Small volume fungal genomic DNA extraction protocol for Illumina genome V.1

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High molecular weight DNA extraction from all kingdoms

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This protocol describes a modified SDS phenol:chloroform method for obtaining high quality DNA from hyphae of filamentous fungi. Resulting DNA can be used for Illumina genome sequencing. Pure fungal cultures are inoculated on 60mm 2% MEA plates with a sterile cellophane overlay to minimize media carry-over and grown for 5-10 days (see dx.doi.org/10.17504/protocols.io.qtedwje). Mycelium is removed with sterile forceps and scalpels and placed in a 1.5mL tube with steel beads. Flash freeze the tissue in liquid N and store at -80 prior to extraction.

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Be very gentle during all mixing and pipetting steps to ensure that DNA does not get sheared.

MATERIALS

- 100% EtOH **Contributed by users**
- SDS Buffer **Contributed by users**
- Liquid Nitrogen **Contributed by users**
- Phenol:Chloroform:IAA (25:24:1) **Contributed by users**
- RNase A **Contributed by users**
- Low salt TE **Contributed by users**

Phenol:chloroform is highly toxic and should only be used in designated fume hood. All waste should be disposed of in proper containers.

- Freeze bead-beating aluminum block in -80 for at least 1 hour
- Ensure you have a lab bench aliquot of 100% molecular grade ethanol, Nuclease free water, SDS Extraction buffer, etc.
- Make fresh 70% ethanol dilution (3mL 70% EtOH x _____ samples = _____ mL 70% EtOH)
  _____mL 70% EtOH needed x 0.7= _____mL 100% EtOH, _____mL H2O
- Turn on 4°C refrigerated centrifuge and allow to chill
- Record samples to extract in notebook
- Record lot numbers of all reagents used in notebook
- Label all tubes needed for DNA extraction
- Turn on dry heat block and set to 65°C

1 Pre-measure SDS Buffer need for all samples (e.g. 500uL SDS Buffer x 3 samples= 1,500uL) into a 5 mL tube.

500uL SDS Buffer x _____samples = _____uL of SDS Buffer pre-aliquoted

2 Bead beat tissue frozen tissue via one 15 second cycle at 1400 RPM in frozen aluminum block. If samples are properly homogenized, remove from bead beater and place in -20C ice box. Repeat one 15 second cycle grinding until homogenization has occurred.

Note: The samples might have to be dropped in liquid nitrogen, or have liquid nitrogen poured over them to ensure the samples don't thaw during the grinding process.

3 Add 500uL SDS Buffer to each sample.
- Note: Vortex gently if needed to homogenize sample in SDS Buffer.

4 Incubate at 65°C for 15 minutes, gently inverting sample 5 times every 5 minutes.

5 Centrifuge samples at maximum speed (14,000 RPM) at 4°C for 5 minutes.

6 Carefully remove the supernatant (~500ul) without disturbing the pellet. Place in a new, labeled 1.5mL centrifuge tube.

7 In the chemical fume hood, add 500uL of Phenol:Chloroform:IAA (or 1:1 of supernatant to P:C:IAA) to each tube of supernatant.

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8 Invert the samples 20X to mix until “milky white.”

9 Spin tube at maximum speed at 4°C for 10 minutes.

10 In chemical fume hood, carefully remove the aqueous layer with 200uL pipette, do not disturb the interface. Place respectively into new 1.5mL microcentrifuge tubes. (The smaller aperture of the 200 ul tips makes it easier to remove the aqueous layer without disturbing the layer below).

11 Add 0.3X volume of absolute molecular grade ethanol to each tube. This high-salt, low ethanol mixture precipitates the excess polysaccharides while gDNA remains in solution.

_____uL supernatant x 0.3= _____uL of ethanol

12 Invert tubes 20 times to mix.

13 Spin at maximum speed at 4°C for 15 minutes.

14 Carefully remove the supernatant without disturbing the polysaccharide pellet. Place supernatant into a new 1.5mL centrifuge tube.

15 Add 1.7X volume of absolute molecular grade ethanol to each tube. The gDNA can be seen as falling out of solution as long strands of gDNA.

_____uL supernatant x 1.7= _____uL of ethanol

16 Invert tube 20 times to mix.

17 Spin tube at maximum speed at 4°C for 15 minutes.

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18 Carefully pour off ethanol into glass beaker.

19 Add 1.5 mL of 70% ethanol to remove the excess salt, do not disturb the pellet.

20 Spin the tube at maximum speed at 4°C for 1 minute.

21 Carefully pour off ethanol into glass beaker.

22 Add 1.5 mL of 70% ethanol to remove the excess salt, do not disturb the pellet.

23 Spin the tube at maximum speed at 4°C for 1 minute.

24 Carefully pour off ethanol into glass beaker.

25 Quick spin tube with pellet to gather residual ethanol at the bottom of the tube and carefully remove with a P20 tip.

26 Let the pellet air dry for 5 minutes at room temperature in the biosafety cabinet, taking care not to over dry.

27 Resuspend pellet in 50uL low salt TE.

Note: Increase volume of low salt TE of 10uL if pellet does not resuspend.

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28 Add 2 uL RNase A and place in 37°C for 1 hour.

29 Dilute samples 1:10 and use dilution to Nanodrop and Qubit samples. Record quantification and purity values with chart below.

Sample name	Nanodrop 1:10 ng/uL	Qubit 1:10 ng/uL	Qubit ng/uL	260/280	260/230	Total TE (uL)	Total DNA (ug)

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