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Yeast gDNA extraction

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1 Works for me dx.doi.org/10.17504/protocols.io.bptbmnin

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ABSTRACT

The purpose of this protocol is to describe how to purify genomic (or chromosomal) DNA from yeast. Here, we use *Saccharomyces cerevisiae* strain DBY10418, though this protocol should apply to other yeast strains. This protocol is adapted from many other protocols (for example, see "Molecular genetic analysis of fission yeast Schizosaccharomyces pombe" by Sergio Moreno, Amar Klar, and Paul Nurse in *Methods in Enzymology* Volume 194, 1991, Pages 795-823, section: Preparing *Schizosaccharomyces pombe* Chromosomal DNA) but we made enough changes to those protocols that made it necessary to publish our own. We used the downstream DNA for whole genome sequencing with Illumina technology, but we anticipate the genomic DNA isolated in this manner can be used for other applications. This protocol uses Zymolyase to digest the yeast cell wall, SDS to lyse the cells, potassium acetate to precipitate proteins, and purifies the resulting genomic DNA via alcohol precipitations and RNase A digestion.

EXTERNAL LINK

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MATERIALS TEXT

YPD agar plate: 50 g Difco™ YPD (BD, USA) and 20 g agar (RPI) dissolved in 1 L of DI water, autoclaved at 120°C for 15 minutes, and poured into 15 x 100 mm plates, about 25 mL per plate

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Culturing yeast

1 Streak out yeast from glycerol stock onto YPD and grow at 30 °C for two or three overnights until visible colonies have formed.

2 Select 3 separate colonies and grow in 40 mL of YPD (50 g Difco™ YPD dissolved in 1 L of DI water and autoclaved at 120 °C for 15 minutes) in a 50 mL conical tube at 30°C overnight (~17 hrs) with shaking at 250 rpm.

3 Centrifuge the overnight culture at 4,000 rpm for 5 minutes to pellet the yeast. Discard supernatant.

Zymolyase treatment

4 Resuspend yeast pellet in 3 mL of 0.9 M sorbitol and 0.1 M Na_2EDTA (pH 7.5).

5 Add 0.1 mL of 2.5 mg/mL (resuspended in 0.9 M sorbitol) solution of Zymolyase 100T (Sigma).

6 Incubate for one hour at 37 °C with gentle shaking.

7 Centrifuge at 4,000 rpm for 5 minutes. Discard supernatant.

Cell lysis

8 First, resuspend cell pellet in 5 mL of 50 mM Tris-Cl and 20 mM Na_2EDTA (pH 7.4).

9 Then, add 0.5 mL of 10% (w/v) SDS, taking care to avoid introducing bubbles. Carefully and thoroughly stir mixture.

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10 Incubate in 65 °C in a water bath for 30 minutes. At the halfway point (i.e., after 15 minutes), gently mix by swirling the tube around.

11 Add 1.5 mL of 5 M potassium acetate and mix thoroughly by gentle inversion. Leave on ice for 1 hour. For every 15 minutes, mix again via gentle inversion.

12 Centrifuge at 10,000 rpm in a Sorvoll SS-34 rotor for 10 minutes to yield a thick, wide pellet.

Purifying genomic DNA

13 Carefully transfer the supernatant into a clean Oak Ridge tube and add two volumes (about 10 mL) of absolute ethanol.

14 Centrifuge at 5,500 rpm for 15 minutes. Discard supernatant.

15 Invert the uncapped Oak Ridge tube to allow the pellet to air-dry for about ten minutes.

16 Resuspend the pellet by gentle mixing in 3 mL of TE buffer (10 mM Tris-Cl, 1 mM Na_2EDTA, pH 7.4).

17 Transfer the solution into two 1.5 mL Eppendorf tubes.

18 Centrifuge at 13,000 rpm at 4 °C for 10 minutes in a tabletop centrifuge to yield a small pale white or pale white-yellow pellet.

19 Combine the supernatant from both tubes back into one 15 mL conical tube.

20 Add 7.5 µL of RNAse A (20 mg/mL from Sigma) and mix gently.

21 Incubate at 37 °C for 30 minutes with gentle shaking.

22 Add two volumes (about 6 mL) of into the 15 mL conical tube and gently mix by inversion to yield a loose “cobweb” of DNA.

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23 Pellet the DNA by centrifugation at 4,500 rpm at 4 °C in tabletop centrifuge. Discard supernatant.

24 Resuspend DNA pellet in 200 µL of DI water.

25 Transfer DNA to new 1.5 mL Eppendorf tube.

26 Add two volumes (400 µL) of absolute ethanol and gently mix by inversion.

27 Centrifuge at 5k rpm for 5 minutes at 4 °C in tabletop centrifuge.

28 Remove supernatant by careful pipetting.

29 Invert tube and leave uncapped for 15 minutes to allow remaining ethanol to air-dry.

30 Resuspend purified DNA in 50 µL of DI water.

31 Assess purity and integrity of the genomic DNA via Nanodrop and TE-agarose gel electrophoresis.

32 Store DNA in freezer (-20°C) until sequencing.

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