Introduction

This protocol describes a method to extract high molecular weight genomic DNA from fever tree (*Cinchona pubescens*) leaves, as an example of plant leaves. After collection, the leaves were 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 MinION™. Prior to library preparation, 3 µg of extracted DNA was size selected using the size selection of HMW DNA be semi-selective DNA precipitationprotocol. 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.

Materials

- 2 g of fever tree 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
- NaCl
- PEG (Polyethylene glycol) 8000
- EDTA
- β-mercaptoethanol
- Chloroform
- Isopropanol
- Ethanol
- Mortar and pestle
- Vortex
- 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
- 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

Method

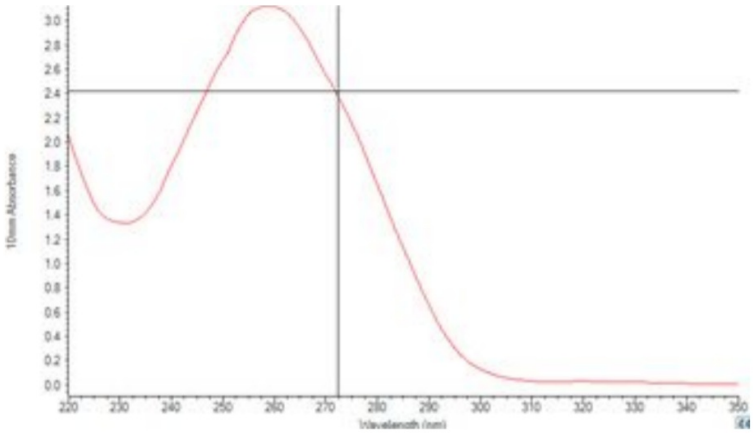
1. Prepare 50 ml of Carlson lysis buffer (100 mM Tris-HCl, pH 9.5, 2% CTAB, 1.4 M NaCl, 1% PEG 8000, 20 mM EDTA) and mix the solution overnight on a magnetic stirrer.
2. Pre-cool the mortar and pestle at -80°C for at least 30 minutes before starting the extraction.
3. Transfer 40 ml of Carlson lysis buffer to a 50 ml Falcon tube. In a fume hood, add 100 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 maintain 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. It is not recommended to exceed 1 g of leaves as it will take longer to grind the material, leading to an increase in the temperature, which could result in the activation of endonucleases.

6. Transfer the powder to one 50 ml Falcon tube and place on ice.
7. Repeat Step 5 and add the powdered leaves to a separate 50 ml Falcon tube.
8. In a fume hood, add 20 ml of the pre-warmed Carlson lysis buffer to each of the 50 ml Falcon tubes containing the powdered leaf material.
9. Add 40 µl of RNase A to each of the tubes, and vortex for 5 seconds.
10. 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.
11. Allow the tubes to cool to room temperature for at least 5 minutes.
12. In a fume hood, add 20 ml of chloroform to each tube and vortex for two pulses of 5 seconds each.
13. Centrifuge the tubes at 3500 x g for 15 minutes at 4°C.
14. In a fume hood, transfer the top layer (i.e. the lysate), of each tube to a new 50 ml Falcon tube, without disturbing the interphase layer.
    Note: It is recommended to use wide-bore tips to avoid DNA fragmentation.
15. Add 0.7x volumes of isopropanol to the lysate and invert 10 times. Incubate at -80°C for 15 minutes.
16. Centrifuge the sample at 3500 x g for 45 minutes at 4°C.
17. 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.
18. Add 19 ml of G2 buffer, from the QIAGEN Blood and Cell Culture DNA Maxi Kit, to the Falcon tubes with the pellets. Incubate at 50°C for 15 minutes, or until the pellet is dissolved; it should not take more than 30 minutes.
    Note: Do not try to pipette or vortex the pellet, the G2 incubation will dissolve it.
19. Equilibrate a QIAGEN Genomic-tip 500/G column with 10 ml of Buffer QBT.
20. Pour one tube with the fully dissolved DNA in G2 buffer through the equilibrated QIAGEN Genomic-tip 500/G column and allow it to go through by gravity flow. Once the first tube’s contents has passed through the column, apply the dissolved pellet of the second tube to the same QIAGEN Genomic-tip 500/G column.
21. Once all the lysate has passed through the column via gravity flow, wash the QIAGEN Genomic-tip 500/G column with 20 ml of Buffer QC. Wait until all the buffer has flowed through the column and repeat the wash with another 20 ml of Buffer QC.
22. Place the QIAGEN Genomic-tip 500/G over a fresh 50 ml Falcon tube, and elute the genomic DNA with 15 ml of Buffer QF, pre-warmed to 55°C into the fresh 50 ml Falcon tube.
23. Allow the eluate to cool to room temperature.
24. Add 10.5 ml of isopropanol to the eluted DNA and mix by inverting the tube 10 times.
25. Incubate the tube at -20°C for 30 minutes.
26. Centrifuge at 3500 x g for 30 minutes at 4°C.
27. Discard the supernatant without disturbing the pellet.
28. Add 4 ml of ice-cold 70% ethanol to the pelleted DNA and invert the tube 10 times.
29. Centrifuge at 3500 x g for 10 minutes at 4°C.
30. Discard the supernatant without disturbing the pellet.
31. Use a sterile wipe to dry the tube walls.

32. Resuspend the DNA in 150 µl of TE buffer and incubate at room temperature overnight.
33. **Optional Step:** 3 µg of extracted DNA was size selected using the Size selection of HMW DNA by semi-selective DNA precipitation protocol.
    Note: ~50% of input DNA was recovered after size selection.

Results

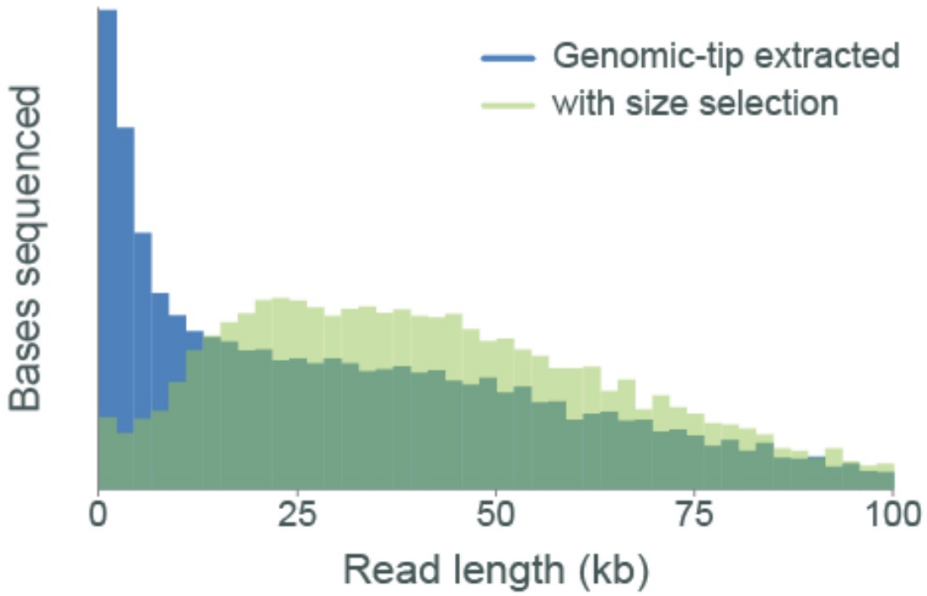
- **Yield:** 30-40 µg
- **OD 260/280:** 1.89
- **OD 260/230:** 2.34



Sequencing performance

Libraries were prepared using the Ligation Sequencing Kit.

- Read length profile:



Change log

Version	Change
v4, August 2023	Updated QIAGEN blood and cell product name
v3, 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.
v2, September 2021	Updated protocol to size select DNA using the size selection of HMW DNA by semi-selective DNA precipitation protocol
v1, June 2019	Initial protocol publication