Introduction

This protocol describes a method to extract high molecular weight genomic DNA from Paragon wheat (*Triticum aestivum L*) leaves, as an example of plant leaves. The leaves were collected and cut into ~5 mm² chunks, weighed and stored at −80°C in bags each containing 1 g of leaf material. We recommend extracting the DNA with Carlson lysis buffer followed by purification using the QIAGEN Genomic-tip 500/G. Sequencing performance was determined by PromethION. Prior to library preparation, 3 µg of extracted DNA was size selected using the Size selection of HMW DNA by semi-selective DNA precipitationprotocol. The Ligation Sequencing Kit was used to generate sequencing libraries from 1 µg of size selected gDNA.

Materials

- 1 g of wheat leaves, cut into ~5 mm² chunks (frozen at -80°C)
- QIAGEN Blood and Cell Culture DNA Maxi kit with Genomic-tip 500/G
- RNase A
- Tris-HCl, pH 9.5
- CTAB (cetrimonium bromide)
- NaCl
- PEG (Polyethylene glycol) 8000
- EDTA
- β-mercaptoethanol
- Chloroform
- Isopropanol
- 70% ethanol
- Mortar and pestle
- Vortex mixer
- 50 ml Falcon tubes
- Refrigerated centrifuge with capacity for 50 ml tubes
- Incubator or water bath with capacity for 50°C, 55°C, and 65°C
- TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0)
- Magnetic stirrer and magnet
- Ice bucket and crushed ice
- Qubit dsDNA BR Assay Kit (ThermoFisher Scientific)
- 70% ethanol in nuclease-free water
- 2X “size selection buffer” (2.5% w/v PVP 360000 1.2 M NaCl, 20 mM Tris.HCl pH 8)

Method

1. Prepare an appropriate amount of Carlson lysis buffer (100 mM Tris-HCl, pH 9.5, 2% CTAB, 1.4 M NaCl, 1% PEG 8000, 20 mM EDTA) and mix overnight on the magnetic stirrer.
2. Pre-cool the mortar and pestle at −80°C for at least 30 minutes before starting the extraction.
3. Transfer 20 ml of Carlson lysis buffer to a 50 ml Falcon tube. In a fume hood, add 50 µl β-mercaptoethanol to the Carlson buffer, mix by vortexing, and pre-warm to 65°C in a water bath or incubator for 30 minutes before starting the extraction.
4. Transfer the pre-cooled mortar and pestle to an ice bucket with crushed ice to keep a low temperature.
5. Add 1 g of frozen leaves to the mortar and grind to a fine powder.
   Note: It is recommended to use frozen leaf material to aid the grinding process. However, it is not recommended to exceed 1g of leaves as it will take longer to grind and cause the material to increase in temperature, which could result in the activation of endonucleases.

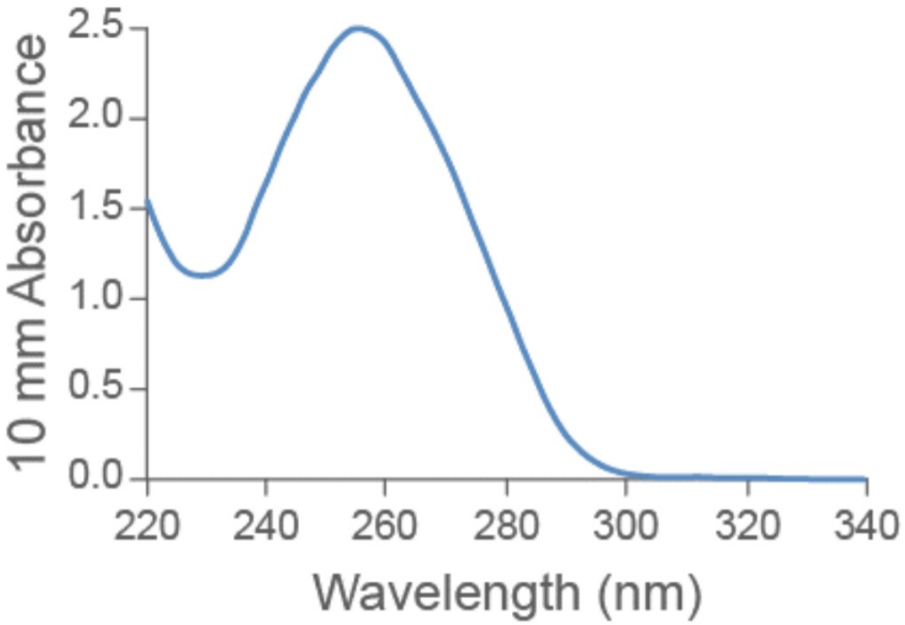
6. Transfer the powder to a fume hood and add to the 50 ml Falcon tube containing 20 ml of the pre-warmed Carlson lysis buffer.
7. Add 40 µl of RNase A to the tube, and vortex for 5 seconds.
8. Incubate at room temperature for 2 minutes.
9. Transfer the tube to a water bath or incubator at 65°C and incubate for 1 hour. After 30 minutes, gently invert the tubes 10 times.
10. Allow the tube to cool down to room temperature for a minimum of 5 minutes.
11. In a fume hood, add 20 ml of chloroform to the tube and vortex for two pulses of 5 seconds each.
12. Centrifuge the tube at 3500 x g for 15 minutes at 4°C.
13. In a fume hood, transfer the top layer (lysate) of each tube to a new 50 ml Falcon tube without disturbing the interphase layer. Transfer 1 ml at a time with a Gilson P1000 using wide-bore tips.
14. Add 0.7 volumes of isopropanol to the lysate and invert 10 times. Incubate at −80°C for 15 minutes.
15. Centrifuge the sample at 3500 x g for 45 minutes at 4°C.
16. Discard the supernatant without disturbing the pellet. Use sterile wipes to absorb the liquid on the tube walls, being careful not to disturb the pellet.
17. To the pellet, add 20 ml of G2 buffer from the QIAGEN Blood and Cell Culture DNA Maxi Kit. Incubate at 50°C for 15 minutes, or until the pellet has dissolved.
    Note: Dissolving the pellet should not take more than 30 minutes. Do not try to pipette or vortex as the G2 incubation will dissolve the pellet.
18. Equilibrate a QIAGEN Genomic-tip 500/G column with 10 ml of Buffer QBT.
19. Pour the fully dissolved DNA in G2 buffer through the equilibrated QIAGEN Genomic-tip 500/G column and allow it to go through gravity flow.
20. Once all the lysate has passed the column through gravity flow, wash the QIAGEN Genomic-tip 500/G column with 15 ml of Buffer QC. Wait until all the buffer flows through the column and repeat the wash with another 15 ml of Buffer QC.
21. Place the QIAGEN Genomic-tip 500/G over a clean 50 ml Falcon tube, and elute the genomic DNA with 15 ml of Buffer QF, pre-warmed to 55°C.
22. Allow the eluate to cool down to room temperature.
23. Add 10.5 ml of isopropanol to the eluted DNA and mix by inverting the tube 10 times.
24. Incubate the tube at −20°C overnight. If the leaves were stored in an RNA stabilisation solution (e.g. RNAlater) prior to extraction, incubate for a maximum of 3 hours, otherwise salt formation may occur.
25. Centrifuge at 3500 x g for 45 minutes at 4°C.
26. Discard the supernatant without disturbing the pellet.
27. Add 4 ml of ice-cold 70% ethanol to the pelleted DNA and invert the tube 10 times.
28. Centrifuge at 3500 x g for 10 minutes at 4°C.
29. Discard the supernatant without disturbing the pellet.
30. Use a sterile wipe to dry the tube walls.
31. Resuspend the DNA in 200 µl of TE buffer and incubate at room temperature until the pellet is fully resuspended. This may take a few hours and we recommend leaving overnight.

32. **Optional Step:** Size-select 3 µg of extracted DNA using the Size selection of HMW DNA by semi-selective DNA precipitation protocol.
    Note: ~70% of input DNA was recovered after size selection.

Results

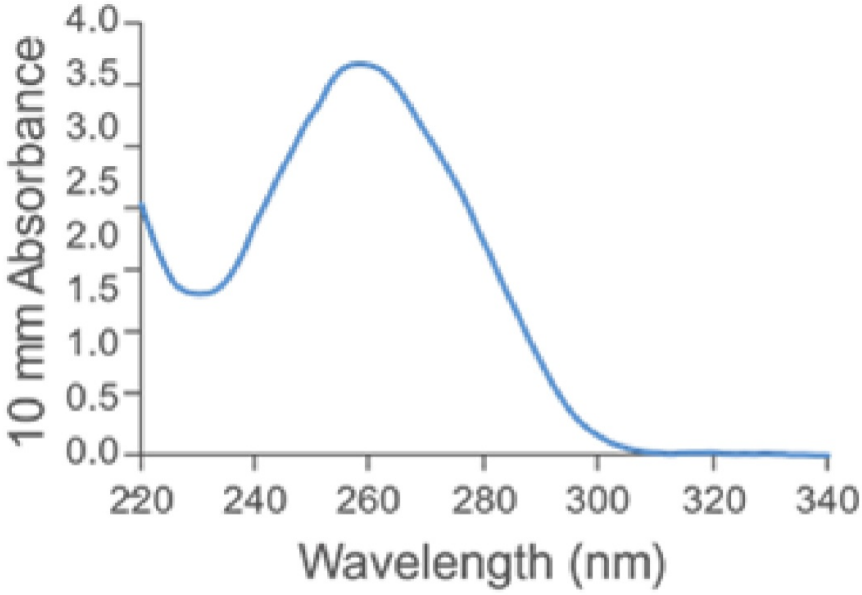
Before size selection:

- **Yield:** 120-180 µg
- **A260/280:** 1.85
- **A260/230:** 2.21



After size selection:

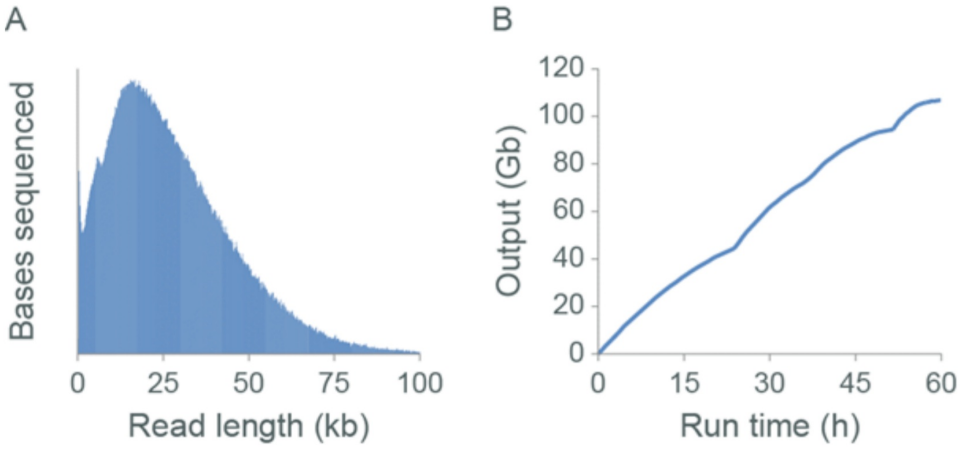
- **Recovery:** 60-70%
- **A260/280:** 1.83
- **A260/230:** 2.41



Sequencing performance

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

- Read length profile:



Change log

Version	Change
v5, August 2023	Updated QIAGEN blood and cell product name
v4, June 2023	Updated materials to clarify Genomic-tip 500/G are required with with Blood & Cell Culture DNA Kit, and updated the URL. Removed specific reference to SQK-LSK109.
v3, January 2023	Updated error on step 21 to use Buffer QF
v2, September 2021	Updated protocol to size select DNA using the size selection of HMW DNA by semi-selective DNA precipitation protocol

Version	Change
v1, June 2019	Initial protocol publication