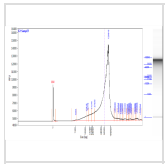

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Extraction of high molecular weight DNA from aphids and other sap-feeding insects for long-read sequencing.

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1 *Works for me* dx.doi.org/10.17504/protocols.io.bhftj3nn

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ABSTRACT

Chromosome-scale genome assembly usually requires the use of long-read sequencing technologies such as PacBio or ONT MinION. However, the development of suitable DNA extraction methods that can yield high molecular weight DNA suitable these platforms can be problematic and time-consuming. We found that some traditional DNA extraction methods that are suitable for short-read sequencing platforms did not produce satisfactory results using long-read sequencing technologies with aphids or other sap-feeding hemipteran insects. We hypothesised that plant-derived components of the aphid's diet might inhibit the sequencing chemistry.

Here we present a method suitable for extraction of high-molecular DNA for long-read sequencing of aphids and other sap-feeding insects. We employed the Illustra Nucleon Phytopure DNA extraction kit designed to remove plant-specific contaminants. We were able to consistently recover very high molecular weight DNA that was compatible with library preparation and sequencing by PacBio or ONT MinION. The protocol presented here describes the use of this plant-DNA extraction kit optimised to obtain high molecular weight DNA from insect samples.

We have used this method across a range of aphid species, leafhoppers and froghoppers (spittlebugs). It has enabled sequencing and - in most cases -chromosome-level assembly of genomes from the green peach aphid *Myzus persicae* (1), the pea aphid *Acyrthosiphon pisum* (1), the woolly apple aphid, *Eriosoma lanigerum* (2), and the common meadow spittlebug *Philaenus spumarius* (3).

1. Chromosome-scale genome assemblies of aphids reveal extensively rearranged autosomes and long-term conservation of the X chromosome
Thomas C.Mathers, Roland H. M.Wouters, Sam T.Mugford, DavidSwarbreck, CockVan Oosterhout, Saskia A.Hogenhout
bioRxiv 2020.03.24.006411; doi: https://doi.org/10.1101/2020.03.24.006411

2. A chromosome-level genome assembly of the woolly apple aphid, *Eriosoma lanigerum* (Hausmann) (Hemiptera: Aphididae)
RobertoBiello, ArchanaSingh, Cindayniah J.Godfrey, FelicidadFernández Fernández, Sam T.Mugford, GlenPowell, Saskia A.Hogenhout, Thomas C.Mathers
bioRxiv 2020.05.29.121947; doi: https://doi.org/10.1101/2020.05.29.121947

3. Draft genome assembly version 1 of the meadow spittlebug Philaenus spumarius (Linnaeus, 1758) (Hemiptera, Aphrophoridae) (Version 1) [Data set].
Roberto Biello, Thomas C. Mathers, Sam T. Mugford, Qun Liu, Ana S. B. Rodrigues, Ana Carina Neto, Maria Teresa Rebelo; Octávio S. Paulo; Sofia G. Seabra; Saskia A. Hogenhout. (2020). Zenodo.
http://doi.org/10.5281/zenodo.3368385

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GUIDELINES

The optional steps in the protocol improve the purity of the DNA. We have found these steps are necessary for spittlebugs, but not for aphids.

DNA should be extracted from samples that have been kept frozen at -80° C or lower since collection and have not undergone any freeze-thaw cycles.

Mix by gentle shaking and inversion, and not by vortexing.

Use wide-bore tips at all stages to prevent shearing of DNA.

Avoid repeated freeze-thaw cycles of the purified DNA. DNA can be stored at 4° C for several weeks.

Using this method we can recover 4-5 µg DNA from 15-20 aphids, or 3-15 µg DNA from a single spittlebug, depending on size and species. DNA extractions routinely have a median molecular weight >100kb.

Using DNA isolated in this method, we can typically recover 10Gb data per ONT Minion run with a read N50 around 20-25kb, or around 140Gb per Pacbio Sequel II cell with a read N50 of 35-40kb.

MATERIALS TEXT

Illustra Nucleon PhytoPure Genomic DNA extraction kit (GE Healthcare; RPN8510 or RPN8511)

Add the following fresh to reagent 1 before use:
0.2 mg/ml RNAse (DNAse-free, Qiagen, 100mg/ml stock) 2 µl per ml

(Optional) Proteinase K (600U/ml)

(Optional) 3M Sodium Acetate (pH 5.2)

Pre-cool isopropanol (2-propanol) in a freezer at -20° C

Pre-cool chloroform in a freezer at -20° C

Freshly made 70% ethanol.

2069G 200 µL ART Wide Bore Filtered Pipette Tips (Thermofisher)

SAFETY WARNINGS

Liquid nitrogen: Warning Hazard Statement: When spilled the liquid will vaporise rapidly forming an oxygen-deficient vapour cloud. Contact with cold liquid or gas may cause frostbite.

Dry Ice: Warning Hazard Statement: May displace oxygen and cause rapid suffocation. Contact with product may cause severe cold burns or frostbite.

Chloroform: Warning Hazard Statement: Harmful if inhaled or swallowed. Causes respiratory tract, eye and skin irritation. Suspect cancer hazard - may cause cancer. May cause damage to the following organs: kidneys, liver, heart, skin, eyes, central nervous system

BEFORE STARTING

Pre-cool isopropanol (2-propanol) in a freezer at -20° C

Pre-cool chloroform in a freezer at -20° C

1 Grind approx. 10mg (20 frozen aphids; originated from a single female, one froghopper) in a 1.5ml Eppendorf tube, with a plastic pestle, pre-cooled in liquid nitrogen. Ensure that tissue remains frozen throughout. When grinding the samples do not turn the pestle, but crush the aphids via an up-down movement.

2 Add 600 µl of reagent 1, with RNAse added. Leave the plastic pestle in the tube during the addition of buffer to ensure tissue stuck to the pestle is not lost, mix thoroughly by agitating the pestle. Remove the pestle, taking care to leave as much material inside the tube as possible. Incubate at 37° C for 30 minutes, with occasional mixing by inversion.

3 Add 200 µl of reagent 2 and mix by inversion until a consistent solution in acquired.

4 (Optional) Add 10 µl proteinase K (600U/ml), incubate at 55° C for 2 hours with occasional mixing by inversion.

5 Alternatively, if not using proteinase K, incubate at 65° C for 10 minutes with regular mixing by inversion.

6 Place samples on ice for 20 minutes.

7 Add 500 µl of pre-cooled chloroform at -20° C, and 100 µl of Nucleon PhytoPure DNA extraction resin suspension, mix

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by gentle shaking and inversion and mix the sample on a rotator disk for 10 minutes on room temperature.

8 Centrifuge in a bench-top microfuge (1,300 G) for 10 minutes. Transfer the upper aqueous phase to a new tube. Take care not to disturb the interface. Be conservative, and leave some of the upper phases behind rather than carrying over chloroform or resin contamination.

9 (Optional) Add the upper phase to 0.05 X volumes of 3M Na OAc (pH 5.2)

10 Add 1 volume of pre-cooled isopropanol at -20° C. Add one drop at a time. Mix the tube by inversion. Mix by inversion and incubate at -20° C for 15 minutes.

11 Centrifuge in a bench-top microfuge (4,000 G) for 10 minutes. Discard the supernatant without disturbing the pellet. Put the Eppendorf top-down on a paper roll to dry the sample further.

12 Wash the pellet in 1ml of freshly made 70% ethanol. Inverse the tube five times and centrifuge in a bench-top microfuge (4,000 G) for 20 minutes to collect the pellet.

13 (Optional) repeat the wash step 2 more times.

14 Pour off the ethanol without disturbing the pellet and dry the sample by putting the Eppendorf top-down on a paper roll.

15 Resuspend the pellet in 100 µl water, or TE.

16 Assess the quality of DNA by electrophoresis and spectrophotometry. A single, high-molecular-weight band should be visible, a smear at lower molecular weight indicates fragmentation and/or RNA contamination. Absorbance ratio of 260:280 and 260:230 should both be 1.8 or greater. If you suspended the pellet in TE, ensure than you blank the spectrophotometer with TE. Determine the concentration of the DNA using a fluorescent dye-binding based method such as the Qubit (Invitrogen).

17 Asses the molecular weight of the DNA sample by checking the DNA fragment sizes on the Agilent FEMTO-pulse Fragment Analyzer, or by PFGE.

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