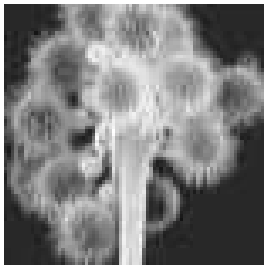
Oct 26, 2020

Fungal CTAB DNA Extraction



In 1 collection

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High molecular weight D... | Chytrid Fungi - Molecula... | 1 more workspace

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

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Protocol Integer ID: 38624

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Abstract

This protocol is a CTAB DNA extraction method for filamentous fungi. Its purpose is to extract high molecular weight genomic DNA for genome sequencing.

Materials

MATERIALS

RNase A (10 mg/mL) **Thermo Fisher Scientific Catalog #**EN0531

PVP **Sigma**

Sodium Acetate 3M, pH 5.2 **Thermo Scientific Catalog #**R1181

potassium acetate **Sigma Aldrich Catalog #**P1190

Proteinase K **Sigma Aldrich Catalog #**P2308

BUFFER A: 0.35 M sorbitol; 0.1 M Tris-HCl, pH 9; 5 mM EDTA, pH 8

BUFFER B: 0.2 M Tris-HCl, pH 9; 50 mM EDTA, pH 8; 2 M NaCl; 2% CTAB

BUFFER C: 5% Sarkosyl (N-lauroylsarcosine sodium salt SIGMA L5125)

Polyvinylpyrrolidone (PVP) 1 %

Proteinase K (20 mg/ml)

Potassium Acetate 5M (KAc precipitate polysaccharides) pH 7.5

RNAse A (10 mg/ml)

(PCI) Phenol:Chloroform:Isoamyl alcohol (25:24:1)

(CI)Chloroform:Isoamyl alcohol (24:1)

Sodium Acetate (NaAc) 3M

Isopropanol 100%

Ethanol 70%

Protocol materials

- RNase A (10 mg/mL) **Thermo Fisher Scientific** **Catalog #**EN0531
- PVP **Merck MilliporeSigma (Sigma-Aldrich)**
- Sodium Acetate 3M, pH 5.2 **Thermo Scientific** **Catalog #**R1181
- potassium acetate **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**P1190
- Proteinase K **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**P2308
- 3M Sodium Acetate solution
- Ethanol
- Ethanol
- Qubit™ dsDNA BR Assay Kit **Thermo Fisher** **Catalog #**Q32853
- Ethidium bromide [EB, EtBr] **Bio Basic Inc.** **Catalog #**EB0195.SIZE.1g

DNA Extraction steps

1m

1 Each tube of lysis buffer will be split in half so prepare one tube of lysis buffer for two samples.
Prepare Lysis Buffer by adding to each 2mL microcentrifuge tube 650 µL µL Buffer A, 650 µL µL Buffer B, 260 µL µL Buffer C, 175 µL µL [M] 1 % (v/v) PVP, and 10 µL Proteinase K to microcentrifuge tube, mix, and then split equally into two 2 mL tubes.

> Note
>
> 65 degree preheated solutions are much less viscous and allow for better mixing and equal splitting.

2 Place in hot plate and heat to 65 °C .

3 Heat a water bath to 37 °C for use in step 12 of this protocol

4 Take your liquid nitrogen, carefully and completely immerse the inside of your mortar. 1m

Add your pestle inside the liquid to super cool it as well.

Add your fungi using a spatula cleaned with ethanol, make sure to grab only the fungi and as little agar as possible.

Grind fungal tissue in liquid nitrogen, add 100 mg of tissue to each tube.

> Note
>
> Take your time on this step by slowly crushing the pieces of frozen fungi.
>
> Make sure the mortar stays cold by carefully adding more liquid nitrogen. (Add a little at a time to ensure the crushed fungi remains in the mortar).
>
> Grinding can take up to a minute, make sure to put enough force to get a fine powder.

5 Incubate 00:30:00 at 65 °C mixing by inversion frequently (00:02:00 - 00:05:00 min).

Note

Sometimes this step can generate gas due to heat expansion. As you are mixing the inversion, make sure to open the cap a bit to relieve the pressure.

6 Add 280 µL 5M Potassium Acetate to each tube, mix by inversion, incubate on ice for 00:05:00 .

7 Add 500 µL - 700 µL (as much as the microcentrifuge tube can reasonably hold) Phenol:Choloform:Isoamyl alcohol, 25:24:1 (PCI), mix by inversion (>5 min) or vortex briefly (5-10 seconds) then incubate for 2 min at room temp (RT).

Note

DO NOT overfill the tube though. The phenol:chloroform:isoamyl alcohol can seep out of the edges and make it very slippery to hold. (Vortexing will be difficult).

If you have over-filled, tip out a bit of your solution onto a napkin and throw it away.

8 Spin at 6000 x g, 00:10:00

9 Take supernatant, transfer to new 2 ml microcentrifuge tube add equal volume Choloform:Isoamyl alcohol, 24:1 (CI) (usually about 1000ul).

Note

If you can, use cut pipette tips here to ensure DNA doesn't shred due to the force of pipetting through a small opening.
Cut pipette tips must be autoclaved before use.

10 Mix by inversion (00:05:00 at least) then incubate at room temperature for 00:02:00

11 6000 x g, 00:10:00

12 Take supernatant (usually 700 µL) and add it to a new 2 ml tube then:

a. RNAse treatment (2.5 µL RNAse A, 37 °C , 01:30:00 - 02:00:00)

b. Optional 1-2 additional CI washes

Note

Additional CI washes will result in cleaner DNA but will also have a lower yield.

13 Add 1/10 vol 3M Sodium Acetate, mix, add 1 vol Isopropanol

3M Sodium Acetate solution

Note

How to tell what 1/10 volume will be? Check the side of the eppendorf tube with your liquid inside. Is the line at 750uL? Divide this by 10 and that will be the volume of Sodium Acetate you add. (in this case 75uL)

14 **Gently** mix by inversion and incubate at Room temperature 00:05:00 , should start to see lots of DNA threads.

Note

This may look like a super sugary solution when you add water to it. It'll have ripples inside. Don't worry if this ends up dissolving after some time.

15

Equipment

Centrifuge	NAME
Benchtop Centrifuge	TYPE
Eppendorf	BRAND
5405000441	SKU
https://online-shop.eppendorf.us/US-en/Centrifugation-44533/Centrifuges-44534/Centrifuge-5425-PF-243560.html	LINK
Any benchtop centrifuge will suffice	SPECIFICATIONS

3000 x g, 00:02:00 **pour out the supernatant.**

Note

A tip here is to align your tubes all in the same orientation. This will help with clear DNA or very low yields of DNA.
The next step requires you to pour our the supernatant. It's good to have a general idea of where the DNA is as you pour out.
Make sure to pour slowly and in one step (vs pouring a little and orienting the tube to stand, back and forth which essentially mixes your DNA and dislodges it from the edge of the tube).

16

Wash with 1 mL freshly prepared, cold [M] 70 % (v/v) Ethanol

17

Equipment

Centrifuge	NAME
Benchtop Centrifuge	TYPE
Eppendorf	BRAND
5405000441	SKU
https://online-shop.eppendorf.us/US-en/Centrifugation-44533/Centrifuges-44534/Centrifuge-5425-PF-243560.html	LINK
Any benchtop centrifuge will suffice	SPECIFICATIONS

at 3000 x g, 00:02:00 , pipette out the Ethanol
Remove as much EtOH as possible before drying.

Note

Pour ethanol out slowly and remove excess EtOH.

18 Dry pellet at RT for 00:10:00 - 00:15:00 and/or 65 °C for < 00:02:00 to dry any leftover ethanol OR if you have a vacuum chamber, place your samples there for 10-20 minutes under constant vacuum.

a. Resuspend in 50 µL - 100 µL TE (adjusted to pH 9) at 65 °C

If DNA still has high protein:
Optional CI wash add 600-800 TE buffer at 65 °C , resuspend DNA, add equal volume CI*, mix as directed in step 9, carry on protocol from there without the RNAse and CI steps.

Note

*For example, take 500-600 uL supernatant if added 800 uL CI

19 Assess DNA quality on NanoDrop.
DNA concentration may not be accurate but the absorbance values will determine the purity of the sample.

Equipment

NanoDrop™ 3300 Fluorospectrometer — NAME

Fluorospectrometer — TYPE

NanoDrop™ 3300 — BRAND

nd-3300 — SKU

https://www.thermofisher.com/order/catalog/product/ND-3300#/ND-3300 — LINK

Note

Remember:
Ranges for high quality DNA
260:280 - 1.80-2.00
260:230 - 2.00-2.20

20 Assess DNA concentration with Qubit Fluorometer with DNA Broad Range (BR) assay.

Note

* Dillute 2 µL of DNA with water and use all 10 µL for the Qubit Assay.

Qubit™ dsDNA BR Assay Kit **Thermo Fisher Catalog #**Q32853

21 Assess DNA quality on Agarose gel

a. Make 0.8- 1% agarose gel with 3 µL EtBr solution per 100mL of gel

Ethidium bromide [EB, EtBr] **Bio Basic Inc. Catalog #**EB0195.SIZE.1g

b. Mix equal parts loading dye and DNA sample and load into wells (leaving space for DNA ladder)

c. Add equal amount of ladder as loading dye + DNA to the first and last well

d. Run the gel (75-120v) for 00:45:00 to 01:00:00 or until samples have run 3/4 of the way through the gel

e. Visualize bands to assess size of DNA fragments

Note

What you are looking for is high quality DNA.

Most should stay in the well.

What you don't want to see is shearing of DNA, which will have a large blur down your entire well.

Also a thing to look for is a general smearing at the bottom of the gel, indicating protein contamination. If you have this, follow **Part A** in **Step 18**.

22 For reference genome sequencing or other applications where a high volume of DNA is required, tubes of the same sample can be combined. Be sure to check the concentration of the final sample.

For applications requiring very clean DNA, it may be helpful to perform additional chloroform:isoamyl washes (Step 12b). As this reduces final yield, it may be helpful to perform multiple DNA extractions of the same organism/sample.