Improved DNA Yield and Quality from Diverse Plant Material

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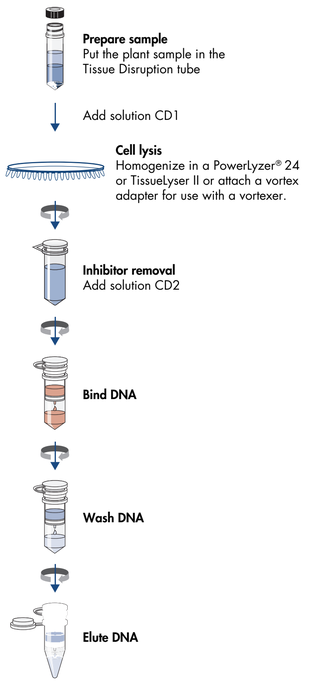
Introduction and Experimental Workflow

Isolating DNA from plant material can be a tedious process with significant hands-on time. Furthermore, dependable results are difficult to obtain due to differences in consistency and inhibitor presence between plant species and between parts of individual plants.

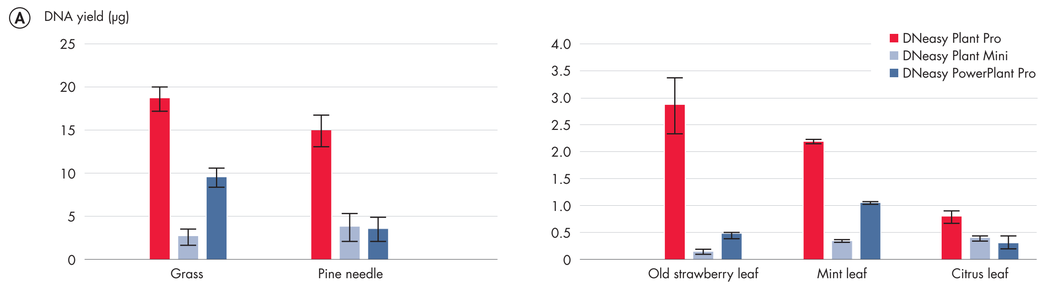
Here, we present a workflow using the DNeasy® Plant Pro Kit for efficient extraction of DNA from different plant types. Its novel bead-beating method and lysis chemistry resulted in more efficient sample lysis, less hands-on time and significantly better DNA yields than conventional methods.

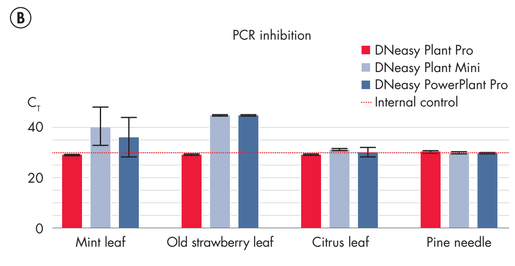
The workflow also leverages multiple technologies to improve the removal of secondary metabolites that could inhibit downstream applications, such as polyphenols, complex polysaccharides, alkaloids and tannins. The resulting DNA has high quality and purity and is suitable for immediate use in downstream reactions, including PCR, qPCR and next-generation sequencing. The workflow is well suited for plant microbiome and plant pathology studies. It is also an excellent fit for plant breeding and engineering work.



Improved DNA Yield and Quality (I)

Overall DNA yields were higher with the DNeasy Plant Pro Kit than with the DNeasy Plant Mini and DNeasy PowerPlant Pro kits. Samples isolated with the DNeasy Plant Pro Kit also had fewer inhibitors co-isolated with the DNA.

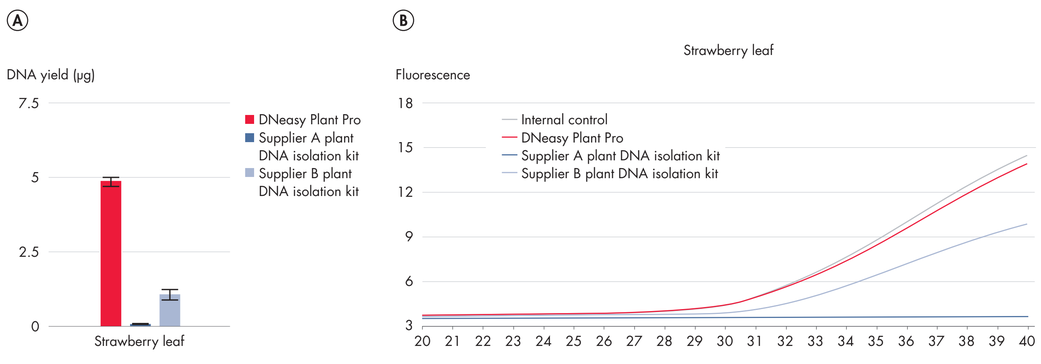




Significantly increased DNA yields and reduced inhibitor co-isolation. **A** DNA was isolated from 50 mg samples of plant material (4 replicates) using the DNeasy Plant Pro, DNeasy Plant Mini and DNeasy PowerPlant Pro kits. The DNA yields were measured with a Qubit Fluorometer. The overall yields were consistently higher. **B** The internal control (IC) from the QuantiFast® Pathogen +IC Kit was spiked with 8 µl eluates of the resulting plant DNA (3 replicates). After IC amplification via PCR, the C_T values were compared to the C_T values for PCR with water added to the IC (red dotted line, representing no inhibition). The eluates from the DNeasy Plant Pro Kit showed no inhibition, even when the original plant sample was high in inhibitors (e.g., old strawberry leaf).

Improved DNA Yield and Quality (II)

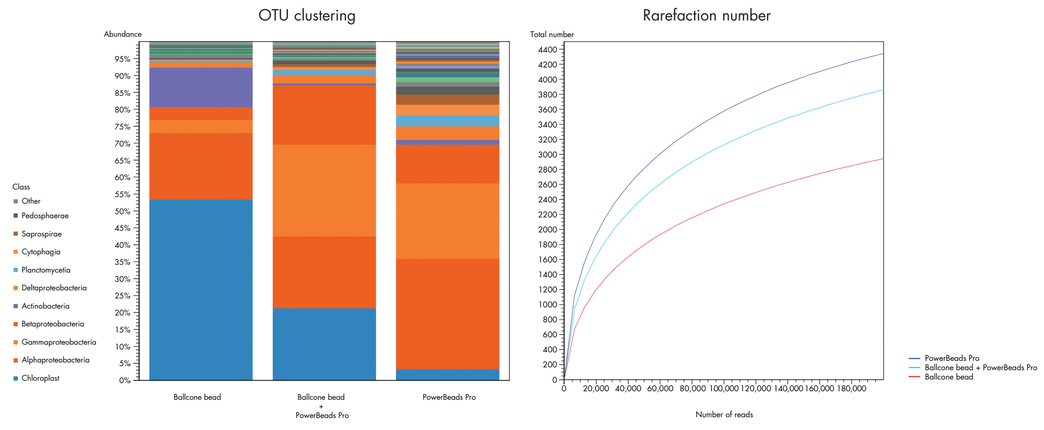
The DNeasy Plant Pro Kit also gave better DNA yield and inhibitor removal compared to various kits from other suppliers.



Significantly higher DNA yields and lower inhibitor co-isolation. A DNA was isolated from 50 mg samples of strawberry leaves using the DNeasy Plant Pro Kit and two kits from other suppliers. Yields were measured using a Qubit Fluorometer. **B** The internal control (IC) from the QuantiFast® Pathogen +IC Kit was spiked with 8 µl eluates of the resulting plant DNA (3 replicates). PCR-generated fluorescence, which is proportional to the product levels, was measured with a Rotor-Gene® Q and compared. Distilled water added to the IC was used as a control. The eluate from the DNeasy Plant Pro Kit showed no inhibition.

Studying the Root-associated Plant Microbiome

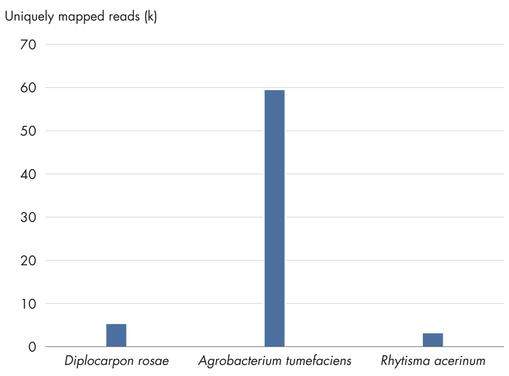
To assess the disruption efficiency of plant-associated bacteria, we used the DNeasy Plant Pro Kit protocol with three different disruption tubes to isolate DNA from the roots of apple trees (*Malus domestica*). This was followed by library preparation, sequencing and bioinformatics analyses.



Identification of root-associated bacteria. DNA was extracted from 50 mg samples of apple tree roots using the DNeasy Plant Pro Kit with: Ballcone beads (according to the DNeasy Plant Pro protocol); a mix of Ballcone beads and PowerBeads Pro; or PowerBeads Pro alone. A 16S rRNA gene library was prepared with the QIAseq® FX DNA Library Kit and sequenced using the Illumina® MiSeq® system (2 x 250 bp run). The resulting reads were analyzed with the CLC Genomic Workbench (QIAGEN Microbial Genomics Pro Suite). The results show that plant cells are more efficiently lysed using the Ballcone beads and bacterial cells are more efficiently lysed using PowerBeads Pro.

Detecting Plant Pathogens in Plant Samples

Fungal and bacterial plant pathogens can have a large economic impact. We used the DNeasy Plant Pro Kit as part of a workflow to identify commonly found plant pathogens *Diplocarpon rosae, Agrobacterium tumefaciens* and *Rhytisma acerinum*.



Diplocarpon rosae (Fungus)
Disease: Rose black spot disease
Host: Rose species

Agrobacterium tumefaciens (Bacteria)
Disease: Crown gall
Host: > 60 different plant families

Rhytisma acerinum (Fungus)
Disease: Tar spot
Host: Maple and sycamore trees

Co-purification of plant and pathogen DNA. Using the DNeasy Plant Pro Kit, DNA was extracted from 50 mg samples of plant material infected with one of the three pathogens. A whole genome library was prepared with the QIAseq FX DNA Library Kit and sequenced using the Illumina MiSeq system (2 x 250 bp run). The resulting reads were analyzed with the CLC Genomic Workbench (QIAGEN Microbial Genomics Pro Suite). Each plant pathogen was successfully identified.

Conclusions

The new DNeasy Plant Pro Kit:

- Gives higher DNA yields and better inhibitor removal than other plant DNA isolation kits from QIAGEN and other suppliers
- Isolates large amounts of high-quality DNA from a variety of samples, including grass, pine needles, strawberry leaves, citrus leaves, grapevine leaves, tomato stems, coffee seeds, and cotton seed and roots
- Provides DNA of sufficient quality for downstream applications, including PCR, qPCR and NGS
- Was successfully used as part of workflows to identify various common plant pathogens and to study the root-associated plant microbiome
- Has a disruption protocol adjustable for plant or bacterial cells

These data show that the new DNeasy Plant Pro Kit can successfully isolate DNA from samples that are both difficult to lyse and high in inhibitors

The DNeasy Plant Pro Kit is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.

1115584 11/2018

Sample to Insight