

Rapid DNA Purification from Different Plant Samples with the Thermo Scientific KingFisher Pure DNA Plant Kit

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Goal

This technical note describes the purification of genomic DNA from 50 mg of fresh plant samples with the Thermo Scientific™ KingFisher™ Pure DNA Plant Kit. The purified DNA is free of RNA, contaminants and inhibitors, thus providing intact DNA for various downstream applications.

Introduction

The KingFisher Pure DNA Plant Kit in combination with Thermo Scientific™ KingFisher™ magnetic particle processors comprises an efficient solution for purifying genomic DNA (gDNA) from variable plant material with minimal hands-on work. Highly pure DNA can be obtained from fresh or frozen plant samples. The Thermo Scientific™ KingFisher™ Duo and Thermo Scientific™ KingFisher™ Flex instruments enable automation for medium- or high-throughput and large-volume sample preparation. The optimized combination of kit reagents, plastic consumables, the new Thermo Scientific™ BindIt™ Software 3.2, and patented magnetic particle handling constitutes an exceptional purification system for obtaining a high yield and purity of DNA.

Materials and Methods

Homogenization and lysis

The homogenization step must disrupt plant cell walls rapidly and completely in order to ensure high DNA yield. Plant tissue can be homogenized, for example by grinding it with a pestle, using bead beating, or with a homogenizer device. 350 μL of Lysis Buffer A was added to each homogenized sample and mixed for 20 s, and then 50 μL of Lysis Buffer B was added. For samples containing large amounts of RNA, 20 μL of RNase A was also added. The samples were then incubated at 65 °C for 10 mins and then centrifuged for 5 mins to clear the lysate.

The KingFisher process

The gDNA was isolated from 50 mg samples of fresh thale cress (*Arabidopsis thaliana*), tobacco leaves, wheat leaves, rapeseed leaves, tomato leaves, barley seedlings, rice seedlings, maize (corn) leaves, spinach leaves, and potato stems, using the KingFisher Pure DNA Plant Kit. The purification protocol was optimized for both the KingFisher Duo and KingFisher Flex with BindIt



Software 3.2. The purified DNA was eluted into 150 μL of Elution Buffer on the KingFisher Flex instrument.

Comparisons

The KingFisher Pure DNA Plant Kit in combination with the KingFisher Flex was compared to four competitive magnetic particle-based purification kits. The starting material for all isolations was 50 mg of fresh *Arabidopsis*, tobacco, and wheat leaf tissue. Purification was performed in accordance with the respective instruction manuals of each kit.

Results

The results of gDNA purification using the KingFisher Pure DNA Plant Kit in combination with the KingFisher Flex are listed in Table 1. gDNA was purified intact from all samples, and without RNA co-purification (Figure 1).

Table 1. Typical DNA yields from 50 mg plant samples

No.	Sample	DNA yield
1	Thale cress " <i>Arabidopsis th.</i> "	2 µg
2	Tobacco	4 µg
3	Wheat	15 µg
4	Rapeseed (canola)	4.5 µg
5	Tomato	7.5 µg
6	Barley	7 µg
7	Rice	5 µg
8	Maize (corn)	4.5 µg
9	Spinach	3 µg
10	Potato	1.5 µg

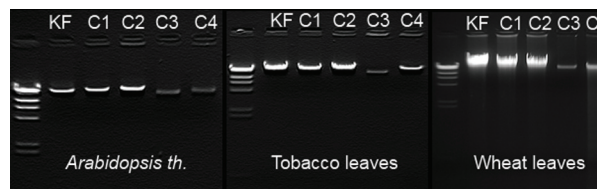


Figure 2: gDNA from *Arabidopsis th.*, tobacco, and wheat leaf tissues purified with the KingFisher Pure DNA Plant Kit (KF) and four other plant DNA purification kits (C1-C4) using the KingFisher Flex instrument.

Conclusions

High-quality DNA free of proteins, salt, and other inhibitors was purified from several different plant samples with the KingFisher Pure DNA Plant Kit. The purification process provides an optimized system with the KingFisher Pure DNA Plant Kit, the KingFisher Duo or KingFisher Flex, and BindIt Software 3.2. The KingFisher Pure DNA Plant Kit performed excellently in comparison to four other magnetic particle kits.

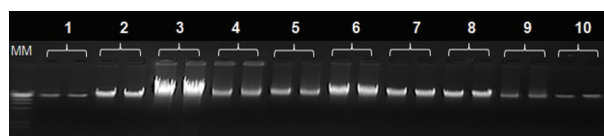


Figure 1: gDNA purified from various plant materials with the KingFisher Pure DNA Plant Kit using the KingFisher Flex instrument. Bands represent: fresh thale cress (*Arabidopsis th.*) (1); tobacco (2); wheat (3); rapeseed (canola) (4); tomato (5); barley (6); rice (7); maize (corn) (8); spinach (9); and potato (10). See Table 1 for measured DNA yields.

Purification using the KingFisher Pure DNA Plant Kit was shown to be better than with the four other magnetic particle-based kits, all run on the KingFisher Flex instrument (Figure 2).

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