Isolation Method for Ultra-HMW DNA from Plants for Next-Generation Mapping Applications

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Abstract

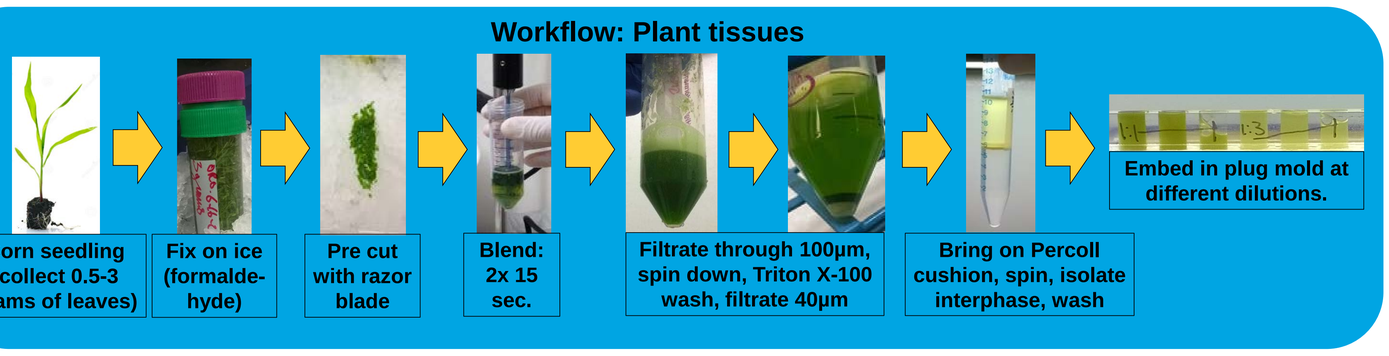
Next-generation mapping (NGM) and long-read sequencing technologies are becoming necessary tools for revealing the complex plant genomes. However, these tools require starting material that is pure, long, and more intact compared to what is required for next-generation sequencing. The challenges associated with achieving pure and long starting material arise from limited availability of protocols and commercial solutions for such applications. The new IrysPrep® method for improving DNA extraction and isolation not only provides purer and longer DNA starting material for utilization with NGM, but could also become very useful for long-read sequencing applications.

Traditional methods that are meant to isolate high molecular weight (HMW) DNA from plants for cloning typically use liquid nitrogen grinding with a mortar and pestle, and usually require from 10 to 30 grams of starting material, yet the process often results in low DNA yield and quality. Generating high-quality ultra-high molecular weight (U-HMW) DNA is key to successful analysis and assembly using NGM on the Irys® System[2] and helping to generate more complete assemblies using complementary long-read sequencing technologies.

We present a novel U-HMW DNA isolation method using a commercially available tissue homogenizer and IrysPrep Plant Homogenization Buffers that requires significantly less starting material and provides a significant increase in DNA yield and quality. Only 0.5 to 3 grams of young leaves from dark-treated seedlings are required for the starting material, yielding 1.5 to 9 micrograms of DNA (species dependent). Standard processing begins with fixing the nuclei with formaldehyde, and then blending using a handheld rotor-stator tissue homogenizer using the IrysPrep® Plant Homogenization Buffers, destroying polyphenols, chloroplasts and mitochondria, while preserving high-quality nuclei after releasing from the cells. Percoll density cushions are then used to purify and separate the nuclei from the debris[1]. Standard filtration and centrifugation purification steps are followed before agarose embedding, and the treating of the DNA plugs overnight with Proteinase K and detergents. After treating with RNase and washing in TE_{50} and TE buffers, the plugs are melted, treated with ß-Agarase and dialyzed. Finally, the DNA is assessed using a quality control process to determine concentration (using the Quant-iT™ PicoGreen® dsDNA Assay Kit measured against Lambda DNA standards), viscosity, and clarity prior to being labeled using the IrysPrep NLRS kit. We have demonstrated that this method can achieve an approximate 10x improvement in percent yield. Furthermore, in a comparison study of five samples that were processed by traditional methods and the novel IrysPrep method, the IrysPrep method resulted in far superior molecule length when analyzed on Irys. This novel method has been successfully performed on numerous plant species, such as Papaya, Tobacco, Asparagus, Goat grass, *Eucalyptus*, and a desiccation-tolerant grass[3].

Methods

Workflow: Plant tissues



Species	DNA quality and assembly results	
	Traditional method	Rotor-stator method *)
Tobacco	*Throughput and N50 too low for assembly*	Pass
Duckweed	*DNA too short, conc. low*	Pass
Eucalyptus	*DNA too short, conc. low*	Pass

Table 1: DNA isolation methods compared for several plant species. Part of the QC process is to determine the concentration, viscosity and the clarity of the melted DNA plug.

Other successful DNA isolations:

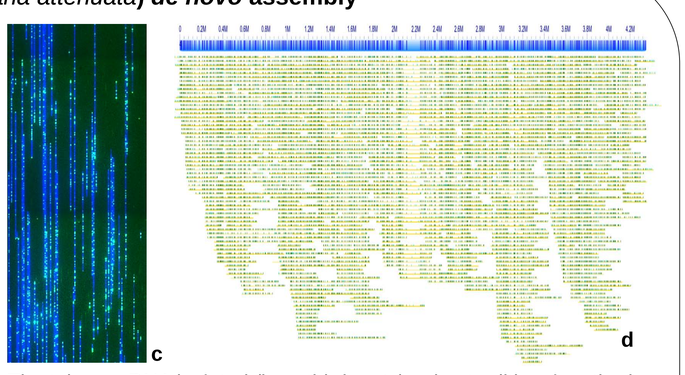
Lettuce, Basil, Papaya, Goat grass, Soy bean, Maize, Sugar beet, Asparagus, *Arabidopsis* and Eggplant.

Tobacco (*Nicotiana attenuata*) *de novo* assembly

Method	Traditional	Rotor-stator
Single Molecule N50	115	250 kb
Assembly size	NA	2.19 Gb
Genome map N50	NA	1.42 Mb

a

b



c **d**

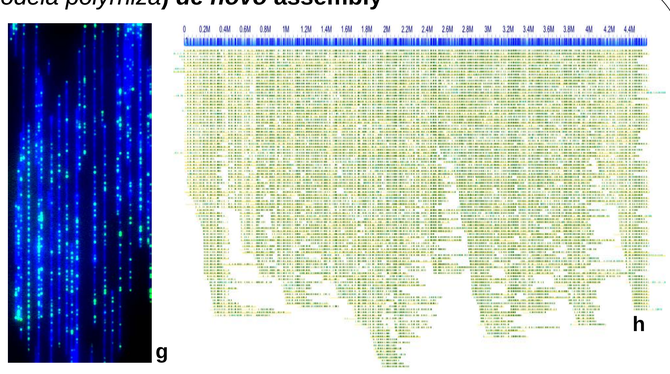
The tobacco DNA isolated (by a third party) using traditional methods passed QC but resulted in a small N50 (**a**). Due to the small molecule length, it failed to assemble. After isolating DNA using the rotor-stator method, the N50 of the collected data was 250 kb (**a-c**) and *de novo* assembly was performed. **Figures:** Molecule distribution (**b**), image of single molecules (**c**) and an example of assembly result (**d**).

Duckweed (*Spirodela polyrhiza*) *de novo* assembly

Method	Traditional	Rotor-stator
Single Molecule N50	Failed	253 kb
Assembly size	NA	146 Mb
Genome map n50	NA	1.02Mb

e

f



g **h**

The duckweed DNA isolated using traditional methods failed QC so no data could be collected (**e**). After isolating DNA using the rotor-stator method, the DNA passed QC. Sufficient data was collected and *de novo* assembly was performed (**h**). **Figures:** Molecule distribution (**f**)**,** image of single molecules (**g**) and an example of assembly result (**h**).

Conclusions

This novel U-HMW DNA isolation method utilizing a rotor-stator tissue homogenizer and IrysPrep Plant Homogenization Buffers have allowed us, and our customers, to successfully isolate high-quality U-HMW DNA from various plant species where traditional HMW DNA isolation methods have failed. These species we have evaluated include, but are not limited to, Tobacco, Duckweed and *Eucalyptus* (Table 1). Furthermore, this methodology opens up new avenues for NGM's Irys System in QC standards, such as high percent yield achieved (~10x higher than standard protocols) and increased N50, compared to other HMW DNA isolation methods, and significantly increases accessibility of NGM to new tissues. The BioNano Genomics NGM Irys System utilizes high-quality U-HMW DNA to aid in the analysis of known genomes and *de novo* assemble novel genomes, and will be especially useful for *de novo* assembly of polyploid plant genomes where larger N50 is required. See also Posters: P02078, P0207, P0702, and P1272.

Reference

- [1]Šimková, et al. (2003). Preparation of HMW DNA from plant nuclei and chromosomes isolated from root tips. Biologia Plantarum (3rd ed., Vol. 46, pp. 369-373). Olomouc.
- [2]Anfora et al. (2015). Making platinum genomes feasible: purification of chromosomal-length plant DNA molecules. GEN Vol. 35, No. 14
- [3]VanBuren et al. (2015). Single-molecule sequencing of the desiccation-tolerant grass Oropetium thomaeum. Nature doi:10.1038/nature15714