Rapid, High Performance and Cost-Effective Plant DNA Extractions

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Introduction

The incredible diversity of the plant kingdom comes with a wide range of DNA extraction challenges. These challenges include extensive variations in plant structures and in their chemical composition of polysaccharides, polyphenolic compounds, and humic substances, all of which can interfere with extraction efficiency and/or inhibit downstream applications. In this context, Omega Bio-tek has developed a highly versatile DS line of plant DNA extraction kits encompassing both manual and automated extraction realms. The kits utilize the well-established properties of the cationic detergent, cetyltrimethylammonium bromide (CTAB) in conjunction with our proprietary silica column chemistries (manual) and magnetic bead (automated) to extract high quality DNA from a variety of plants.

Materials and Methods

To demonstrate Omega Bio-tek's plant extraction capabilities, a variety of plant samples were subjected to automated and manual extraction performance testing. The manual extractions were performed with Omega Bio-tek's E.Z.N.A.® Plant DNA DS (D2411) spin column-based kit alongside a similar product from a leading competitor (Company Q). The automated extractions were performed using Omega Bio-tek's Mag-BIND® Plant DNA DS 96 Kit (M1130) ported onto an open-ended, programmable, robotic liquid handler (Qiagen's BioSprint® 96). Plant specimens used for the experiments were either acquired commercially or grown at Omega Bio-tek. All the extractions for each method tested were performed in triplicate following manufacturer's recommended protocols. Each sample consisted of approximately 40-50 mg (wet weight) of plant leaf material and was disrupted at 1,500 rpm.

The workflows for both manual and automated extractions are shown in Figure 1. Resulting purified DNA was quantified via Promega's QuantiFluor® dsDNA system and normalized per mg of input plant material. The quality of the purified DNA extracted from corn samples using silica spin column-based kits from Omega Bio-tek (D2411) and Company Q was assessed by real-time PCR. Briefly, undiluted, 10-fold and 100-fold dilutions of purified DNA were isolated and amplified using Agilent's Brilliant III 2X SYBR® mix and corn-specific primers following a standard amplification protocol on the ABI 7900.

Results

The DNA yields obtained following manual protocols using Omega Bio-tek's kit was significantly higher than the leading competitor's kit on all 5 different plant samples tested (Figure 2). Table 1 shows the average C_t values obtained on serial dilutions of purified DNA from corn using both the kits. On average across the dilutions, the C_ts with the Omega extraction were lower by ~4.9 cycles compared to Company Q's. The C_t values suggest that there is at least 16x more DNA using the

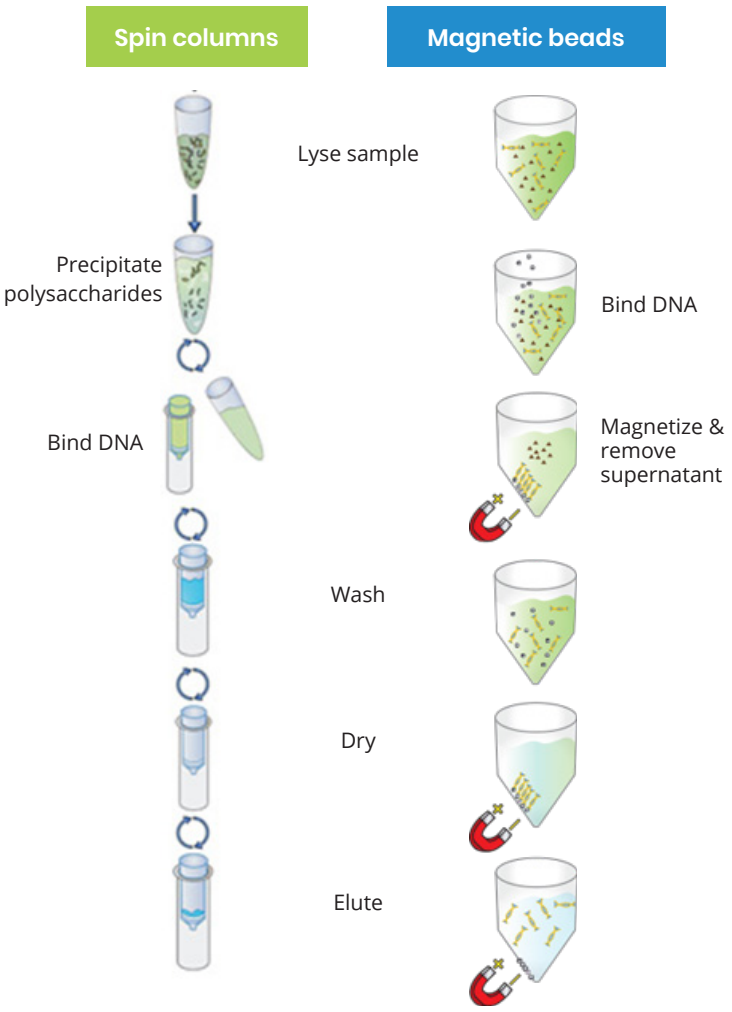

Figure 1. The E.Z.N.A.® Plant DNA DS Kit (D2411) uses silica spin columns and is the manual process shown above. The Mag-BIND® Plant DNA DS Kit (M1130) uses magnetic beads and is the automated process shown above. Omega Bio-tek's recommendation is to use magnetic bead-based protocols for automated processing.

Omega kit over Company Q's kit. Also, the ΔC_t values with the Omega kit were closer to 3.3 that of Company Q (~2.94 vs. ~1.87 difference between 10-fold and undiluted) suggesting less qPCR inhibition in spite of more template DNA that seems to have been present. The results from the qPCR indicate that Omega Bio-tek's kit was not only able to extract more DNA, but was also able to remove potential plant-related inhibitors that can hamper various downstream applications.

Twenty-three of the top agricultural and biofuel crops were subjected to automated extraction using Omega Bio-tek's M1130 kit on Qiagen's BioSprint® 96 and compared to the manual protocol of Company Q's.

The results shown in Table 2 reveal that the automated methods resulted in significantly higher recoveries of DNA than the competitor's manual method of 21 of the 23 plants

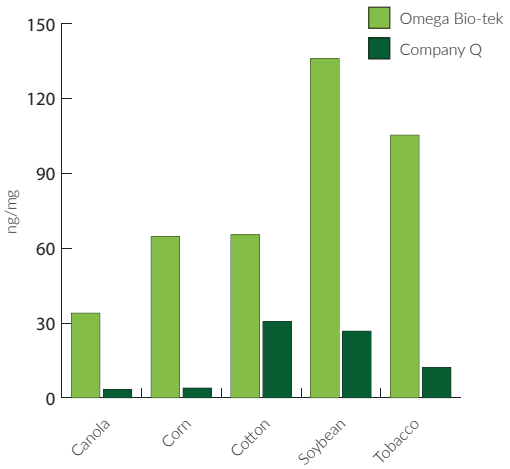

Figure 2. 40-50 mg of respective fresh leaf tissue was extracted in triplicate according to the E.Z.N.A.® Plant DNA DS Kit protocol and Company Q's recommended protocols and eluted in 100 µL. DNA analyzed with fluorescent DNA-based quantification method. Total yield was divided by total tissue amount to show ng of DNA per mg of leaf tissue.

tested. Because the extraction methods being tested are based on different chemistries, real-time PCR for the beta-tubulin gene was performed on a subset of 100-fold dilutions of plant extracts from both the methods. The differences in the resulting real-time PCR cycle threshold values were consistent with the amount of template measured to be present in each extract, or when adjusted to contain equivalent (1 ng) amounts of template (data not shown). These results indicate that matrix effects have not substantially influenced reported DNA recovery results.

Conclusions

Omega Bio-tek's spin column and magnetic bead-based approaches for plant DNA extraction performed significantly better than its competitor. Overall, automated methods were much faster and required much less hands-on time than either of the manual methods. Up to 96 samples if 50 mg wet tissue (or 15 mg dry tissue) can be processed in parallel in less than 1 hour using Omega Bio-tek's automated approach. Omega Bio-tek has leveraged their plant extraction experience and expertise to develop a robust solution for plant DNA extraction needs. Options are available for automated on open liquid handling and magnetic processor platforms, such as the Hamilton Microlab® STAR™, Hamilton Firefly® NIMBUS 96, and the Thermo KingFisher™ Flex®.

Table 1. Real-time PCR with corn-specific primers was performed on the triplicates of undiluted, 10-fold and 100-fold dilutions of DNA. DNA isolated using Omega Bio-tek's E.Z.N.A.® Plant DNA DS Kit and a comparable column-based kit from Company Q. Omega Bio-tek's kit not only has significantly higher yields, but also less qPCR inhibition when compared to that of Company Q's.

Extraction Method for Corn	Average C_t			ΔC_t	
	1X	10X	100X	(10X-1X)	(100X-10X)
Omega Bio-tek	2.388	26.324	29.978	2.935	3.654
Company Q	28.703	30.573	35.038	1.870	4.465

Table 2. DNA yield comparison from 23 different plant types. 50 mg leaf sample extracted per sample using Omega Bio-tek's Mag-Bind® Plant DNA DS Kit (M1130) and Company Q's recommended protocols. The lysis and binding buffers in the Mag-Bind® Plant DNA DS Kit and the E.Z.N.A.® Plant DNA DS Kits are identical. DNA concentration determined via fluorescence-based nucleic acid quantification. Total yield was divided by total tissue amount to show ng of DNA per mg of leaf tissue.

Plant Type	Company Q	Mag-Bind® Plant DNA DS Kit (M1130)
Hay	27.2	104.6
Tobacco	12.3	19.4
Peanut	6.3	52.9
Sunflower	41.8	89.1
Orange	4.6	31.2
Switchgrass	21.9	7.9
Pepper	6.9	111.0
Sugarcane	10.5	93.1
Jatropha	7.5	19.0
Oats	18.4	270.0
Wheat	0.5	152.3
Barley	9.6	198.1
Canola	3.4	59.0
Tomato	2.6	120.2
Apple	5.7	121.8
Grape	1.9	212.4
Alfalfa	17.9	85.2
Corn	4.0	29.8
Sugar beet	20.2	34.0
Soybean	26.8	25.4
Cotton	30.5	63.5
Sorghum	29.4	72.1
Potato	30.0	206.5

Product Information

Description	Product No.	Preps
E.Z.N.A.® Plant DNA DS Kit	D2411-00	5
	D2411-01	50
E-Z 96® Plant DNA DS Kit	D1411-00	1 x 96
	D1411-01	4 x 96
Mag-BIND® Plant DNA DS 96 Kit	M1130-00	1 x 96
	M1130-01	4 x 96

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