

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 DNA isolation from 1–96 samples



Introduction

The KingFisher Plant DNA Kit together with the Thermo Scientific 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 Flex instrument enables automation of high-throughput or large-volume sample preparation. The optimized combination of kit reagents, plastic consumables, Thermo Scientific BindIt Software and patented magnetic particle handling constitutes an exceptional purification system for obtaining a high yield and purity of DNA.

Material and Methods

Sample material

The best quality and yield of DNA from plants is obtained by using young plant samples. 20–50 mg of fresh plant sample is used for the DNA extraction with the KingFisher Plant DNA Kit. With dried plant material, one fifth (4–10 mg) of the starting material is used compared to the fresh sample. 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 by adding RNase A to the Lysis Buffer at a final concentration of 0.25 mg/ml. The samples were incubated at +56°C for 30 min and centrifuged for 20 min to clear the lysate.

KingFisher procedure

The gDNA was isolated from 50 mg of fresh sunflower and tobacco leaf tissue using the KingFisher Plant DNA Kit in accordance with the instruction manual. The purification protocol has been optimized for the KingFisher Flex with BindIt Software 3.1. The protocol “KF_PlantDNA_Flex96” was run on the KingFisher Flex. The purified DNA was eluted into 150 µl of the Elution Buffer.

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

Comparisons

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

Results

The results of the gDNA purification by using the KingFisher Plant DNA Kit with the KingFisher Flex are listed in Table 1. The purification of gDNA from fresh and dried sunflower and tobacco leaf tissue resulted in a very good quality and quantity of gDNA for any secondary applications. Figure 1 shows the PCR performed from purified gDNA by using universal plant primers.

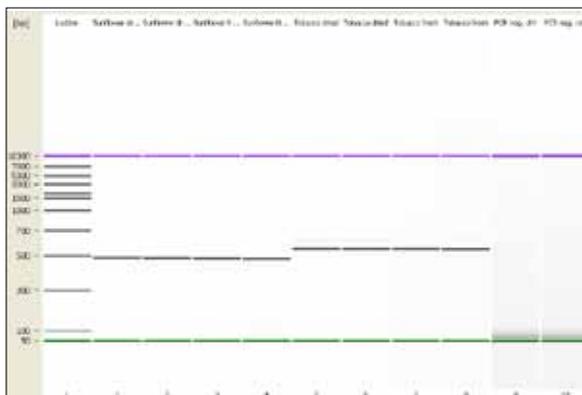


Figure 1: PCR performed from purified gDNA. Lanes 1–2 are dried sunflower, 3–4 fresh sunflower, 5–6 dried tobacco, 7–8 fresh tobacco, and 9–10 negative controls.

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 2 represents the agarose gel image of the DNA elutions from the tested purification systems.

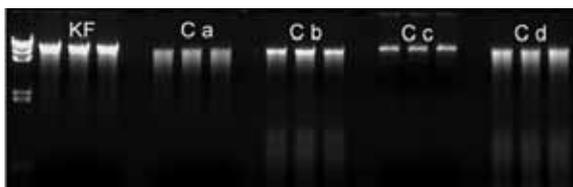


Figure 2: gDNA from tobacco leaf tissue purified with the KingFisher Plant DNA Kit and four other plant DNA purification kits.

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 Flex and BindIt Software.
- The KingFisher Plant DNA Kit showed excellent performance when compared with competing kits.

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