

# peqGOLD

## HP Plant DNA Mini Kit

– Instruction Manual –

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## Introduction

The peqGOLD HP Plant DNA Mini Kit allows rapid and reliable isolation of high-quality genomic DNA from fresh and dried plant tissue samples rich in polysaccharides or lower DNA contents. Up to 100 mg of wet tissue (or 30 mg dry tissue) can be processed in less than 1 hour. The system combines the reversible nucleic acid-binding properties of HiBind<sup>®</sup> 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

The procedure relies on the well established properties of the cationic detergent, cetyltrimethyl ammonium bromide (CTAB), in conjunction with the selective DNA binding of the HiBind<sup>®</sup> matrix. Samples are homogenized and lysed in a high salt buffer containing CTAB and extracted with chloroform to remove polysaccharides and other components that interfere with many routine DNA isolations and downstream applications. Following a rapid alcohol precipitation step, binding conditions are adjusted and DNA is further purified using HiBind<sup>®</sup> DNA spin columns. In this way salts, proteins and other contaminants are removed to yield high quality genomic DNA suitable for downstream applications such as endonuclease digestion, thermal cycle amplification, and hybridization techniques.

\* PCR is covered by patents owned by F. Hoffmann-La Roche Ltd.

## Kit Components

<b>peqGOLD HP Plant DNA Mini Kit</b>	<b>5 Purifications</b>	<b>50 Purifications</b>	<b>200 Purifications</b>
Product Number	12-2486-00	12-2486-01	12-2486-02
<b>Components</b>			
HiBind <sup>®</sup> Columns	5	50	200
2 ml Collection Tubes	10	100	400
Buffer CPL	5 ml	50 ml	180 ml
Buffer CXD	1 ml	10 ml	40 ml
DNA Wash buffer	12 ml	40 ml	3 x 40 ml
Elution buffer	1,5 ml	15 ml	60 ml
Instruction manual	1	1	1

## Storage and Stability

peqGOLD HP Plant DNA Mini Kit components should be stored at room temperature (22 °C – 25 °C). All peqGOLD HP Plant DNA Mini 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 Buffer CPL and CXD. Warm up to 37 °C to dissolve.

## 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 Buffer CPL and CXD. This is normal and the bottle should be warmed (37°C) to dissolve the salt before use.
- ! Prepare an RNase stock solution (20 mg/ml in 10 mM Tris-HCl, pH 9.0) and aliquot into adequate portions. Store each aliquot at –20°C and thaw before use. Each sample will require 20 µl of this solution.
- ! DNA Wash Buffer is concentrated and has to be diluted with absolute ethanol as follows:

Kit 12-2486-00	Add 18 ml 100% EtOH to 12 ml Wash Buffer
Kit 12-2486-01	Add 60 ml 100% EtOH to 40 ml Wash Buffer
Kit 12-2486-02	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).

## peqGOLD HP 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:

- ! RNase A (20 mg/ml)
- ! Chloroform:isoamyl alcohol (24:1)
- ! Isopropanol
- ! 100 % Ethanol
- ! Sterile dH<sub>2</sub>O (optional)
- ! Sterile RNase-free pipet tips and centrifuge tubes (1,5 ml and 2 ml)
- ! Optional: β-Mercaptoethanol

#### 1. Homogenization and lysis

To prepare dried samples place 10-50 mg of dried tissue into a 2 ml microcentrifuge tube 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 900 µl Buffer CPL (optional: add also 10µl β-Mercaptoethanol) to 10-50 mg powdered dry tissue and vortex vigorously to mix. Make sure to disperse all clumps. Incubate at 65°C for 30 min. Mix sample twice during incubation by inverting tube. Add 900 µl chloroform:isoamyl alcohol (24:1) 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

Add 0.7 volume isopropanol and vortex to precipitate DNA.

*In most cases 800 µl supernatant can easily be removed. This will require 560 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 5 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 150 µl of pre-heated (65°C) sterile dH<sub>2</sub>O and vortex to resuspend the pellet. A brief incubation at 65°C may be necessary to effectively dissolve the DNA.

Add 20 µl RNase (20 mg/ml) and mix. Incubate for 2 min at room temperature.

### 4. Load and Bind

Add 150 µl Buffer CXD 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 650 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-through liquid and place the spin column back in the collection tube.

### 6. Wash II

Repeat the washing step as described in step 5 with 650 µl of the completed DNA Wash Buffer. Discard the flow-through 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:

- ! RNase A (20 mg/ml)
- ! Chloroform:isoamyl alcohol (24:1)
- ! Liquid nitrogen for freezing/disrupting samples
- ! Isopropanol
- ! 100 % Ethanol
- ! Sterile dH<sub>2</sub>O
- ! Sterile RNase-free pipet tips and centrifuge tubes
- ! Optional: β-Mercaptoethanol

### 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 800 µl Buffer CPL (optional: add also 10µl β-Mercaptoethanol) to the sample and vortex vigorously to mix. Make sure to disperse all clumps. Incubate at 65°C for 15 min. Mix sample twice during incubation by inverting tube. Add 800 µl chloroform:isoamyl alcohol (24:1) 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

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 5 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 150 µl of pre-heated (65°C) sterile dH<sub>2</sub>O and vortex to resuspend the pellet. A brief incubation at 65°C may be necessary to effectively dissolve the DNA.

Add 20 µl RNase (20 mg/ml) and mix. Incubate for 2 min at room temperature.

### 4. Load and Bind

Add one after the other 150 µl Buffer CPX 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 650 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-through liquid and place the spin column back in the collection tube.

### 6. Wash II

Repeat the washing step as described in step 5 with 650 µl of the completed DNA Wash Buffer. Discard the flow-through 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. Protocol for Samples with Lower DNA Content

This modified method allows rapid isolation of DNA from fresh, frozen, or dried specimens for sample types with lower DNA content or when larger yields are essential. The procedure increases the amount of starting material so that DNA yields will generally be higher than those obtained with Protocols A and B.

Materials required, but not supplied:

- ! RNase A (20 mg/ml)
- ! Chloroform:isoamyl alcohol (24:1)
- ! Liquid nitrogen for freezing/disrupting samples
- ! Isopropanol
- ! 100 % Ethanol
- ! Sterile dH<sub>2</sub>O
- ! Sterile RNase-free pipet tips and centrifuge tubes (1,5 ml, 2 ml **AND** 15 and 50 ml tubes)
- ! Optional: β-Mercaptoethanol

Follow suggestions for preparation of dried or fresh samples as outlined in Sections A and B. Note the following limitations on sample size:

- ! Dry Samples - use a maximum of 200 mg ground tissue
- ! Fresh Samples - use a maximum of 400mg fresh/frozen ground tissue

#### 1. Homogenization and lysis

Collect ground sample in a 15 ml polypropylene tube and add 9.0 ml Buffer CPL. Add 10 µl 2-mercaptoethanol per ml Buffer CPL and vortex to vigorously to mix. Add 4.5 ml chloroform:isoamyl alcohol (24:1) and vortex to mix. . Centrifuge at 3,000 x g for 10 min.

#### 2. Removal of polysaccharides

Carefully transfer top aqueous phase to a new 15 ml tube making sure not to disturb the pellet or transfer any debris. Add 4.5 ml chloroform:isoamyl alcohol (24:1) and vortex to mix. Centrifuge at 3,000 x g for 10 min.

Transfer the top aqueous phase to a new 15 ml tube and add 0.7 volume isopropanol and vortex to precipitate DNA. Immediately centrifuge for 20 min at 3,000 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 400 µl of pre-heated (65°C) sterile dH<sub>2</sub>O and vortex to resuspend the pellet. A brief incubation at 65°C may be necessary to effectively dissolve the DNA. Add 20 µl RNase (20 mg/ml) and mix. Incubate for 2 min at room temperature.

#### 4. Load and Bind

Add 200  $\mu$ l Buffer CXD followed by 400  $\mu$ l absolute ethanol. Vortex thoroughly to mix. This will adjust binding conditions prior to loading the HiBind<sup>®</sup> DNA column.

Place a HiBind<sup>®</sup> DNA spin column into a fresh 2 ml collection tube (supplied) and apply the 700  $\mu$ l of the mixture. Centrifuge the spin column / collection tube assembly at 10,000 x g for 1 min. Add the remainder of the sample (including any precipitate that may have formed) to the 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 650  $\mu$ l 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-through liquid and place the spin column back in the collection tube.

#### 6. Wash II

Repeat washing step as described in step 5 with 650  $\mu$ l of the completed DNA Wash Buffer. Discard the flow-through liquid and keep the spin column for the next step.

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

Place the HiBind<sup>®</sup> 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  $\mu$ l 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  $\mu$ l 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  $\mu$ l of buffer for elution.

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

*Yields vary according to sample size and whether dried or fresh. Between 2-10  $\mu$ g restrictable DNA can usually be obtained with this method.*

## 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  $\mu\text{g}$  DNA/ml. The DNA concentration is calculated as follows:

$$\text{DNA conc. } (\mu\text{g /ml}) = \text{Absorption}_{260} \times 50 \times \text{Dilution Factor}$$

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  $-20\text{ }^{\circ}\text{C}$  in 10 mM Tris-HCl (pH 0.9) or sterile  $\text{dH}_2\text{O}$ . 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	5 Preparations
	12-3486-01	50 Preparations
	12-3486-02	200 Preparations
peqGOLD HP Plant DNA Mini Kit	12-2486-00	5 Preparations
	12-2486-01	50 Preparations
	12-2486-02	200 Preparations
peqGOLD SP Plant DNA Mini Kit	12-5510-00	5 Preparations
	12-5510-01	50 Preparations
	12-5510-02	200 Preparations

## Troubleshooting Tips

<b>Problem</b>	<b>Likely cause</b>	<b>Suggestion</b>
Clogged column.	Carry-over of debris.	Following precipitation with chloroform:isoamyl alcohol, 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 CXD 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 CPL and CXD 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 CPL.
	Poor lysis of tissue.	Decrease amount of starting material or increase amount of Buffers CPL and CXD and of chloroform:isoamyl alcohol.
	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.
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.