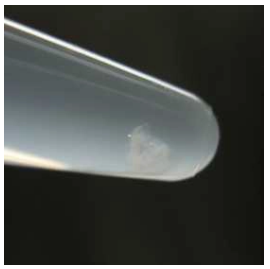
Aug 15, 2022

DNA Extraction from Yeast



This protocol is a draft, published without a DOI.

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Yeast ORFans CURE

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OPEN ACCESS

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Protocol status: Working
We use this protocol and it's working

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Abstract

This is a "quick and dirty" way to get some genomic DNA out of yeast cells. Not pure enough for many things, but should be fine for a PCR template.

This protocol is adapted from

CITATION

Blount BA, Driessen MR, Ellis T (2016). GC Preps: Fast and Easy Extraction of Stable Yeast Genomic DNA.. Scientific reports.
LINK
https://doi.org/10.1038/srep26863

who in turn adapted it from

CITATION

Lõoke M, Kristjuhan K, Kristjuhan A (2011). Extraction of genomic DNA from yeasts for PCR-based applications.. BioTechniques.
LINK
https://doi.org/10.2144/000113672

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Guidelines

The centrifugation steps all specify 21000 x g . If your microcentrifuge doesn't go this high, spin at the fastest speed available.

Materials

Equipment

- Dry bath at 72°C
- Dry bath at 42°C (optional)

Materials

- Sterile water
- Lithium Acetate Dihydrate **Sigma Aldrich Catalog #**L4158 solution, [M] 1 Molarity (M)
- Sodium dodecyl sulfate **Sigma Aldrich Catalog #**436143-25G solution, [M] 10 Mass / % volume
- Ethanol (100%, Molecular Biology Grade) **Fisher Scientific Catalog #**BP2818500
- Ethanol (100%, Molecular Biology Grade) **Fisher Scientific Catalog #**BP2818500 solution, [M] 70 % (v/v)
- TE Buffer **Contributed by users**

Protocol materials

Ethanol (100%, Molecular Biology Grade) **Fisher Scientific Catalog #**BP2818500 In Materials, Materials and 2 steps

TE Buffer Materials, Step 10

Lithium Acetate Dihydrate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**L4158 Materials, Step 1

Sodium dodecyl sulfate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**436143-25G Materials, Step 1

Safety warnings

Both lithium acetate and SDS are irritants, particularly in the eyes. Wear appropriate PPE, including safety glasses, lab coats and gloves.

SDS is particularly gnarly if it's inhaled. If you're making a solution from powdered SDS, use a dust mask and/or weigh it out in a hood.

1 Make 100 µL of yeast lysis solution by mixing the following in a 1.7 ml microcentrifuge tube:

- 70 µL H2O
- 20 µL Lithium Acetate Dihydrate **Sigma Aldrich Catalog #**L4158 solution, [M] 1 Molarity (M)
- 10 µL Sodium dodecyl sulfate **Sigma Aldrich Catalog #**436143-25G solution, [M] 10 Mass / % volume

2 Choose a yeast colony to analyze and circle it on the bottom of the petri dish. A large one is best.

3 Using a micropipette tip, scrape some of the colony off and resuspend it in the lysis solution. Vortex vigorously until the colony is mixed completely into the lysis solution.

Note

THIS IS NOT A CASE WHERE MORE IS BETTER. Your solution should be slightly cloudy. If it is quite "thick", then try again.

4 Incubate at 70 °C for 00:05:00 5m

5 Add 300 µL of 3m
Ethanol (100%, Molecular Biology Grade) **Fisher Scientific Catalog #**BP2818500 and vortex briefly. Centrifuge 21000 x g, 00:03:00 .

Note

Make sure the centrifuge is balanced!

6 Using a P-1000 micropipettor, carefully aspirate the supernatant and discard as biological waste. **Do not disturb the pellet.**

7 Add 500 µL of [M] 70 % (v/v) Ethanol (100%, Molecular Biology Grade) **Fisher Scientific Catalog #**BP2818500 to the microcentrifuge tube. Centrifuge 21000 x g, 00:03:00 . 3m

> **Note**
>
> Make sure the centrifuge is balanced!

8 Using a P-1000 micropipettor, carefully aspirate the supernatant and discard as biological waste. **Do not disturb the pellet. Try to get as much of the ethanol off as you can.**

9 Let the pellet dry by leaving it in a 42 °C dry bath for 00:15:00 . Leave the cap open. 15m

> **Note**
>
> If you don't have a dry bath, just leave the tube (cap open) at room temperature. Extend the time to 00:30:00

10 Add 100 µL of TE Buffer **Contributed by users** and vortex to resuspend the pellet.

11 Centrifuge 21000 x g, 00:00:30 to collect the cellular debris at the bottom. The DNA remains suspended in the supernatant. 30s

12 Label the tube and store at -20 °C , or proceed directly to PCR.

13 If you need to use this sample again: 30s

- Thaw the sample completely.
- Vortex briefly to resuspend everything.
- Centrifuge 21000 x g, 00:00:30 to (re)collect the cellular debris at the bottom of the tube.

Citations

Blount BA, Driessen MR, Ellis T. GC Preps: Fast and Easy Extraction of Stable Yeast Genomic DNA.
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Lõoke M, Kristjuhan K, Kristjuhan A. Extraction of genomic DNA from yeasts for PCR-based applications.
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