High quality DNA extraction from Fungi_small scale

Yiheng Hu

Abstract

Modified from Benjamin Schwessinger: High quality DNA from Fungi for long read sequencing e.g. PacBio. protocols.io

Optimized for DNA extraction from wheat stripe rust spores.

Buffers are best when fresh and not older than 3-6 months.
Buffered Phenol:Chloroform:Isoamylalcohol (25:24:1) should not be older than 3 months.

Critical steps to obtain high quality DNA:

- Do NOT heat samples during DNA extractions! Perform all steps at RT or 4oC as indicated.
- Do NOT incubate samples with KAc for prolonged time periods

Citation: Yiheng Hu High quality DNA extraction from Fungi_small scale. **protocols.io**
dx.doi.org/10.17504/protocols.io.exmbfk6
Published: 26 Oct 2016

Guidelines

Modified from Benjamin Schwessinger: High quality DNA from Fungi for long read sequencing e.g. PacBio. protocols.io

I am grateful for critical suggestion from Benjamin Schwessinger.

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Reagents required

BUFFER A: 0.35 M sorbitol

0.1 M Tris-HCl, pH 9

5 mM EDTA, pH 8

autoclave to sterilize

BUFFER B: 0.2 M Tris-HCl, pH 9

50 mM EDTA, pH 8

2 M NaCl

2% CTAB

autoclave to sterilize

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

Filter-sterilize

Other solutions:

Potassium Acetate 5M (KAc precipitate polysaccharides) pH 7.5

Polyvinylpyrrolidone (40000 MW) 1 % [w/v] (Sigma PVP40)

Filter-sterilize

Isopropanol 100%

Ethanol 70%

Buffered Phenol:Chloroform:Isoamylalcool P:C:I (25:24:1, Sigma P2069)

Metal beads 5mm in diameter

Enzymes

RNAse T1 (1000 U/ml, Thermo Fisher EN0541)

Lysis Buffer for 500 ul for 10-20 mg starting material

2.5 volume of Buffer A 187.5 ul

2.5 volume of Buffer B 187.5 ul

1.0 volume of Buffer C 75 ul

PVP 0.1 % 50 ul

Protocol

Step 1.

Collect tissue/spores (10-20mg) in a 2ml eppendorf tube with one metal bead. lyse tissue/spores with liquid nitrogen in a precooled tissuelycer. using 50Hz for 1min.

ANNOTATIONS
Isabel Distefano 23 Apr 2018

I performed this protocol with dried fungal culture samples of lichens. We don't have much luck with bead beaters, so I ground the tissue manually in a mortar and pestle with liquid N similar to Schwessinger's protocol.

I let the mortar return to RT, added the lysis buffer directly to it, ground some more, and transferred the buffer to a new 1.5mL tube for incubation with a wide-bore pipette tip.

Step 2.

Make lysis buffer by mixing buffer A+B+C (2.5:2.5:1 + 0.1%PVP final) and briefly heat to 64 °C. Let cool to room temperature, add RNAse T1 to lysis buffer according to 1:1750 of RNAse T1:lysis buffer ratio

Step 3.

Add 500ul lysis buffer into the powder, briefly vortex, incubate at 64oC for 30 mins

DURATION
00:30:00

Step 4.

Cool on ice for 5 mins

DURATION
00:05:00

Step 5.

Add 100 ul (0.2 vol) of KAc 5M, mix by inversion, incubate on ice for max 5 mins

DURATION

00:05:00

Step 6.

Spin at 4°C at max speed for 10 mins

DURATION

00:10:00

Step 7.

Transfer supernatant to fresh eppendorf tube containing 500ul (1vol) (P/C/I) and mix by inversion for 2 mins

DURATION

00:02:00

Step 8.

Spin at 4 °C and max speed for 10 mins

DURATION

00:10:00

Step 9.

Transfer supernatant (400ul) to fresh eppendorf tube containing 500ul RT isopropanol

Step 10.

Incubate at RT for 5-10mins

DURATION

00:10:00

Step 11.

Spin at 4 °C and max speed for 30 mins

DURATION

00:30:00

Step 12.

Carefully remove supernatant with pipette and wash with 1mL fresh 70% Ethanol, invert several times to dislodge pellet

Step 13.

Spin in table top centrifuge for 5mins at 13000g

DURATION

00:05:00

Step 14.

Remove supernatant with pipette and wash with 1mL fresh 70% Ethanol, invert several times to dislodge pellet

Step 15.

Spin in table top centrifuge for 5mins at 13000g

DURATION

00:05:00

Step 16.

Remove supernatant with pipette

Step 17.

Spin in table top centrifuge for 1min at 13000g

DURATION
00:01:00

Step 18.

Remove the remaining ethanol with pipette

Step 19.

Air-dry pellet for 7mins

DURATION
00:07:00

Step 20.

Add 50ul of TE buffer and flick the tube slightly for mixing. DO NOT vortex as it shears DNA.

Store at -20oC.

EXPECTED RESULTS

1ul of extracted DNA can be directly used for PCR analysis.

Sometimes, expecially when larger amount of tissue is used, it is better to dillute 10X to 100X of the DNA sample before processing PCR.