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Fungal DNA Isolation with PowerPlant Pro DNA Isolation Kit (MO BIO)

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We are still developing and optimizing this protocol

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Abstract

This is a slightly modified MOBIO PowerPlant Pro DNA Isolation Kit for Bennett Plant Pathology.

Technical information:

Toll free 1-800-606-6246, or 1-760-929-9911 Email: technical@mobio.com Website: www.mobio.com

Introduction

The PowerPlant® Pro DNA Isolation Kit is designed for fast and easy purification of total cellular DNA from plant cells, tissues and seeds. The bead beating technology used in this kit replaces cumbersome DNA isolation procedures such as CTAB, phenol, or chloroform extraction for recovery of high quality DNA from the toughest sample types, including strawberry leaf, cotton leaf, cotton seeds, and pine needles. The PowerPlant® Pro DNA Isolation Kit utilizes our patented Inhibitor Removal Technology® (IRT) for removal of PCR inhibitors from plant extracts during the isolation process, resulting in DNA that is ready to use in any downstream applications including PCR, qPCR and sequencing

Guidelines

Plant samples from 5 - 50 mg are added to a bead tube along with a kit supplied buffer for rapid homogenization. Cell lysis and DNA release occurs by mechanical and chemical methods. Released genomic DNA is cleared of PCR inhibitors using IRT and then DNA is captured on a silica membrane in a spin column format. DNA is washed and eluted from the membrane and ready for PCR and other downstream applications.

Materials

Microcentrifuge (up to 16,000 x g)

Pipettor (volumes required 50 – 600 µl)

Vortex-Genie® 2 Vortex (MO BIO Catalog# 13111-V or 13111-V-220), PowerLyzer™ 24 Homogenizer or similar instrument

Component Catalog# Amount

Solution PD1 13400-50-1 25 ml

Solution PD2 13400-50-2 3 ml

Solution PD3 13400-50-3 14 ml

Solution PD4 13400-50-4 32 ml

Solution PD5 13400-50-5 28 ml

Solution PD6 13400-50-6 2 × 30 ml

Solution PD7 13400-50-7 5.5 ml

RNase A Solution (25 mg/ml) 13400-50-8 165 µl

Phenolic Separation Solution 13400-50-9 2.2 ml

PowerPlant® Bead Tubes 13400-50-BT 50

Spin Filters 13400-50-SF 50

2 ml Collection Tubes 13400-50-T 150

RNase A should be stored at 4oC.

The other kit reagents and components should be stored at room temperature (15-30°C).

Safety warnings

 Please wear gloves when using this product. Avoid all skin contact with kit reagents. In case of contact, wash thoroughly with water. Do not ingest. See Material Safety Data Sheets for emergency procedures in case of accidental ingestion or contact. All MSDS information is available upon request (760-929-9911) or at www.mobio.com. Reagents labeled flammable should be kept away from open flames and sparks.

WARNING: Solutions PD5 & PD6 contain ethanol. They are flammable.

IMPORTANT NOTES FOR USE: Check Solution PD2 for precipitates. If the solution contains precipitates, heat at 37oC-55oC to dissolve.



Before start

Mechanical Lysis Options

The PowerPlant® Pro DNA Isolation Kit may be used with a vortex or high velocity bead beater, such as the PowerLyzer™ 24 homogenizer. The PowerLyzer™ 24 is suitable for fast homogenization of plant materials including stems, roots, seeds or difficult leaf tissue without the need of liquid nitrogen grinding

The PowerLyzer™ 24 is a highly efficient bead beating system that allows for optimal DNA extraction from a variety of plant tissues. The instrument's velocity and proprietary motion combine to provide the fastest homogenization time possible, minimizing the time spent processing samples. The programmable display allows for hands-free, walk-away extraction with up to ten cycles of bead beating for as long as 5 minutes per cycle. This kit provides Bead Tubes prefilled with 2.38 mm stainless steel beads for homogenizing plant tissue for optimal DNA isolation. Alternative pre-filled bead tube options are available for additional applications. Please contact technical service (technical@mobio.com) for details.

For isolation of DNA using this kit with the FastPrep® or Precellys®, the following conversion chart will help you to adapt your current protocol. However, due to the highly efficient motion of beads in the PowerLyzer™ 24, we have found that less cycle numbers are required to generate the same effect. You may want to perform extractions on the PowerLyzer™ 24 at the equivalent speed and number of cycles as your current instrument and compare it to less time or lower speed to determine which settings give the best results.

- The Bennett lab uses the Thermo Savant FastPrep FP120.



Preamble

- 1 Begin this extraction protocol after growing *Pythium* or other fungal isolates on 1/2-strength PDA or full-strength PDA (recommended) for 5-7 days.

Detailed Protocol

10m 10s

- 2 Add  450 μL of **Solution PD1** into each 2 ml PowerPlant® **Bead Tubes** provided.

Note

If your sample is high in phenolics (see step:) and you are using the **Phenolic Separation Solution**, reduce **Solution PD1** to  410 μL and add  40 μL of the **Phenolic Separation Solution**.

Note

What's happening: Plant material is added to the Bead Tube to prepare it for a bead beating homogenization step. The PSS disassociates the phenolics from the nucleic acids so that they can be removed during the Inhibitor Removal Technology® (IRT) process.

- 2.1 To each tube scrape  0.1 mL hyphae into microcentrifuge tube using pipette tip, scalpel or sterile toothpick. Avoid scraping agar.
 - Pipette tips or toothpicks can be useful when initially breaking down the hyphal mat. Scrape the side of the tip or toothpick against the inside of the tube to create an opaque solution.

- 3 Check **Solution PD2** for precipitates, if precipitated, warm at  37 °C -  55 °C until dissolved. Add  50 μL of **Solution PD2**.

Note

What's happening: **Solution PD2** contains SDS. It will form a precipitate if it gets cold. Heating and dissolving the solids will restore it to full efficiency.



- 4 Add  3 μL of **RNase A Solution** to the PowerPlant® Bead Tube and vortex briefly to mix.

Note

What's happening: The RNase A will digest the unwanted RNA during the homogenization step.

- 5 Homogenize using one of the following methods:

30s

Note

Note: See Heating of Samples Prior to Bead Beating in the Hints and Troubleshooting Section.

1. Vortex: Secure PowerPlant® Bead Tubes horizontally using the MO BIO Vortex Adapter (MO BIO Catalog# 13000-V1-24) or on a flat-bed vortex pad with tape. Vortex at maximum speed for 10 minutes.

Note

Most leaf tissues are soft and can be processed for DNA isolation by using a vortex adapter. However, plant tissues such as roots, wood, and plant seeds require pre-grinding with a mortar and pestle before placing on the vortex.

2. PowerLyzer™ 24 Homogenizer: Properly identify each PowerPlant® **Bead Tube** on both the cap and on the side. Place Bead Tubes into the Tube Holder of the PowerLyzer™ 24. The PowerPlant® **Bead Tubes** must be balanced (evenly spaced) on the Tube Holder. Homogenize the tissue for 1 cycle at the chosen speed depending on your sample type for 2 minutes.

Note

Due to the high energies of the PowerLyzer™ 24, the potential for marring of the cap tops is possible, therefore it is recommended to mark the sides of the PowerPlant® Bead Tubes as well as the caps to ensure proper sample identification.

3. Other Homogenizers: The Bennett Lab uses a FastPrep FP120. You may want to perform extractions at the equivalent speed and number of cycles as your current

instrument and compare it to less time or lower speed to determine which settings give the best results.

Fungal mycelium may be best homogenized on the FP120 at  , 00:00:30 , Speed 6

A	B	C	D	E
Plant Tissue Type	PowerLyzer Speed	FastPrep Speed (24 m/s)	No. of Cycles	Time/Cycle
Soft leaf tissues	2000 RPM	NA	1	2 min
Fibrous leaf tissue	2200 RPM	NA	1	2 min
Stems	2200 RPM		1	2 min
Roots	2500 RPM	4	1	2 min
Pine needles	2600 RPM	4	1	2 min
Seeds	2800 RPM	4.5	1	2 min
Fungal mycelium	3700 RPM	6	1	30 sec

Suggested homogenization times for recommended and available equipment.

Note

Homogenization should only be attempted within these guidelines. Exceeding these limits will stress the PowerPlant® Bead Tubes and may result in either tube breakage or leaking.

Note

What's happening: The bead beating step homogenizes plant material without the need for manual grinding. In some cases the plant material will not be completely disintegrated after the specified times of each method. However, there should be sufficient disruption for a good yield of DNA.



6 Centrifuge **Bead Tubes** at  13000 x g, 00:02:00 (RCF).

2m

Note

What's happening: This step will pellet unwanted cell and tissue debris.

7 Transfer the supernatant to a clean  2 mL **Collection Tube** (provided).

Note

With  50 mg of plant tissue and depending upon plant type, expect  450-550 μL of supernatant, which may contain some particles.

Note

What's happening: The supernatant contains DNA and other cell components. Avoid transferring any solid tissue at this point.

8 Add  175 μL of **Solution PD3**. Vortex  00:00:05 . Place on ice or refrigerated rack at  4 $^{\circ}\text{C}$ for  00:05:00 .

5m 5s

Note

For problematic samples you can add up to  250 μL of **PD3** at this step. It is best to start at  175 μL with most sample types.

Note

What's happening: Solution PD3 is a novel formulation of Inhibitor Removal Technology® (IRT) and completes the process for removing PCR inhibitors in one step.



- 9 Centrifuge the Collection Tube at  13.000 x g, 00:02:00 (RCF).

2m

Note

What's happening: This step pellets the proteins and inhibitors.

- 10 Avoiding the pellet, transfer up to  600 μL of supernatant to a clean **2 ml Collection Tube** (provided).

- 11 Add  600 μL of **Solution PD4** and  600 μL of **Solution PD6**. Vortex to mix for  00:00:05 .

5s

Note

What's happening: Solution PD4 is a binding salt. The concentration and amount of salt allows for optimal DNA binding to the silica spin filter membrane. Solution PD6 is an ethanol based buffer that allows for maximal nucleic acid binding to the column.

- 12
1. Load approximately  600 μL of lysate onto the **Spin Filter** and centrifuge at  10.000 x g, 00:00:30 (RCF).
 2. Discard the flow through, place the Spin Filter back into the Collection Tube and add another  600 μL of lysate and centrifuge at  10.000 x g, 00:00:30 (RCF).
 3. Discard the flow-through and repeat a third time until all of the lysate has been passed through the Spin Filter.
 4. Discard the flow-through and place the Spin Filter back into the Collection Tube.

1m

Note

What's happening: In the presence of Solution PD4 & Solution PD6, DNA will bind to the spin filter. Centrifugation of the combined lysate through the spin filter allows the DNA to bind the filter membrane while allowing unwanted salt and impurities to pass through the membrane.



- 13 Add  500 μL of **Solution PD5** to the Spin Filter column. Centrifuge for  10.000 x g, 00:00:30 (RCF). Discard the flow through. Place the Spin Filter back into the same Collection Tube.

30s

Note

What's happening: Solution PD5 is an ethanol containing wash buffer that removes residual salt and other impurities from the spin filter membrane.

- 14 Add  500 μL of **Solution PD6** to the Spin Filter column. Centrifuge for  10.000 x g, 00:00:30 (RCF). Discard the flow through. Place the Spin Filter back into the same Collection Tube.

30s

Note

What's happening: Solution PD6 is an ethanol based buffer to completely remove all metabolites and salt from the spin filter membrane.

- 15 Centrifuge for  16.000 x g, 00:02:00 (RCF) to remove residual **Solution PD6**.

2m

Note

What's happening: This is a critical step. It is very important to remove all traces of the previous wash solutions before continuing.

- 16 Carefully place the Spin Filter into a new clean **2 ml Collection Tube** (provided). This is your final collection tube, label accordingly. Avoid splashing any **Solution PD6** onto the **Spin Filter**.

- 17 Add  50-100 μL of **Solution PD7** (10 mM Tris, pH 8.0) to the center of the white filter membrane and incubate for  00:02:00 at  Room temperature .

2m

- 18 Centrifuge  10.000 x g, 00:00:30 (RCF).

1m



Note

For maximum elution efficiency re-load the flow through once again to the center of the white filter membrane.

Centrifuge  10.000 x g, 00:00:30 (RCF).

Note

What's happening: Solution PD7 is 10 mM Tris, pH 8.0. The bound DNA is re-solubilized from the membrane into the low salt buffer that is neutral pH which protects DNA during storage.

- 19 Discard the **Spin Filter**. DNA in the tube is now ready to use. No further steps are required.

We recommend storing DNA frozen  -20 °C . **Solution PD7** contains no EDTA

Nanodrop

- 20 Use the elution buffer (Solution PD7) as your blank in Nanodrop.

Protocol



NAME

Nanodrop Lite (Shared Equipment Lab)

CREATED BY

Nimalka Weerasuriya

Preview

- 21 Please enter the sample names and DNA concentrations and A260/280 ratios as a note.

Protocol references

<https://www.qiagen.com/us/resources/resourcedetail?id=ceb406b8-0d48-4675-a376-e76709099c74&lang=en>