Quick Fungal DNA Extraction

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Abstract

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Protocol

Grow Fungal Cultures

Step 1.

Inoculate fungal material into 300 µL rich growth medium in a 2 mL micro-centrifuge tube.

Grow at RT for 3-4 days.

DURATION
36:00:00

Freeze Dry Samples

Step 2.

Freeze culture tubes in LN2

Freeze dry over night

DURATION
24:00:00

NOTES
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Open the tubes. Obviously.

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Optional: Place frozen tube in tube rack inside a paper bag to prevent samples flying away when vacuum is released.

Tissue Lysis

Step 3.

Add 50 µL 0.6mm glass beads to freeze-dried sample in 2 mL tube and grind using Tissue-Lyser LT for 5 mins at 50 Hz

Tissue Lysis

Step 4.

Add 500 µL **lysis buffer** to pulverised sample. Vortex to mix

PROTOCOL

. Fungal DNA Lysis Buffer

CONTACT: Adam Taranto

Prepare Stock Solutions

Step 4.1.

10% SDS solution

Dissolve 10 g SDS in 80 mL ddH_2O

Adjust volume to 100 mL

AMOUNT

10 g Additional info:

REAGENTS

- Sodium Dodecyl Sulfate PubChem CID: 3423265 by Contributed by users

Prepare Stock Solutions

Step 4.2.

1M Tris Base, pH 8.0

Dissolve 12.114g Tris-Base in 80 mL ddH_2O

pH to 8.0 with 10 M HCl

Adjust volume to 100mL

AMOUNT

12 g Additional info:

REAGENTS

- Tris Hydroxymethylaminomethane PubChem CID: 6503 by Contributed by users

Prepare Stock Solutions

Step 4.3.

0.5 M EDTA, pH 8.0

Add 18.61 g of disodium EDTA•$2H_2O$ to 80 mL of ddH_2O

Adjust the pH to 8.0 with NaOH (20 NaOH pellets)

Adjust volume to 100 mL

AMOUNT

19 g Additional info:

REAGENTS

- EDTA Disodium Salt PubChem CID: 8759 by Contributed by users

Prepare Stock Solutions

Step 4.4.

3 M NaCl

Dissolve 17.532 g NaCl in 80 mL ddH_2O
Adjust volume to 100 mL

AMOUNT
18 g Additional info:

REAGENTS
Sodium Chloride PubChem CID: 5234 by Contributed by users

Prepare Lysis Solution - 100 mL
Step 4.5.

Add 500µL IGEPAL-CA630 to 82.3 mL ddH_2O

Stir until dissolved

AMOUNT
500 µl Additional info:

REAGENTS
IGEPAL-CA630 I3021 SIGMA-ALDRICH by Sigma Aldrich

NOTES
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Final concentration: 0.5%

Prepare Lysis Solution - 100 mL
Step 4.6.

Add 0.5g Sodium deoxycholate

Stir until dissolved

REAGENTS
Sodium Deoxycholate PubChem CID: 23668196 by Contributed by users

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Final concentration: 0.5%

Prepare Lysis Solution - 100 mL
Step 4.7.

Add 10 mL of 10% SDS stock solution

Stir until dissolved

AMOUNT
10 ml Additional info:

REAGENTS
Sodium Dodecyl Sulfate PubChem CID: 3423265 by Contributed by users

NOTES

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Final concentration: 1%

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1% SDS = 34.67 mM

Prepare Lysis Solution - 100 mL

Step 4.8.

Add 5 mL of 3M NaCl stock solution

AMOUNT

5 ml Additional info:

REAGENTS

Sodium Chloride PubChem CID: 5234 by Contributed by users

NOTES

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Final concentration: 150 mM

Prepare Lysis Solution - 100 mL

Step 4.9.

Add 200 µL of 0.5 M EDTA (pH 8.0) stock solution

AMOUNT

200 µl Additional info:

REAGENTS

EDTA Disodium Salt PubChem CID: 8759 by Contributed by users

NOTES

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Final concentration: 1 mM

Prepare Lysis Solution - 100 mL

Step 4.10.

Add 1 mL Tris-Base (pH 8.0)

Stir until dissolved

AMOUNT

1 ml Additional info:

REAGENTS

Tris Hydroxymethylaminomethane PubChem CID: 6503 by Contributed by users

NOTES

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Final concentration: 10 mM

Prepare Lysis Solution - 100 mL

Step 4.11.

Add 1mL of RNase A (10 mg/mL) stock solution

AMOUNT

1 ml Additional info:

REAGENTS

RNase A (10 mg/mL) EN0531 by Thermo Fisher Scientific

NOTES

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Final concentration: 100 µg/mL

Prepare Lysis Solution - 100 mL

Step 4.12.

Store lysis solution at 4˚C

Prepare Lysis Solution - 100 mL

Step 4.13.

Add PVPP

Add 2g Polyvinylpolypyrrolidone, stir until dissolved.

AMOUNT

2 g Additional info:

REAGENTS

Polyvinylpolypyrrolidone CID 6917 by Contributed by users

NOTES

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Final concentration 2%

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PVPP precipitates phenols, tannins, alkaloids which may inhibit enzymes or damage DNA

Tissue Lysis

Step 5.

Heat to 45 ˚C for 5 mins, shaking at 1200 rpm.

DURATION
00:10:00

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Alternatively: Shaking rack on max for 10 mins @ RT

Tissue Lysis

Step 6.

Add 0.5 vol (250 µL) **3 M Potassium Acetate** to lysis mix.

Vortex to mix.

NOTES
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Potassium salt of SDS is highly insoluble. Will precipitate SDS and protein in the absence of ethanol.

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3 M Potassium Acetate, pH 5.5
Dissolve 29.445 g ***Potassium Acetate*** in 80 mL ddH_2O,
pH to 5.5 with 10 M HCl
Mass: 98.15 g/mol
Target working concentration: 0.3 M

Tissue Lysis

Step 7.

Centrifuge at 12 K rpm for a minimum of 15 minutes @ 4˚C.

DURATION
00:15:00

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Proteins should be precipitated.

Tissue Lysis

Step 8.

Transfer supernatant (650 µL) to fresh 1.5 mL microcentrifuge tube.

DNA Precipitation

Step 9.

Add 50 µL **3M Sodium Acetate** pH 5.5

NOTES
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3 M Sodium Acetate, pH 5.5
Dissolve 24.6103 g ***Sodium acetate*** *in 80 mL* ddH_2O
pH to 5.5 with 10 M HCl
Vol to 100 mL
Mass: 82.0343 g/mol
Target working concentration: 0.3 M
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Sodium ions re-solubilise SDS to prevent excessive precipitation in DNA pellet

DNA Precipitation
Step 10.

Add 1 vol chilled **Isopropanol (700 µL)** to the supernatant, mix well, incubate -20˚C for 1hr.

DURATION
01:00:00

DNA Precipitation
Step 11.

Centrifuge at 12 K rpm for a minimum of 15 minutes at 4°C. Decant gently or aspirate supernatant.

DURATION
00:15:00

NOTES
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Take care not to dislodge DNA pellet

Wash DNA Pellet
Step 12.

To the pellet add 500 µL **70% ethanol** stored at -20°C.

Wash DNA Pellet
Step 13.

Centrifuge at 12 K rpm for a minimum of 3 minutes at 4°C. Discard supernatant.

Wash DNA Pellet
Step 14.

Repeat wash step with 500 µL of cold **70% ethanol.** Spin and discard supernatant.

DURATION
00:05:00

Wash DNA Pellet

Step 15.

Dry pellet in speed-vac at 45˚C for 5-10 minutes.

DURATION
00:10:00

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Or until all ethanol is evaporated.

Re-suspend DNA

Step 16.

Re-suspend pellet in nuclease free water or **TE buffer** (10 mM Tris pH 8.0, 1 mM EDTA pH 8.0).

NOTES
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Some samples may require heated shaking (45˚C @ 1200rpm for 10 mins) followed by manual pipetting to fully suspend pellet. Especially in polysaccharide-rich samples.