Bionano Prep Plant Protocol

Selection and Troubleshooting Guide

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Revision History

Revision	Notes
A	Initial Release

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).

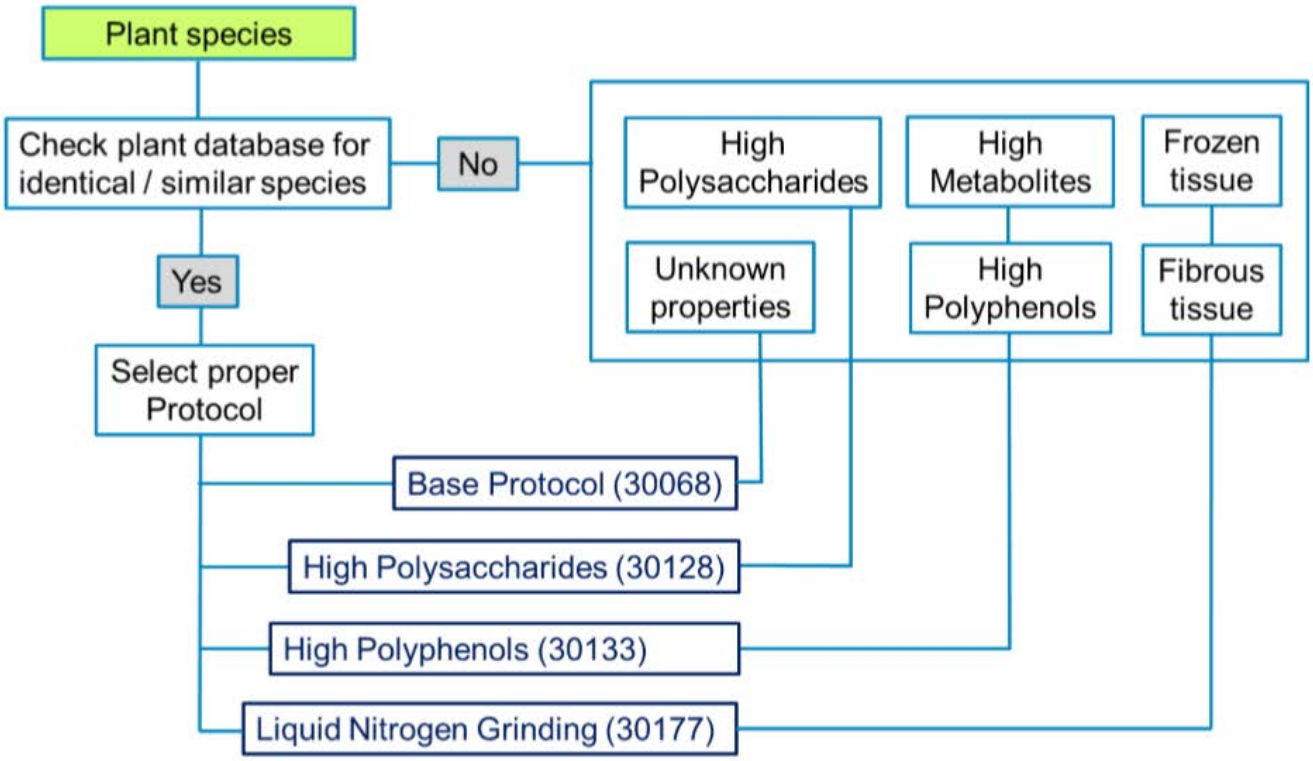

Figure 1. Plant Tissue DNA Isolation Protocol Selection Flowchart

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 the 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 Protocol

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 on how to choose the right protocol for your plant tissue DNA isolation. Our on-line Bionano Plant Database 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 next section, Plant DNA Sample Prep Troubleshooting Guide.

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 cotton, 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 next section, Plant DNA Sample Prep Troubleshooting Guide.

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 portion 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 method for plants high in polysaccharides or polyphenols, or when all three other protocols fail to isolate high quality HMW DNA.

This guide will help users select a Plant DNA Isolation Protocol that will have the highest likelihood of success from a target plant species; however, we cannot guarantee that users will obtain 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.

Plant DNA Sample Prep Troubleshooting Guide

This section provides a general troubleshooting guide to users who are encountering issues when isolating DNA from plant tissues. In the list below, the issues are highlighted in blue. For each issue, we include possible causes and questions/comments to assist you in troubleshooting. This guide may be used to determine the next steps when the initial protocol selection failed to yield high quality gDNA.

Issue: Raw DNA concentration obtained is low (<35 ng/ul) following Qubit Broad Range Assay.

Possible causes:

- Fully mature plants used
- Bruised plant tissue used (Figure 2)
- Plant tissue had undergone multiple freeze-thaw cycles or improper handling and storage
- Poor disruption and grinding resulted in fewer cells released, due to:
    1. insufficient blending time,
    2. not chopping the plant tissue into small enough sections,
    3. not grinding the frozen plant tissue into a fine powder
- No nuclei pellet observed before starting density gradient centrifugation
- Swing bucket rotor was not used during centrifugation
- No interface bands observed following density gradient centrifugation

Figure 2. Images of fresh young leaves (left) and bruised leaves (right).

Troubleshooting questions:

1. Did you refer to Bionano's Plant Database to see if your plant is mentioned?
2. Was the recommended plant protocol used? If the DNA extraction for that plant tissue has been successful with an existing protocol, was that protocol used? If the plant tissue is not in the Bionano Plant Database, is not frozen, or has not been tested with an existing protocol, was the Base Protocol for DNA extraction followed?
3. Were fresh young leaves used? Did you ensure that no bruised leaves were used? If you are not performing DNA extraction protocol immediately, did you snap-freeze the plant sample in liquid nitrogen (then stored at -80 deg C)? Did the plant tissue undergo freeze-and-thaw cycles?
4. Was the plant tissue chopped into approximately 2 x 2 mm pieces with a sharp razor blade? Was the plant tissue blended for up to 2 minutes with a maximum of 6 blending cycles on ice (each blending cycle contains 20 seconds of blending followed by 30 seconds resting on ice) to get the desired puréed consistency of homogenate? If following the Liquid Nitrogen Grinding Protocol, was the frozen plant tissue ground into a find powder?
5. Did you perform centrifugation using a swing bucket rotor at the appropriate speed and time? Was the centrifuge *g* force set based on the plant's genome size?
6. Did you take note of the size of the nuclei pellet? If the nuclei pellet is large (>6 mm in diameter) and young tissue material from a plant with a genome size greater than 1 Gbp was used, was titration performed?
7. If too few nuclei were embedded, did you increase the sample input amount?

Issue: DNA sample does not contain Ultrahigh Molecular Weight (UHMW) DNA.

Possible causes:

- Incorrect handling and storage of starting material
- Plant tissue was mature. Use only new growth.
- Starting material had undergone multiple freeze-thaw cycles
- Plant tissue was not fixed
- Residual nuclease remains

Troubleshooting questions:

1. Did you confirm if UHMW DNA was obtained via pulsed field gel electrophoresis (PFGE)?
2. Did you use a previously frozen plant tissue within 3 months of storage at -80 deg C?
3. Did you perform crucial steps during DNA extraction (e.g. tissue disruption) on ice?

4. Was the plant tissue fixed to protect the DNA/nuclei against mechanical shearing during disruption? Was a wide bore tip, not a regular tip, used to homogenize the DNA after the completion of DNA extraction and prior to labeling?
5. Did you ensure that nuclease-free water was used to dilute 5x Wash Buffer to 1x?
6. If the plant is rich in Polyphenols, did you follow the High Polyphenols Protocol?

Issue: DNA sample is white or opaque.

Figure 3. Image of tube containing white or opaque DNA.

Possible causes:

- Metabolite (polysaccharide) contamination
- Too much starting material

Troubleshooting questions:

1. If you observed precipitates in the DNA sample after long-term storage at 4 deg C, was a 200 µl wide bore tip used to resuspend the DNA? Did you make sure to subsequently leave the DNA at room temperature overnight?
2. If re-extraction of the DNA was performed, did you try reducing the sample material amount?
3. Did you increase Proteinase K incubation time during plug lysis? Alternatively, did you perform an additional Proteinase K treatment step? Did you ensure Proteinase K and agarase have not lost enzymatic activity and are within expiration?

4. Did you perform additional wash steps or longer wash step with 1x Wash Buffer on the agarose plug? For extended wash, did you incubate the agarose plug at 4 deg C in 1x Wash buffer?
5. Did you try to extend drop dialysis incubation time to 1.5 hours?
6. When doing titration, did you use the agarose plug with the lowest or middle yield within the specification (35-200 ng/μl) as that will have a lower concentration of any potential contaminants?
7. Did you follow the High Polysaccharides Protocol?
8. If the High Polysaccharides Protocol was still unsuccessful after DNA re-extraction, did you try following the Liquid Nitrogen Grinding Protocol?

Issue: DNA sample is colored.

Figure 4. Image of tubes containing brown DNA samples.

Possible causes:

- Metabolite (polyphenols) contamination
- Too much starting material
- Note: Polyphenols will tend to darken to a brown or reddish-brown color over time after DNA isolation.

Troubleshooting questions:

1. Did you follow the High Polyphenols Protocol? If so, did you ensure Bionano Prep Triton was added to HB+ at Step 5 and/or Step 29? Did you perform up to four additional washes to get a nuclei suspension that is light greenish-yellow to white in color?
2. Did you adjust the Lysis Buffer to pH 9.0 by adding sodium hydroxide solution before adding 200 μl Proteinase K enzyme and 5 μl βME to 2.5 ml Lysis Buffer?
3. Overloading the prep amount is common and works against you ultimately due to inability to remove contaminants. Did you try to reduce the sample material amount?
4. Did you use the plug with the lowest or middle yield within the specification (35-200 ng/μl) as that will have a lower concentration of any potential contaminants?

5. If High Polyphenols Protocol is still unsuccessful, did you follow the Liquid Nitrogen Grinding Protocol?

Issue: Plant material is tough or fibrous (hard to chop or blend).

- Follow the Liquid Nitrogen Grinding Protocol when processing tough or fibrous plant material to ensure successful disruption and homogenization.
- Use fresh young leaves only (if you had initially used mature leaves).

Issue: Low label density (3 labels/100 kbp difference between predicted label density following *in-silico* digestion and observed label density).

Possible causes:

- The DNA sample does not contain UHMW gDNA.
- Metabolite (polysaccharide, polyphenol) contamination
- Raw DNA is not homogeneous
- High DNA concentration (>150 ng/µl)

Troubleshooting questions:

1. Did you evaluate the size of starting gDNA by pulsed-field gel electrophoresis (PFGE) before labeling?
2. Did you confirm that the raw DNA is homogeneous (CV<0.3)?
3. Did you ensure accuracy of the volume of raw DNA pipetted, either by using a marked pipette tip or using a positive displacement pipette?
4. If the plant is high in polyphenols, did you follow the High Polyphenols Protocol?
5. If the plant has high polysaccharide levels, did you follow the High Polysaccharides Protocol?

Additional Training Resources

Additional training videos for each critical step are available on the Plant DNA Isolation Kit webpage (click "Videos"). Video tutorials include "Tissue Disruption with TissueRuptor and Liquid Nitrogen", "Pellet Resuspension", "Density Gradient", "Transfer Nuclei Band" and "Liquid Nitrogen Grinding."

Technical Assistance

For technical assistance, contact Bionano Genomics Technical Support.

You can retrieve documentation on Bionano products, SDS's, certificates of analysis, frequently asked questions, and other related documents from the Support website or by request through e-mail and telephone.

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