Bioplatforms Australia Genomics for Australian Plants Initiative
Activity: Reference genomes
Species: *Hibbertia scandens*
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DNA extraction protocol used for *Hibbertia scandens*

Staring material: 8 g of fresh young leaves (tips of vines)
We then proceeded with 4x 50mL Falcon tube extractions (~2g per Tube)

I followed the protocol from A. Jones et al.
'Scalable high-molecular weight DNA extraction for long-read sequencing'
https://www.protocols.io/view/scalable-high-molecular-weight-dna-extraction-for-dm6gpwwj1lzp/v1 with some adjustments.

- Step 1, 2 and 3
- Skipped step 4 and 5

A Sorbitol wash was performed before proceeding to cell lysis (Step 6).
'Sorbitol washing complex homogenate for improved DNA extractions'
By A Jones et al.

- We used DTT instead of β-Mercaptoethanol
- In step **2** of the Sorbitol Wash, a NUTRIBULLET Pro 1200 was used to grind up the leaf material using the smallest cup.
- 200mL of Sorbitol solution was added to the blender with a small scoop of DTT powder.
- The mixture was blended until everything turned to liquid, no solid plant fragments.
- The liquid was distributed over 4 Falcon tubes (50mL each); excess of liquid was discarded.
- 2 centrifugations were performed, and the pellets were used in following DNA extraction (Step 7)
- Lysis buffer was added to 20mL.
- At Step 13, 7mL of Potassium Acetate was added.
- At Step 18 Volumes were brought to 50mL with the Binding Buffer.
- A 15mL magnetic rack was used in this extraction, so the extraction solution was distributed over 4 x 15mL tubes, and separations were performed multiple times by removing the supernatant and adding more solution to the tube with beads.
- The tubes with beads were left for hours or overnight to separate.
- After the Ethanol wash, the beads were resuspended in 70% Ethanol and collected in one 15mL tube.

- The tube was placed on magnet again and the beads were separated. Finally, all the beads were collected in 2mL Ethanol.

- The beads were separated on a magnet for small tubes (1.5-2mL) and left to dry for ~5min.

- The DNA solution was very viscous (we believe there are polysaccharides in solution still) and elution of DNA was done in 500uL 10mM Tris HCL.

- The DNA was quantified with Qubit, or similar, to estimate how much DNA you have (and how much DNA you will have lost after the next step).

- After Step 34, multiple Salt:Chloroform Washes were performed (Protocol by Pacific Biosciences, See Supplemental 1, also on page 3 below).

- The DNA was quantified with Qubit and NanoDrop to determine the quality and quantity.

- Proceeded with PippinBlue or Pippin HT (Step 36 of the DNA extraction protocol) and performed a 20kb high pass separation.

- If all is OK, you should have plenty of HMW DNA.

- Preform a Pulse electrophoresis run to estimate size distribution, a Qubit for DNA quantity and a Nanodrop for DNA purity.

References

Ashley Jones, Cynthia Torkel, David Stanley, Jamila Nasim, Justin Borevitz, Benjamin Schwessinger 2020. Scalable high-molecular weight DNA extraction for long-read sequencing. protocols.io https://dx.doi.org/10.17504/protocols.io.bnjhmcj6

Ashley Jones, Benjamin Schwessinger 2020. Sorbitol washing complex homogenate for improved DNA extractions. protocols.io https://dx.doi.org/10.17504/protocols.io.beuvjew6

Guidelines for Using a Salt:Chloroform Wash to Clean Up gDNA

This protocol can be used to clean up high-molecular-weight genomic DNA (gDNA) prior to the SMRTbell™ library preparation. It describes how to use a high-salt low-ethanol percentage wash to remove polysaccharides before DNA is precipitated from the solution.

1. Prepare a buffer of 1 M NaCl and 2 mM EDTA. Label tube as **Buffer A** and set aside.
   Note that 500 µL of buffer will need to be prepared for each sample.

Buffer A	Volume (µL)
5 M NaCl	100
500 mM EDTA	2
PacBio® EB	398
TOTAL	**500**

2. Bring the volume of DNA up to 200 µL with Elution Buffer (EB) – label as **TUBE 1**.
3. Add the following reagents to **TUBE 1**.

Tube 1	Volume (µL)
gDNA in EB	**200**
5 M NaCl	**100**
500 mM EDTA	**2**
PacBio EB	**198**
TOTAL	**500**

4. Add 400 µL of Phenol:Chloroform:Isoamyl Alcohol (25:24:1) to **TUBE 1**.
5. Invert the tube 20 times to mix.
6. Spin the tube at maximum speed (at least 10 g) for 10 minutes.
7. Carefully remove the aqueous layer, do not disturb the interface. Place into a clean 2 mL microcentrifuge tube. Label the tube as **TUBE 2.**
8. Add 400 µL of **Buffer A** (from step 1) to **TUBE 1**.
9. Invert tube 20 times to mix.
10. Spin tube at maximum speed (at least 10 g) for 10 minutes.
11. Carefully remove the aqueous layer, do not disturb the interface. Place into **TUBE 2.**
12. Measure the volume in **TUBE 2**: _____ µL. It should be close to 800 µL.