Bionano Prep™ Plant Tissue DNA Isolation Protocol Selection Guide

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Bionano Prep Plant Tissue DNA Isolation Protocol Selection Guide

Product and protocols associated with this guide:
Bionano Prep Plant DNA Isolation Kit (Part # 80003)

Bionano Prep Plant Tissue DNA Isolation Base Protocol (Part # 30068)
Bionano Prep High Polysaccharides Plant Tissue DNA Isolation Protocol (Part # 30128)
Bionano Prep High Polyphenols Plant Tissue DNA Isolation Protocol (Part # 30133)
Bionano Prep Plant Tissue DNA Isolation, Liquid Nitrogen Grinding Protocol (Part # 30177)

The above Bionano Prep Plant Tissue DNA Isolation Protocols provide detailed instructions for utilizing the Bionano Prep Plant Tissue DNA Isolation Kit (Part # 80003) to isolate high-quality HMW gDNA from plants. This document describes the major differences between the plant DNA isolation protocols (Table 1) and assists the user in selecting the proper DNA isolation protocol for their plant sample (Figure 1).

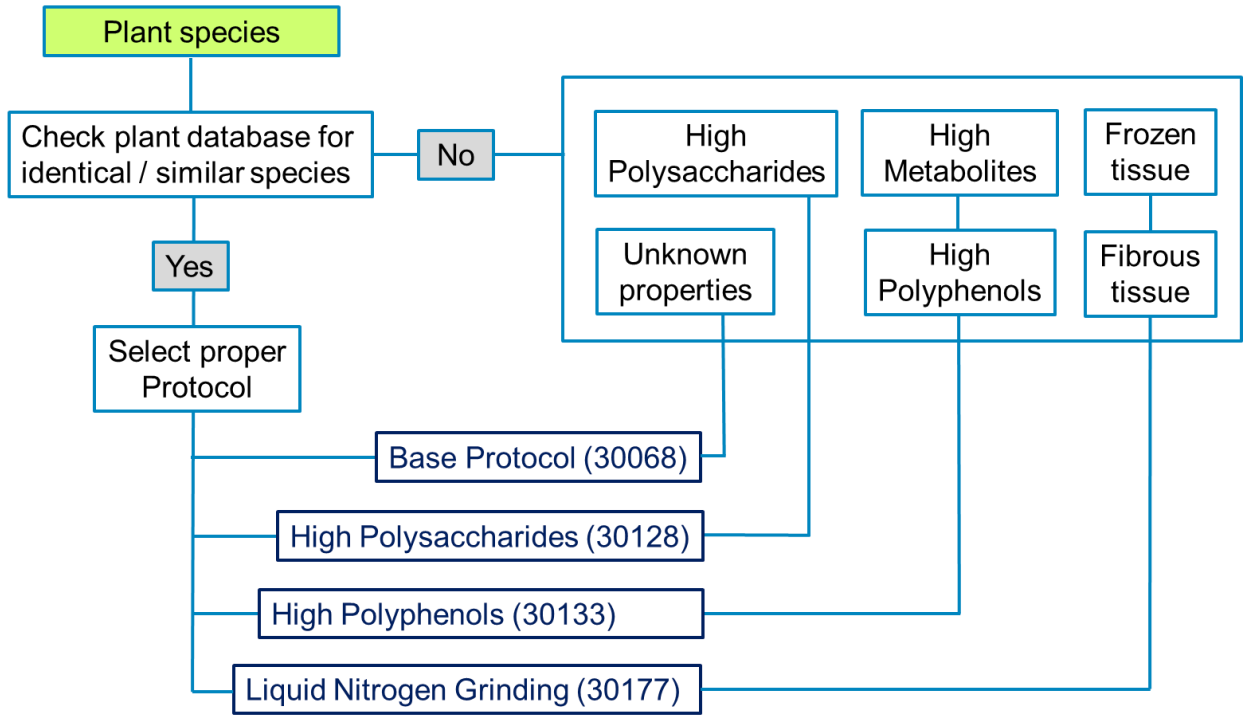

Figure 1. Plant Tissue DNA Isolation Protocol Selection Flowchart

Basic steps to follow for initial protocol selection:

1. Is the plant tissue only available frozen? If yes, follow Liquid Nitrogen Grinding Protocol.
2. Has the plant tissue been successful with an existing protocol? If yes, follow that protocol.
3. Is the plant tissue known to have high polysaccharides, metabolites, polyphenols or is it extremely fibrous? If yes, follow corresponding protocol.
4. If the plant does not meet any of the above criteria, start with the Base Protocol.

Major Protocol Differences for the Existing Bionano Plant DNA Isolation Protocols-

Steps \ Protocol	Base Protocol (BP, 30068)	High Polysaccharides (PS, 30128)	High Polyphenols (PP, 30133)	Liquid Nitrogen Grinding (LNG, 30177)
When to use	1) Previous success with similar plant species using the protocol. 2) Process for the first time. 3) No close relatives in the on-line database. 4) Little information is known about the chemical components and physical characteristics of the species.	1) Plant has high polysaccharides content, such as soybean. 2) Previous success with similar plant species using the protocol. 3) Fail BP due to no band at interface and no pellet on the bottom of the tube.	1) Plant has high polyphenols and / or high metabolites content, such as cotton and *Eucalyptus*. 2) Previous success with similar species using the protocol. 3) Observation of oxidation during the nuclei isolation, PK digestion, DNA recovery, and low label density on Irys or Saphyr System.	1) Plant tissue has been previously snap frozen and stored at ultra-low temp freezer for less than 3 months. 2) Very tough / fibrous plant tissue for a rotor-stator homogenizer to handle, such as weed grasses. 3) Previous success with similar species using the protocol. 4) Plant tissues of high polysaccharides and high polyphenols. 5) Fail BP, PS and PP protocols. 6) Experienced users frequently use LNG for all HMW DNA isolation in the lab.
Tissue disruption method	Rotor stator	Rotor stator	Rotor stator	Liquid nitrogen
Tissue status & storage	Fresh young	Fresh young	Fresh young	Fresh young or frozen young
Tissue input (wet weight)	0.5 - 3.0 g	0.5 g	0.5 g	0.5 - 1.0 g
Fix FA / chop / blend	√	√	√	-
Transfer 0.1 - 0.3 g tissue eq.	-	√	-	√
HB plus with additional Triton	Yes if oxidation (high polyphenols content) is observed during chopping and / or blending.	-	Yes, add additional Bionano Prep Triton to HB+.	Yes. For high polyphenols plant species, add additional Bionano Prep Triton to HB+.
Transfer 0.1-0.3g tissue eq.	-	√	-	√
Filter nuclei & pellet - genome size	√	√	√	√
Low speed spin	Optional with Conditions (refer to protocol for details)	√	√	√
Up to four washes	Yes if cloudy / dark green color supernatant or dark green pellet			
Density gradient nuclei purification	√	-	√	√
Plug lysis DNA purification	√	√	√	√
Plant examples	maize, tomato, spinach	soybean, diploid strawberry	cotton, *Eucalyptus*, grapevine, oak tree	cotton, *Eucalyptus*, soybean, tomato, maize
FA = Formaldehyde				

Table 1 shows the differences between the four plant protocols and gives general guidelines of how to choose the right protocol for your plant tissue DNA isolation. Our on-line database (https://bionanogenomics.com/wp-content/uploads/2017/01/Bionano-Plant-Database.pdf) also lists a selection of plant species that have successfully been processed with the Bionano Prep Plant Tissue DNA Isolation Kit. If a certain protocol was utilized successfully for a plant species of interest, please use the same protocol for your DNA isolation.

Bionano Prep Plant Tissue DNA Isolation Protocol Descriptions

Bionano Prep Plant Tissue DNA Isolation Base Protocol (Part # 30068)
Designed for use with up to 3.0 g of fresh young leaves from various plant species. It is recommended to use the Base Protocol under the following conditions:

- Previously successful with similar species using this protocol (including on-line database);
- Performing plant DNA isolation on an unfamiliar species for the first time;
- No closely related species in the on-line database; and
- Little information is known about the chemical components and physical characteristics of the species.

Bionano Prep High Polysaccharides Plant Tissue DNA Isolation Protocol (Part # 30128)
Designed for use with up to 0.5 g of starting fresh young leaves (carrying forward only 0.1 – 0.3 g equivalent for nuclei purification) from plant species with high polysaccharide content, such as soybeans and diploid strawberry. This protocol is recommended when users fail to isolate nuclei using **"Bionano Prep Plant Tissue DNA Isolation Base Protocol."** Please refer to the Plant Tissue DNA Isolation Troubleshooting Guide (Figure 2).

Bionano Prep High Polyphenols Plant Tissue DNA Isolation Protocol (Part # 30133)
Designed for use with 0.5 – 1.0 g of starting fresh young leaves from plants with high polyphenols content, such as cottons, grapevines, roses, blueberry, trees, woody plants, *etc*. This protocol is also recommended if oxidation (color change) occurred during nuclei isolation, Proteinase K digestion, or DNA recovery when using **"Bionano Prep Plant Tissue DNA Isolation Base Protocol"**. Please refer to the Plant Tissue DNA Isolation Troubleshooting Guide (Figure 2).

Bionano Prep Plant Tissue DNA Isolation, Liquid Nitrogen Grinding Protocol (Part # 30177)
Designed to use liquid nitrogen grinding method to disrupt plant tissue for nuclei isolation. This protocol starts with 0.5 g to 1.0 g of snap frozen young tissues or very tough/fibrous tissues (fresh or frozen). However, only a very small amount of the ground tissue powder (as little as 0.1 g equivalent) is carried forward for high-quality nuclei purification. This protocol is also an alternative isolation methods for high polysaccharides and high polyphenols plant; or when all other 3 protocols fail to isolate high-quality HMW DNA from the plant tissue. Please refer to the Plant Tissue DNA Isolation Troubleshooting Guide (Figure 2).

This guide will help our customers select a Plant DNA Isolation Protocol that will have the highest likelihood of success from a target plant species; however, we cannot guarantee that customers will get the desirable gDNA for Bionano applications with the very first attempt. Optimization may be required before obtaining high-quality gDNA depending on plant tissue status and other unforeseen circumstances. The troubleshooting guide below should be used to determine the next steps when the initial protocol selection failed to yield high quality gDNA.

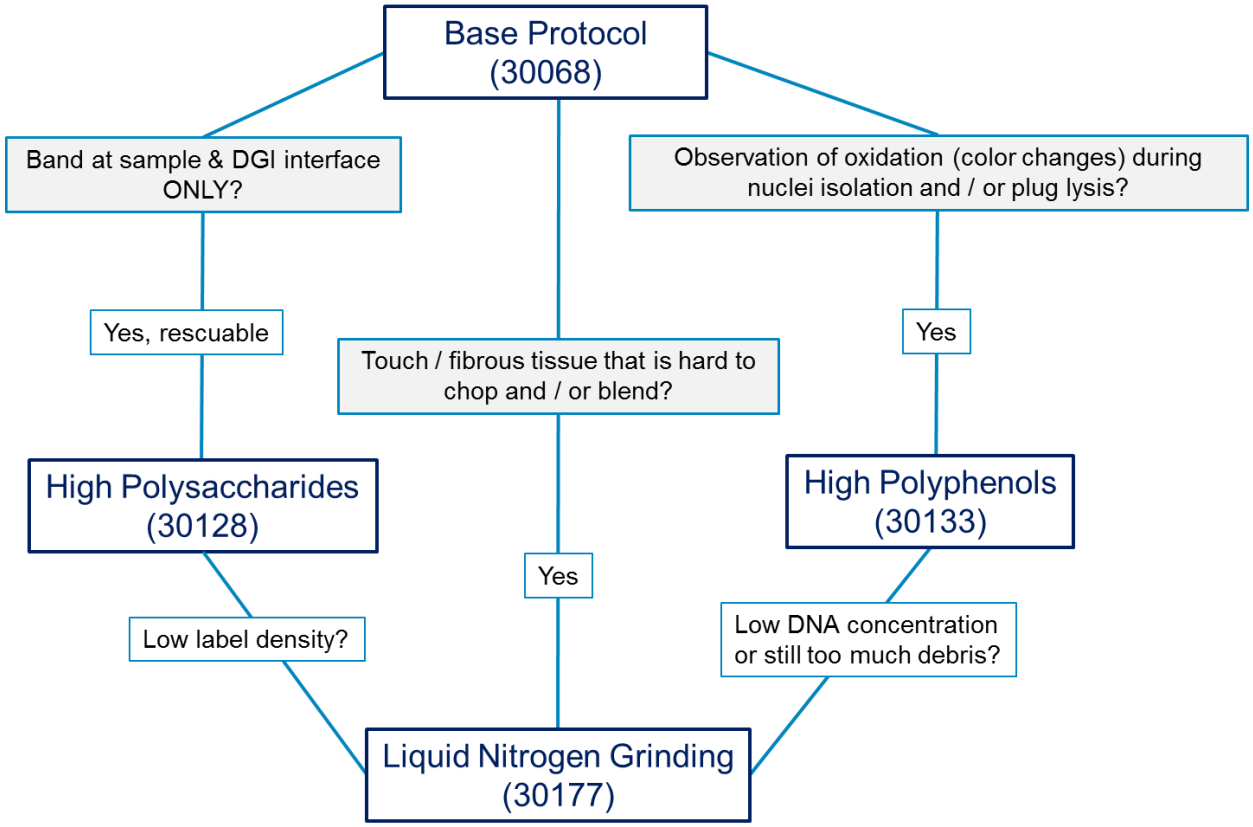

Figure 2. Plant Tissue DNA Isolation Troubleshooting Guide

Additional Training Resources-

Additional training videos for each critical step (with sign) are available at the bottom of the Bionano Genomics Support Webpage on the Plant DNA Isolation Kit Support webpage. Video tutorials include "Tissue Disruption with TissueRuptor and Liquid Nitrogen", "Pellet Resuspension", "Density Gradient", "Transfer Nuclei Band" and "Liquid Nitrogen Grinding."

Bionano Genomics, Inc.
9540 Towne Centre Drive, Suite 100
San Diego, CA 92121
Phone: (858) 888-7600
www.bionanogenomics.com