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

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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 achieve the desired amount of genomic DNA

### IMAGE ATTRIBUTION

*Metarhizium pingshaense* (M-1000)

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

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**Keywords:** DNA extraction, PacBio HiFi, Long read sequencing, High molecular weight DNA extraction, Fungi

## MATERIALS

### Samples

Fungal spores

### Consumable

Tris-hydrochloride (Tris-HCL)  
Ethylenediaminetetraacetic acid (EDTA)  
Sodium dodecyl sulfate (SDS)  
2-mercaptoethanol ( $\beta$ -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)

## 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  $\mu$ l of 1.0 mm zirconia (ceramic) beads and 600  $\mu$ l 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  $\mu$ m.

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 ( $\geq 19,000g$ ) for 10-15 minutes or longer if required

15m



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



## RNA and protein removal

3h 30m

7 Add 20  $\mu$ l 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  $\mu$ l 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 ( $\geq 19,000g$ ) or until the supernatant have no visible cell debris of 10m protein 

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

## DNA precipitation 1h

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

16 Centrifuge for 10-15 minutes at high speed ( $\geq 19,000g$ ) 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 ( $\geq 19,000g$ ) 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 ( $56^{\circ}\text{C}$ ) 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  $56^{\circ}\text{C}$  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.

