Dec 15, 2021

Fungal DNA extraction for Nanopore sequencing

Eri Ogiso-Tanaka[1], Hiyori Itagaki[2], Muneyuki Ohmae[3], Tsuyoshi hosoya[4], Kentaro Hosaka[4]

[1]Center for Molecular Biodiversity Research, National Museum of Nature and Science, 4-1-1, Amakubo, Tsukuba, Ibaraki, 305-0005, Japan;
[2]Department of Biological Science, Graduate School of Science, The University of Tokyo, 7-3-1, Hongo, Bunkyo-ku, Tokyo, 113-0033, Japan;
[3]Hokken Co. Ltd., 7-3 Ekihigashimachi, Mibu-machi, Shimotsuga-gun, Tochigi 321-0222, Japan;
[4]Department of Botany, National Museum of Nature and Science, 4-1-1, Amakubo, Tsukuba, Ibaraki, 305-0005, Japan

1

dx.doi.org/10.17504/protocols.io.b2vfqe3n

Eri Ogiso-Tanaka

This protocol is intended for extraction of high molecular weight DNA from fungal samples.

DOI

dx.doi.org/10.17504/protocols.io.b2vfqe3n

Eri Ogiso-Tanaka, Hiyori Itagaki, Muneyuki Ohmae, Tsuyoshi hosoya, Kentaro Hosaka 2021. Fungal DNA extraction for Nanopore sequencing. **protocols.io** https://dx.doi.org/10.17504/protocols.io.b2vfqe3n

fungi, DNA extraction, HMW, long read sequencing, Nanopore, National Museum of Nature and Science

protocol ,

Dec 15, 2021

Dec 15, 2021

55943

Cut the tip of the pipette man tips in advance to make the outlet wider.

1 The frozen mycelium samples are ground to fine powders using a mortar and pestle in liquid nitrogen to avoid raising the temperature of the samples for high molecular weight DNA extraction.

2 Add **20 mL** of pre-warmed (**60 °C**) lysis buffer with **800 µL** ProteinaseK (FUJIFILM Wako Pure Chemical Co., Ltd. Japan) and **50 µL** RNase A (Nippon Gene Material Co., Ltd. Japan) in a glass beaker.

20 mL lysis buffer [2% CTAB, 100mM TrisHCl, 20mM EDTA, 1.4M NaCl, 1% PVP)]

800 µL ProteinaseK

50 µL RNase A

60 °C

3 The mixture was incubated at 55 °C in a water bath with shaking for 5 min. 5m

55 °C

00:05:00 .

4 Add 1 vol phenol/chloroform/isoamylic alcohol (PCI) 25:24:1 and mix by inversion.

4.1 Centrifuge 12000 rpm (13000 g) with soft/slow break function for 20 min at RT. 20m

12000 rpm **13000 x g**

00:20:00

Room temperature

4.2 Remove aqueous layer to new 50 mL tube.

4.3 Repeat step 4

5 Add 1 vol chloroform and mix by inversion.

Citation: Eri Ogiso-Tanaka, Hiyori Itagaki, Muneyuki Ohmae, Tsuyoshi hosoya, Kentaro Hosaka Fungal DNA extraction for Nanopore sequencing https://dx.doi.org/10.17504/protocols.io.b2vfqe3n

Always work with chloroform in a fume hood.

5.1 Centrifuge 14000 rpm (17800 g) with soft/slow break function for 10 min at RT. 10m

14000 rpm **17800 x g**

00:10:00

Room temperature

5.2 Remove aqueous layer to new 50 mL tube.

5.3 Repeat step 5

6 Add 1 vol isopropanol and mix gently by inversion.

7 Centrifuge 14000 rpm for 5 min at 4 °C. Remove and discard the supernatant. 5m

14000 rpm **17800 x g**

00:05:00

4 °C

8 Rinse pellet with 1mL 75% ethanol at RT.

1 mL 75% ethanol

9 All aqueous and pellet to new 2 mL tube.

10 Spin down and remove supernatant.

Citation: Eri Ogiso-Tanaka, Hiyori Itagaki, Muneyuki Ohmae, Tsuyoshi hosoya, Kentaro Hosaka Fungal DNA extraction for Nanopore sequencing https://dx.doi.org/10.17504/protocols.io.b2vfqe3n

11 Air dry DNA pellet.

If you have a good nose, you can use the smell to determine if the ethanol is gone.

12 Resuspend DNA in sterile 50uL 10mM Tris-HCl for 1 hr~ at RT in dark. 2h

50 µL Tris-HCl

Room temperature

01:00:00 ~ **Overnight**

DO NOT vortex or pipet to resuspend. Gently flick the tube and leave room temperature in dark for up to overnight.

13 Check the quality and concentration of DNA using spectrophotometrically with NanoDrop (Thermo Fisher Scientific, USA) and in a Qubit 2.0 fluorometer (Thermo Fisher Scientific).

14 Check the DNA degradation using Genomic DNA ScreenTape assay with 2200 TapeStation system (Agilent Technologies, Germany).

15 Store at 4 °C until library preparation. (at -20 °C for long-term storage)

Citation: Eri Ogiso-Tanaka, Hiyori Itagaki, Muneyuki Ohmae, Tsuyoshi hosoya, Kentaro Hosaka Fungal DNA extraction for Nanopore sequencing https://dx.doi.org/10.17504/protocols.io.b2vfqe3n