

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-mercaptoethanol
- Triton X-100
- Sucrose
- ddH2O
- 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
Na ₂ EDTA	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.

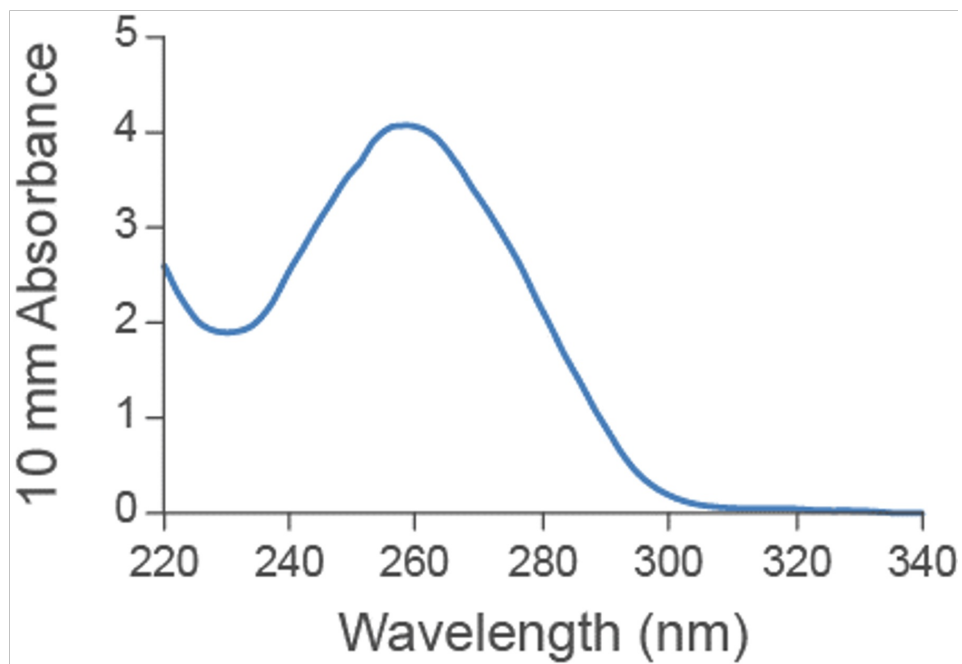
2. Add 9 ml HB washing solution to both tubes, and gently invert 10 times to mix.
3. Centrifuge the tubes at 3,100 x g for 15 minutes at 4°C.
4. Discard the supernatant.
5. Repeat steps 15-18.
6. Add 500 µl of 1X HB working solution and gently resuspend the pellet with a wide-bore pipette tip.

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 the [size 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:

