HiPer® Plant Genomic DNA Extraction Teaching Kit (Column Based)

Product Code: HTBM004

Number of experiments that can be performed: 10

Duration of Experiment:
Protocol: 1 hour
Agarose Gel Electrophoresis: 1 hour

Storage Instructions:

- The kit is stable for 6 months from the date of manufacture
- Store Control DNA at -20°C
- Store 6X Gel Loading Buffer at 2-8°C
- Other kit contents can be stored at room temperature (15-25°C)

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Aim:

To extract and analyze genomic DNA from leaf tissue (using spin columns).

Introduction

Extraction of plant cell DNA (enclosed in the nucleus) essentially requires mechanical method for breaking down the cell wall and membranes, allowing access to nuclear material, without its degradation. DNA extraction from plant tissue can vary depending on the material used.

There are three basic steps in DNA extraction. The cell must be lysed to release the nucleus, the nucleus must be ruptured to release the DNA and the DNA must be protected from enzymes that will degrade it, causing shearing. Once the DNA is released, it must then be precipitated in alcohol.

Plant Genomic DNA Extraction Teaching Kit (Column Based) has many advantages over the crude method of DNA extraction. It is fast, simple and does not require harmful organic compounds such as phenol and chloroform. This kit contains HiElute Miniprep Spin Column format, which allows rapid processing of multiple samples. The columns have a high binding capacity and high quality DNA is obtained from various species.

The DNA purification procedure using the Miniprep Spin Columns comprises of three steps:

- Adsorption of DNA to the membrane
- Removal of residual contaminants
- Elution of pure genomic DNA

The DNA obtained is compatible with down stream applications such as restriction endonuclease digestion, PCR and southern blotting.

Principle:

HiPer® Plant Genomic DNA Extraction Teaching Kit (Column Based) simplifies isolation of DNA from fresh leaves using spin column procedure. The procedure is optimized for a maximum of 100 mg of wet-weight starting leaf material. The fresh leaves are cut and ground in Lysis Buffer. Protein precipitation is followed by removal of other contaminants such as cell debris, salt precipitates using HiShredder.

The HiShredder removes all cell debris and precipitates making the preparation of a clear lysate rapid and efficient. Preparation of the clear lysate is essential to prevent clogging of the HiElute Miniprep Spin Column used in the subsequent steps. The flow through fraction is then mixed with a solution that enhances the binding of DNA to the column. The solution is then passed through HiElute Miniprep Spin Column, which is based on the advanced silica binding principle presented in a microspin format.

This column eliminates the need for alcohol precipitation, expensive resins, and harmful organic compounds such as phenol and chloroform, otherwise employed in traditional DNA isolation techniques. DNA binds specifically to the advanced silica gel membrane while contaminants pass through it. PCR inhibitors such as divalent cations and proteins are completely removed in two efficient washing steps, leaving DNA to be eluted in the Elution buffer.

Kit Contents:

This kit can be used to perform extraction of plant genomic DNA from leaves.

Table 1: Enlists the materials provided in this kit with their quantity and recommended storage

Sr. No.	Product Code	Materials Provided	Quantity 10 expts	Storage
1	TKC007	Control DNA	0.11 ml	-20°C
2	DS0016	Lysis Buffer (PL)	5 ml	R T
3	DS0017	Precipitation Buffer (PS)	2 ml	R T
4	DS0018	Binding Buffer	10 ml	R T
5	DS0019	Wash solution	12 ml	R T
6	DS0040	Elution Buffer (ET) [10mM Tris-Cl, pH 8.5]	2.5 ml	R T
7	DS0003	RNase A solution	0.250 ml	R T
8	MB002	Agarose	4.8 g	R T
9	ML016	50X TAE	120 ml	R T
10	ML015	6X Gel Loading Buffer	0.05 ml	2-8°C
11	DBCA03	HiElute Miniprep Spin Column (Capped) [in DBCA016 Collection Tube]	10 Nos.	R T
12	DSCA01	HiShredder™	10 Nos.	R T
13	PW1139	Collection Tubes, Polypropylene (2.0 ml)	20 Nos.	R T
14	DBCA016	Collection Tubes(Uncapped), Polypropylene (2.0 ml)	10 Nos.	R T

Materials Required But Not Provided:

Sample: Plant leaves (100 mg wet weight)
Glass wares: Conical flask, Measuring cylinder, Beaker
Reagents: Distilled water, Ethidium bromide (10 mg/ml)
Other requirements: Mortar and Pestle, Electrophoresis apparatus, UV Transilluminator, Heating Block or Water Bath, Vortex Mixer, Micropipettes, Tips, Adhesive tape, Crushed ice

Storage:

HiPer® Plant Genomic DNA Extraction Kit (Column Based) can be stored for up to 6 months from the date of manufacture without showing any reduction in performance. On receipt, store Control DNA at -20°C and 6X Gel Loading Buffer at 2-8°C. Other kit contents can be stored at room temperature (15-25°C).

Important Instructions:

1. Preheat the water bath or heating block to 65°C.
2. Thoroughly mix the reagents. Examine the reagents for precipitation. If any kit reagent forms a precipitate (other than enzymes), warm at 55-65°C until the precipitate dissolves and cool it down to room temperature (15-25°C) before use.
3. Ensure that clean & dry eppendorf tubes and tips are used for the procedure.
4. Clean the mortar and pestle with distilled water before use. Prechill the same before starting the experiment

Procedure:

It is preferable to use young and tender plant parts especially leaves, needles, since they contain more cells per weight and therefore result in higher yields. Also, young leaves and needles contain less polysaccharides and polyphenolics and are therefore easier to handle.

A) Sample Preparation:

Take young and tender leaves (for e.g. mint, spinach, tulsi, ginger etc) and wash them with distilled water. Finely cut the leaf material. Midrib and petiole should be removed from the leaf material before grinding, as they are a major source of carbohydrate contamination. Weigh 100 mg of the finely cut leaves and grind properly in 400 µl Lysis Buffer using a mortar and pestle. Proceed immediately according to the DNA isolation protocol.

NOTE: Delay in continuing DNA isolation after sample preparation may result in DNA degradation and yield loss. Do not grind very coarsely as it may cause shearing of DNA.

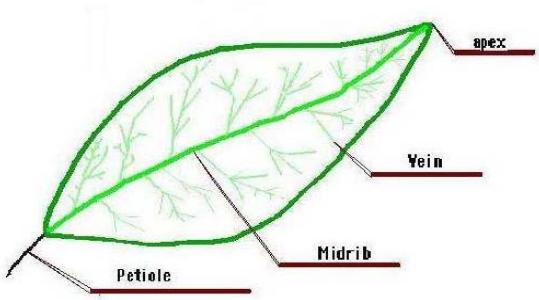


Fig 1: Leaf showing the midrib and petiole

B) DNA Extraction:

1. Transfer the above mixture to 2.0 ml collection tube using a clean spatula. Vortex vigorously.
2. Add 20µl of RNaseA Solution (20mg/ml) to the above tube and incubate the mixture for 10 minutes at Room Temperature (15-25°C).
3. Incubate the mixture for 10 minutes at 65°C, mix the contents 2-3 times by inverting the tube.
4. Add 130 µl of Precipitation Buffer to the above mixture, mix and incubate for 5 minutes on ice.

5. **Load sample on HiShredder**
   Add the entire sample onto the HiShredder placed in a 2.0 ml collection tube and centrifuge for 2 minutes at 14,000 rpm.

6. Transfer the flow through fraction from step 4 to a new 2.0 ml collection tube without disturbing the cell debris pellet.

7. **Binding**
   Add 1.5 volumes of Binding Buffer to the above mixture obtained from step 5 and mix by pipetting.

   NOTE: E.g.: To 500 µl of the mixture, add 750 µl of Binding Buffer. Adjust the volume of Binding Buffer according to the mixture obtained from step 5. A precipitate may form after the addition of Binding Buffer but this will not affect the DNA isolation procedure.

8. **Load lysate onto HiElute Miniprep Spin Column (Capped) [DBCA03]**
   Add 650 µl of the mixture from step 6, including any precipitate that may have formed, onto the HiElute Miniprep Spin Column placed in a 2.0 ml collection tube. Centrifuge for 1 minute at 8,000 rpm. Discard the flow through fraction.

9. Repeat step 7 with the remaining sample. Transfer the column in a new 2.0 ml collection tube and discard the flow through fraction and the 2.0 ml collection tube.

10. **Wash**
    To the HiElute Miniprep Spin Column placed in a new 2.0 ml collection tube, add 500 µl of Wash Solution. Centrifuge for 1 minute at 8,000 rpm.

    NOTE: Discard the flow through and reuse the 2.0 ml collection tube in step 10.

11. Add another 500 µl of the Wash Solution to the HiElute Miniprep Spin Column and centrifuge for 2 minutes at 14,000 rpm. Discard the flow through.

12. Centrifuge the tube with HiElute Miniprep Spin Column for an additional 2 minutes at 14,000 rpm to remove traces of Wash Solution.

13. **DNA Elution**
    Pipette 200 µl of the Elution Buffer directly onto the column without spilling to the sides of the column. Incubate for 1 minute at room temperature. Centrifuge at 10,000 rpm for 1 minute to elute the DNA.

    Storage of the eluate with purified DNA: The eluate contains pure genomic DNA. For short term storage (24-48 hours) of the DNA, 2-8°C is recommended. For long-term storage, -20°C or lower temperature (-80°C) is recommended. The Elution Buffer will help to stabilize the DNA at these temperatures. Avoid repeated freezing and thawing of the sample which may cause denaturation of the DNA.

Agarose Gel Electrophoresis:

Preparation of 1X TAE: To prepare 500 ml of 1X TAE buffer, add 10 ml of 50X TAE Buffer to 490 ml of sterile distilled water*. Mix well before use.

Preparation of agarose gel: To prepare 50 ml of 0.8% agarose gel, add 0.4 g agarose in 50 ml 1X TAE buffer in a glass beaker or flask. Heat the mixture on a microwave or hot plate or burner by swirling the glass beaker/flask occasionally, until agarose dissolves completely (ensure that the lid of the flask is loose to avoid buildup of pressure). Allow the solution to cool down to 55-60°C. Add 0.5 µl Ethidium bromide, mix well and pour the gel solution into the gel tray. Allow the gel to solidify for about 30 minutes at room temperature.

NOTE: Ethidium bromide is a powerful mutagen and is very toxic. Appropriate safety precautions should be taken by wearing latex gloves; however, use of nitrile gloves is recommended.

Loading of the DNA samples: To prepare sample for electrophoresis, add 2 µl of 6X gel loading buffer to 10 µl of DNA sample. Mix well by pipetting and load the sample into the well. Load the Control DNA after extracting the DNA sample.

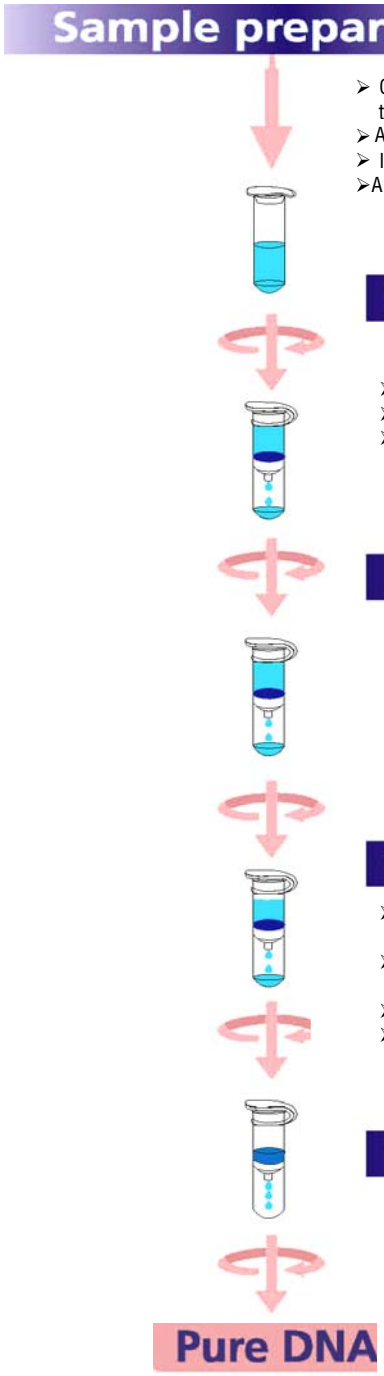
Electrophoresis: Connect the power cord to the electrophoretic power supply according to the conventions: Red-Anode and Black- Cathode. Electrophorese at 100-120 volts and 90 mA until dye markers have migrated an appropriate distance, depending on the size of DNA to be visualized.

* Molecular biology grade water is recommended (Product code: ML024).

Quantitation of DNA:

Spectrophotometric analysis and agarose gel electrophoresis will reveal the concentration and the purity of the genomic DNA. Use Elution Buffer to dilute samples and to calibrate the spectrophotometer, measure the absorbance at 260 nm, 280 nm, and 320 nm using a quartz microcuvette. Absorbance readings at 260 nm should fall between 0.1 and 1.0. The 320 nm absorbance is used to correct background absorbance. An absorbance of 1.0 at 260 nm corresponds to approximately 50 µg/ml of DNA. The $A_{260} - A_{320} / A_{280} - A_{320}$ ratio should be 1.6 –1.9. Purity is determined by calculating the ratio of absorbance at 260 nm to absorbance at 280 nm. The concentration of DNA is calculated by the following formula:
Concentration of DNA sample (µg/ml) = 200 x A_{260} x dilution factor

Flowchart:



- Grind 100 mg of finely cut leaves in 400 µl of Lysis Buffer and transfer the mixture to 2.0 ml collection tube.
- Add 20 µl of RNase A Solution, vortex and incubate at 15-25°**C for 10 minutes**
- Incubate at 65ºC for 10 minutes with intermittent vortexing
- Add 130 µl of Precipitation Buffer, m ix and incubate on ice for 5 minutes

- Load the entire sample onto the HiShredder
- Centrifuge for 2 minutes at 14,000 rpm
- Transfer the flow through in 2.0 m l collection tube without disturbing the pellet

- Add 1.5 volumes of Binding Buffer to above mixture, mix by pipetting
- Add 650 µl of above mixture to HiElute Column
- Centrifuge for 1 minute at 8,000 rpm and discard flow through.
- Repeat above 2 steps for remaining sample
- Discard flow-through and reuse same collection tube

- Add 500 µl Wash Solution and centrifuge at 8,000 rpm for 1 minute and discard flow through
- Add another 500 µl Wash Solution and centrifuge at 14,000 rpm for 2 minutes, discard flow through
- Centrifuge for additional 2 minutes at 14,000 rpm
- Place column in new collection tube

- Add 200 µl of Elution Buffer
- Incubate at room temperature for 1 minute
- Centrifuge for 1 minute at 10,000 rpm to elute the pure DNA

Observation and Result:

Perform Agarose Gel Electrophoresis. Visualize the DNA bands using UV Transilluminator and calculate the yield and purity using UV Spectrophotometer.

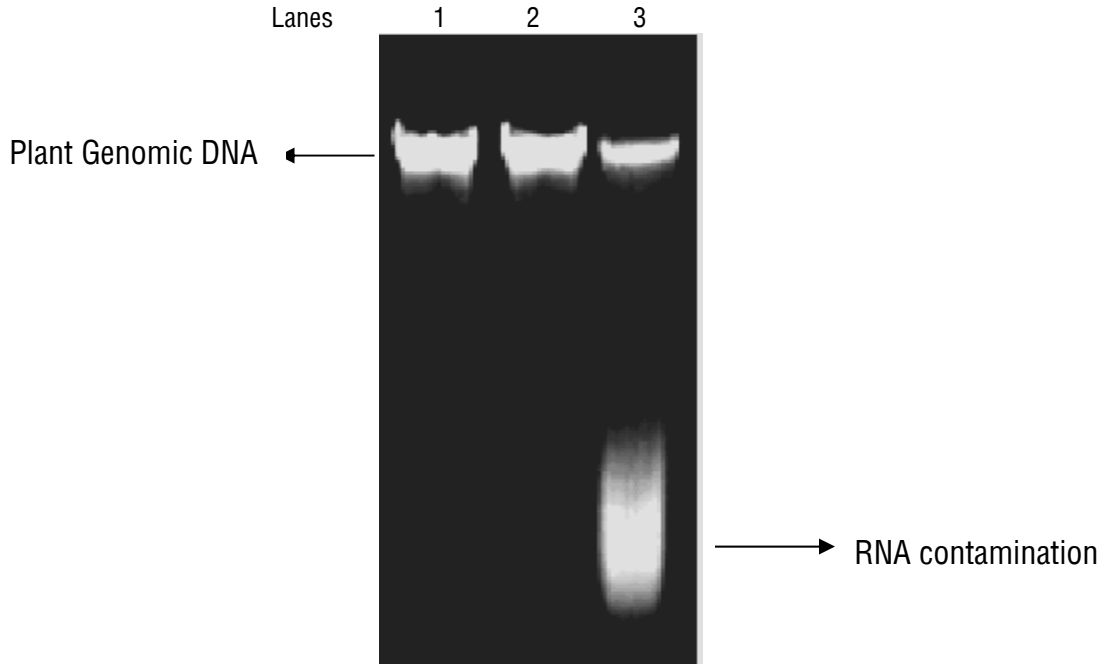


Fig 2: Gel image of isolated plant genomic DNA

Lane 1: Control DNA
Lane 2: Plant Genomic DNA
Lane 3: Plant Genomic DNA with RNA contamination

Table 2: Absorbance of the extracted genomic DNA at 260 nm and 280 nm

Sample	Dilution Factor	A_{260}	A_{280}	A_{260}/A_{280}	Concentration (µg/ml)
1					
2					
3					

Calculate the concentration of isolated DNA using following formula:

Concentration of DNA sample (µg/ml) = 200 x A_{260} x dilution factor

Interpretation:

The lanes 1 and 2 demonstrate that highly purified DNA has been obtained with no visible RNA contamination when electrophoresed on agarose gel. If RNA contamination is present, one would see a faint and smeary RNA band below the genomic DNA as shown in Lane 3. RNA being of lower molecular weight than DNA runs faster than the genomic DNA. RNA contamination is observed when the RNase treatment has either been skipped or not been carried out properly.

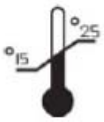
An absorbance of 1.0 at 260 nm corresponds to approximately 50 µg/ml of DNA. If the A_{260}/A_{280} ratio is 1.6-1.9, then the isolated DNA sample is considered to be pure. If higher A_{260}/A_{280} ratio is observed it indicates the possibility of RNA contamination.

Troubleshooting Guide:

Sr. No.	Problem	Possible Cause	Solution
1	Clogged HiElute Miniprep Spin Column	Carryover of the particulate material	Ensure that no particulate material is transferred following centrifugation through the HiShredder
2	Lower yields of DNA	Insufficient disruption of the plant tissue	Ensure that the plant material is disrupted properly using a mortar and pestle
		Improper binding	Ensure that the binding conditions are adjusted correctly by accurately determining the amount of lysate recovered (as per step 6)
3	Shearing of DNA	Precipitation of Lysis Buffer	Examine the solution for any kind of precipitation, if the solution forms a precipitate, warm at 55-65°C until the precipitate dissolves completely, allow it to cool to room temperature (15–25°C) before use
4	Darkly colored membrane or green/ yellow eluate after washing with wash solution	Insufficient washing of the membrane	After washing with the Wash Solution an additional wash with 500 µl ethanol (96-100%) should be performed. Centrifuge for 2 minutes at 14,000 rpm to dry the membrane
		Amount of starting material is more than recommended	Reduce the amount of starting material for future preps
5	Poor performance of the DNA in downstream experiments	Wash Solution carryover	Ensure that during the second wash with Wash Solution, the column is centrifuged for 2 minutes at 14,000 rpm. Following the spin, remove the column carefully from the collection tube so that it does not come in contact with the flow through as this will result in carryover of Wash Solution

Technical Assistance:

At HiMedia we pride ourselves on the quality and availability of our technical support. For any kind of technical assistance, mail at mb@himedialabs.com



Storage temperature

Do not use if package is damaged

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