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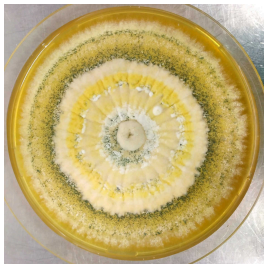
High molecular weight DNA extraction from fungal spores for long read sequencing V.2



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Nonthakorn (Beatrice) Apirajkamol[1,2], Wee Tek Tay[1,2], Bishwo Mainali[2], Phillip Taylor[2], Thomas Kieran Walsh[1,2]
[1]CSIRO; [2]Macquarie University

Nonthakorn (Beatrice) Apirajkamol
CSIRO; Macquarie University

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

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Abstract

A modified extraction protocol is required to extract high quantity and quality DNA from fungal spores. We optimised DNA extraction protocols to obtain a sufficient amount of high molecular weight DNA from fungal spores for long read sequencing i.e., PacBio HiFi.

Note: If required, the DNA extraction protocol can be scaled up to achieved the desired amount of genomic DNA

Image Attribution

Metarhizium pingshaense (M-1000)

Materials

Samples

Fungal spores

Consumable

Tris-hydrochloride (Tris-HCL)
Ethylenediaminetetraacetic acid (EDTA)
Sodium dodecyl sulfate (SDS)
2-mercaptoethanol (β-mercaptoethanol)
1.0 mm zirconia (ceramic) beads
1.5 and 2ml microcentrifuge tubes
Protease K
RNase A
Sodium acetate
Isopropanol
Ethanol
Auto pipette and pipette tips
Paper towel
TE buffer

Equipment

Tissue homogeniser
Incubator (set for 56-57 and 37˚C)
Vortex
Centrifuge
Heat block (optional)

Troubleshooting

Cell disruption

30m

1 **Note:** to obtain the best outcome, freshly made lysis buffer should be used.
Make cell lysis buffer: 50mM Tris-HCL pH8.5, 50mM EDTA, 5% SDS, and 1% 2-mercaptoethanol

2 Add 250 µl of 1.0 mm zirconia (ceramic) beads and 600 µl of cell lysis buffer in a 2ml microcentrifuge tube

Note: 1.0mm zirconia (ceramic) disruptor beads suit for fungal spores size from 2-3.5 µm.

3 Add spore sample (~50-200 mg)

4 Homogenise with tissue homogeniser (5,000 rpm for 15 seconds) 15s

5 To precipitate cell debris, centrifuge at high speed (≥19,000g) for 10-15 minutes or longer if required 15m

6 Collect supernatants to a new 1.5ml microcentrifuge tube (avoiding cell debris pallet)

RNA and protein removal

3h 30m

7 Add 20 µl of protease K (20 mg/ml, invitrogen(TM), cat. #25530049) and vortex briefly

8 Incubate at 56-57°C for a maximum of 3 hours or until the mixture turns clear 3h

9 Cool it to 22-24°C (room temperature)

10 Add 3 µl of RNase A (100 mg/ml, Qiagen cat. # 19101) and incubate at 37°C for 5 minutes 5m
Note: If different concentrations of protease K and RNase A were used, the manufacturer's recommended volume will need to be adjusted accordingly.

11 To precipitate protein, add half of volume of 3M sodium acetate (pH5.2) to the supernatant

12 Vortex for 30 seconds (make sure to vortex well, it should get cloudy) 30s

13 Centrifuge for 5-10 minutes at high speed (≥19,000g) or until the supernatant have no visible cell debris or protein 10m

14 Transfer supernatant to a new tube (avoiding the precipitated protein pallet)

DNA precipitation 1h

15 To precipitate DNA, add equal volume of isopropanol (≥99.8%) and invert the tube 10x **DO NOT VORTEX**

16 Centrifuge for 10-15 minutes at high speed (≥19,000g) 15m

17 Remove the supernatant using a pipette making sure to avoid disturbing the DNA pallet; invert the tube over a piece of clean absorbant paper to dry the tube and DNA pellet

18 To wash the DNA pallet, add 1000 µl of freshly made 70% w/v (80% v/v) ethanol (from ≥99.5% undenatured ethanol) and invert the tube gently 10 times **DO NOT VORTEX**

19 Centrifuge for 10-15 minutes at high speed (≥19,000g) 15m

20 Remove the supernatant and dry the tube over paper as described in step# 18

21 To ensure there is no alcohol residue, dry tubes at room temperature for an hour or in a heat block (56ºC) for no longer than 15 minutes 15m

22 Add 20-50µl of TE buffer (Invitrogen(TM), cat. #12090015) and leave the DNA pallet to resuspend at room temperature overnight or at 56ºC for 10 minute

Results

23 Examples of pooled genome of four Metarhizium species. The total amount of DNA extracted per sample ranged between 23-43µg (derived from approximately 500 - 1,000 mg of starting fungal material. 5 times scaled up) and was submitted to Genomics WA (Perth, Australia) for whole genome sequencing. The genomes were sequenced using PacBio HiFi Sequel® II sequencer with SMRTBell technology.