Nov 03, 2021

Large Volume Fungal Genomic DNA Extraction Protocol for PacBio

Jana M U'Ren[1], Lilly Moore[1]

[1]University of Arizona

dx.doi.org/10.17504/protocols.io.qtjdwkn

High molecular weight DNA extraction from all kingdoms

U'Ren lab - Ecosystem Genomics

Jana M U'Ren
University of Arizona

This protocol describes a modified SDS phenol:chloroform method for obtaining high molecular weight DNA from hyphae of filamentous fungi. Resulting DNA can be used for PacBio sequencing. Pure fungal culture are inoculated into liquid culture (taking care to limit agar), grown for 3-5 days, and vacuum filtered to remove liquids. Mycelium is rinsed with molecular grade water during filtration to remove excess media. Flash freeze the tissue in liquid N and store at -80 prior to extraction.

DOI

dx.doi.org/10.17504/protocols.io.qtjdwkn

Jana M U'Ren, Lilly Moore 2021. Large Volume Fungal Genomic DNA Extraction Protocol for PacBio. **protocols.io** https://dx.doi.org/10.17504/protocols.io.qtjdwkn

protocol ,

Jun 07, 2018

Nov 03, 2021

Jun 07, 2018 Megan Nickerson University of Arizona

Jun 15, 2018 Jana M U'Ren University of Arizona

12875

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

MATERIALS

- 100% EtOH **Contributed by users**
- 70% EtOH **Contributed by users**
- Nuclease free water **Contributed by users**
- Liquid Nitrogen **Contributed by users**
- Molecular Grade Isopropanol **Contributed by users**
- SDS Buffer **Contributed by users**
- Potassium Acetate **Contributed by users**
- Centrifuge Tubes **Contributed by users**
- RNase **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.

- Make aliquot of molecular grade 100% Ethanol
- Determine amount of 70% molecular grade Ethanol needed and make a fresh dilution
- _____mL 70% EtOH needed x 0.7= _____mL EtOH + _____mL nuclease free water
-

- Put liquid Nitrogen in dewar
- Ensure mortar and pestles are clean and autoclaved
- Estimate molecular grade isopropanol needed and place in -20C to become ice cold

1 Using sterile mortar and pestle, grind mycelium in liquid nitrogen. If multiple different samples, grind one at a time. Once done place the tissue in -80C and grind the next sample until all are complete and ready to begin the extraction process.

Helpful tips regarding grinding samples: Utilize the small red insulated box to pour liquid nitrogen into the mortar and pestle to pre-chill them. After the mortar and pestle are chilled, add half the volume of the mortar of liquid nitrogen along with the mycelium from the -80C tube. Chip away at the mycelium before really starting to grind (this limits the amounts of pieces of mycelium that will fly during grinding.) Add as much liquid nitrogen (in intervals) that is needed to ensure the sample does not thaw during the grinding process. Once the mycelium has been broken apart, it can be ground to a fine powder (making sure to only grind to a fine powder--any further and it can be destructive to DNA.)

Citation: Jana M U'Ren, Lilly Moore Large Volume Fungal Genomic DNA Extraction Protocol for PacBio https://dx.doi.org/10.17504/protocols.io.qtjdwkn

2 Quickly add ground mycelium to 50mL Falcon tube that originally held the mycelium.
- Note: Only do this if all of the non ground mycelium was removed from the tube during the grinding process. If there is still non ground mycelium in the tube, get a new 50mL tube, quickly submerge it in liquid nitrogen to chill it, and add the ground mycelium to it.

3 Once all desired samples have been ground, remove them from the freezer and place them on ice.

4 Estimate the amount of SDS Buffer needed. Add _____mL SDS Buffer to each sample.
- Note: Around 14mL per ~3-4g of ground mycelium proved to be a sufficient ratio to ensure an evenly homogenized sample.

5 Incubate at 65°C for 30 minutes, gently inverting sample 5 times every 10 minutes.

6 Add 0.5X volume of potassium acetate (5M KOAc, pH 7.5) and invert to mix.
_____mL SDS Buffer x 0.5= _____mL KOAc to add

7 Place on ice for 30 minutes.

8 Centrifuge at 4500 RPM for 10 minutes at 4°C. Remove supernatant (~_____mL) and place in new tube. Repeat 3 times.

9 Add 0.7X volume of cold isopropanol and invert to mix.
_____mL supernatant x 0.7= _____mL of ice cold isopropanol

10 Centrifuge at 4500 RPM for 20 minutes at 4°C to pellet precipitated DNA.

11 Remove supernatant by pouring into a glass beaker.

Citation: Jana M U'Ren, Lilly Moore Large Volume Fungal Genomic DNA Extraction Protocol for PacBio
https://dx.doi.org/10.17504/protocols.io.qtjdwkn

12 Wash pellet with 5 mL 70% EtOH, centrifuge for 5 minutes at 4500 RPM, and pour off supernatant.

13 Quickly spin tubes with pellet to gather residual ethanol and the bottom of the tube. Carefully remove residual EtOH with a P1000 tip.

14 Allow the pellet to dry for 5 minutes in the biosafety cabinet. Do not overdry the pellet!

15 Resuspend pellet in 2mL TE.

16 Add 10uL RNase (20mg/mL) and heat in 37°C water bath for 60 minutes.

17 In chemical fume hood, add 2mL of Phenol:Chloroform:IAA (1:1) to sample.

18 Very gently invert the samples 20X to mix until mixture turns a “milky" white color.

19 Centrifuge tube at 4500 RPM for 25 minutes at 4°C.

20 In chemical fume hood, carefully remove as much of the aqueous layer as possible and place in new 5mL microcentrifuge tube.
- Note: If a white, stringy substance is pulled up with the supernatant, that is okay--but do not disturb the interface.

21 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.
_____mL supernatant x 0.3= _____mL of ethanol

Citation: Jana M U'Ren, Lilly Moore Large Volume Fungal Genomic DNA Extraction Protocol for PacBio https://dx.doi.org/10.17504/protocols.io.qtjdwkn

22 Very gently invert tubes 20 times to mix.

23 Centrifuge tube(s) at maximum speed 4500 RPM for 35 minutes.

24 For each sample, carefully remove the supernatant without disturbing the polysaccharide pellet. Place the supernatant into a new 5mL centrifuge tube (FYI: you might have to split into two 5mL microcentrifuge tubes.)

25 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.
_____mL supernatant x 1.7= _____mL of ethanol

26 Very gently invert tube 20 times to mix.

27 Centrifuge tube at maximum speed 4500 RPM for 35 minutes.

28 Carefully pour off the supernatant; do not disturb the pellet.

29 Add 4 mL of 70% ethanol to remove the excess salt, centrifuge maximum speed 4500 RPM for 5 minutes, and carefully pour off the supernatant. Repeat once more.

30 Quickly centrifuge tube with pellet to gather residual ethanol and the bottom of the tube and carefully remove with a P20 tip.

31 Let the pellet air dry for 5 minutes in the biosafety cabinet, taking care not to over dry.

32 Resuspend pellet in 25 uL low salt TE. (Increase volume of low salt TE in intervals of 10uL if

Citation: Jana M U'Ren, Lilly Moore Large Volume Fungal Genomic DNA Extraction Protocol for PacBio
https://dx.doi.org/10.17504/protocols.io.qtjdwkn

pellet does not resuspend.)

33 Gently agitate samples by flicking the tube with your finger, place in 65°C water bath in increments of 5-10 minutes, add more low salt TE if needed and return sample to 65°C to ensure complete resuspension. Samples should not be in 65°C water bath for more than one hour.

34 Transfer 1.5mL microcentrifuge tube with wide bore P1000 pipette.

35 Prepare 1:20 dilution of samples to ensure the sample will fall within Qubit HS range and provide an accurate assessment of DNA purity.

DNA Quantification and QC

36 Nanodrop and Qubit 1:20 dilution of samples and record information in the chart below.

Sample name	Nanodrop ng/uL	Qubit ng/uL	260/280	260/230

Citation: Jana M U'Ren, Lilly Moore Large Volume Fungal Genomic DNA Extraction Protocol for PacBio https://dx.doi.org/10.17504/protocols.io.qtjdwkn