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High molecular weight DNA extraction for long read sequencing

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High molecular weight DNA extraction from all kingdoms
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Protocol status: Working
We use this protocol and it's working

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Abstract

Adaptation of the protocol **dx.doi.org/10.17504/protocols.io.k6qczdw**
Optimized for DNA extration from Bipolaris sorokiniana spores.

Guidelines

Optimized for DNA extraction from Bipolaris sorokiniana

Buffers are best when fresh and not older than 3-6 months. Buffered Phenol:Chloroform:Isoamylalcohol (25:24:1) should not be older than 3 months.

Critical steps to obtain high quality DNA:

- Do NOT heat samples during DNA extractions! Perform all steps at RT or 4°C as indicated.
- Do NOT incubate samples with KAc for prolonged time periods
- Perform two steps of buffered Phenol:Chloroform:Isoamylalcohol purification to reduce co-purifying metabolites.

Materials

MATERIALS

N-lauryl sarcosine **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**L5125-50G

Reagents required
BUFFER A: 0.35 M sorbitol
0.1 M Tris-HCl
5 mM EDTA, pH 8
autoclave to sterilize

BUFFER B: 0.2 M Tris-HCl
50 mM EDTA, pH 8
2 M NaCl
2% CTAB
autoclave to sterilize

Other solutions:
Potassium Acetate 5M (KAc precipitate polysaccharides) pH 7.5
Polyvinylpyrrolidone (40000 MW) 10 % [w/v] (Sigma PVP40)
Polyvinylpyrrolidone (10000 MW) 10% [w/v] (Sigma PVP10)
Sodium Acetate (NaAc) 3M pH 5.2
5% Sarkosyl N-lauroylsarcosine sodium salt (SIGMA L5125)

Filter-sterilize

Isopropanol 100%
Ethanol 70%
Buffered Phenol:Chloroforme:Isoamylalcool P:C:I (25:24:1, Sigma P2069)
Autoclave acid washed Sand

Enzymes
RNAse A or T1 (1000 U/ml, Thermo Fisher EN0541)
Proteinase K (800U/ml, NEB P81072)

Troubleshooting

Extraction

1

Note

This protocol is an adaptation from https://www.protocols.io/view/high-quality-dna-from-fungi-for-long-read-sequenci-k6qczdw

2.5 volume of Buffer A 5 mL
2.5 volume of Buffer B 5 mL
1.0 volume of Buffer C 2 mL
PVP 40 10% 1mL
PVP 10 10% 1mL

Extraction

2 Grind tissue/spores with liquid nitrogen in a mortar with sand, use 1g of sand per 100mg of starting material. Grind for 2 mins in 4× 15 sec bursts adding liquid nitrogen after each 15 sec grinding burst.

Note

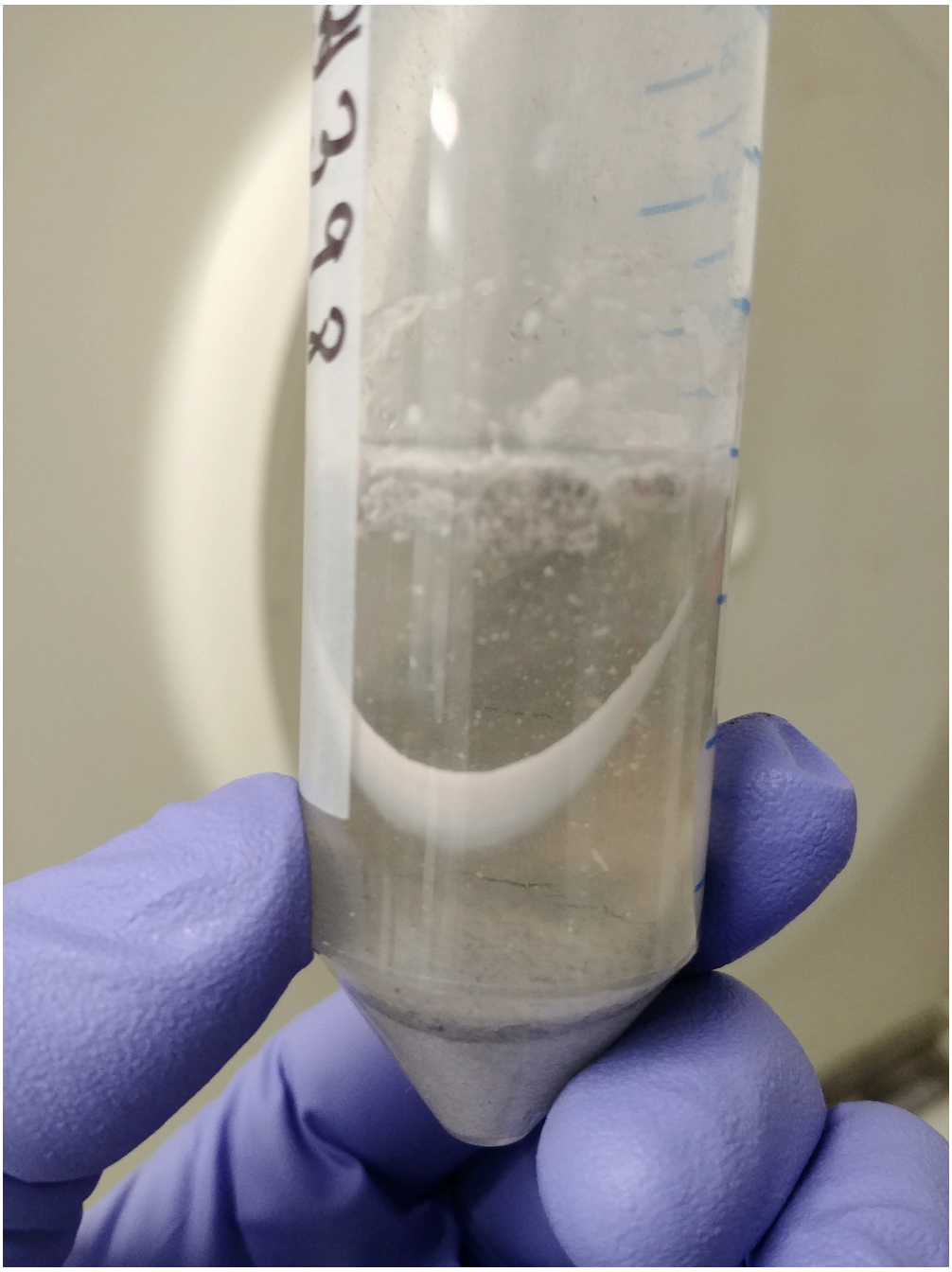
Using a sharp scalpal, I scrape the mycelia and the spores from two fully covered petri dishes (~90mm diameter).

3 Transfer ground tissue with sand into 50mL Falcon containing lysis buffer. Add 10 uL of RNAse A and 100 uL of Proteinase K and mix by inversion until no clumps are visible. Incubate in rotator at 30 rpm for 30 min at RT. 00:30:00

4 Cool in ice for 5 minutes 00:05:00

5 Add 2.8 mL (0.2 vol) of KAc 5M, mix by inversion, incubate on ice for max 5 mins 00:05:00

6 Spin at 4°C and 5000g for 12 mins 00:12:00



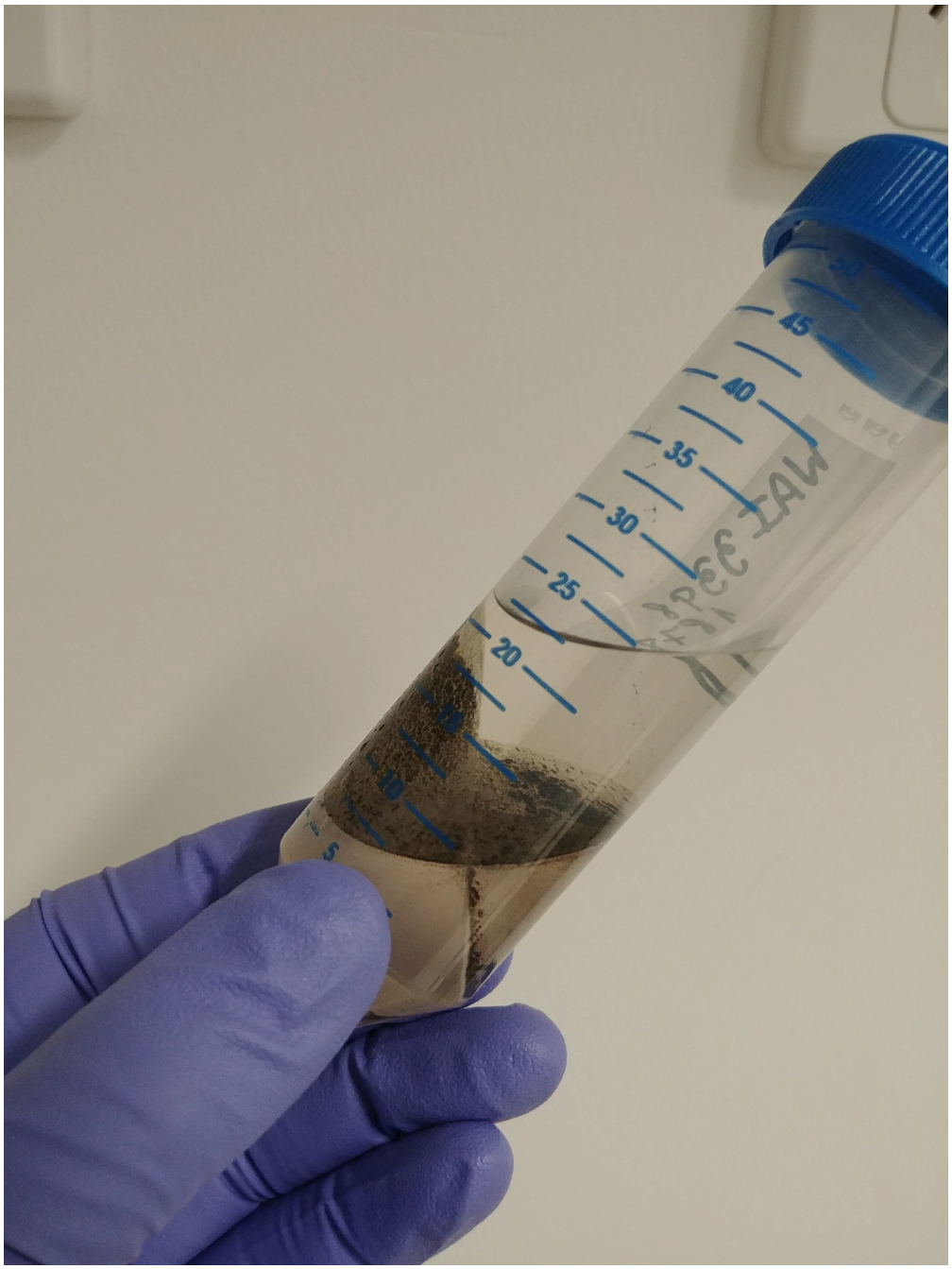
View of Falcon tube with sand after centrifugation

7 Transfer supernatant to fresh Falcon tube containing 17 mL (~1vol) (P:C:I) and mix by inversion.

8 Spin at 4°C and 5000g for 12 mins 00:12:00

Note

With this fungus, there is always an interphase with debris. I take most of the supernatant without taking the phenol phase.
Even if there is still debris at the end of the purification, the AMpure beads will not bind the debris, so to increase your final DNA yield it is better to take as much as the supernatant as possible.



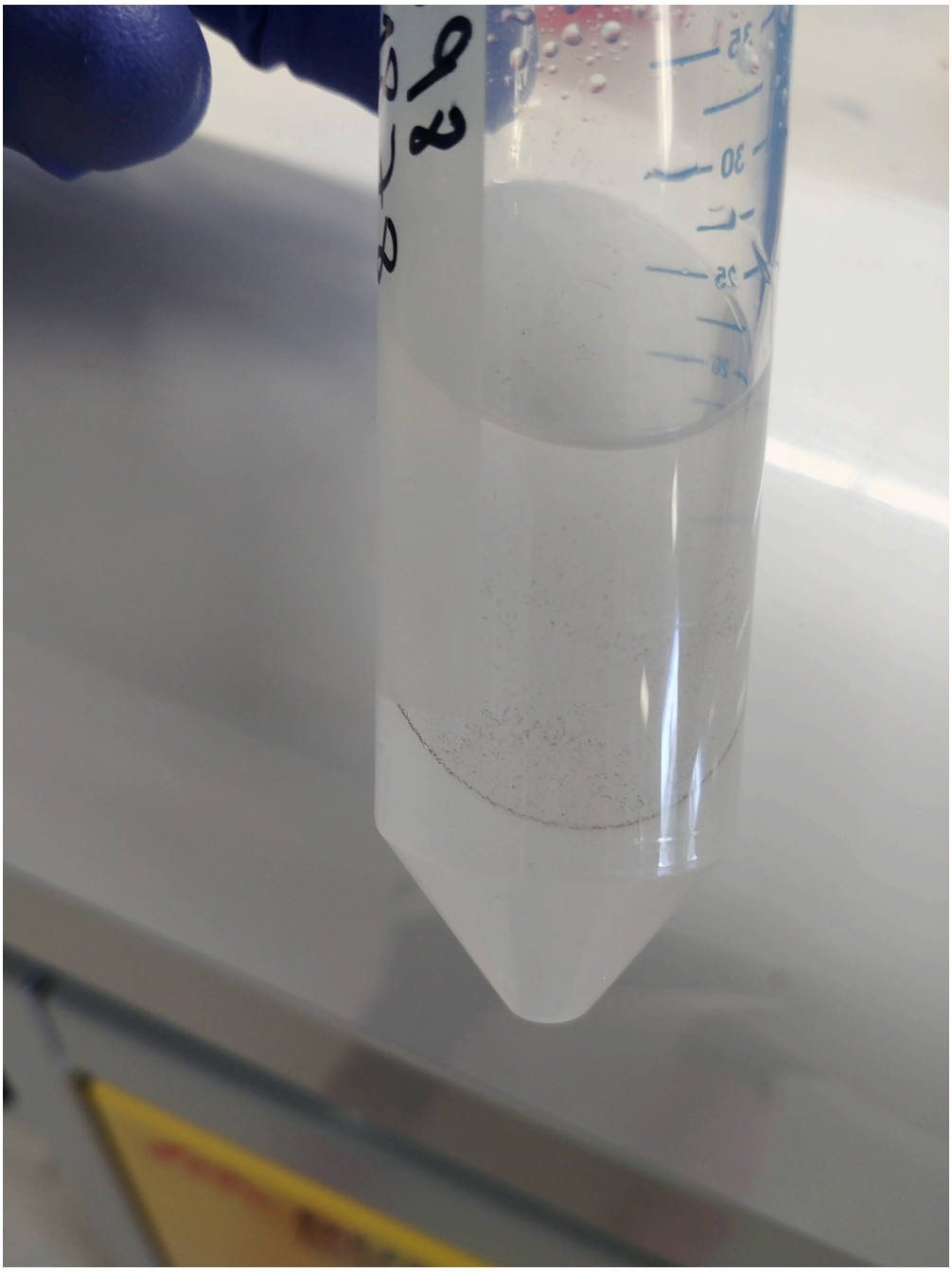
Fungal spore debris visible at the interphase between the phenol (bottom) and aqueous (upper) layers.

9

go to step #7 undefined

Note

Debris is mostly at the interphase, but it also attaches to the tube wall, so it is carried over to the next step.



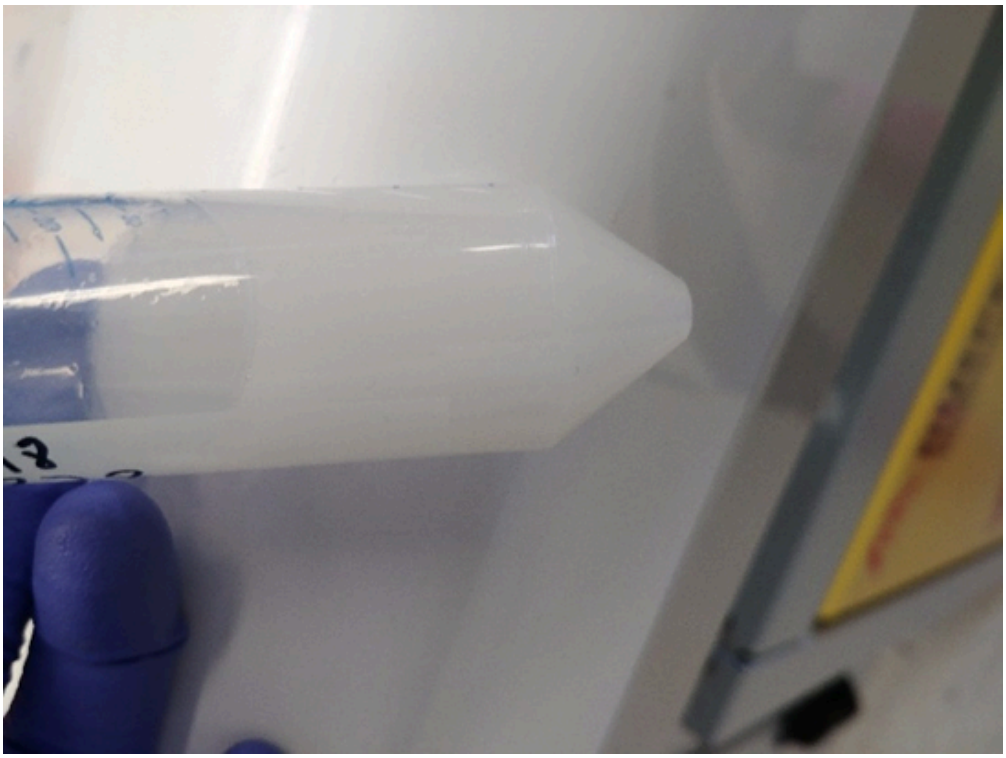
View of fungal debris after centrifugation of second P:C:I wash

10 Transfer supernatant to a new 50 mL falcon and add 10 µL of RNAse A. Incubate in rotator at 30 rpm for 30 min at RT.

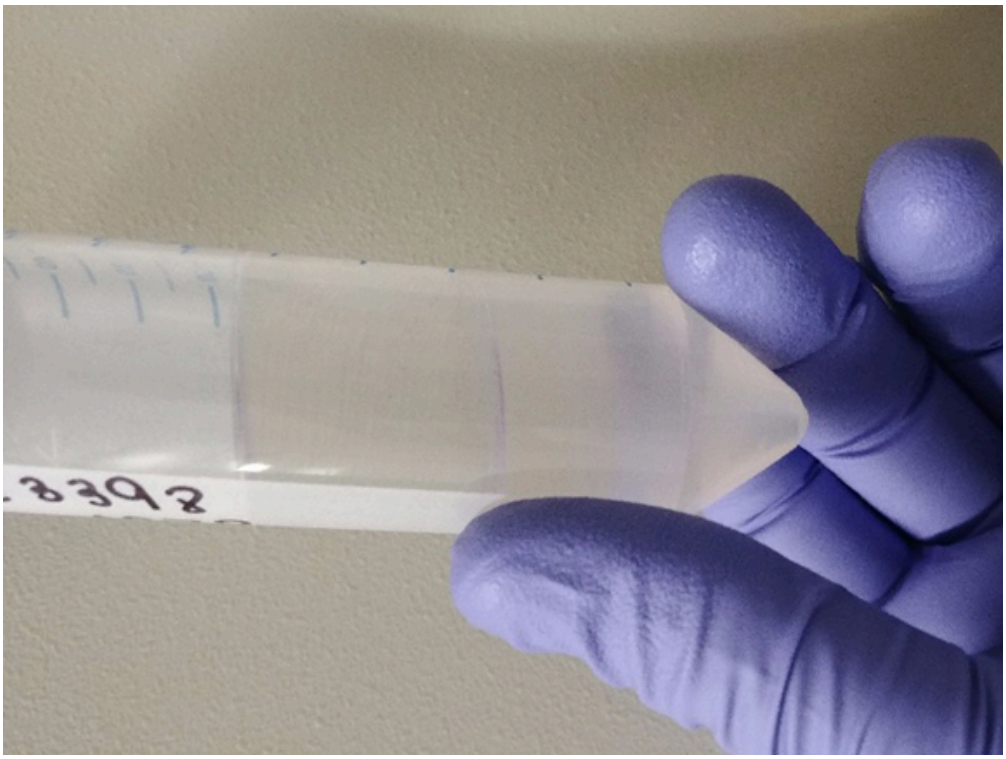
11 Add 100 µL of Proteinase K and incubate in rotator at 30 rpm for 30 min at RT.
00:30:00

12 Add 1 vol (17mL) of P:C:I, mix by inversion and spin at 4°C and 5000g for 12 mins
00:12:00

Solution will look milky like this.



After centrifugation, there should be little to no debris in the aqueous layer, as in here.



DNA precipitation

13 Transfer supernatant to a new 50 mL falcon and add 0.1 vol (~1.5 mL) of NaAc and mix by inversion.

14 Add 1 vol (~ 17 mL) of cold isopropanol and mix well by inversion.

15 Spin at 4°C and 8000g for 30 mins. 00:30:00

Depending on the fungus, the pellet will appear brown, yellow.

16 Pour off supernatant, keeping around 1 mL. Use it to dislodge the pellet and transfer it to a 1.5 mL tube.

17 Spin in a microcentrifuge at 15 000g for 3 min. 00:03:00

18 Discard supernatant and add 1 mL of cold 70% ethanol, flick the tube to help wash the pellet and spin in a microcentrifuge at 15 000g for 3 min. 00:30:00

go to step #18 undefined

19 Discard the supernatant, spin again to get the remaining ethanol out. Air dry the pellet for 1 min.

Re-dissolve DNA

20 Add 200 µL of 10mM Tris pH 8.5 and flick the tube to dislodge the pellet.
Let it dissolve over night.

Measure concentration with BR Qubit and absorbance with Nanodrop.

Expected result

	Sample*	Qubit (ng/µl)	Nanodrop (ng/µl)	A260/A230	A260/A280	Total amount (µg)
	WAI2411	72.9	1923	2.022	2.161	14.58
	WAI3382	89.4	2353	2.006	2.129	17.88

*Bipolaris sorokiniana isolates

AMPure bead cleanup to remove small DNA Fragments

21 Vortex AMPure beads and add 0.6 vol of beads to the DNA sample. Mix beads and DNA by flicking the tube.
DO NOT VORTEX BEAD/DNA MIXTURE
Incubate in a rotator 30 rpm for 10min 00:10:00

22 Spin the beads/DNA mix for 1-2 sec in a microcentrifuge or table top centrifuge. Place the tube on a magnetic rack. Wait for the beads to pellet against the magnet. Slowly pipet off the supernatant.

Note

DNA should now be bound to the AMPure beads, however I often save this supernatant, just in case something goes wrong. If your final elution contains little or no DNA, you can come back to this supernatant and "rescue" the DNA from there by adding an equal volume of beads. If all goes well this supernatant is waste that can be thrown away at the end of the extraction

23 Wash beads with fresh cold 70% ethanol, using enough volume to fill the tube. Pipette the ethanol in the tube on the wall opposite of the bead pellet.

24 Pipette off ethanol and repeat wash.
go to step #23 undefined

25 Remove from magnet and spin the beads for 1-2 sec in a microcentrifuge or table top centrifuge.
Place back on the magnet and let the bead pellet stick. (Should be very quick 1-2 secs).
Pipette off the remaining ethanol. Do NOT let the ethanol dry.

26 Take the tube from the rack and add 49 µL of nuclease-free water and flick the tube to ressuspend the beads. Incubate at 37°C for 5 min. 00:05:00

27 Spin the tube and place it at the magnetic rack. Wait for the beads to pellet and recover supernatant.
Measure concentration with BR Qubit and absorbance with Nanodrop.

Note

At this step, the ratio A260/A230 falls abruptly, but sequencing occurs without any issue. Repeat clean up if you loose more than 30% of your DNA.
From here we follow with Oxford Nanopore protocol "1D genomic DNA by ligation (SQK-LSK108)", version GDE_9002_v108_revU_10Oct2016. For the elution, at each step in the library preparation, we incubate the beads with water/elution buffer at 37°C (5 minutes instead of 2 at the DNA repair and the End-prep steps).