Introduction

This protocol describes a method to extract high molecular weight genomic DNA from button mushrooms (*Agaricus bisporus*) using nuclei isolation, performed as described in Zhang, M. et al., 2012, followed by QIAGEN Genomic-tip purification. Prior to sequencing, 8 µg of genomic DNA was size-selected using the size selection of HMW DNA by semi-selective DNA precipitation protocol. Sequencing performance was determined using the MinION, using the Ligation Sequencing Kit to generate sequencing libraries.

Materials

For nuclei isolation:

- 8 g mushroom gills
- TissueRuptor II and disposable probes
- Trizma base
- KCl
- Na2EDTA
- Spermidine trihydrochloride
- Spermine tetrahydrochloride
- NaOH
- B-mercaptoehanol
- Triton X-100
- Sucrose
- ddH20
- Miracloth
- Cheesecloth
- Funnel
- 50 ml Falcon tubes
- Refrigerated centrifuge with capacity for 50 ml Falcon tubes
- Ice bucket with ice
- P20, P100, P200 and P1000 pipettes, tips and wide-bore pipette tips

For gDNA extraction and purification:

- QIAGEN Blood and Cell Culture DNA Midi Kit
- Isopropanol
- 70% ethanol
- Proteinase K
- Vortex
- 50 ml Falcon tubes
- Refrigerated centrifuge with capacity for 50 ml tubes
- Incubator or water bath with agitation capability and temperature control for 50°C
- TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0)
- P20, P100, P200 and P1000 pipettes, tips and wide-bore pipette tips

Method

Nuclei isolation:

1. Prepare 10X Homogenisation Buffer (HB) stock as below. Adjust the pH to 9.0-9.4 with NaOH, and store the solution at 4°C. **Tip**: When developing this protocol, we made up 1 litre of HB stock and stored it at 4°C for up to a year.

HB stock:

Reagents	Concentration (mM)
Trizma base	100 mM
KCl	800 mM
Na2EDTA	100 mM
Spermidine trihydrochloride	10 mM
Spermine tetrahydrochloride	10 mM

1. Prepare approximately 90 ml of 1x Homogenisation Buffer (HB) working solution. Keep the solution at 4°C.

HB working solution:

Reagents	Concentration
HB stock	1x
Sucrose	0.5 M

1. Prepare two 50 ml tubes per sample and add 20 ml of HB working solution to each.
2. Add 4 g of mushroom gills to each tube.
3. Homogenise the mushroom sample using the TissueRuptor II at the lowest speed. This will take between 45 seconds to a minute to homogenise
4. Add another 20 ml of HB working solution to each tube and invert a few times.
5. Transfer the tubes to a fume hood and continue the next steps inside the hood until step 20 due to the β-mercaptoethanol toxicity.
6. Add 0.15% (v/v) of β-mercaptoethanol and invert the tubes 10 times to mix.
7. Incubate the tubes in a HulaMixer (or equivalent) at 4°C for 10 minutes. If there is no mixer available at 4°C, keep the tubes on ice and gently invert them five times every minute.
8. Put a funnel on top of two fresh 50 ml Falcon tubes and add a layer of miracloth and two layers of cheesecloth.
9. Pass the solutions from step 9 through the funnel prepared in step 10. We recommend squeezing the miracloth to maximise the nuclei recovery.
10. Centrifuge the tubes at 4,000 x g for 20 minutes at 4°C.
11. Discard the supernatant.
12. Prepare approximately 45 ml of HB washing solution as below and cool to 4°C.
    HB washing solution:

Reagents	Concentration
HB working solution	1X
β-mercaptoethanol	0.15% (v/v)
Triton X-100	0.2% (v/v)

1. Add 1 ml HB washing solution to the pellets and gently resuspend the pellets by pipetting up and down with a wide-bore pipette

tip.

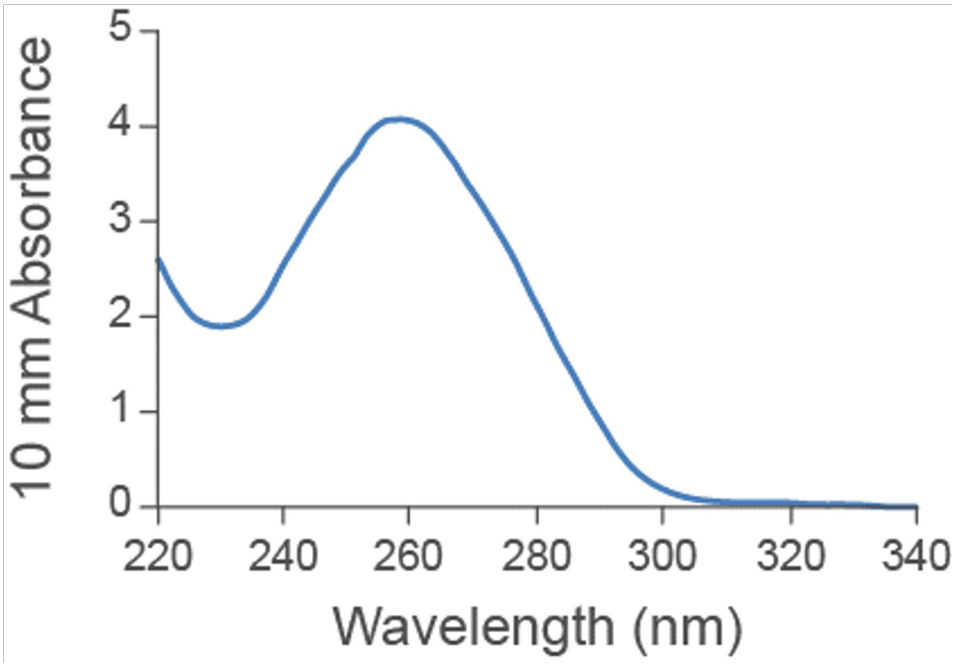
2. Add 9 ml HB washing solution to both tubes, and gently invert 10 times to mix.
3. Centrifuge the tubes at 3,100 x g for 15 minutes at 4°C.
4. Discard the supernatant.
5. Repeat steps 15-18.
6. Add 500 µl of 1X HB working solution and gently resuspend the pellet with a wide-bore pipette tip.

DNA extraction:

1. To each tube with isolated nuclei, add 10 ml of buffer G2 and 100 µl of proteinase K,before inverting the tube 10 times to mix.
2. Incubate at 50°C for 1 hour with gentle mixing (100 RPM). The tube contents should appear homogeneous but if solid particles are still visible, centrifuge the tubes for a minute at 2,000 x g and transfer the supernatant to fresh tubes.
3. Equilibrate a Genomic-tip 100/G with 4 ml of buffer QBT.
4. Pour one of the tubes with lysate into the Genomic-tip 100/G.
5. Once the content of the first tube has passed through, pour the second tube into the Genomic-tip 100/G.
6. Purify the lysate according to the standard QIAGEN protocol (steps 3-5, pages 50-51).
7. After adding the isopropanol, invert the tubes 10 times and incubate it overnight at −20°C.
8. Centrifuge the tube at 4,000 x g for 30 minutes at 4°C.
9. Discard the supernatant and add 5 ml of ice-cold 70% ethanol.
10. Invert the tube five times and centrifuge at 4,000 x g for 3 minutes at 4°C.
11. Discard the supernatant and use a clean tissue to dry the walls of the tube.
12. Elute the pellet in 150 µl TE buffer.
13. **Optional step:** Take 60 µl of the gDNA and size select your sample using thesize selection protocol. About 60% of gDNA is expected to be recovered.

Results

- **Yield:** 15-25 µg
- **A260/280:** 1.92
- **A260/230:** 2.14



Sequencing performance

The library for nanopore sequencing was prepared using the Ligation Sequencing Kit.

The flow cell was washed using the Flow Cell Wash Kit (EXP-WSH004) and the library re-loaded after ~20 hours of sequencing to maximise flow cell output.
Read length profile: