High molecular weight gDNA extraction from Arabidopsis leaves (*Arabidopsis thaliana* L*E*r)

February 2019

This protocol describes a method to extract high molecular weight genomic DNA from Arabidopsis (*Arabidopsis thaliana* Landsberg *erecta*) leaves, as an example of plant leaves. The plants were grown in a lab with LED lights and the leaves were collected when they were large enough to cut into ~5 mm^2 chunks. The samples were stored at -80°C in bags containing 1 g of leaf material. We recommend extracting the DNA with Carlson lysis buffer followed by purification using the QIAGEN Genomic-tip 100/G. The size selection of HMW DNA by semi-selective DNA precipitation protocol was used to size select 3 µg of extracted DNA before library preparation. The Ligation Sequencing Kit was used to generate sequencing libraries from both 1 µg of the extracted DNA and 1 µg of size selected DNA. Sequencing performance was determined using the PromethION.

Materials

- 2 g of Arabidopsis leaves, cut into ~5 mm^2 chunks (frozen at -80°C)
- QIAGEN Blood and Cell Culture DNA Midi Kit
- QIAGEN RNase A
- Tris-HCl, pH 9.5
- CTAB
- NaCl
- PEG 8000
- EDTA
- -mercaptoethanol
- Chloroform
- Mortar and pestle
- 2X “size selection buffer” (2.5% w/v PVP 360000 1.2 M NaCl, 20 mM Tris.HCl pH 8)
- Qubit dsDNA BR Assay Kit (ThermoFisher Scientific)
- 70% ethanol in nuclease-free water

- Isopropanol
- TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8)
- 50 ml Falcon tubes
- 1.5 ml Eppendorf DNA LoBind tubes
- Refrigerated centrifuge with capacity for 50 ml Falcon tubes
- Vortex mixer
- Incubator or water bath with capacity for 37°C, and 50°C
- Magnetic stirrer and magnet
- Ice bucket and ice
- Sterile wipes

Method

Prior to DNA extraction:

Step 1

Prepare 50 ml 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 a magnetic stirrer.

Step 2

Pre-cool the mortar and pestle at -80°C for at least 30 minutes before starting the extraction.

Step 3

Transfer 40 ml of Carlson lysis buffer to a 50 ml Falcon tube.

Step 4

In a fume hood, add 100 µl β-mercaptoethanol to the Carlson buffer and mix by vortexing. Pre-warm the solution to 65°C in a water bath or incubator for 30 minutes before starting the extraction.

Step 5

Transfer the pre-cooled mortar and pestle to an ice bucket with crushed ice to keep a low temperature.

DNA extraction:

- Step 1

  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. Do not exceed the recommended 1 g of leaves as it will take longer to grind, causing a temperature increase and the activation of endonucleases.

- Step 2

  Transfer the powder to an empty fresh 50 ml Falcon tube and keep on ice.

- Step 3

  Grind another 1 g of frozen leaves to a fine powder and add to another empty fresh 50 ml Falcon tube.

- Step 4

  In a fume hood, add 20 ml of the pre-warmed Carlson lysis buffer to each of the 50 ml Falcon tubes containing the powered leaf material.

- Step 5

  Add 40 µl of RNase A to each of the tubes, and vortex 5 seconds.

- Step 6

  Transfer the tubes to a water bath or incubator at 65°C and incubate for 1 hour. After 30 minutes, gently invert the tubes 10 times.

- Step 7

  Allow the tubes to cool down to room temperature for a minimum of 5 minutes.

Step 8

In a fume hood, add 20 ml of chloroform to each tube and vortex for two pulses of 5 seconds each.

Step 9

Centrifuge the tubes at 3500 x g for 15 minutes at 4°C.

Step 10

In a fume hood, transfer the top layer of lysate from each tube to a new 50 ml Falcon tube, without disturbing the interphase layer.

Note: The lysate layer should be several millilitres of solution, but it is recommended to use a Gilson P1000 with wide-bore tips to transfer the lysate to a new 50 ml Falcon tube, 1 ml at a time.

Step 11

Add 0.7x volumes of isopropanol to the lysate and invert 10 times. Incubate at -80°C for 15 minutes.

Step 12

Centrifuge the sample at 3500 x g for 45 minutes at 4°C.

Step 13

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.

Step 14

To each pellet, add 10 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 is dissolved (it should not take more than 30 minutes). Do not try to pipette or vortex the pellet.

Step 15

Equilibrate a QIAGEN Genomic-tip 100/G column with 4 ml of Buffer QBT.

Step 16

Pour one tube with the fully dissolved DNA in G2 buffer through the equilibrated QIAGEN Genomic-tip 100/G column and allow it to go through with just gravity. Once the tube of dissolved DNA has passed the column, repeat with the second tube of dissolved pellet with the same QIAGEN Genomic-tip 100/G column.

Step 17

Once all the lysate has passed through the column, wash the QIAGEN Genomic-tip 100/G column with 8 ml of Buffer QC. Wait until all the buffer flows through the column and repeat the wash with another 8 ml of Buffer QC.

Step 18

Place the QIAGEN Genomic-tip 100/G over a clean 50 ml Falcon tube, and elute the genomic DNA with 5 ml of Buffer QF, pre-warmed to 55°C.

Step 19

Allow the eluate to cool down to room temperature.

Step 20

Add 3.5 ml of isopropanol to the eluted DNA and mix by inverting the tube 10 times.

Step 21

Incubate the tube at -20°C overnight.

Note: If the leaves were stored in RNA*later* prior to extraction, incubate for a maximum of 3 hours, otherwise salt formation may occur.

Step 22

Centrifuge the tube at 3500 x g for 45 minutes at 4°C.

Step 23

Discard the supernatant without disturbing the pellet.

Step 24

Add 4 ml of ice-cold 70% ethanol to the pelleted DNA and invert the tube 10 times.

Step 25

Centrifuge at 3500 x g for 10 minutes at 4°C.

Step 26

Discard the supernatant without disturbing the pellet. Use sterile wipes to dry the tube walls, being careful not to disturb the pellet.

Step 27

Resuspend the DNA in 100 µl of TE buffer and incubate at room temperature, typically overnight.

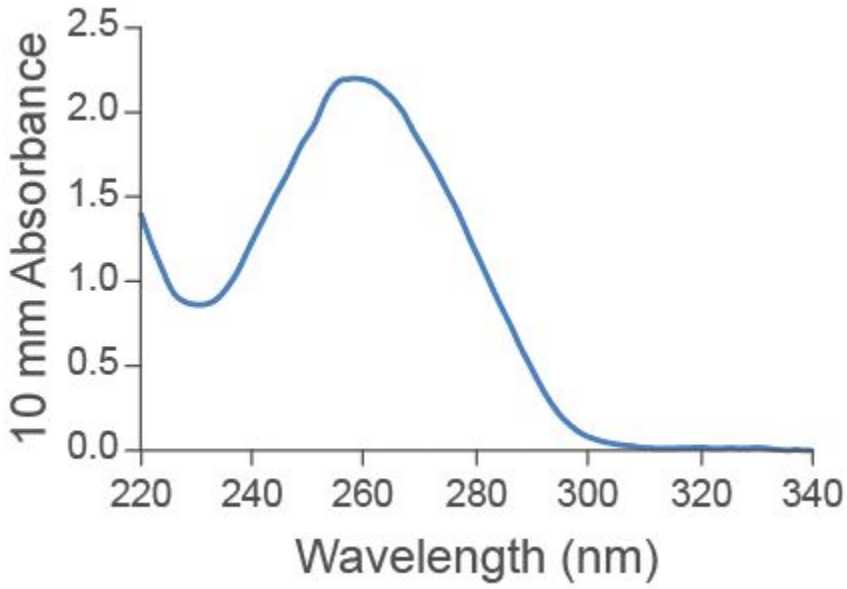
Optional step

Size-select 3 µg of extracted DNA was size selected using the size selection of HMW DNA by semi-selective DNA precipitation protocol.

Note: ~70% of input DNA was recovered after size selection.

Results

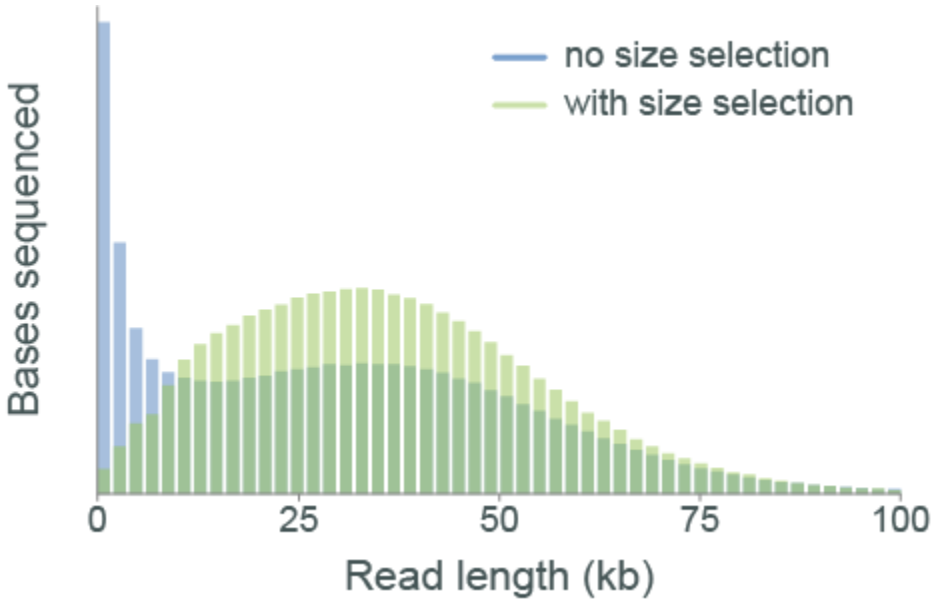
- **Yield:** 10-12 µg
- **OD 260/280:** 1.88
- **OD 260/230:** 2.54



Sequencing performance

Libraries were prepared using the Ligation Sequencing Kit (SQK-LSK109):

- Typical throughput: ★★★ (60+ Gb in 48 h on FLO-PRO002) for the Ligation Sequencing Kit, equivalent to the Lambda DNA supplied in the Control Expansion pack (EXP-CTL001).
- Read length profile:



Date	Change note
September 2021	Updated protocol to size select DNA using the size selection of HMW DNA by semi-selective DNA precipitation protocol.