Purification of high-quality DNA from a wide variety of plant samples using the BioSprint 96 workstation

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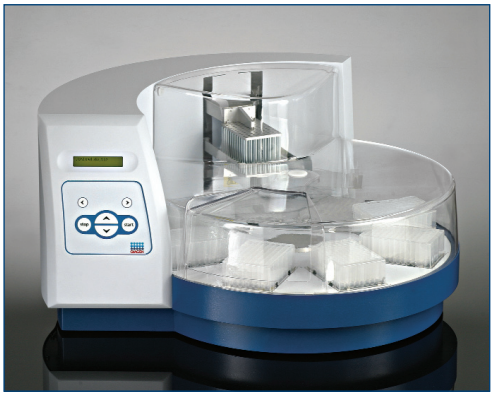
Total DNA was successfully purified from a range of plant samples, including difficult plant material, using the BioSprint 96 workstation and the BioSprint 96 DNA Plant Kit. The purified DNA was of high quality and gave excellent results in downstream analysis.

Introduction

The BioSprint 96 workstation allows automated, high-throughput purification of DNA, which provides standardized processing, better sample-to-sample reproducibility, and a more efficient workflow. The purified DNA is ready to use in sensitive downstream applications, such as PCR. In this study, total DNA (i.e., genomic, chloroplast, and mitochondrial DNA) was purified from a range of plant samples using the BioSprint 96 workstation with the BioSprint 96 DNA Plant Kit. Samples included difficult plant material such as colza (*Brassica napus*) seeds, rose leaves, apple seeds, and grape seeds. The purified DNA performed well in real-time PCR, endpoint PCR, and restriction enzyme digestion.

BioSprint 96 Workstation



Materials and methods

DNA was purified from the sample types shown in Table 1. Each sample was placed in a microtube, with 1 or 2 stainless steel or tungsten carbide beads, and cooled in liquid nitrogen. Samples were then disrupted and homogenized with a TissueLyser for 2 x 1 minute at 30 Hz with cooling in between. Next, 300 µl Buffer RLT was added to each sample before vortexing and centrifugation to remove debris. Cleared lysates were loaded into the BioSprint 96, which performed all DNA purification steps using the BioSprint DNA Plant protocol and reagents and plasticware from the BioSprint DNA Plant Kit. Purified DNA was eluted in 200 µl Buffer AE. The BioSprint system uses proven MagAttract® magnetic particle technology for DNA purification. For more details, visit www.qiagen.com/Products/Automation/BioSprint96.aspx .

DNA yield and purity were determined by measuring the absorbance at 260 nm (A_{260}) and the ratio of absorbance at 260 nm and 280 nm (A_{260}/A_{280}). The quality of DNA was confirmed by running 5 µl or 10 µl eluted DNA on a 0.8% agarose gel.

Endpoint PCR analysis was performed using the universal trnL PCR system which amplifies an intergenic region of a tRNA encoding gene in the chloroplast genome. 5 µl, 3 µl, 0.5 µl, or 0.05 µl purified DNA from each sample was used as template in a final reaction volume of 25 µl.

Real-time PCR analysis of the maize β-amylase gene was carried out using 5 µl, 0.5 µl, or 0.05 µl purified maize DNA from fresh leaf discs in a final reaction volume of 20 µl.

Restriction enzyme digests consisted of 16 µl DNA eluate, 2 µl restriction enzyme (XhoI or SacI), and 2 µl buffer.

Table 1. DNA Yields from a Range of Sample Types

Sample type	Amount	Typical yield (µg)*
Fresh leaf tissue/needles		
Wheat	50 mg	8–49
Soy	50 mg	39–80
Maize	50 mg	10–41
Cotton	50 mg	10–26
Arabidopsis	50–120 mg	13–41
Rose	100–140 mg	11–51 (old, dark green leaves) 40–100 (small, light green leaves)
Pine	100–190 mg	5–18
Spruce	130–160 mg	5–13
Lyophilized leaves		
Sunflower	5–10 mg	57–93
Colza (*Brassica napus*)	5–10 mg	13–23
Maize	5–10 mg	10–16
Dried seed powder		
Maize	20–55 mg	10–34
Seeds		
Wheat	3–4 seeds	85–122
Soy	1 seed	80–105
Colza (*Brassica napus*)	1–4 seeds	8–48
Linen	1–10 seeds	1–38
Tomato	15–30 seeds	12–16
Apple	1–6 seeds	5–30
Orange	1 large and 1 small seed	1–35
Lupine	2–7 seeds	18–97
Turnip radish	4–18 seeds	11–41
Swedish turnip	5–50 seeds	21–52
French bean	1 bean	13–55
Grape	3 seeds	18–19

* OD measurements used to calculate yields can be misleading. See gels and PCR results (Figures 1–6) for more accurate indications of yield and quality.

Results

DNA purification from a variety of plant samples

DNA was purified from a wide range of plant samples, including fresh and lyophilized leaves, and seeds from many different plant types. DNA yields and amounts of starting material are shown in Table 1. Absorbance measurements showed that the purified DNA was highly pure.

Purified DNA successfully used in endpoint PCR

DNA purified from plant samples was visualized on agarose gels which confirmed that it was of high quality. Purified DNA gave excellent results in endpoint PCR analysis of the trnL gene (Figures 1–3).

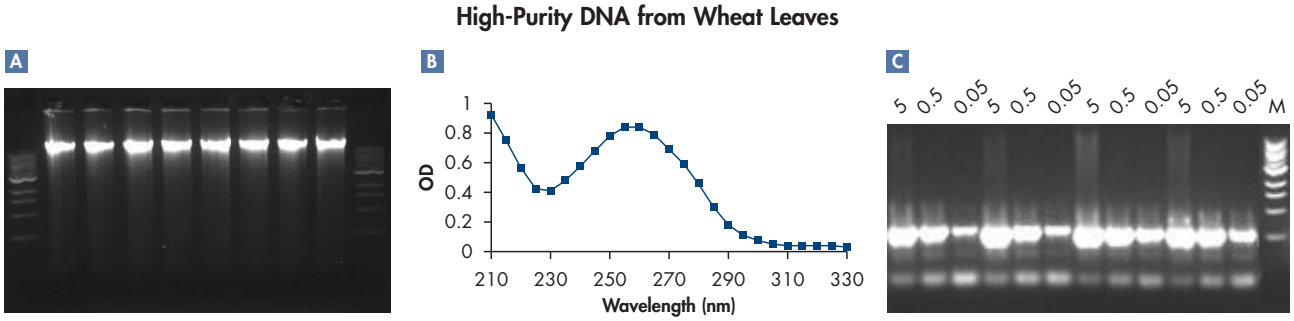


Figure 1 DNA was purified from 50 mg fresh leaves and eluted in 200 µl Buffer AE. A Eluates (5 µl) were visualized on a 0.8% agarose gel. B A typical OD curve is shown. C Amplification (25 µl reaction volume) was performed using the trnL PCR system with **5 µl**, **0.5 µl**, or **0.05 µl** wheat leaf DNA from 4 samples. PCR products (5 µl) were visualized on a 0.8% agarose gel. The marker (**M**) was the 1 kb ladder.

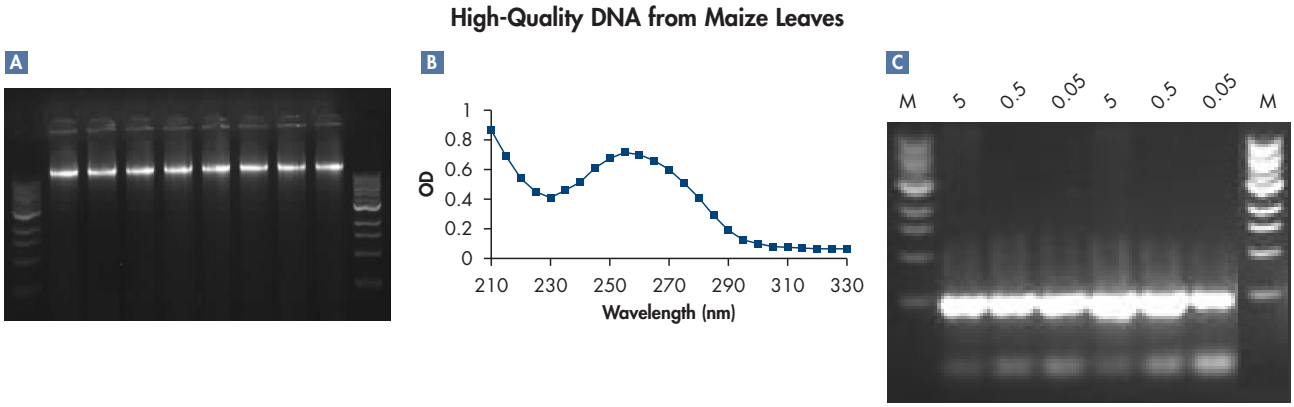


Figure 2 DNA was purified from 50 mg maize leaves and eluted in 200 µl Buffer AE. A Eluates of maize leaf DNA (5 µl) were visualized on 0.8% agarose gels. B A typical OD curve for maize leaf DNA is shown. C Amplification (25 µl reaction volume) was performed using the trnL PCR system with **5 µl**, **0.5 µl**, or **0.05 µl** maize leaf DNA from 2 maize leaf DNA samples. PCR products (5 µl) were visualized on 0.8% agarose gels. The marker (**M**) was the 1 kb ladder.

Highly pure DNA from difficult material

The BioSprint 96 DNA Plant Kit allows purification of DNA from many plant species, including plants that are considered difficult raw material for DNA purification. In the example shown in Figure 3, Rose DNA gave reliable results in endpoint PCR analysis of the trnL gene.

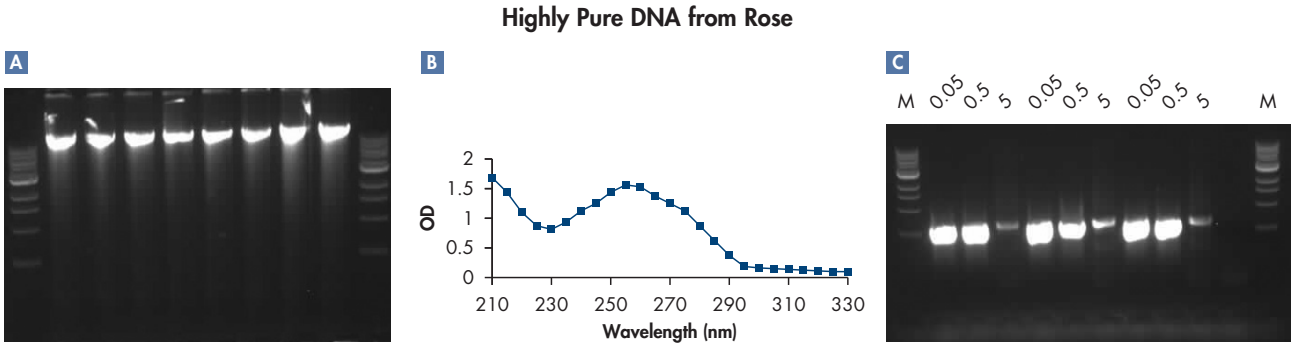


Figure 3 DNA was purified from 50 mg light green leaves and eluted in 200 µl Buffer AE. A Eluates (5 µl) were visualized on a 0.8% agarose gel. B A typical OD curve is shown. C Amplification (25 µl reaction volume) was performed using the trnL PCR system with **0.05 µl**, **0.5 µl**, or **5 µl** eluted DNA from 3 samples. PCR products (5 µl) were visualized on a 0.8% agarose gel. The marker (**M**) was the 1 kb ladder.

Reliable results in sensitive downstream analysis

DNA isolated from fresh maize leaves performed well in real-time PCR analysis of the maize β-amylase gene. Threshold cycle (C_T) values were comparable between replicates and linearly decreased with increasing amounts of template (Figure 4).

Restriction enzyme digestion also confirmed the DNA purity. DNA isolated from maize and wheat was successfully digested with the restriction enzymes XhoI and SacI, producing smears of digested DNA when visualized on an agarose gel (Figure 5).

Eluting in volumes of 100 µl and 50 µl showed that elution is very efficient even for the smaller 50 µl volume. Yields were maintained and the concentrations linearly increased (Figure 6 and Table 2).

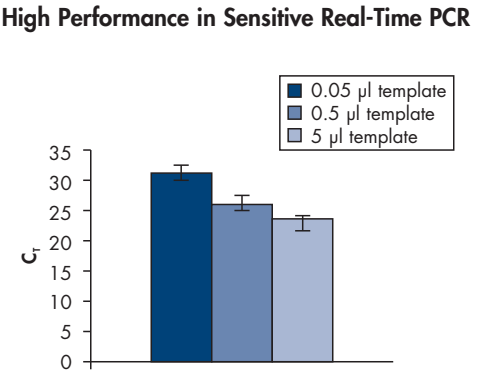


Figure 4 Quantitative, real-time PCR of the maize β-amylase gene was performed using DNA purified from maize leaves. Observed C_T values decreased with increasing template amount (0.05 µl, 0.5 µl, or 5 µl).

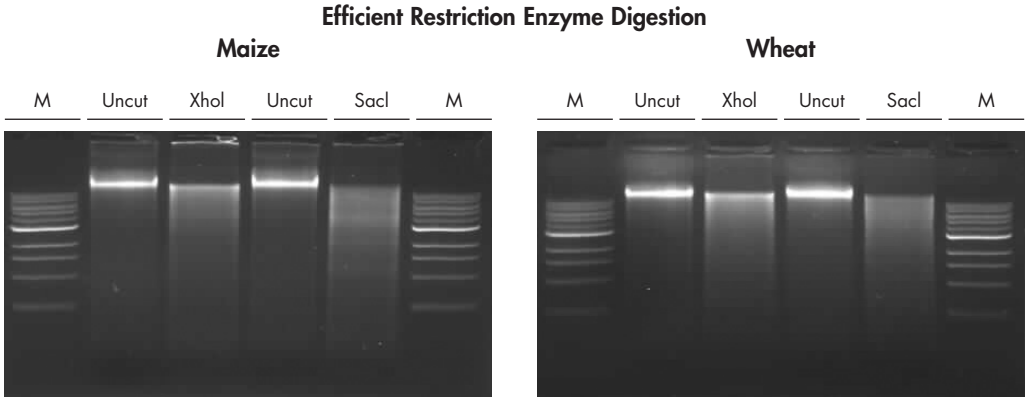


Figure 5 DNA from maize and wheat eluates was digested with XhoI and SacI as described in "Materials and methods". For all digested samples, a smear of DNA can be seen. Eluates (8 µl of uncut DNA or 10 µl of a restriction digest) were visualized on a 0.8% agarose gel. The marker (**M**) was the 1 kb ladder.

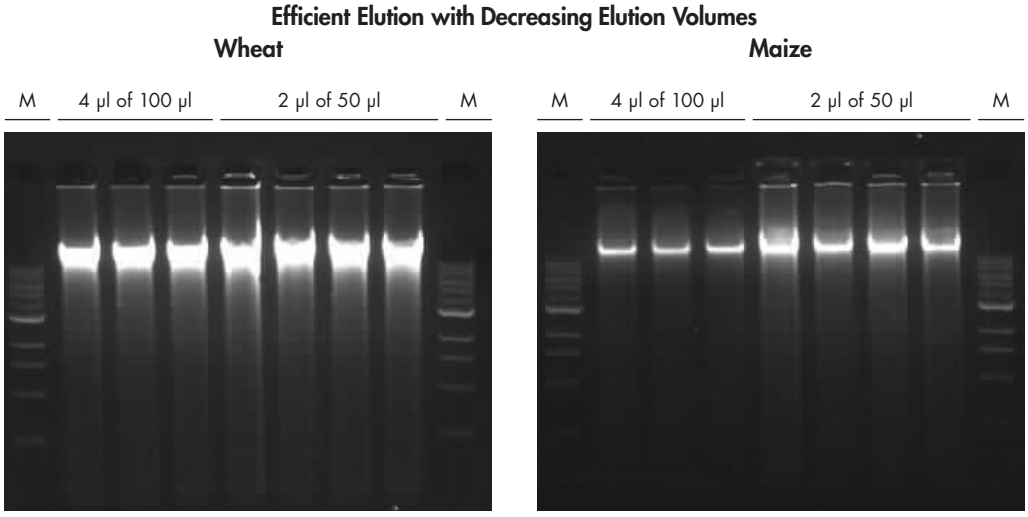


Figure 6 Purified DNA from maize and wheat was eluted in either 100 µl or 50 µl Buffer AE. Eluates (4 µl of 100 µl eluates and 2 µl of 50 µl eluates) were visualized on a 0.8% agarose gel. High yields were maintained irrespective of the elution volume. The marker (**M**) was the 1 kb ladder.

Table 2. DNA Concentrations with Decreasing Elution Volumes

	Elution volume	Concentration	Total yield
Maize	100 µl	400 +/- 52 ng/µl	40 µg
	50 µl	808 +/- 4 ng/µl	40.4 µg
Wheat	100 µl	434 +/- 3 ng/µl	43.4 µg
	50 µl	840 +/- 70 ng/µl	42 µg

Conclusions

Using the BioSprint 96 workstation and BioSprint 96 DNA Plant Kit enabled:

- Automated, high-throughput purification of DNA from a wide range of plant samples, including difficult materials
- Purification of high-quality DNA that performed well in sensitive downstream analyses
- A streamlined workflow with reduced hands-on time

Ordering Information

Product	Contents	Cat. no.
BioSprint 96	Robotic workstation for automation of magnetic-particle purification technology	9000852
BioSprint 96 DNA Plant Kit (576)	For 576 preps: Large 96-Rod Covers, 96-Well Microplates MP, S-Blocks, MagAttract Suspension G, Buffers and Reagents	941557
BioSprint 96 DNA Plant Kit (1536)	For 1536 preps: Large 96-Rod Covers, 96-Well Microplates MP, S-Blocks, MagAttract Suspension G, Buffers and Reagents	941558

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.

Discover more about automated DNA purification using the BioSprint 96 system at www.qiagen.com/automation!

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