown in YPD. If you wish to isolate a plasmid, grow the cells in media that selects for the plasmid marker.
2. If cells were grown in liquid, collect cells in a screw-cap microcentrifuge tube (*see Note 5*) by centrifuging at 16,000g for 15 s and discarding the liquid. You may wish to repeat this step to collect additional cells, especially if the cells were grown in selective media. When you have collected enough cells, add 230 μL of DNA lysis buffer and resuspend the cell pellet. If cells were grown on a plate, collect cells with a sterile toothpick or loop, and resuspend them into 230 μL of DNA lysis buffer in a screw-cap microcentrifuge tube.
3. Add 0.4 g of acid-washed glass beads, and 200 μL phenol:chloroform:isoamyl alcohol. The phenol:chloroform mixture is hazardous, so gloves should be worn for any steps involving its use. Cap the tube, and make sure that it is tight enough that it will not leak during vortexing.
4. Vortex at top speed for 2 min if isolating plasmid DNA, and for 3 min if isolating genomic DNA (*see Note 6*).
5. Centrifuge at 16,000g for 5 min. Transfer aqueous phase (top layer) to a new microcentrifuge tube. Take care not to disturb the interface. Discard tubes of phenol:chloroform according to the requirements of your institution.
6. Add 600 μL of cold 95% ethanol (*see Note 7*) to precipitate the DNA, and keep the tube at -20°C for 30 min. Pellet the DNA by centrifugation at 16,000g for 15

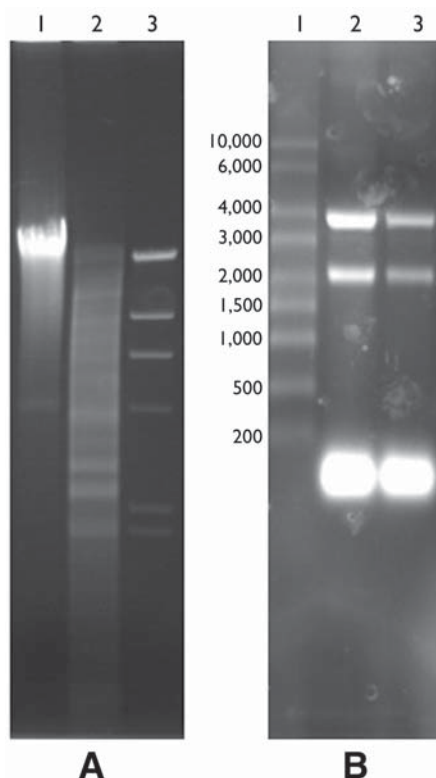


Fig. 1. (A) Yeast genomic DNA isolated by the protocol described. Lane 1, undigested DNA representing half of one isolated sample. Lane 2, the same quantity of DNA, digested with *Eco*R1. Lane 3, λ DNA digested with *Hind*III, used as a size marker. **(B)** Total yeast RNA isolated by the protocol described. Lane 1, RNA size marker with transcript sizes listed in base pairs (Transcript RNA Markers 0.2–10 kb, Sigma, St. Louis, MO). Lanes 2 and 3, 10 μ L of isolated RNA (one-quarter of the isolated sample).

min, and discard ethanol. Tubes may be placed upside down to air-dry for 30 min, or may be dried under vacuum for a few minutes. If you are isolating plasmid DNA, this DNA pellet may be resuspended in water or TE and directly used to transform yeast or bacterial cells. If you are isolating genomic DNA, proceed to **step 7**.

7. Resuspend the DNA pellet in 200 μ L of TE. Add 5 μ L of RNase A, and incubate at 37°C for 10 min.
8. Add 8 μ L of 5 M NaCl and 2 vol (about 430 μ L) of cold 95% ethanol. Place at –20°C for 30 min. Pellet the DNA by centrifugation at 16,000g for 15 min, and discard ethanol. Tubes may be placed upside down to air-dry for 30 min, or may be dried under vacuum for a few minutes. Resuspend the DNA pellet in water or TE (*see Notes 8 and 9*).

3.2. RNA Extraction (see Fig. 1)

This protocol is for the isolation of total RNA, and is based on the protocol in **ref. 4**. There are other methods to isolate particular types of RNA, such as polyA RNA (**5**). Wear gloves throughout this protocol.

1. Culture yeast cells overnight at 30°C in 4 mL of the appropriate liquid medium.
2. Transfer culture to a 15-mL conical tube, and collect cells by centrifuging at 3000g for 4 min at room temperature. Discard the liquid medium, and add 2 mL of 0.1% DEPC water. Resuspend the cell pellet by vortexing briefly.
3. Centrifuge at 3000g for 4 min and discard the DEPC water.
4. Add 350 μ L of Lysis Buffer and resuspend the cells. Transfer this mixture to a microcentrifuge tube (*see Note 5*), then add 0.4 g of acid-washed glass beads and 350 μ L of phenol:chloroform:isoamyl alcohol. Vortex the tubes at top speed for approx 2.5 min.
5. Centrifuge the tubes at 16,000g for 4 min, then transfer the aqueous phase into a new microcentrifuge tube. Add 2.3 vol of 95% ethanol (about 0.8 mL; *see Note 10*). Mix well and centrifuge immediately at 16,000g for 4 min.
6. Discard the supernatant and wash the RNA pellet with 200 μ L of 70% ethanol. Dry briefly under vacuum.
7. Dissolve the RNA pellet in 40 μ L of DEPC water (*see Note 9*).

4. Notes

1. Hydroxyquinilone aids in inhibiting RNase, and it is both an antioxidant and a weak chelator of metal ions (**6**). It has the added benefit of coloring the phenol to make it readily distinguishable from the Tris buffer.
2. The final pH of the phenol is extremely important to the successful isolation of nucleic acids. If the phenol is at too acidic a pH, the nucleic acid will partition into the phenol and be discarded!
3. The pH must be adjusted after the boiling step, otherwise the RNase will precipitate during this step.
4. DEPC should be handled in a fume hood. The water should be allowed to incubate with the DEPC for at least 1 h (we usually leave it overnight) before autoclaving. DEPC in water will decompose into carbon dioxide and ethanol at room temperature with a chemical half-life of about 30 min. Any DEPC remaining after overnight incubation will be inactivated by autoclaving.
5. A screw-cap tube is recommended for this protocol rather than a snap-cap tube because while vortexing with phenol, a snap-cap tube is more likely to leak.
6. If you are isolating from several samples at once, a floater or other holder may be employed so that all tubes may be vortexed at once.
7. In our lab we routinely use 95% ethanol; however, 100% ethanol can be used instead.
8. The quantity of water or TE added depends on the number of cells isolated from and the desired final concentration of DNA. We usually use 25–50 μ L.
9. A spectrophotometer may be used to determine DNA or RNA concentration in the isolated sample, if the sample is quite pure and sufficiently concentrated (**3**).

An optical density (OD) of 1 at 260 nm means that your sample contains approx 50 µg/mL of DNA, or approx 40 µg/mL of RNA. The ratio between readings at 260 nm and 280 nm is also important as an indicator of sample purity. Pure DNA should have an OD_{260}/OD_{280} value of 1.8, and a pure RNA sample should yield a value of 2.0. If the values are significantly lower, your sample is likely contaminated with proteins or phenol; in this case, spectrophotometric determination of the amount of DNA or RNA is not possible. If the sample is not pure enough or concentrated enough for spectrophotometric measurement, the amount of DNA or RNA can be estimated by gel electrophoresis of the sample alongside a sample of known concentration.

10. Various protocols call for a wide range of ethanol volumes, but the range of 2 to 2.5 vol is the most commonly used.

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