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High-throughput DNA extraction for amplicon sequencing of infected plant samples

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Magnaporthe Protocols



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We tested this protocol for PCR-based methods

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Abstract

Pathogen surveillance and characterization from field samples often includes PCR-based high-throughput diagnostic methods, e.g., short amplicon-sequencing. Therefore, high-throughput DNA extraction from field samples is required. Here we present a protocol for high-throughput DNA extraction for amplicon sequencing of field samples. The protocol was optimized for dried, infected plant material from grasses. A yield of 4 – 20 µg of DNA per sample with A260/A280 ratio of 2.1 and A260/A230 ratio of 2.2 can be expected.

Guidelines

The protocol was optimized for high-throughput DNA extraction from field samples. Hence, all steps are performed in 96-well-format. While proper grinding of the samples is crucial to obtain a good quantity of DNA, tight attachment of lids to the 96-well plate is as important to avoid cross-contamination. We also tested other plates and plate-lid combinations for grinding (e.g. 2 ml deepwell plate, Axygen, P-DW-20-C) than the ones recommended in the protocol. However, in these tests either the plate broke or lids opened during grinding. When using other systems for grinding, we highly recommend to test grinding with a few samples, first.



Materials

Materials

- 96-tube plate (e.g. Quiagen 19560)
- 8-strips to refill tube-holder (Brand, 781525)
- Lids for 8-strips (19566, Quiagen for Quiagen 8-strips; 781535, Brand, for Brand 8-strips)
- Sticky aluminium foil
- Laboratory tape
- 2 ml deep well plate (e.g. Axygen P-DW-20-C)
- 1.1 ml deep well plate (e.g. Axygen P-DW-11-C)
- Econospin 96 well filter plate (2020-001, EpoChlifescience), alternative: AHN myPlate FP 96-Wells, 1.0 mL, GF/N 1.6 µm Filter
- 96 well PCR plate

Reagents

Extraction buffer

- 8 M Guanidine Hydrochloride (152.8 g for 200 ml)
- 20 mM MES (0.78 g for 200 ml)
- 20 mM EDTA (8 ml 0.5 M EDTA, pH = 8.0 for 200 ml)

Add MilliQ water to 200 ml, autoclave.

Add 1 ml RNase A per 200 ml buffer directly before starting DNA extraction.

96 % Ethanol

70 % Ethanol

MilliQ water (for elution)

Preparation of starting material

- 1 Collect infected leaf material and dry.
- 2 Prepare a 96 collection tube plate (e.g. Quiagen 19560) and fill with clean, Ethanol-washed 4 mm steel beads, 1 bead per tube.

Note

Note: Tube holders can be reused and refilled with 8-strips (we used 8-strip collection tubes, Brand, 781525 with lids, Brand, 781535).

- 3 Cut out 2-3 lesions per leaf (2-3 cm in total) using sterilized scissors, cut into 0.5 cm pieces and place in tube.

Note

Avoid cross-contamination between samples when placing samples into 96-tube plate. This can be achieved by taping wells with laboratory tape.

- 4 Close tightly with corresponding lids (19566, Quiagen for Quiagen 8-strips; 781535, Brand, for Brand 8-strips).
- 5 Freeze the sample in liquid nitrogen. Grind the sample using a ball mill (we used TissueLyser II, Quiagen) at 20 Hz for 1 min. Freeze again in liquid nitrogen and repeat grinding.

Note

The right combination of lids and strips is important to avoid opening of lids and cross-contamination during the grinding process. In addition, a quick spin-down after grinding helps to remove the material from the upper part of the tubes which reduces the risk of carryover when opening the tubes later.

- 6 Store samples at -80 °C or proceed immediately.



DNA Extraction with Econospin filter plates

- 7 Preheat water bath to 60 °C. Add RNase A (3 µl/600 µl volume) to extraction buffer.

Note

When samples were stored at -80 °C prior to extraction, we recommend taking them out a few minutes before starting as lids stick to the tubes very tightly when frozen. When thawed a bit, the lids can be removed more easily and do not break. We used Purelink RNase A (Invitrogen 12091039; Stock concentration 20 mg/ml).

- 8 Add 600 µl extraction buffer with RNase A to each well. Remove liquid from the top of the tubes with tissue. Close the plate with sticky aluminium foil and vortex thoroughly.

- 9 Incubate in 60 °C water bath for at least 10 min.

- 10 Centrifuge at 6200 g for 15 min.

Note

If the centrifuge cannot reach 6200 g, centrifugation at 4000 g for 25 minutes can be performed instead.

- 11 Transfer 350 µl of the clear supernatant to 2 ml deep well plate (e.g. Axygen P-DW-20-C). When using an electrical multichannel pipette, we recommend using setting "manual pipet" at speed 1.

- 12 Add 1x volume of 96 % Ethanol (350 µl) and mix by pipetting. When using an electrical multichannel pipette, we recommend using setting "pipet/mix" at speed 5, mixing volume 500 µl, mix cycles 6.

- 13 Transfer 700 µl to an Econospin 96 well filter plate (2020-001, EpoChlifescience). Place filterplate on top of the used 2 ml deep well plate.

Note

The protocol has also successfully been tested with the AHN myPlate Microfilter Plate.

14 Centrifuge at 6200 g for 2 min.

Note

If the centrifuge cannot reach 6200 g, centrifugation at 4000 g for 5 minutes can be performed as an alternative. This also applies to all following centrifugation steps.

15 Add 450 µl 70 % Ethanol. Optional: Incubate for 5 min, especially if columns are clogged. Centrifuge at 6200 g for 2 min.

16 Add 350 µl 70 % Ethanol. Centrifuge at 6200 g for 2 min.

Note

If the DNA samples contain any contamination, you can increase the volume of 70 % Ethanol to 700 µl or repeat this wash step.

17 Discard flowthrough. Place Econospin 96 well filter plate on top of the 2 ml deep well plate and dry spin at 6200 g or 4000 g for 4 min.

18 Place Econospin 96 well filter plate on top of a 96 well PCR plate. For stabilization during centrifugation, put this PCR plate into a 1.1 ml deepwell plate (e.g. Axygen P-DW-11-C).

19 Add 40 µl MilliQ water (optional: preheated to 55 °C). Incubate for 2 min. Centrifuge at 6200 g for 1 min. The expected yield should be in the range of 200 – 500 ng/µl.

Note

The amount of MilliQ water added to the columns can be adapted according to specific needs: To maximize total yield, increase the volume to 80 µl. To maximize concentration, use 40 µl.