Technical Note:
TN-PSKFPLANTKIT_0212

Rapid DNA purification with the Thermo Scientific KingFisher Plant DNA Kit from variable plant material

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- Efficient purification of genomic DNA
- Isolation from variable plant material
- Flexible processing from 1 to 96 samples

Introduction

The Thermo Scientific KingFisher Plant DNA Kit in conjunction with the KingFisher® magnetic particle processor provides 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, frozen, dried or lyophilized plant samples. The Thermo Scientific KingFisher Duo and KingFisher Flex instruments enable automation for medium- or high-throughput and additionally large-volume sample preparation. The optimized combination of kit reagents, plastic consumables, 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.

Material and methods

Typical sample material

The best quality and yield of DNA from plants is obtained by using young plant samples. 20 to 50 mg of fresh plant sample is used for the DNA extraction with the KingFisher Plant DNA Kit (Cat. No. 97050196). With dried plant material, 4 to 10 mg of the starting material per sample is recommended. With demanding plant species, optimization of the purification protocol may require titrating an amount of the sample material to gain the best possible DNA yield.



Homogenization and lysis

The homogenization step must disrupt the structures of the plant material rapidly and completely in order to ensure a high yield of DNA. Plant tissue can be homogenized, for example, by grinding with a pestle, using bead beating, or with a homogenizer device. 500 µl of Lysis Buffer is added to a homogenized sample and mixed for 30 seconds. RNase A treatment is recommended for the samples containing large amounts of RNA. RNase A is added to the Lysis Buffer at a final concentration of 0.25 mg/ml. The samples are incubated at +56°C for 30 min and centrifuged for 20 min to clear the lysate.

KingFisher process

The gDNA was isolated from 20 to 50 mg of fresh sunflower, tobacco, wheat, maize, pepper and rice leaf tissue or 10 mg of dried sunflower and tobacco leaf tissue using the KingFisher Plant DNA Kit. The purification protocol has been optimized for both KingFisher Duo and KingFisher Flex with BindIt® Software 3.2. The purified DNA was eluted into 100 µl or 150 µl of the Elution Buffer, depending on the instrument.

TABLE 1. Typical DNA yields from plant samples		
Sample	Sample input (fresh leaf tissue)	Typical yield
Tobacco	50 mg	2—15 μg
Wheat	50 mg	10—20 μg
Sunflower	50 mg	2–5 μg
Maize	45 mg	5—20 µg
Pepper	45 mg	4–8 μg
Rice	20 mg	1.5–2.5 µg



Figure 1: gDNA purified from rice plant samples with KingFisher Duo.

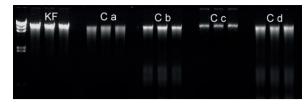


Figure 3: gDNA from tobacco leaf tissue purified with the KingFisher Plant DNA Kit (KF) and four other plant DNA purification kits (Ca-Cd).

Comparisons

The KingFisher Plant DNA Kit in conjunction with KingFisher Flex was compared to four comparable magnetic particle based purification kits. The starting material for all isolations was 50 mg of fresh tobacco leaf tissue. Purification was performed in accordance with the instruction manuals.

Results

The results of the gDNA purification, by using the KingFisher Plant DNA Kit in conjunction with either KingFisher Duo or KingFisher Flex, are listed in Table 1. gDNA purified from fresh rice samples was intact and no RNA was co-purified (Figure 1). The purification of gDNA from fresh and dried sunflower and tobacco leaf tissue resulted in a very good quality and quantity of gDNA. Figure 2 shows the PCR performed from purified gDNA by using universal plant primers.

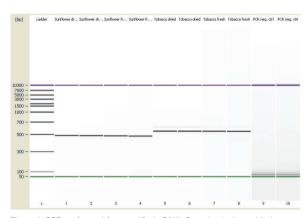


Figure 2: PCR performed from purified gDNA. Samples 1-2 are dried sunflower samples, 3-4 fresh sunflower, 5-6 dried tobacco, 7-8 fresh tobacco, and 9-10 negative controls.

Comparing the performance of the KingFisher Plant DNA Kit with four other magnetic particle based kits showed excellent performance of the purification process with the KingFisher Plant DNA Kit together with the KingFisher Flex instrument. Figure 3 represents the agarose gel image of the DNA elutions from the tested purification systems.

Conclusions

- . High-quality DNA free of proteins, salt and other inhibitors was purified from several different plant samples with the KingFisher Plant DNA Kit.
- The purification process offers an optimized system with the KingFisher Plant DNA Kit, the KingFisher Duo or KingFisher Flex and Bindlt Software 3.2.
- The KingFisher Plant DNA Kit showed excellent performance in comparison to competing magnetic particle kits.



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