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Endophytic fungal DNA extraction from seeds using Qiagen DNeasy Plant Mini

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Eve Kenny[1]

[1]University of Victoria

Eve Kenny
University of Victoria

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We use this protocol and it's working

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Disclaimer

Much of this protocol is based on the Qiagen DNeasy Plant Mini extraction protocol. I added and modified what was necessary to use this kit for seed endophytes, but all reagents and centrifugation parameters come directly from Qiagen.

Abstract

This protocol was modified from the QIAGEN Plant Mini Kit Handbook to extract fungal DNA from within the seed coat of small (<1mm diameter) seeds. It can be followed by amplification with fungal specific primers (ITS-1F KY02 and ITS-4 KY01) to optimize concentration of fungal DNA. Together, this maximizes the ratio of fungal DNA to plant DNA for metagenomic analysis of the fungal community.

Guidelines

Refer to Qiagen DNeasy Plant Mini handbook for troubleshooting. I have written what worked best for my needs, but Qiagen provides detailed information about how to modify the procedure for different purposes.

The handbook recommends that less than 20 mg dry weight be used with the kit, but I have found that with fungal extraction from seeds, I have never exceeded this limit.

Materials

QIAGEN DNeasy Plant Mini Kit:

- DNeasy spin columns (white) in 2 mL tubes
- QIAshredder spin columns (lilac) in 2 mL tubes
- Collection tubes (2 mL)
- Buffer AP1 - tissue disruption
- Buffer P3 - precipitates proteins, polysaccharides, detergents
- Buffer AW1 - binds DNA
- Buffer AW2 - washes DNA
- Buffer AE - elution

Note: in older kits: P3 = AP2, AW1 = AP31E/AP3, AW2 = AW

Other:

- >96% ethanol
- Microcentrifuge tubes - 5 per sample
- Microcentrifuge
- Heating Block
- Mini beads
- Microspatula
- Screw cap tubes - 1 per sample
- ddH_20
- Micropipettes (P200, P1000) and tips
- Autoclaved micro-pestle
- Ice bath

Troubleshooting

Before start

This protocol takes approximately 2 hours to process 8 seeds. Additional time per sample depends mostly on the capacity of the centrifuge and beadbeater. For example, as my beadbeater could only process 8 tubes at a time, anymore than 8 seeds doubled my time for "Surface sterilization and lysis", which was typically the longest section.

1. If Buffer AP1 and Buffer AW1 have formed precipitate in storage, redissolve at 65°C. Do not heat Buffer AW1 once ethanol has been added.
2. Add ethanol (>96%) to Buffer AW1 and AW2 as indicated on buffer bottle.
3. Preheat heat block to 65°C
4. Prepare ice bath

Surface sterilization and lysis

1h

1 Rinse seeds in microcentrifuge tube with ddH_20 and finger vortex for 1 minute. Replace water and repeat two more times. Move seeds to screw cap tubes and add a large spatula scoop of mini beads (~100 µg).

2 Add 400 µg Buffer AP1 to each tube and use micro-pestle to grind seed coat until seed interior is exposed (1 minute).

3 Add tubes to the beadbeater and run for 1 minute. Follow with 1 minute in the centrifuge at 12,000 rpm. Repeat three times for a total of four rounds of beadbeating and spinning.

Note: This is the top speed of the Sigma 112 Mini Centrifuge. I have not tested if a different speed yields different results.

4 Incubate the mixture for 10 minutes at 65°C. Mix three times during incubation by inverting tube.

5 Repeat Step 3. Incubate for 10 minutes, or until foam has been brought down.

6 Spin the tubes briefly. Remove lysate and place in a new microcentrifuge tube.

Lysate purification

20m

7 Add 130 µL Buffer P3 to the lysate. Mix and incubate for 5 minutes on ice

8 Centrifuge the lysate for 5 minutes at 14,000 rpm.

9 Pipet the lysate into the QIAsheredder Mini spin column (lilac) placed in the supplied 2 mL collection tube. Discard pellet and old microcentrifuge tube. Centrifuge spin column in collection tube for 2 minutes at 14,000 rpm.

10 Carefully pipet flow-through into a new microcentrifuge tube without disturbing the cell-debris pellet. Record amount of flow-through transferred (typically about 350 µL).

Lysate wash

20m

11 Add 1.5 x lysate volume of Buffer AW1 to lysate. Mix IMMEDIATELY by pipetting.

Example: 350 µL lysate recovered = 525 µL Buffer AW1

12 Pipet 650 µL mixture (including precipitate) into the DNeasy Mini spin column (white) placed in the supplied 2 mL collection tube. Centrifuge for 1 minute at 8000 rpm.

13 Discard flowthrough and add 650 µL remaining mixture to the same column and collection tube. Repeat centrifugation for 1 minute at 8000 rpm. Discard flowthrough and repeat until all lysate is used up. Discard collection tube.

14 Place DNeasy Mini spin column into a new supplied 2 mL collection tube. Add 500 µL Buffer AW2 and centrifuge for 1 minute at 8000 rpm.

15 Discard flowthrough and add 500 µL Buffer AW2 to same column and collection tube. Centrifuge for 2 minutes at 14,000 rpm.

Note: This step dries the membrane.

Elution

20m

16 Transfer the DNeasy Mini spin column to a new microcentrifuge tube. Pipet 50 µL Buffer AE directly onto spin column membrane. Incubate for 5 minutes at room temperature and centrifuge for 1 minute at 8000 rpm.

Note: you can elute with 100 µL Buffer AE for a greater overall yield of DNA, yet this dilutes the final DNA concentration for each elution. Refer to DNeasy Plant Mini Handbook for more information.

17 Repeat step 16 in a new microcentrifuge tube.

Note: You can elute into the same tube used in step 16, but I found that this diluted my DNA too much.

Protocol references

Extraction Method:
QIAGEN DNeasy Plant Mini Handbook
March 2016
https://www.qiagen.com/us/resources/resourcedetail?id=6b9bcd96-d7d4-48a1-9838-58dbfb0e57d0&lang=en

Primer Selection:
Paul Y. de la Bastide, Terrie Finston, Luise Hermanutz, and Will Hintz. 2022. Fungal endophytes affecting the health and recovery of Long's Braya (*Braya longii*) and Fernald's Braya (*Braya fernaldii*), endangered endemic species of Newfoundland, Canada. *Botany*. **100**(12): 869-883. https://doi.org/10.1139/cjb-2022-0058