USER GUIDE

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PureLink® Plant Total DNA Purification Kit

For purification of DNA from plant

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Experienced Users Procedure

Introduction

This quick reference sheet is included for experienced users of the PureLink® Plant Total DNA Purification Kit. If you are a first time user, refer to the details included in this manual.

Step	Action
Preparing Plant Lysate	1. For hard plant tissue, freeze the tissue in liquid nitrogen and grind the tissue to a powder. For soft, non-fibrous plant tissue, cut the tissue into small pieces. For lyophilized samples, proceed to Step 2. 2. Add 250 µL Resuspension Buffer (R2) to 100 mg plant tissue. Prepare lysate by homogenizing the soft tissue with a homogenizer/tissue grinder or vortexing the ground tissue/lyophilized sample until the sample is completely resuspended. 3. Add 15 µL 20% SDS and 15 µL RNase A (20 mg/mL) to lysate. 4. Incubate the lysate at 55°C for 15 minutes to complete lysis. 5. Centrifuge the lysate at high speed for 5 minutes to remove insoluble materials. 6. Transfer supernatant to a sterile microcentrifuge tube and add 100 µL Precipitation Buffer (N2) supplied with the kit to the clear lysate. Mix well and incubate **on ice** for 5 minutes. 7. Centrifuge at maximum speed in a microcentrifuge for 5 minutes at room temperature to produce a clear lysate. 8. Transfer 250 µL clear lysate to a new, sterile microcentrifuge tube and add 375 µL Binding Buffer (B4) with ethanol (page 12) to the lysate. Mix well. 9. Proceed to **Purification Procedure** (step 1, page 5).

Experienced Users Procedure, Continued

Step	Action
Purification Procedure	The purification procedure is designed for use with a microcentrifuge capable of centrifuging >10,000 × *g*. 1. **Add** sample from Step 8, page 4 to a PureLink® Spin Cartridge in a collection tube. 2. Centrifuge the cartridge at 10,000 × *g* for 30 seconds at room temperature. Discard the flow through and place the spin column into the Wash Tube supplied with the kit. 3. **Wash** the cartridge with 500 µL Wash Buffer (W4). 4. Centrifuge the cartridge at 10,000 × *g* for 30 seconds at room temperature. Discard the flow through and place the column into the tube. 5. **Wash** the cartridge with 500 µL Wash Buffer (W5) with ethanol (page 12). 6. Centrifuge the column at 10,000 × *g* for 30 seconds at room temperature. Discard the flow through and place the column into the tube. 7. **Repeat** Steps 5–6 one more time. 8. Centrifuge the column at maximum speed for 2 minutes at room temperature to remove any residual Wash Buffer (W5). Discard the collection tube. 9. Place the spin column in a sterile DNase-free 1.5-mL microcentrifuge tube. 10. **Elute** with 100 µL Elution Buffer (E1) or sterile, distilled water (pH >7.0). 11. Incubate at room temperature for 1 minute. Centrifuge the column at maximum speed for 1 minute. *The elution tube contains the purified DNA.* 12. To recover more DNA, perform a second elution step using 100 µL Elution Buffer. Centrifuge the column at maximum speed for 1 minute at room temperature. *The elution tube contains the purified DNA.* Remove and discard the column. 13. Store the purified DNA at –20°C or use DNA for the desired downstream application.

Kit Contents and Storage

Shipping and Storage

All components of the PureLink® Plant Total DNA Purification Kit are shipped at room temperature. Upon receipt, store all components at room temperature.

Contents

The components included in the PureLink® Plant Total DNA Purification Kit are listed in the following table.

Sufficient reagents are provided in the kit to perform 50 reactions.

Component	Amount
Resuspension Buffer (R2)	12.5 mL
Precipitation Buffer (N2)	5 mL
Binding Buffer (B4)	7.5 mL
Wash Buffer (W4)	25 mL
Wash Buffer (W5)	10 mL
Elution Buffer; 10 mM Tris-HCl, pH 8.5 (E1)	10 mL
20% SDS	0.75 mL
RNase A (20 mg/mL RNase A in 50 mM Tris-HCl, pH 8.0, 10 mM EDTA)	0.75 mL
PureLink® Spin Cartridges with Collection Tubes	50 each
PureLink® Wash Tubes (2.0 mL)	50 each

Introduction

Overview

Introduction

The PureLink® Plant Total DNA Purification Kit allows rapid and efficient isolation of plant total DNA.

The kit is designed to efficiently isolate total DNA from a variety of plant samples in ~40 minutes using centrifugation of spin columns without the use of organic solvents.

The isolated DNA is 20–50 kb in size and is suitable for PCR, restriction enzyme digestion, and Southern blotting.

System Overview

The PureLink® Plant Total DNA Purification Kit is based on the selective binding of dsDNA to silica-based membrane in the presence of chaotropic salts.

The plant lysate is prepared using Resuspension Buffer (R2) with RNase and SDS. After lysis, the proteins, polysaccharides, and protein-associated photosynthetic pigments are precipitated with the Precipitation Buffer (N2) resulting in a clear, non-viscous lysate. The use of precipitation buffer and short incubation on ice promotes efficient precipitation of proteins which are completely removed with high-speed centrifugation providing an easy method to clarify the lysate without the use of additional clarification steps.

The clear lysate is mixed with Binding Buffer (B4) to adjust conditions for subsequent DNA binding to the PureLink® Spin Cartridge. The DNA binds to the silica-based membrane in the column and impurities are removed by thorough washing with Wash Buffers. The DNA is then eluted in low salt Elution Buffer (E1) or water.

Overview, Continued

Advantages

The PureLink® Plant Total DNA Purification Kit has the following advantages:

- Rapid and efficient purification of total DNA from a variety of plant samples such as spinach, tomato, soy, wheat, *Arabidopsis*, and mushroom
- Designed to purify high-quality total DNA in less than an hour
- Minimal contamination from RNA
- Reliable performance of the purified DNA in PCR, restriction enzyme digestion, and Southern blotting

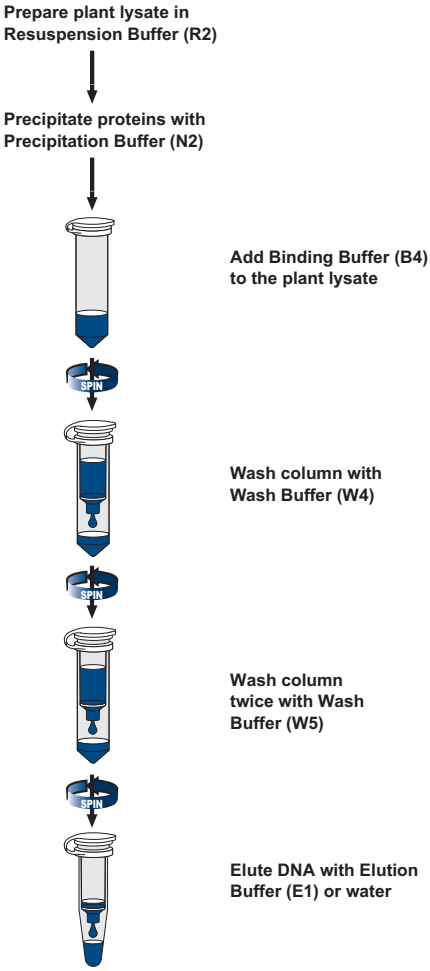
System Specifications

Starting Material:	100 mg plant tissue
Binding Capacity:	~1 mg nucleic acid
Column Reservoir Capacity:	700 µL
Wash Tube Capacity:	2.0 mL
Centrifuge Compatibility:	Capable of centrifuging >10,000 × *g*
Elution Volume:	2 × 100 µL
DNA Recovery:	>80%
DNA Yield:	Varies (see page 15)
DNA Size:	20–50 kb

Experimental Overview

Introduction

The flow chart for purifying plant DNA using the PureLink® Plant Total DNA Purification Kit is outlined in the following diagram.



Methods

Purification Procedure

Introduction

The purification procedure is designed for purifying plant DNA using a centrifuge in a total time of **~40 minutes**.

WARNING! GENERAL CHEMICAL HANDLING.
For every chemical, read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **www.lifetechnologies.com/support**.

The PureLink® Plant Total DNA Purification Kit buffers contain guanidine hydrochloride. Contact with acids or bleach liberates toxic gases. **Do not add** acids or bleach to any liquid wastes containing this product.

Plant Samples

The PureLink® Plant Total DNA Purification Kit is suitable for isolating plant DNA from a large variety of plant tissues including alfalfa sprouts, sunflower sprouts, corn husks, soybeans, mushroom, tomato leaves, wheat grass, and *Arabidopsis thaliana* leaves.

The kit is designed to purify DNA from ≤100 mg plant tissue using the protocol described page 12. If you wish to use <100 mg plant tissue for purification, use the purification protocol described in this manual without changing the volume of buffers used, except the Elution Buffer. The Elution Buffer volume can be adjusted accordingly. Note that if you start with less amount of sample, the DNA yield may be lower.

To obtain high yield of DNA and minimize DNA degradation, use young plant samples such as leaves and freeze the sample in liquid nitrogen immediately after collection. If you are using lyophilized samples, resuspend the lyophilized sample in Resuspension Buffer (R2) as described on page 12.

Purification Procedure, Continued

Elution Volume

The DNA is eluted in 2 aliquots of 100 µL each to obtain greater DNA yield. The DNA recovery in the first elution is 65–80% and after second elution is >90%.

You may perform the two-elution steps using the same elution tube or different tubes. Performing elution in different tubes prevents dilution of the DNA sample.

Follow the recommendations below to obtain the best results:

- To minimize DNA degradation, freeze the tissue in liquid nitrogen and avoid repeated freezing and thawing of DNA samples
- Maintain a sterile environment when handling DNA to avoid any contamination from DNases
- Ensure that no DNases are introduced into the sterile solutions supplied with the kit
- Make sure all equipment that comes in contact with DNA is sterile including pipette tips and tubes
- Do not vortex the samples for more than 5–10 seconds at each vortexing step to avoid extensive shearing of DNA
- Perform the recommended wash steps during purification to obtain the best results
- Perform a 1 minute incubation with Elution Buffer (E1) or water prior to elution
- Always use sterile water with pH 7–8.5, if you are using water for elution

Materials Needed

Components supplied by the user

- 96–100% ethanol
- Plant tissue
- Water bath or heat block set at 55°C
- Sterile, DNase-free microcentrifuge tubes
- Microcentrifuge capable of centrifuging >10,000 × *g*

Components supplied with the kit

- Binding Buffer (B4)
- Wash Buffers (W4) and (W5)
- Elution Buffer (E1)
- PureLink® Spin Cartridges and Wash Tubes

Purification Procedure, Continued

Before Starting

Binding Buffer (B4)

Add 11.5 mL 96–100% ethanol to 7.5 mL Binding Buffer (B4) included with the kit. Mix well and store the Binding Buffer (B4) with ethanol at room temperature.

Wash Buffer (W5)

Add 40 mL 96–100% ethanol to 10 mL Wash Buffer (W5) included with the kit. Store the Wash Buffer (W5) with ethanol at room temperature.

Preparing Plant Lysate

Use the following procedure to prepare lysate from ≤100 mg plant tissue.

1. For hard plant tissue, freeze the tissue in liquid nitrogen and grind the tissue to a powder.

   For soft, non-fibrous plant tissue, cut the tissue into small pieces.

   For lyophilized samples, proceed to Step 2.
2. Add 250 µL Resuspension Buffer (R2) supplied in the kit to the tissue from Step 1 at room temperature.
3. Prepare lysate by homogenizing the soft tissue with a tissue homogenizer or vortexing the ground tissue/lyophilized sample until sample is completely resuspended.
4. Add 15 µL 20% SDS and 15 µL RNase A (20 mg/mL) supplied in the kit to the lysate.
5. Incubate the lysate at 55°C for 15 minutes to complete lysis.
6. Centrifuge the lysate at high speed for 5 minutes to remove insoluble materials.
7. Transfer the clear supernatant to a sterile, 1.5-mL microcentrifuge tube without disturbing the pellet.
8. Add 100 µL Precipitation Buffer (N2) supplied with the kit to the clear lysate. Mix by vortexing and incubate **on ice** for 5 minutes.

   The proteins and polysaccharides are precipitated and any photosynthetic pigments that are bound to proteins are also removed with this step. The pigments can stain the PureLink® spin cartridge material that may produce colored eluate.

Purification Procedure, Continued

Preparing Plant Lysate, continued

9. Centrifuge at maximum speed in a microcentrifuge for 5 minutes at room temperature to produce a clear lysate. **Note:** The supernatant should be clear and not viscous after this precipitation step.
10. Transfer 250 µL clear lysate to a new, sterile microcentrifuge tube and add 375 µL Binding Buffer (B4) with ethanol (previous page) to the lysate. Mix well. Proceed to **Binding DNA**.

Binding DNA

1. Remove a PureLink® Spin Cartridge in a Collection Tube from the package.
2. Add sample with Binding Buffer from Step 10, previous page to the PureLink® Spin Cartridge.
3. Centrifuge the cartridge at 10,000 × *g* for 30 seconds at room temperature.
4. Discard the flow through and place the spin cartridge into the Wash Tube supplied with the kit. Proceed to **Washing DNA**.

Washing DNA

1. Add 500 µL Wash Buffer (W4) supplied in the kit to the cartridge.
2. Centrifuge the cartridge at 10,000 × *g* for 30 seconds at room temperature. Discard the flow through from the Wash Tube and place the column back into the tube.
3. Add 500 µL Wash Buffer (W5) with ethanol (page 12) to the column.
4. Centrifuge the cartridge at 10,000 × *g* for 30 seconds at room temperature. Discard the flow through from the Wash Tube and place the cartridge back into the tube.
5. **Repeat** Steps 3–4 one more time.
6. Centrifuge the cartridge at maximum speed for 2 minutes at room temperature to remove any residual Wash Buffer (W5). Discard the Wash Tube. Proceed to **Eluting DNA**, page 14.

Purification Procedure, Continued

Eluting DNA

1. Place the spin cartridge in a sterile, DNase-free 1.5-mL microcentrifuge tube.
2. Add 100 µL of Elution Buffer (E1) or sterile, distilled water (pH >7.0).
3. Incubate at room temperature for 1 minute. Centrifuge the cartridge at maximum speed for 1 minute. *The elution tube contains the purified DNA.*
4. To recover more DNA, perform a second elution step using 100 µL Elution Buffer (E1) or sterile water. You may perform the second elution using the same elution tube or in a different tube.
5. Centrifuge the column at room temperature at maximum speed for 1 minute. *The elution tube contains the purified DNA.* Remove and discard the cartridge.

   Based on the volume of elution buffer used for elution, the recovery of the elution volume will vary and is usually >95% of the elution buffer volume used.

Storing DNA

- Store the purified DNA at –20°C or use DNA for the desired downstream application.
- For long-term storage, store the purified DNA in Elution Buffer (E1) at –20°C as DNA stored in water is subject to acid hydrolysis.
- To avoid repeated freezing and thawing of DNA, store the purified DNA at 4°C for immediate use or aliquot the DNA and store at –20°C for long-term storage.

The Next Step

The DNA isolated using the PureLink® Plant Total DNA Purification Kit is suitable for use in any downstream application of choice such as PCR, restriction enzyme digestion, or Southern Blotting without the need to perform any additional steps.

Estimating DNA Yield and Quality

Introduction

Once you have isolated DNA, use the following procedure to determine the quantity and quality of the purified DNA.

DNA Yield

Perform DNA quantitation using UV absorbance at 260 nm or Quant-iT™ DNA Assay Kits.

UV Absorbance

1. Measure the A_{260} of the solution on a spectrophotometer blanked against 10 mM Tris-HCl, pH 7.5.
2. Calculate the amount of DNA using formula:

DNA (µg) = A_{260} × 50 µg/(1 A_{260} × 1 mL) × dilution factor × total sample volume (mL)

For DNA, A_{260} = 1 for a 50 µg/mL solution measured in a cuvette with an optical path length of 1 cm.

Note: Any contamination from RNA will inflate the DNA content measured at 260 nm. To avoid any interference from RNA, use the Quant-iT™ Kits for DNA quantitation.

Quant-iT™ DNA Assay Kits

The Quant-iT™ DNA Assay Kits (see page 19 for ordering information) provide a rapid, sensitive, and specific method for dsDNA quantitation with minimal interference from RNA, protein, ssDNA (primers), or other common contaminants that affect UV absorbance.

The kit contains a state-of-the-art quantitation reagent, pre-diluted standards for standard curve, and a pre-made buffer. The assay is performed in a microtiter plate format and is designed for reading in standard fluorescent microplate readers. Follow manufacturer's recommendations to perform the assay.

Estimating DNA Quality

Typically, DNA isolated using the PureLink® Plant Total DNA Purification Kit has an A_{260}/A_{280} >1.80 when samples are diluted in Tris-HCl (pH 7.5) indicating that the DNA is reasonably clean of proteins that could interfere with downstream applications. Absence of contaminating RNA may be confirmed by agarose gel electrophoresis.

DNA isolated with the PureLink® Plant Total DNA Purification Kit is usually in the size range of 20–50 kb. To determine the exact size of DNA, perform Pulse-Field Gel Electrophoresis (PFGE) on an agarose gel according to the manufacturer's recommendations.

Expected Results

Introduction

The DNA yield from various plant samples is described below.

DNA Yield

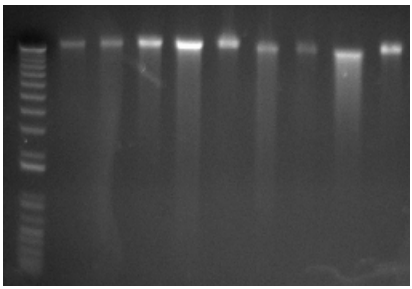
The yield of total DNA obtained from various plant samples (100 mg) using the PureLink® Plant Total DNA Purification Kit is listed in the following table. The DNA quantitation was performed with the Quant-iT™ DNA Assay Kits (page 15).

Material	DNA Yield (µg)
Spinach	2.0–2.5
Arabidopsis thaliana	1.8–3.1
Alfalfa	2.1–3.0
Sunflower	1.6–4.0
Mushroom	1.1–1.4
Soy	0.3–2.0
Wheat	9.2–14.6
Corn	4.4–6.6
Tomato	1.0–2.3

Note: The DNA yield varies with the plant sample, DNA content of the sample, and age of the sample.

Results

Total DNA isolated using the PureLink® Plant Total DNA Purification Kit from various plant samples was analyzed on an 0.8% E-Gel® agarose gel.



Each lane contains ~3–10 ng DNA.
Lane 1: 1 Kb Plus DNA ladder
Lane 2: Soy
Lane 3: Sunflower
Lane 4: Alfalfa
Lane 5: Mushroom
Lane 6: Wheat
Lane 7: Corn
Lane 8: Tomato
Lane 9: *Arabidopsis thaliana*
Lane 10: Spinach

Troubleshooting

Introduction

Review the information below to troubleshoot your experiments with PureLink® Plant Total DNA Purification Kit.

Problem	Cause	Solution
Low DNA yield	Incomplete lysis	Reduce the amount of starting material. Be sure to add SDS during lysis.
	PureLink® Spin Cartridge is clogged	Make sure that the lysate is clear when loaded on the spin cartridge. Remove any particulate material by centrifugation prior to loading the lysate on to the spin cartridge.
	Incorrect binding conditions	Always maintain the ratio of lysate: Binding Buffer to 1.5 for optimal binding. Add 96–100% ethanol to the Binding Buffer (B4) as described on page 12.
	Ethanol not added to Wash Buffer	Be sure to add 96–100% ethanol to Wash Buffer (W5) as described on page 12.
	Incorrect elution conditions	Perform incubation for 1 minute with elution buffer before centrifugation. To recover more DNA perform a second elution step.
	DNA is sheared or degraded	Avoid extensive pipetting to facilitate lysis/homogenization or vortexing to prevent any DNA damage. Maintain a sterile environment while working to avoid any contamination from DNases. Avoid repeated freezing and thawing of DNA samples.

Troubleshooting, Continued

Problem	Cause	Solution
RNA contamination	—	Be sure to add RNase included in the kit during lysis (page 12).
Inhibition of downstream enzymatic reactions	Presence of ethanol in purified DNA	Traces of ethanol from the Wash Buffer W5 can inhibit downstream enzymatic reactions. To remove Wash Buffer (W5), discard Wash Buffer flow through from the collection tube. Place the spin cartridge into the collection tube and centrifuge the spin cartridge at maximum speed for 2–3 minutes to completely dry the cartridge.

Appendix

Accessory Products

Additional Products

The following products are also available from Life Technologies. For more details on these products, visit our website at **www.lifetechnologies.com** or contact Technical Support (page 19).

Product	Quantity	Cat. No.
Easy-DNA™ Kit	1 kit	K1800-01
UltraPure™ DNase/RNase-free Distilled Water	500 mL	10977-015
Quant-iT™ DNA Assay Kit, High Sensitivity	1000 assays	Q33140
Quant-iT™ DNA Assay Kit, Broad-Range	1000 assays	Q33130
DNAzol® Reagent	100 mL	10503-027
DNAzol® BD Reagent	100 mL	10974-020
Plant DNAzol® Reagent	100 mL	10978-021

E-Gel® Agarose Gels and DNA Ladders

E-Gel® Agarose Gels are bufferless pre-cast agarose gels designed for fast, convenient electrophoresis of DNA samples. E-Gel® agarose gels are available in different agarose percentage and well formats for your convenience.

A large variety of DNA ladders are available from Life Technologies for sizing DNA.

For more details on these products, visit our website at **www.lifetechnologies.com** or contact Technical Support (page 19).

Technical Support

Obtaining Support

For the latest services and support information for all locations, go to **www.lifetechnologies.com**

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support (**techsupport@lifetech.com**)
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

Safety Data Sheets (SDS)

Safety Data Sheets (SDSs) are available at **www.lifetechnologies.com/support**.

Certificate of Analysis

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on our website. Go to **www.lifetechnologies.com/support** and search for the Certificate of Analysis by product lot number, which is printed on the box.

Technical Support, Continued

Limited Product Warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

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Notes

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