Protocol

Small-Scale Preparations of Yeast DNA

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In this protocol, yeast DNA is prepared by digestion of the cell wall and lysis of the resulting spheroplasts with SDS. This method reproducibly yields several micrograms of yeast DNA that can be efficiently cleaved by restriction enzymes and used as a template in polymerase chain reaction (PCR). Note that yeast colonies can also be used directly in PCR, without purifying yeast DNA.

MATERIALS

It is essential that you consult the appropriate Material Safety Data Sheets and your institution's Environmental Health and Safety Office for proper handling of equipment and hazardous materials used in this protocol.

RECIPES: Please see the end of this protocol for recipes indicated by <R>. Additional recipes can be found online at http://cshprotocols.cshlp.org/site/recipes.

Reagents

Potassium acetate <R> (5 M)
SDS (10%, w/v)
Sodium acetate (3 M, pH 7.0)
Sorbitol buffer (1 M sorbitol, 0.1 mM EDTA at pH 7.5)
TE buffer, 10× <R> (pH 7.4)
TE buffer, 10× <R> (pH 8.0), containing 20 µg/mL RNase
Yeast cells
Yeast resuspension buffer (50 mM Tris-Cl at pH 7.4, 20 mM EDTA at pH 7.5)
YPD <R>

The use of rich YPD medium is recommended for the propagation of yeast artificial chromosome (YAC)-bearing yeast strains before isolation of genomic DNA. Because YPD contains uracil and tryptophan, there is no selection for retention of the YAC. However, provided that the culture is grown for a short period of time (overnight) and that the yeast strain grows well, there is little danger of selecting variants that have lost their YAC. When working with a strain that grows slowly, either because of the particular YAC carried or because of the genotype of the host, it is a good idea to grow the yeast in uracil tryptophan drop-out medium (also known as $^{-}$*Ura* $^{-}$*Trp drop-out medium, referring to minimal medium lacking uracil and tryptophan) or acid-hydrolyzed casein (AHC) medium to apply selection for the retention of YAC DNA. Some strains of yeast harboring YAC clones grow better in AHC medium (Burke and Olson 1991). This is a complete medium containing adenine, which inhibits the reversion of* ade *mutants. Adenine is added at either a low concentration (20 mg/L) or a high concentration (100 mg/L) depending on the experiment. Low concentrations are used in the initial construction of YAC libraries to select for insert-containing YAC vectors. High concentrations of adenine are used when a YAC strain is to be grown for DNA isolation.*

Zymolyase 100T

From the Molecular Cloning collection, edited by Michael R. Green and Joseph Sambrook.

Cite this protocol as *Cold Spring Harb Protoc*; doi:10.1101/pdb.prot098152

Equipment

Sorvall SS-34 rotor or equivalent
Water bath preset to 65°C

METHOD

Growth of Cells and Extraction of DNA

1. Set up 10-mL cultures of yeast in YPD medium. Incubate the cultures with moderate agitation overnight at 30°C.
2. Transfer 5 mL of the cells to a centrifuge tube. Collect the cells by centrifugation at 2000*g* (4100 rpm in a Sorvall SS-34 rotor) for 5 min. Store the unused portion of the culture at 4°C.
3. Resuspend the cells in 0.5 mL of sorbitol buffer. Transfer the suspension to a microcentrifuge tube.
4. Add 20 µL of a solution of zymolyase 100T (2.5 mg/mL in sorbitol buffer), and incubate the cell suspension for 1 h at 37°C.
5. Collect the cells by centrifugation in a microcentrifuge for 1 min. Remove the supernatant by aspiration.
6. Resuspend the cells in 0.5 mL of yeast resuspension buffer.
7. Add 50 µL of 10% SDS. Close the top of the tube, and mix the contents by rapidly inverting the tube several times. Incubate the tube for 30 min at 65°C.
8. Add 0.2 mL of 5 M potassium acetate, and store the tube for 1 h on ice.

Isolation of DNA

9. Pellet the cell debris by centrifugation at maximum speed for 5 min at 4°C in a microcentrifuge.
10. Use a wide-bore pipette tip to transfer the supernatant to a fresh microcentrifuge tube at room temperature.
11. Precipitate the nucleic acids by adding an equal volume of room-temperature isopropanol. Mix the contents of the tube, and store it for 5 min at room temperature.

    Do not allow the precipitation reaction to proceed for >5 min.

12. Recover the precipitated nucleic acids by centrifugation at maximum speed for 10 sec in a microcentrifuge. Remove the supernatant by aspiration, and allow the pellet to dry in the air for 10 min.
13. Dissolve the pellet in 300 µL of 1× TE (pH 8.0) containing 20 µg/mL pancreatic RNase. Incubate the digestion mixture for 30 min at 37°C.
14. Add 30 µL of 3 M sodium acetate (pH 7.0). Mix the solution and then add 0.2 mL of isopropanol. Mix once again, and recover the precipitated DNA by centrifugation at maximum speed for 20 sec in a microcentrifuge.
15. Remove the supernatant by aspiration, and allow the pellet to dry in the air for 10 min. Dissolve the DNA in 150 µL of 1× TE (pH 7.4). Store the DNA at 4°C.

RELATED INFORMATION

Alternative methods for preparing yeast DNA are provided in Protocol: **Growth of *Saccharomyces cerevisiae* and Preparation of DNA** (Heintz and Gong 2020) and Protocol: **Rapid Isolation of Yeast DNA** (Green and Sambrook 2018).

RECIPES

Potassium Acetate

5 M potassium acetate, 60 mL
glacial acetic acid, 11.5 mL
H_2O, 28.5 mL

The resulting solution is 3 M with respect to potassium and 5 M with respect to acetate. Store the buffer at room temperature.

TE Buffer, 10×

100 mM Tris-Cl (desired pH)
10 mM EDTA (pH 8.0)

Sterilize solutions by autoclaving for 20 min at 15 psi (1.05 kg/cm^2) on liquid cycle. Store the buffer at room temperature.

YPD

Peptone, 20 g
Glucose, 20 g
Yeast extract, 10 g
H_2O to 1000 mL

YPD (YEPD medium) is a complex medium for routine growth of yeast.
To prepare plates, add 20 g of Bacto Agar (2%) before autoclaving.

REFERENCES

Burke DT, Olson MV. 1991. Preparation of clone libraries in yeast artificial chromosome libraries. *Methods Enzymol* **194:** 251–270.

Green MR, Sambrook J. 2018. Rapid isolation of yeast DNA. *Cold Spring Harb Protoc* doi: 10.1101/pdb.prot093542.

Heintz N, Gong S. 2020. Growth of *Saccharomyces cerevisiae* and preparation of DNA. *Cold Spring Harb Protoc* doi: 10.1101/pdb.prot098145.

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