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

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

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Protocol status: In development
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 □l)
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 x 30 ml
Solution PD7 13400-50-7 5.5 ml
RNase A Solution (25 mg/ml) 13400-50-8 165 □l
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 INSTRUCTIONS

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.

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

	A	B	C	D	E
	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 °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 1m

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.

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 30s

10.000 x g, 00:00:30 (RCF). Discard the flow through. Place the Spin Filter back into the same Collection Tube.

> 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 M Weerasuriya

PREVIEW

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