

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

SP&A Application Laboratory, Thermo Fisher Scientific, Vantaa, Finland

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

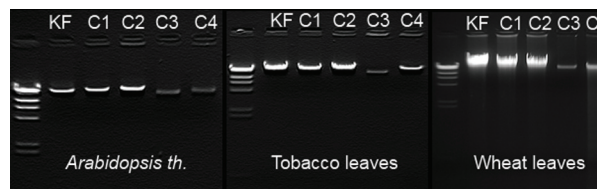


Figure 2: gDNA from *Arabidopsis th.*, tobacco, and wheat leaf tissues purified with the KingFisher Pure DNA Plant Kit (KF) and four other plant DNA purification kits (C1-C4) using the KingFisher Flex instrument.

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.

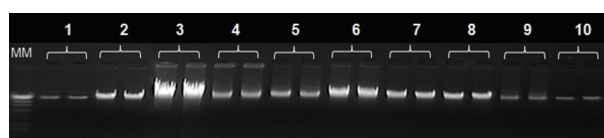


Figure 1: gDNA purified from various plant materials with the KingFisher Pure DNA Plant Kit using the KingFisher Flex instrument. Bands represent: fresh thale cress (*Arabidopsis th.*) (1); tobacco (2); wheat (3); rapeseed (canola) (4); tomato (5); barley (6); rice (7); maize (corn) (8); spinach (9);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

thermoscientific.com/kingfisherinfo

© 2013 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific Inc. and its subsidiaries. Specifications, terms and pricing are subject to change. Not all products are available in all countries. Please consult your local sales representative for details.

North America:

USA/Canada +1 603 595 0505
USA toll free 800 345 0206

Europe:

Austria +43 1 801 40 0
Belgium +32 53 73 42 41
Finland +358 9 3291 0200
France +33 2 2803 2000
Germany national toll free 08001-536 376

Germany international +49 6184 90 6940

Italy +39 02 95059 552
Netherlands +31 76 571 4440
Nordic/Baltic countries +358 9 329 100
Russia/CIS +7 (495) 739 76 41
Spain/Portugal +34 93 223 09 18
Switzerland +41 44 454 12 12
UK/Ireland +44 870 609 9203

Asia:

Australia +613 9757 4474
China +86 21 6865 4588 or +86 10 8419 3588
China toll free 800-810-5118, 400-650-5118
India +91 22 6716 2200
Japan +81-3-5826-1616
Korea +82 11 796 7771
Other Asian countries +65 6872 9717
Countries not listed:
 +49 6184 90 6940 or +33 2 2803 2000

Thermo
 SCIENTIFIC

Part of Thermo Fisher Scientific