MAR 14, 2023

OPEN ACCESS

**DOI:**
dx.doi.org/10.17504/protocols.io.rm7vzb1b4vx1/v1

**Protocol Citation:** Lois L. Hoyer 2023. Extraction of Yeast High-Molecular-Weight Genomic DNA . **protocols.io** https://dx.doi.org/10.17504/protocols.io.rm7vzb1b4vx1/v1



**Protocol status:** Working
This protocol is in active use and works well.

**Created:** Feb 09, 2023

**Last Modified:** Mar 14, 2023

**PROTOCOL integer ID:** 76750

**Keywords:** genomic DNA extraction, long-read DNA preparation, yeast DNA extraction, fungal DNA extraction, Zymolyase

# Extraction of Yeast High-Molecular-Weight Genomic DNA

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ABSTRACT

This protocol is used for extraction of high-molecular-weight genomic DNA from yeast cells such as *Candida albicans*. We have used the protocol successfully for other fungal species such as *Emydomyces testavoran*s that have considerably different incubation conditions and growth kinetics than *C. albicans*. The resulting DNA is suitable for long-read genome sequencing as well as many other applications.

The protocol uses Zymolyase to spheroplast fungal cells, followed by phenol/chloroform extraction and isopropanol precipitation to collect the genomic DNA. The protocol is scaled for cultured yeast cells in a 50-ml volume. Starting with a large number of cells allows DNA yield to be sacrificed at each step in favor of purity and gentle handling of the preparation.

The protocol was based on the method from the Laboratory Course Manual for Methods in Yeast Genetics (Cold Spring Harbor Laboratory, 1986; see References). Modification and addition of protocol steps were made over the years, evolving into the protocol presented here which is a standard method in the Hoyer Laboratory.

## MATERIALS

### Equipment

Autoclave
Chemical fume hood
Balance
Purified water (i.e. distilled or deionized)
Shaking incubator
Centrifuge
Water baths or heating blocks
Pipettes
Pipette tips
Pipettemen
Sterile glass Erlenmeyer flasks
Sterile centrifuge tubes
Electrophoresis gel box
Electrophoresis power supply

### Reagents/Chemicals

Fungal growth medium appropriate for the species
0.5 M EDTA pH 8.0
2- mercaptoethanol (stock bottle from supplier)
1 M sorbitol
1 M dithiothreitol (DTT, Cleland's reagent)
Zymolyase (yeast lytic enzyme)
1 M Tris-HCl pH 8.0
5 M sodium chloride
1 M $MgCl_2$
10% sodium dodecyl sulfate (SDS; filter sterilize, do not autoclave)
Buffer-saturated phenol for molecular biology
Chloroform for molecular biology
Isopropanol
10 mM Tris-HCl/1 mM EDTA pH 8.0 (1x TE buffer)
RNase for molecular biology
Proteinase K for molecular biology
Agarose gel for electrophoresis
Electrophoresis gel buffer

SAFETY WARNINGS

Use chemicals according to manufacturer's safety data sheets. For example, phenol causes skin burns. It should be used in a chemical fume hood. Standard personal protective equipment such as a lab coat, closed-toe shoes, gloves, and safety glasses should be used when working with this protocol.

## Preparation of Reagents

1 The protocol requires four different solutions (called Solution 1 through Solution 4 below). Prepare each as directed and filter sterilize them. Solutions 1 through 3 are stored at 4 °C. Solution 4 is stored at room temperature.

Safety information

**Work with 2-mercaptoethanol (2-ME), dithiothreitol (DTT), phenol, and chloroform in a chemical fume hood. Wear Personal Protective Equipment (PPE) such as a lab coat, closed-toe shoes, gloves, and eye protection when working with these chemicals.**

The recipes below assume that you have various stock solutions available. These stocks are listed in the Materials section of the protocol and will need to be made first. The recipes also may be recalculated to accommodate different starting materials.

**Solution 1: EDTA and 2-ME**

| | A | B | C |
|---|---|---|---|
| | **Final Solution** | **Stock Solutions** | **For 100 ml** |
| | 40 mM EDTA | 0.5 M EDTA pH 8.0 | 8 ml |
| | 90 mM 2-ME | Stock bottle acquired from supplier | 0.69 ml |
| | Purified water | | To 100 ml total volume |

**Solution 2: Sorbitol/EDTA/DTT (add Zymolyase just before use)**

| | A | B | C |
|---|---|---|---|
| | **Final Solution** | **Stock Solutions** | **For 100 ml** |
| | ~ 1 M Sorbitol | 1 M Sorbitol | 100 ml |
| | 1 mM EDTA | 0.5 M EDTA, pH 8.0 | 200 µl |
| | 3 mM DTT | 1 M DTT | 300 µl |
| | 2 mg/ml Zymolyase | Powder | Weigh as much Zymolyase as needed for that day. Dissolve in Solution 2 just before use. |

**Solution 3: Tris-HCl/NaCl/$MgCl_2$/2-ME**

| | A | B | C |
|---|---|---|---|
| | **Final Solution** | **Stock Solutions** | **For 100 ml** |
| | 10 mM Tris-HCl, pH 8.0 | 1 M Tris-HCl, pH 8.0 | 1 ml |
| | 100 mM NaCl | 5 M NaCl | 2 ml |
| | 10 mM $MgCl_2$ | 1 M $MgCl_2$ | 1 ml |
| | 2 mM 2-ME | Stock bottle acquired from supplier | 15 µl |
| | Purified water | | To 100 ml total volume |

**Solution 4: Tris-HCl/NaCl/Sodium Dodecyl Sulfate (SDS)/EDTA**

| | A | B | C |
|---|---|---|---|
| | **Final Solution** | **Stock Solutions** | **For 100 ml** |
| | 50 mM Tris-HCl, pH 8.0 | 1 M Tris-HCl, pH 8.0 | 5 ml |
| | 1 M NaCl | 5 M NaCl | 20 ml |
| | 2% SDS | 10% SDS | 20 ml |
| | 50 mM EDTA, pH 8.0 | 0.5 M EDTA, pH 8.0 | 10 ml |
| | Purified water | | To 100 ml total volume |

**Buffer-Saturated Phenol/Chloroform (1:1) Mixture**

Add equal volumes of buffer-saturated phenol and chloroform to a clean glass bottle. Close the

bottle tightly and mix by inversion. Ensure that the cap does not have a liner that will be dissolved by either phenol or chloroform. Store the bottle at 4 °C .

## Culture of the Yeast Strain

2

Note

This protocol will work for fungi that can be spheroplasted using Zymolyase. Volumes in the recipe can be increased or decreased according to the size of the cell pellet. The protocol volumes are geared toward a 50-ml culture of *Candida albicans* in a 250-ml Erlenmeyer flask. Many yeast strains will grow well in Yeast Extract/Peptone/Dextrose medium (per liter: 10 g Bacto Yeast Extract, 20 g Bacto Peptone, 20 g Dextrose). Typical incubation conditions are 16 h at 30 °C and 200 rpm shaking.

Grow the yeast strain on an agar plate, streaking for isolated colonies. Incubate the plate as appropriate for the species. Store the stock plate at 4 °C for no more than 1 week.

Inoculate a single, representative colony into a sterile Erlenmeyer flask that contains appropriate liquid growth medium. Incubate the flask to reach early stationary phase of the growth curve. Incubation conditions will vary with the species. Zymolyase digestion is more efficient for cells recovered from earlier stages of the growth curve.

## Setting up Equipment

3 On the day of the extraction, set up a 30 °C water bath as well as a 45 °C water bath or heating block.

Warm the bottle containing Solution 4 at 45 °C to dissolve the SDS.

Place tube racks, pipettes, solutions and other small equipment in the fume hood. Solutions that contain 2-ME, DTT, phenol or chloroform should be opened in the fume hood.

## DNA Extraction

4 Collect the yeast cells from the culture flask by centrifugation. Many yeast species will pellet after 1h 5m centrifugation at 3000 x g for 00:05:00 . Adjust the g force and time according to the species.

Remove the culture supernatant into a biohazard waste beaker for autoclaving prior to disposal.

Resuspend the cell pellet in 5 mL Solution 1. Incubate 00:05:00 at room temperature.

Repeat the centrifugation step to pellet the cells. Remove the culture supernatant and set aside for appropriate disposal.

Resuspend the cell pellet in 5 mL of Solution 2 (into which 10 mg Zymolyase was dissolved). Place the tube into a 30 °C water bath. Incubate for at least 00:40:00 with gentle shaking (approximately 100 rpm). Gentle shaking is intended to discourage the cells from pelleting.

**Optional step:** Place 2 µL of the Zymolyase prep onto a microscope slide with and without 2 µL of 10% SDS. Spheroplasts will burst in the presence of SDS, making the SDS-treated sample look quite different than the untreated sample. If the samples look alike, spheroplasts have not been formed yet. Continue the 30 °C incubation step.

Centrifuge to collect the spheroplasts. Carefully pour off the supernatant and set aside for appropriate disposal.

Gently resuspend the spheroplasts in 2 mL of Solution 3. Spheroplasts are fragile. Prematurely releasing the DNA and harsh pipetting will shear the DNA.

Add 2 mL of Solution 4 and mix gently by inversion. Solution 4 contains SDS and will burst the spheroplasts. Handling the prep roughly or pipetting the sample at this point in the protocol will shear the DNA and result in a lower-quality preparation. Your patience and gentle handling of the prep will be rewarded with beautiful high-molecular-weight genomic DNA for procedures such as long-read genome sequencing. If all you want is a genomic DNA prep for PCR amplification of short sequences, it is fine to rush through these steps.

Incubate the tube at 45 °C for 00:10:00, gently inverting the tube several times over the course of the incubation period.

Add 2 mL of phenol/chloroform to the tube. Invert gently until the mixture is homogeneous. Centrifuge the tube for 00:05:00 at 3000 x g. The phenol/chloroform extraction step may be repeated or completed just once.

Remove the upper aqueous phase to a fresh tube sacrificing yield for the sake of purity. Add 2-3 volumes of isopropanol to the aqueous phase to precipitate the genomic DNA. Mix gently by inversion. If you started the prep with 50 ml of yeast cells, there is a sufficient quantity of DNA that you should see the DNA fall out of solution. Mixing by inversion will create a stringy precipitate or pellet that can be scooped out of the tube using a sterile pipet tip or sterile wooden stick. If you started the prep with a smaller number of cells, centrifuge to pellet the DNA and

discard the isopropanol supernatant into the correct waste container.

Add 0.5 mL Tris-EDTA pH 8.0 (1x TE buffer) to the DNA pellet. Allow the DNA to dissolve into the solution over the course of several hours or overnight. Do not pipet the DNA to resuspend it. Pipetting will cause shearing and a lower-quality DNA prep.

RNase treatment is needed to remove RNA from the genomic DNA prep. RNase may be added to the DNA pellet as it is dissolving in the TE buffer.

**Optional step:** Proteinase K treatment is often required before genomic DNA will be accepted for long-read DNA sequencing. Proteinase K treatment may be completed after the RNase treatment following manufacturer's instructions.

Extract the sample with phenol/chloroform and precipitate with isopropanol as described above. Resuspend the DNA pellet in a buffer appropriate for the next steps in your experimental plan.

## Checking DNA Size by Agarose Gel Electrophoresis

5 Agarose gel electrophoresis can be used to check the quality of your DNA prep. Select an appropriate size marker that can help you to demonstrate the size and quality of your DNA. My laboratory routinely gets DNA >50 kb without evidence of shearing from this protocol. The preps are used successfully for long-read genome sequencing.