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peqGOLD
Plant DNA Mini Kit

– Instruction Manual –

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Introduction

The peqGOLD Plant DNA Miniprep Kits allow rapid and reliable isolation of high-quality total cellular DNA from a wide variety of plant species and tissues. Up to 200 mg of fresh or frozen tissue (or 50 mg dry tissue) can be processed in less than 1 hour. The system combines the reversible nucleic acid-binding properties of HiBind® matrix with the speed and versatility of spin column technology to eliminate polysaccharides, phenolic compounds, and enzyme inhibitors from plant tissue lysates. Purified DNA is suitable for PCR*, restriction digestion, and hybridization techniques. There are no organic extractions thus reducing plastic waste and hands-on time to allow multiple samples to be processed in parallel.

Theory

Dry or fresh plant tissue is disrupted and then lysed in a specially formulated buffer containing detergent. Proteins, polysaccharides, and cellular debris are subsequently precipitated. Contaminants are further removed by isopropanol precipitation of DNA. Binding conditions are then adjusted and the sample is applied to a HiBind® DNA spin-column. Two rapid wash steps remove trace contaminants such as residual polysaccharides and pure DNA is eluted in water or low ionic strength buffer. Purified DNA can be directly used in downstream applications without the need for further purification.

Kit Components

peqGOLD Plant DNA Mini Kit	5 Purifications	50 Purifications	200 Purifications
Product Number	12-3486-00	12-3486-01	12-3486-02
Components			
HiBind® Columns	5	50	200
2 ml Collection Tubes	10	100	400
P1 Buffer	5 ml	50 ml	180 ml
P2 Buffer	1 ml	10 ml	40 ml
P3 Buffer	2 ml	20 ml	80 ml
RNase A	40 µl	250 µl	1 ml
Elution buffer	1.5 ml	15 ml	50 ml
DNA Wash buffer	12 ml	40 ml	3 x 40 ml
Instruction manual	1	1	1

Storage and Stability

peqGOLD Plant DNA Mini Kit components should be stored at room temperature (22 °C – 25 °C). All peqGOLD Total RNA Kit components are stable for at least 12 months from the date of purchase when stored at 22-25 °C. During shipment crystals may form in the P3 Buffer. Warm up to 37 °C to dissolve.

Binding Capacity

The binding capacity of a HiBind® DNA Mini column is 50 µg DNA. Do not use more cell or tissue material as indicated.

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Before Starting

Briefly examine this booklet and become familiar with each step. Prepare all components and have the necessary materials ready before starting.

! Under cool ambient conditions, crystals may form in the P3 Buffer. This is normal and the bottle should be warmed (37°C) to dissolve the salt before use.

! DNA Wash Buffer is concentrated and has to be diluted with absolute ethanol as follows:

Kit 12-3486-00	Add 18 ml 100% EtOH to 12 ml Wash Buffer
Kit 12-3486-01	Add 60 ml 100% EtOH to 40 ml Wash Buffer
Kit 12-3486-02	Add 3 x 60 ml 100% EtOH to 3 x 40 ml Wash Buffer

Store diluted DNA Wash Buffer at room temperature.

! All steps must be carried out at room temperature (22 – 25°C).

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peqGOLD Plant DNA Isolation Protocol

A. Dry Specimens

Drying allows storage of field specimens for prolonged period of time prior to processing. Samples can be dried overnight in a 45°C oven, powdered, and stored dry at room temperature.

Materials required, but not supplied:
- ! ß-Mercaptoethanol
- ! Isopropanol
- ! 100 % Ethanol
- ! Sterile dH_2O
- ! Sterile RNase-free pipet tips and centrifuge tubes

1. Homogenization and lysis

To prepare dried samples place 10-50 mg of dried tissue into a 1,5 ml microcentrifuge tube (2 ml tubes are recommended for processing >50 mg tissue) and grind using a pellet pestle. A fine powder will ensure optimal DNA extraction and yield.

For critical work such as PCR and cloning, pestles are best used a single time then soaked in a dilute bleach solution immediately after use until cleaning. Disposable pestles may be autoclaved several times. For standard Southern analysis, the same pestle can be reused several times to grind multiple tissue samples by rinsing with ethanol and wiping the surface clean between samples.*

Add one after the other 800 µl P1 Buffer and 10 µl ß-Mercaptoethanol to 10-50 mg powdered dry tissue and vortex vigorously to mix. Make sure to disperse all clumps. Incubate at 65°C for 10 min. Mix sample twice during incubation by inverting tube. Add 140 µl P2 Buffer and vortex to mix. Centrifuge at 10,000 x g for 10 min. Transfer supernatant carefully to a new microcentrifuge tube making sure not to disturb the pellet or transfer any debris.

2. Removal of polysaccharides (Optional!)

Add 0.7 volume isopropanol and vortex to precipitate DNA.

In most cases 700 µl supernatant can easily be removed. This will require 490 µl isopropanol. Note that depending on the sample, the volume of supernatant may vary. After transferring to a fresh tube, measure the volume and add the correct amount of isopropanol.

This step removes much of the polysaccharide content and improves spin-column performance by increasing DNA binding capacity in the steps that follow.

Immediately centrifuge for 2 min at 10,000 x g to pellet the DNA. A longer centrifugation does not improve yields.

Pour off the supernatant making sure not to dislodge the DNA pellet. Invert the microfuge tube on a paper towel for 1 min to allow residual liquid to drain. Drying the pellet is not recommended.

3. RNA Digest

Add 100 µl of pre-heated (65°C) sterile dH_2O and vortex to resuspend the pellet. A brief incubation at 65°C may be necessary to effectively dissolve the DNA.

Add 4 µl RNase A and mix. Incubate for 2 min at room temperature.

4. Load and Bind

Add 150 µl P3 Buffer first, followed by 300 µl absolute ethanol. Vortex thoroughly to mix. This will adjust binding conditions prior to loading the HiBind® DNA column.
Place a HiBind® DNA spin column into a fresh 2 ml collection tube (supplied) and apply the entire sample, including any precipitates that may form, to the spin column. Centrifuge the spin column / collection tube assembly at 10,000 x g for 1 min. Discard the flow-through liquid and the old collection tube and place the spin column into a new collection tube.

5. Wash I

Add 750 µl of the completed DNA Wash Buffer to the column and centrifuge the spin column / collection tube assembly for 1 min at 10,000 x g. Discard the flow-throw liquid and place the spin column back in the collection tube.

6. Wash II

Repeat the washing step as described in step 5 with 750 µl of the completed DNA Wash Buffer. Discard the flow-throw liquid and keep the spin column for the next step.

7. Dry (Important, do not skip this step!)

Place the HiBind® spin column containing your DNA in the collection tube used in step 6 and centrifuge for 2 min at maximum speed to dry the column matrix.

8. Elution

Transfer the spin column to a fresh 1.5 ml Centrifuge tube. Apply 100 µl Elution buffer or sterile deionized water pre-warmed to 65°C and incubate at room temperature for 1 min. Centrifuge at 10,000 x g for 1 min to elute DNA. Repeat elution with additional 100 µl of buffer. This may be performed using another 1.5 ml tube to obtain a higher DNA concentration in the first eluate. Smaller volumes will significantly increase DNA concentration but give lower yields. It is not recommended to use more than 200 µl of buffer for elution.

To increase DNA concentration add buffer and incubate the column at 60°C - 70°C for 5 min before elution.

Total DNA yields vary depending on type and quantity of sample. Typically, 10-50 µg DNA can be isolated using 50 mg dried tissue.

B. Fresh and frozen Specimens

This protocol is suitable for most fresh or frozen tissue samples allowing more efficient recovery of DNA. However, due to the tremendous variation in water and polysaccharide content of plants, sample size should be limited to <200 mg. Best results are obtained with young leaves or needles.

Materials required, but not supplied:

! ß-Mercaptoethanol
! Isopropanol
! 100 % Ethanol
! Sterile dH_2O
! Sterile RNase-free pipet tips and centrifuge tubes

1. Homogenization and lysis

Collect tissue (start with 100 mg, increase later up to 200 mg) in a 1.5 ml or 2 ml microcentrifuge tube and freeze by dipping in liquid nitrogen with a pair of tweezers to fill the tube. Grind the tissue using disposable pellet pestles.

Alternatively, one can allow liquid nitrogen to evaporate and then store samples at -70°C for later use. For critical work such as PCR and cloning, pestles are best used a single time, then soaked in a dilute bleach solution immediately after use until cleaning. Disposable pestles may be autoclaved several times. For standard Southern analysis, the same pestle can be reused several times to grind multiple tissue samples by rinsing with ethanol and carefully wiping the surfaces clean between samples.*

Add one after the other 600 µl P1 Buffer and 10 µl ß-Mercaptoethanol and vortex vigorously to mix. Make sure to disperse all clumps. Incubate at 65°C for 10 min. Mix sample twice during incubation by inverting tube. Add 140 µl P2 Buffer and vortex to mix. Centrifuge at 10'000 x g for 10 min. Transfer supernatant carefully to a new microcentrifuge tube making sure not to disturb the pellet or transfer any debris.

2. Removal of polysaccharides (Optional!)

Add 0.7 volume isopropanol and vortex to precipitate DNA.

In most cases 600 µl supernatant can easily be removed. This will require 420 µl isopropanol. Note that depending on the sample, the volume of supernatant may vary. After transferring to a fresh tube, measure the volume and add the correct amount of isopropanol.

This step removes much of the polysaccharide content and improves spin-column performance by increasing DNA binding capacity in the steps that follow.

Immediately centrifuge for 2 min at 10,000 x g to pellet DNA. A longer centrifugation does not improve yields.

Pour off the supernatant making sure not to dislodge the DNA pellet. Invert the microfuge tube on a paper towel for 1 min to allow residual liquid to drain. Drying the pellet is not recommended.

3. RNA Digest

Add 100 µl of pre-heated (65°C) sterile dH_2O and vortex to resuspend the pellet. A brief incubation at 65°C may be necessary to effectively dissolve the DNA.

Add 4 µl RNase A and mix. Incubate for 2 min at room temperature.

4. Load and Bind

Add one after the other 150 µl P3 Buffer and 300 µl absolute ethanol. Vortex thoroughly to mix. This will adjust binding conditions prior to loading the HiBind® DNA column.

Place a HiBind® DNA spin column into a fresh 2 ml collection tube (supplied) and apply the entire sample, including any precipitates that may form, to the spin column. Centrifuge the spin column / collection tube assembly at 10,000 x g for 1 min. Discard the flow-through liquid and the old collection tube and place the spin column into a new collection tube.

5. Wash I

Add 750 µl of the completed DNA Wash Buffer to the column and centrifuge the spin column / collection tube assembly for 1 min at 10'000 x g. Discard the flow-throw liquid and place the spin column back in the collection tube.

6. Wash II

Repeat the washing step as described in step 5 with 750 µl of the completed DNA Wash Buffer. Discard the flow-throw liquid and keep the spin column for the next step.

7. Dry (Important, do not skip this step!)

Place the HiBind® spin column containing your DNA in the collection tube used in step 6 and centrifuge for 2 min at maximum speed to dry the column matrix. This step is essential to remove ethanol from the column.

8. Elution

Transfer the spin column to a fresh 1.5 ml Centrifuge tube. Apply 100 µl Elution buffer or sterile deionized water pre-warmed to 65°C and incubate at room temperature for 1 min. Centrifuge at 10,000 x g for 1 min to elute DNA. Repeat elution with additional 100 µl of buffer. This may be performed using another 1.5 ml tube to obtain a higher DNA concentration in the first eluate. Smaller volumes will significantly increase DNA concentration but give lower yield. It is not recommended to use more than 200 µl of buffer for elution.

To increase DNA concentration add buffer and incubate the column at 60°C - 70°C for 5 min before elution.

Total DNA yield vary depending on type and quantity of sample. Typically, 20-50 µg DNA can be isolated using 200 mg fresh or frozen tissue.

C. Short protocol

This simplified method allows rapid isolation of DNA from fresh, frozen, or dried specimens for use in PCR* reactions. The procedure limits the amount of starting material, so that DNA yield will generally be lower than those obtained with protocols A and B. Thus in most cases there may not be sufficient material for Southern analysis or cloning work.

Materials required, but not supplied:
- ß-Mercaptoethanol
- 100 % Ethanol
- Sterile dH_2O
- Sterile RNase-free pipet tips and centrifuge tubes

1. Homogenization and lysis

Use up to 10 mg dry or up to 40 mg fresh or frozen plant material and homogenize as described in protocol A or B in a 1.5 ml microcentrifuge tube. Add 600 µl P1 Buffer and 4 µl RNase A. Vortex vigorously to mix and incubate at room temperature for 1 min. Add 10 µl ß-Mercaptoethanol and vortex to mix. Incubate at 65°C for at least 5 min. Mix sample once during incubation by inverting tube. Add 140 µl P2 Buffer and vortex to mix. Centrifuge at 10,000 x g for 10 min.

2. Load and Bind

Carefully transfer 600 µl supernatant to a new microcentrifuge tube making sure not to disturb the pellet or transfer any debris. Add first 0.5 volume of P3 Buffer and then one volume of absolute ethanol.

Volume of supernatant will vary, and is usually lower with dried samples. For 600 µl of supernatant add 300 µl Buffer P3 followed by 600 µl absolute ethanol.

Vortex thoroughly to obtain a homogeneous mixture

A precipitate may form but will not affect the procedure.

Apply 800 µl of the mixture to a HiBind® DNA spin column assembled in a 2 ml collection tube (supplied). Centrifuge at 10,000 x g for 1 min to bind DNA. Discard flow-through liquid and reuse collection tube.

Add the remainder of the sample (including any precipitate that may have formed) to the column. Centrifuge at 10,000 x g for 1 min and discard both the 2 ml collection tube and the flow-through liquid and place the spin column into a new collection tube.

3. Wash I

Add 750 µl of the completed DNA Wash Buffer to the column and centrifuge the spin column / collection tube assembly for 1 min at 10,000 x g. Discard the flow-throw liquid and place the spin column back in the collection tube.

4. Wash II

Repeat washing step as described in step 5 with 750 µl of the completed DNA Wash Buffer. Discard the flow-throw liquid and keep the spin column for the next step.

5. Dry (Important, do not skip this step!)

Place the HiBind® spin column containing your DNA in the collection tube used in step 6 and centrifuge for 2 min at maximum speed to dry the column matrix. This step is essential to remove ethanol from the column.

6. Elution

Transfer the spin column to a fresh 1.5 ml Centrifuge tube. Apply 100 µl Elution buffer or sterile deionized water pre-warmed to 65°C and incubate at room temperature for 1 min. Centrifuge at 10'000 x g for 1 min to elute DNA. Repeat elution with an additional 100 µl of buffer. This may be performed using another 1.5 ml tube to maintain a higher DNA concentration in the first eluate. Smaller volumes will significantly increase DNA concentration but give lower yield. It is not recommended to use more than 200 µl of buffer for elution.

To increase DNA concentration add buffer and incubate the column at 60°C - 70°C for 5 min before elution.

Total DNA yield using this short protocol is about 2-10 µl DNA

Quantitation and storage of DNA

Determine the absorption of an appropriate dilution (10- to 50-fold) of the sample at 260 nm and then at 280 nm.

One A_{260}-unit is about 50 µg DNA/ml. The DNA concentration is calculated as follows:

DNA conc. (µg /ml) = $Absorption_{260} \times 50 \times$ Dilution Factor

The ratio of $A_{260/280}$ is an indication of nucleic acid purity. A value higher than 1.8 indicates > 90% nucleic acid.

Phenol has an absorption maximum at 275 nm and can interfere with absorption readings of DNA or RNA. However, the peqGOLD Plant DNA Kit eliminates the use of phenol and avoids this problem.

Store DNA samples at –20 °C in 10 mM Tris-HCl (pH 0.9) or sterile dH_2O. Under such conditions DNA prepared with the peqGOLD system is stable for years.

Ordering information

For DNA isolation from plants species and tissues

peqGOLD Plant DNA Mini Kit	12-3486-00 12-3486-01 12-3486-02	5 Preparations 50 Preparations 200 Preparations
peqGOLD HP Plant DNA Mini Kit	12-2486-00 12-2486-01 12-2486-02	5 Preparations 50 Preparations 200 Preparations
peqGOLD SP Plant DNA Mini Kit	12-5510-00 12-5510-01 12-5510-02	5 Preparations 50 Preparations 200 Preparations

Troubleshooting Tips

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Problem	Likely cause	Suggestion
Clogged column.	Carry-over of debris.	Following precipitation with P2 Buffer, make sure no particulate material is transferred.
	DNA pellet not completely dissolved before applying sample to column.	In protocols A and B, ensure that DNA is dissolved in water before adding Buffer P3 and ethanol. This may need repeated incubation at 65°C and vortexing.
	Sample too viscous.	In protocol C, do not exceed suggested amount of starting material. Alternatively, increase amounts of Buffers P1 and P2 and use two or more columns per sample.
Low DNA yield.	Incomplete disruption of starting material.	For both dry and fresh samples, obtain a fine homogeneous powder before adding Buffer P1.
	Poor lysis of tissue.	Decrease amount of starting material or increase amount of Buffers P1 and P2.
	DNA remains bound to column.	Increase elution volume to 200 µl and incubate on column at 65°C for 5 min before centrifugation.
	DNA washed off.	Dilute Wash Buffer Concentrate by adding appropriate volume of absolute ethanol prior to use (page 3).
Problems in downstream applications.	Salt carry-over.	Wash Buffer must be at room temperature.
	Ethanol carry-over.	Following the second wash spin, ensure that centrifuging 2 min at maximum speed dries the column.