Text S2. *Coprinopsis cinerea* high-molecular-weight genomic DNA isolation protocol.

Equipment checklist:

- A water bath at 50°C
- A microcentrifuge
- A magnetic plate
- Pipettes (P1000, P200, P10)
- A rotator (optional, can be replaced by inverting the tube manually)
- A thermostat (optional)
- A pot of water at ~ 65°C
- A pot of ice

Reagent checklist:

- Buffer AP1, buffer P3 and RNase A (DNeasy Plant Mini Kit, Qiagen, can be replaced by other cell/tissue lysing reagent)
- AMPure XP beads (Beckman Coulter, USA) or other equivalent magnetic beads
- Ethanol absolute
- Binding buffer (20 g PEG 8000 and 17.5 g NaCl in 100 ml water with DEPC treatment, autoclave and aliquot into several tubes)
- DEPC water (for magnetic beads washing, 70 % ethanol solution preparation and DNA elution, 5 ml times number of reactions)

Consumable checklist:

- Eppendorf tube (1.7 ml and 2 ml)
- 15 ml/50 ml falcon or clean bottle (for 70 % ethanol solution preparation, 2 ml times number of reactions)
- Pipette tips (P1000, P200, P10)

Before start:

1) Preheat buffer AP1 at 65°C before sample homogenization
2) Preheat the water bath to 50°C
3) Cool down the centrifuge at 4°C (or place the centrifuge in a 4°C refrigerator)

Part 1: Cell lysis and contaminants removal (~1.5 h)

1) Collect fresh mycelia and carefully grid with liquid N_2.
2) Add 600 µl of 65°C preheated lysis buffer AP1 and 4 µl RNase A to a 2 ml Eppendorf tube. (A 2 ml tube would make a better contact of tissue powder and buffer.)
3) Add ~ 100 mg of homogenized tissue powder to the above lysis buffer and water bathed at 50°C for 1 hour. During the lysis process, place the tube on the rotator for 1 min or manually invert the tube 20 times every 10 min incubation. After the first 30 min water bath, add another 4 µl RNase A to the homogenous solution to fully remove the RNA.

- During the cell lysing period, prepare pre-treated magnetic beads. Aliquot 450 µl AMPure XP beads (1.8 × volume of 250 µl homogenous solution) to each 1.7 ml Eppendorf tube. Place the tube on a magnetic plate and collect the buffer to another tube. Adding DEPC water to the tube and resuspend the beads, and then return the tube to the magnetic plate and remove the water. Wash the beads with DEPC water for four times and resuspend the beads in the original buffer.

4) Add 200 µl (1/3 of lysis buffer volume) buffer P3 to the homogenous.
5) Place the tube on the rotator for 2 min to allow fully contact of solution and then place the tube on ice for 5 min to precipitate the proteins and polysaccharides.
6) Centrifuge at 5,000 g for 10 min at 4°C to remove the cell pellets and contaminants.

7) Transfer the supernatant to a new 1.7 ml Eppendorf tube and centrifuge at 5,000 g for another 10 min at 4°C to fully remove the precipitate. (Centrifuge at higher speed might cause damage to the DNA.)

Part 2: Genomic DNA purification (~45 min)

8) Transfer 250 µl of the supernatant in step 7 directly to the tube with pre-treated magnetic beads and add 250 µl binding buffer as well.
9) Resuspend the solution by gentle pipetting. Place the tube on the rotator for 10 min to ensure DNA molecules are fully bound to the magnetic beads.

- During the DNA binding period, prepare 70 % ethanol solution and preheat the elution buffer (DEPC water) at 50°C. 2 ml 70 % ethanol solution and 100 µl DEPC water for each tube reaction.

10) Spin down the droplets by short centrifuge at the maximum force of 2,000 g.
11) Place the tube on the magnetic plate for 5 min or until the solution become clear. Remove the supernatant without disturbing the beads pellet.
12) Add another 250 µl of lysis supernatant and 250 µl of binding buffer to the beads and repeat step 9 to 11.
13) Add 1 ml 70 % ethanol solution to wash the beads pellet. Place the tube on the rotator for 2 min.
14) Spin down the droplets by short centrifuge at the maximum force of 2,000 g.
15) Place the tube on the magnetic plate for 5 min or until the solution become clear. Remove the supernatant without disturbing the beads pellet.
16) Repeat step 13 to 15.
17) Spin down the droplets, place the tube on the magnetic rack, remove the remaining solution with a P10 pipette.
18) Air dry for 30 – 60 sec.

Part 3: DNA elution (~15 min)

19) Add 80 µl of DEPC water to the beads and resuspend the beads by pipetting.

20) Incubate the tube at 50°C for 10 min to ensure the good elution efficiency of DNA.

21) Place the tube back to the magnetic plate to separate the beads and the DNA solution. Transfer the DNA solution to a new tube.

Part 4 (optional): Extra DNA purification, DNA size selection and DNA concentration (~30 min)

22) Pool several tubes of DNA solution together.

23) Add 1 × volume AMPure XP beads (not pre-treated) and purify the DNA following the manufacture's protocol (or following step 9 to 11 and 13 to 21).

24) Elute at 50°C for 10 min and adjust the concentration of DNA solution.

The quality and quantity of genomic DNA solution was measured using Nanodrop (Thermofisher, USA). Final DNA solution was quantified using Qubit® dsDNA HS Assay Kits and Qubit® 2.0 fluorometer (Invitrogen, USA). DNA fragment size was evaluated with gel electrophoresis on a 0.8 % agarose gel. Pulse field gel electrophoresis and Bioanalyzer sample analysis were not performed because of the shortage of equipment, therefore, the adequate number of mean molecular weight of genomic DNA sample remained unknown. In this study, the Nanopore sequencing library had the mean read length of over 13 Kbp and N50 read length of 24.6 Kbp (see **Results**).