

TECHNICAL DATA SHEET 712

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SNARe™ Plant Genomic DNA Purification System

Description

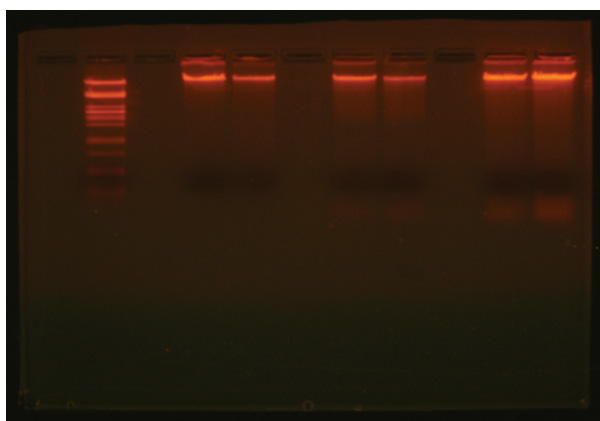
While many procedures are available for the isolation of genomic DNA from plant material, these methods often present challenges with regard to yield, purity, and utility in downstream applications. For example, DNA prepared via traditional CsCl gradient centrifugation is usually of good quality, but yields are generally low. Genomic DNA prepared using cetyltrimethylammonium bromide (CTAB) precipitation methods can contain significant amounts of polysaccharide and requires the use of organic solvents.

SNARe Plant Genomic DNA Purification System offers a rapid, cost-effective method for isolating DNA. The procedure does not require the use of organic solvents and is adaptable to different plant species, tissue types, and sample sizes. DNA Separation Particles are a suspension of superparamagnetic iron oxide particles that bind double-stranded DNA. Once bound, the DNA-particle complex is stable and can be washed to remove any impurities or unwanted protein from the sample to provide a clean DNA preparation. The DNA is eluted from the particles with an elution buffer for use in downstream reactions such as PCR, labeling, sequencing, transfection, cloning, and restriction digest.

SNARe (Simple Nucleic Acid Recovery) products are based on a new patent pending technology. SNARe offers the following advantages over other DNA purification methods:

- Easy-to-use procedures
- No need for columns or filters
- Reduces/eliminates repetitious centrifugation steps
- Reduces time required for DNA purification
- Eliminates use of toxic phenol/chloroform extraction of DNA
- Scalable - easily adjusts for sample size and automation
- DNA immediately available for PCR, restriction digestion

1 2 3 4 5 6 7 8 9 10 11



Lane 1: NA
Lane 2: Molecular Weight Standards Lambda DNA PST-1
Lane 3: NA
Lane 4: Competitor Magnetic Separation Protocol
Lane 5: Competitor Magnetic Separation Protocol
Lane 6: NA; **Lane 7:** SNARe™ DNA Purification System
Lane 7: SNARe™ DNA Purification System
Lane 8: SNARe™ DNA Purification System
Lane 9: NA
Lane 10: SNARe™ DNA Purification System
Lane 11: SNARe™ DNA Purification System

Figure 1

Characteristics

Sample Source: Plant tissue
Sample Size: 50-100mg

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Format: Microcentrifuge tube
Number of Isolations per Kit: 100
Approximate Time Required: ~90 minutes for purification

Material

Material Supplied

SNARe Plant Genomic DNA Purification System contains DNA Separation Particles and all the buffers required to isolate DNA. Magnetic separators must be purchased separately, see "Magnetic Separators." All components are tested and are free of nucleases and contaminating DNA. *Note:* Store all solutions at 4°C, except where noted below.

- DNA Separation Particles (in Prep Buffer): 5.5ml
Note: For handling instructions, see the following section: Preparation of DNA Separation Particles.
- Prep Buffer: 11ml
Note: Use to prewash DNA Separation Particles before use. Prewashing DNA Separation Particles is recommended for best results.
- Plant Lysis Buffer: 50ml
Note: Helps to lyse cells and solubilize proteins and other cell components.
- Proteinase K: 11mg
Note: Store at 4°C. When ready to use, resuspend in 550µl DEPC water to a 20mg/ml aliquot, and store at -20°C.
- DNA Binding Buffer: 7.2ml
- DNA Wash Solution: 33ml (for a final volume of 110ml)
Note: Washes away unwanted proteins to give a cleaner DNA preparation. Prior to use, add 77ml of 100% EtOH to the DNA Wash Solution. Mix well. Mark the label that ethanol has been added.
- DNA Elution Buffer: 22ml
Note: Elutes DNA from DNA Separation Particles.
- RNase A: 1.1ml, 10mg/ml
Note: Removes RNA from the DNA preparation.

Material Required

- 55°C incubator or water bath
- Centrifuge capable of 12,000 x G
- 100% and 70% EtOH (in DEPC treated water)
- Liquid nitrogen or dry ice
- Mortar and pestle, or tissue homogenizer

For microcentrifuge tube format:

- Precision pipets with disposable tips to deliver 1-20µl, 20-200µl, 200-1,000µl
- 2ml microcentrifuge tubes
- One of the following magnetic separators:
BioMag Solo-Sep Microcentrifuge Separator, single sample (Catalog Code 8MB4112S)
BioMag Multi-6 Microcentrifuge Tube Separator, six samples (Catalog Code 8MB4111S)

For the 96-well format:

- Multi-well precision pipets with disposable tips to deliver 1-20µl, 20-200µl, 200-1,000µl
- Centrifuge for 96-well plates capable of 3500 rpm
- 2 x 96-well rigid plates (1.2ml polypropylene wells)
- BioMag 96-Well Plate Separator (Catalog Code 8MB4109S)
- Plate sealer

Procedure

Researchers are advised to optimize the use of particles in any application.

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A. Preparation of DNA Separation Particles

The DNA Separation Particles may be prewashed up to 24 hours in advance. Store at 4°C for up to 24 hours. After 24 hours, repeat the wash step prior to use. This procedure prepares 50µl DNA Separation Particles. Scale up or down according to the number and type of preparations needed. (Recommendation for 96-well format: 25µl particles.)

1. Shake the DNA Separation bottle to ensure a uniform suspension.
2. Transfer 50µl of DNA Separation Particles to a microcentrifuge tube.
3. Place the tube on a magnetic separator until the supernatant is clear.
4. While the tube is on the separator, aspirate and discard the supernatant.
5. Resuspend in 50µl of Prep Buffer.
6. Shake to ensure a uniform suspension.

Helpful Tips for Aspirating Supernatant

It is important not to contaminate the supernatant with particles. To aspirate the most supernatant without particles:

1. Hold the separator with the tube at eye level.
2. Tilt the tube such that the magnet wall of the separator faces up.
3. Hold the tube firmly against the wall. Angle the pipet tip away from the wall of the tube which the magnetic particles rest.

B. Preparing Samples

1. Harvest 0.05-0.100g fresh plant tissue. Rinse tissue with DI water to remove adhering debris and blot dry.
2. Freeze tissue with liquid nitrogen or dry ice and grind to a fine powder using a mortar and pestle.
3. Transfer mixture to a nuclease free microcentrifuge tube. Add 0.5ml of Plant Lysis Buffer to the frozen powder and mix.
4. Add Proteinase K (5µl) to a final concentration of 100µg/ml. Mix well, but do not vortex.
5. Incubate for 1 hour at 55°C.
6. Centrifuge for 5 minutes at 10,000-12,000 x G.
7. Remove the supernatant to a fresh tube.

C. Binding the DNA

1. Add 50µl of prewashed DNA Separation Particles to the 550µl of tissue homogenate.
2. Add 60µl of the DNA Binding Buffer and mix gently.
3. Add 1.5ml of 100% EtOH and mix gently.
4. Incubate at room temperature for 10 minutes with occasional mixing.
5. Place the tube on a magnetic separator until the supernatant is clear.
6. While the tube is on the separator, aspirate and discard the supernatant.

D. Washing the DNA

If not already added, add 77ml of 100% EtOH to the DNA Wash Solution, as directed on the bottle label, and mix. Mark the label that ethanol has been added. For additional purity of your DNA preparation, transfer the vial of RNase A into the DNA Wash Solution or add 10µl of RNase A per test.

First Wash Series:

1. Add 500µl of DNA Wash Solution to each sample tube. If adding the RNase A separately, add 10µl per test. Resuspend the DNA-particle complex by pipette mixing. Incubate for 5 minutes at room temperature to allow the RNase A to work on the RNA. Do not vortex.
2. Place the tube on a magnetic separator until the supernatant is clear.
3. While the tube is on the separator, aspirate and discard the supernatant.
4. Add 500µl DNA Wash Solution and mix by gentle inversion.
5. Repeat Steps 2-3.

Second Wash Series:

1. Add 500µl of 70% EtOH. Resuspend the DNA-particle complex by pipette mixing. Do not vortex.

2. Place the tube on a magnetic separator until the supernatant is clear.
3. While the tube is on the separator, aspirate and discard the supernatant.
4. Repeat Steps 1-3, two times.
5. Air dry the DNA Separation Particles for 2-5 minutes. Remove any residual ethanol.

E. Eluting the DNA

Note: Do not substitute lab water for DNA Elution Buffer. Some lab water is less than pH 4.0. Low pH can interfere with downstream reactions involving enzymes.

1. Resuspend the DNA Separation Particles in 200µl of DNA Elution Buffer. Pipette mix.
2. Incubate at room temperature for 10 minutes. Pipette mix.
3. Place the tube on a magnetic separator until the supernatant is clear.
4. While the tube is on the separator, transfer the DNA-containing supernatant to a new tube.

DNA Yield

This procedure isolates approximately 40µg of plant genomic DNA based on tomato leaves. It is recommended that 20-50µl of the eluted DNA be run on a 1% agarose gel to quantitate the yield. Yield can vary and is dependent on the type and amounts of starting material.

Storage and Stability

Store particles at 4°C. Freezing, drying, or centrifuging particles may result in irreversible aggregation and loss of binding activity.

Safety

Before handling any chemicals in this System, refer to the Material Safety Data Sheets provided. Observe all relevant precautions, and follow all state, local, and federal regulations for chemical handling and disposal.

This product is for research use only and is not intended for use in humans or for *in vitro* diagnostic use.

Ordering Information

Catalog Code	Description	Size
85082	SNARe™ Plant Genomic DNA Purification System	1 kit / 100 isolations

Related Products

Catalog Code	Description	Size
85080	SNARe™ Genomic DNA Purification System (Technical Data Sheet 710)	1 kit / 100 isolations
85081	SNARe™ Plasmid DNA Purification System (Technical Data Sheet 711)	1 kit / 100 isolations

Magnetic Separators

Catalog Code	Description	Size
8MB4112S	BioMag® Solo-Sep Microcentrifuge Tube Separator	1 each
8MB4111S	BioMag® Multi-6 Microcentrifuge Tube Separator	1 each
8MB4109S	BioMag® 96-Well Plate Separator	1 each

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