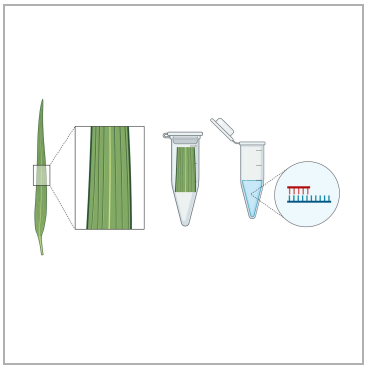

FEB 14, 2023

OPEN ACCESS

**External link:**
https://www.qiagen.com/us/products/discovery-and-translational-research/dna-rna-purification/dna-purification/genomic-dna/dneasy-plant-pro-and-plant-kits?catno=69104

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**Protocol status:** Working
We use this protocol and it's working

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# DNA extraction from infected or not wheat leaf tissue

Benjamin Schwessinger[1]

[1]Australian National University

Benjamin Schwessinger
Australian National University

ABSTRACT

This protocols is part of the ANU Biosecurity mini-research project #1 "Plant Pathogen Diagnostics: Visuals, subcultures, and genomics".

You will be provided four pots of 3-4 week old wheat plants that have been infected with different wheat pathogens. Each pot has been infected with one major pathogen. You will not know which pot has been infected with which pathogen. However, you will be provided a compendium of 10-15 wheat pathogens that will guide you to identify the infective agent for each treatment group. The fifth treatment group will be uninfected wheat plants which will be clearly identified. You can use treatment group #5 as negative control for your experiments.

In total, each group will obtain five pots each:

| | A | B |
|---|---|---|
| | Treatment group 1 | Unknown infective agent |
| | Treatment group 2 | Unknown infective agent |
| | Treatment group 3 | Unknown infective agent |
| | Treatment group 4 | Unknown infective agent |
| | Treatment group 5 | Uninfected control |

This specific protocol is a step by step guide to sample representative leaf material for each treatment group and to extract DNA from these samples with the Qiagen DNeasy Plant Mini Kit. We use a kit in the course to speed up the process and make it reliable. Though it is important to understand the rough principles of DNA extraction as this process might influence the outcome of pathogen detection and identification in a biosecurity setting. We provide a short description of each step and its function in the protocol. In addition, please consult the handbook of the DNeasy Plant Mini Kit provider for further details as required.

The final goal is to achieve the following:

- sample representative leaves for each treatment group that contain enough infective agent for DNA based pathogen identification.
- extract pure and high quality DNA that allows for PCR amplification of marker gene regions for two kingdoms (bacteria and fungi).

This protocol is applicable for week 2 and 3.

Protocols progress overview:

- Week 2 Sampling of two representative leaves and subsections thereof.
- Wee 2-3 tissue rupture with metal beads when tissues is frozen (performed by demonstrator).
- Week 3 DNA extraction from ruptured leaf tissue.

The whole process follows the following major steps.

Sampling -> Tissue rupture -> Tissue lysis -> Clearance of material -> DNA precipitation -> DNA binding to silica matrix -> Washing of impurities -> DNA elution from column and collection.

## ATTACHMENTS

DNeasyPlantMiniKitHandbook.pdf DNeasyPlantMiniKitQuickStart.pdf DNeasyPlantMiniKitSafetyDataSheet.pdf

## IMAGE ATTRIBUTION

The icon was created with BioRender.com.

## GUIDELINES

You must have read, understood, and follow the health and safety instructions provided in the "Overview Mini-Research Project #1 BIOL3106/6106" provided on Wattle (ANU learning portal).

You must have signed and returned one copy of the "Student Safety Declaration Form For Practical Class Work" before starting the any laboratory work.

You must have read and understood the Hazard Sheets (Risk assessment) of all chemicals listed bellow in the "Safety Warnings" section. These Hazard Sheets are provided on Wattle as part of the "Overview Mini-Research Project #1 BIOL3106/6106" document.

## MATERIALS

As always you need to bring a lab notebook, a printed version of this protocol, and a pen to record your adventures in the lab.

Consumables and culture material needed:

- Five sets of 2 ml Eppendorf tubes with 2-3 steal beads.

- For each extraction (five in total) a set of the following;

1. QIAshredder spin column (pinkish color) placed in a 2 ml collection tube. Five in total.
2. One additional 2m collection tube. Five in total.
3. Two additional 1.5ml eppendorf tubes. Ten in total.
4. DNeasy Mini spin column (blueish color) placed in a 2 ml collection tube. Five in total.

- The following buffers and enzymes for five extractions.

1. Buffer AP1 2.1 ml
2. Rnase A 22 ul
3. Buffer P3 0.75 ml
4. Buffer AW1 3.4 ml
5. Buffer AW2 5.5 ml
6. Buffer AE 180 ul

- Ice bucket with ice.

Equipment needed:

- Benchtop centrifuge for 1.5ml/2ml tubes.
- Waterbath/heating block at 64$^{o}$C.
- Vortex.

## SAFETY WARNINGS

This protocol requires the following hazardous substances:

- RNAse A
- Solution AW1
- Solution P3

You need to wear safety equipment at all times including lab coats, gloves, and safety goggles when handling chemicals and biological agents. While the major biological agents used for the wheat infection are pathogens commonly found in Australia, you must treat them as they were infective agents of general concern. Treat them with care. Do not remove them from the laboratory. Do not spread them via clothing. Use a dedicated notebook and pen to make notes during the mini-research project. Do not put anything into your mouth while in the laboratory. Wash your hands each time you leave the laboratory.

## BEFORE START INSTRUCTIONS

You must study the protocol carefully before you start. If anything is unclear post questions directly here on protocols.io.

Additional protocol specific notes:

- Perform all centrifugation steps at room temperature (15–25°C).
- If necessary, redissolve any precipitates in Buffer AP1 and Buffer AW1 concentrates (done by demonstrators).
- Add ethanol to Buffer AW1 and Buffer AW2 concentrates (done by demonstrators).
- Preheat a water bath or heating block to 65°C (done by demonstrators).

# Week 2: Sampling of representative leaf tissue and tissue r..

**1** Label each 2ml Eppendorf tube containing 2-3 steal beads with the treatment group name and your research group name. Label both the top and the side of each tube clearly. 5m

**2** Carefully, study the plants in each pot for potential disease symptoms. Select two leaves for each treatment group that show representative symptoms [TG#1-4] or not in the case of the negative control [TG#5]. 5m

**2.1** Cut 1.5- 2 cm long leaf pieces from each TG from two independent leaves. Cut the middle part 5m

of the leaves. Combine both leaf pieces into the correctly labelled tube.

**2.2** Put the tube containing the leaf material on ice.

**2.3** Once done with all five TGs, hand your five tubes over to the demonstrator.

**2.4** Now the leaf tissues sampling part of the protocol is complete.

**3** The demonstrator will freeze your sample on dry ice.
The demonstrator will rupture your tissue using a TissueLyzer II https://www.qiagen.com/us/products/human-id-and-forensics/automation/tissuelyser-ii at 25 Hz for 1 min.
Now the leaf rupture part of the protocol is complete.

## Week 3: DNA extraction from ruptured leaf tissue

2h 5m

**4** Label all provided tubes and spin columns with your treatment group name and your research group name. 5m
This implies to label the following set of tubes for each TG.

1. QIAshredder spin column (pinkish color) placed in a 2 ml collection tube.
2. One additional 2m collection tube.
3. Two additional 1.5ml eppendorf tubes.
4. DNeasy Mini spin column (blueish color) placed in a 2 ml collection tube.

Now you will have five sets of clearly labeled tubes. This readies you to perform the DNA extraction for all five TG in parallel.

**5** You will receive your five tubes from week 2 that now contain ruptured tissue on ice. Proceed to the following sub-step as soon as possible to avoid degradation of the DNA by DNAses released during tissue lysis. The DNA will be protected in the AP1 buffer. Process all five samples at the same time.

**5.1** Add 400 µl Buffer AP1 to each of 2ml tubes containing your ruptured tissue. 5m

Steps 5.1 to 5.7 will lyse the cells of the ruptured tissue and bring the DNA into solution.

**5.2** Vortex and completely re-suspended the tissue in the AP1 buffer. 2m

**5.3** Collect content of the tube with a short "hand centrifuge" as demonstrated by the demonstrator.

**5.4** Add 4 µl RNase A to each tube and vortex again. 3m
This enzyme will digest RNA that gets released during cell lysis. It performs best at 65°C.

**5.5** Incubate for 10 min at 65°C in the waterbath or heating block. 10m
Invert the tube 2–3 times during incubation.

**5.6** Collect content of the tube with a short "hand centrifuge" as demonstrated by the demonstrator.

**5.7** Cool tubes on ice for 2 min. 2m

**5.8** To each tube add 130 µl Buffer P3. Mix by inversion for 10 to 20 times immediately after adding the P3 buffer. 3m
Steps 5.8 to 5.16 will clear the lysate so it is ready to precipitate the DNA without precipitating too many other contaminants.

**5.9** Incubate the five tubes on ice for 5 min. Slightly longer incubation will not affect the outcome negatively. 5m

**5.10** Team up with other research groups to centrifuge your samples. Make sure all your tubes are clearly labelled with your research group name and treatment group name. Make sure the centrifuge is balanced before you start. Check with a demonstrator about centrifuge usage if you are unclear. 2m

**5.11** Centrifuge the lysate for 5 min at maximum speed. 5m
This steep will clear the DNA in solution from larger undissolved particles.

**5.12** For each of your TG, pipett the 480 ul of the lysate into a QIAshredder spin column placed in a 2 ml collection tube. To not disturb the pellet when transferring the lysate. Remember! All tubes and spin columns must be labelled clearly. 5m

**5.13** Team up with other research groups to centrifuge your samples. Make sure all your tubes are clearly labelled with your research group name and treatment group name. Make sure the centrifuge is balanced before you start. Check with a demonstrator about centrifuge usage if you are unclear. 1m

**5.14** Centrifuge for 2 min at max speed. 2m

**5.15** Keep the flow through in the lower collection tube. 1m

**5.16** Discard the QIAshredder spin column. 1m

**5.17** For each of your TG, transfer 400 ul of the flow-through into a new clearly labelled 1.5 ml tube without disturbing the pellet if present. 5m
The steps 5.17 and 5.18 will precipitate the DNA.

**5.18** For each TG, add 600 ul Buffer AW1, and mix by pipetting. 5m

**5.19** Transfer 500 µl of the mixture into a DNeasy Mini spin column placed in a 2 ml collection tube. 2m
Remember! All tubes and spin columns must be labelled clearly.
The steps 5.19 to 5.22 will bind the DNA to the silica membrane that is part of the DNeasy Mini spin column.

**5.20** Team up with other research groups to centrifuge your samples. Make sure all your tubes are clearly labelled with your research group name and treatment group name. Make sure the centrifuge is balanced before you start. Check with a demonstrator about centrifuge usage if you are unclear. 2m

**5.21** Centrifuge for 1 min at max speed. 1m

**5.22** Discard the flow-through by pouring it into the waste collection container. 1m

**5.23** Place the DNeasy Mini spin column in the same 2ml collection tube. 1m
The steps 5.23 to 5.45 wash the bound DNA from co-precipitated and bound impurities.

**5.24** For each TG, add the remaining sample of about 500 ul onto the correct DNeasy Mini spin column. 3m

**5.25** Team up with other research groups to centrifuge your samples. Make sure all your tubes are clearly labelled with your research group name and treatment group name. Make sure the centrifuge is balanced before you start. Check with a demonstrator about centrifuge usage if you are unclear. 2m

**5.26** Centrifuge for 1 min at max speed. 1m

**5.27** Discard the flow-through by pouring it into the waste collection container. 4m

**5.28** Place the spin column into a new clearly labeled 2 ml collection tube. 2m

**5.29** Discard the old collection tube.

**5.30** For each TG, add 500 µl Buffer AW2 on top of the DNeasy Mini spin column. Do not touch the spin column with your pipette tip. 3m

**5.31** Team up with other research groups to centrifuge your samples. Make sure all your tubes are clearly labelled with your research group name and treatment group name. Make sure the centrifuge is balanced before you start. Check with a demonstrator about centrifuge usage if you are unclear 2m

**5.32** Centrifuge for 1 min at max speed. 1m

**5.33** Discard the flow-through by pouring it into the waste collection container. 2m

**5.34** Tap the collection tube on a paper towel to remove the remaining liquid. 2m

**5.35** Place the DNeasy Mini spin column in the same 2ml collection tube.

**5.36** For each TG, add 500 µl Buffer AW2 on top of the DNeasy Mini spin column. Do not touch the spin column with your pipette tip. 4m

**5.37** Team up with other research groups to centrifuge your samples. Make sure all your tubes are clearly labelled with your research group name and treatment group name. Make sure the centrifuge is balanced before you start. Check with a demonstrator about centrifuge usage if you are unclear. 2m

**5.38** Centrifuge for 2 min at max speed. 2m

**5.39** Discard the flow-through by pouring it into the waste collection container. 2m

**5.40** Tap the collection tube on a paper towel to remove the remaining liquid.

**5.41** Place the DNeasy Mini spin column in the same 2ml collection tube.

**5.42** Team up with other research groups to centrifuge your samples. Make sure all your tubes are clearly labelled with your research group name and treatment group name. Make sure the centrifuge is balanced before you start. Check with a demonstrator about centrifuge usage if you are unclear. 2m

**5.43** Centrifuge for 1 min at max speed. 1m

**5.44** Remove the DNeasy Mini spin column from the collection tube carefully so that the column does not come into contact with the flow-through.

**5.45** Transfer the spin column to a new clearly labelled 1.5 ml tube.

**5.46** Add 75 µl Buffer AE into the centre of the DNeasy Mini spin column for elution. Make sure to pipette the buffer directly onto the membrane in the centre of the tube. 5m
The steps 5.46 to 5.56 elute the bound DNA from the silica membrane and bring it back into solution.

**5.47** Incubate for 5 min at room temperature (15–25°C). 5m

**5.48** Team up with other research groups to centrifuge your samples. Make sure all your tubes are clearly labelled with your research group name and treatment group name. Make sure the centrifuge is balanced before you start. Check with a demonstrator about centrifuge usage if you are unclear. 2m

**5.49** Centrifuge for 1 min at max speed. 1m

**5.50** Add another 75 µl Buffer AE into the centre of the DNeasy Mini spin column for elution. Make sure to pipette the buffer directly onto the membrane in the centre of the tube. 5m

**5.51** Incubate for 5 min at room temperature (15–25°C).

**5.52** Team up with other research groups to centrifuge your samples. Make sure all your tubes are clearly labelled with your research group name and treatment group name. Make sure the centrifuge is balanced before you start. Check with a demonstrator about centrifuge usage if you are unclear. 2m

**5.53** Centrifuge for 1 min at max speed. 1m

**5.54** Discard the DNeasy Mini spin column.

**5.55** Close and keep the collection tube.

**5.56** The DNA is now contained in your in your elute.

**6** You should have now five clearly labelled collection tubes with about 150ul DNA in elution buffer. Each tube contains the DNA of one TG.

**6.1** Hand over your five clearly labelled tubes to the demonstrator for storage till next week.