

Isolate high quality DNA from varieties of plant specimens with E.Z.N.A.™ plant DNA systems from Omega Bio-Tek

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The isolation of genomic DNA from plants is important for many applications in plant molecular biology. The emergences of PCR®, RAPD, and transgenic plant technology have greatly enhanced the speed and efficiency of crop improvement and breeding. A prerequisite for taking advantage of these methods is to isolate DNA of adequate of quality and quantity.

Although numerous DNA extraction methods or commercial kits for plants have been reported the preparation of plant DNA are still encountered a lot of questions because some plant contains high polyphenolic compounds, long-chain sugars, polysaccharides and other secondary metabolites (1). The polysaccharides presented in plants have a similar structure to nucleic acids and cannot be efficiently removed by most home-brew DNA isolation (2). These impurities in nucleic acid preparation can inhibit or reduce the efficiency of downstream applications such as PCR®, RT-PCR®, RAPD analysis and restriction digestion (3). Also there is an increase need for automated high throughput purification of genomic DNA from wide variety of plant species due to large-scale plant genetics and plant breeding projects are undertaken.

Plant scientists often use "homebrew" methods that require organic solvents such as phenol to inactivate the enzymes that degrade genomic DNA. In addition to the time and materials required for these techniques, the solvents pose a health hazard and a considerable expense in their disposal. Omega Bio-Tek has developed a completed plant DNA isolation system for single preparation (E.Z.N.A.™ Plant DNA system, SP Plant DNA system and HP Plant DNA system) and high throughput preparation (E-Z 96® Plant DNA system and Mag-Bind® Plant DNA system). E.Z.N.A.™ Plant DNA system, SP Plant DNA system and HP Plant DNA system use silica-member based spin column to isolate highly pure nucleic acids from a wide variety of plants. Mag-Bind Plant Kit use magnetic particles to bind DNA, which provided high throughput method to isolate genomic DNA with adaptation to automated robotic protocols. In this report below, we used three plant DNA kits from Omega Bio-Tek to isolate DNA from variety of plant species.

DNA quality

DNA quality is evaluated by agarose gel electrophoresis and OD reading from spectrophotometer.

PCR®

To further illustrate the importance of high-quality DNA for amplification, we analysed DNA purified from the leaf of tomato, rice, maize, and sweet potato. PCR® was performed in a thermal cycler PC-701 (Astec, Fukuoka, Japan) in 20 µl containing 1 x PCR® buffer (Takara), 200 µm of dNTPs (Takara), 0,5 U Taq DNA polymerase (Takara), 0,5 µm each of the primer pair, and an appropriate amount of the brain cDNA template. The PCR® cycle protocol was as follows: 94 °C for 1 min, 40 cycles of 94 °C for 30 sec, 52 °C for 30 sec, 72 °C for 30 sec, and 72 °C for 7 min. The PCR® products were analyzed on 1,5% agarose gels.

Results and Discussion

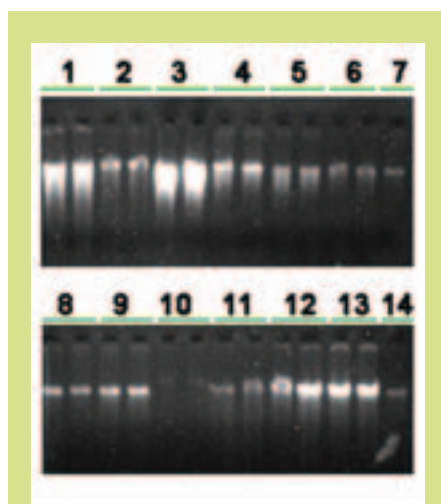
We have successfully isolated total DNA from 50 different plant species using three selected plant DNA kits. (E.Z.N.A.™ Plant DNA kit, SP Plant DNA kit, and E-Z 96 Mag-Bind Plant DNA System) and demonstrated that the DNA could be used for PCR® or RAPD analysis (Table 1). Selected test results are demonstrated below:

Table 1. Plant Sample Types Processed Using Omega Bio-Tek Plant DNA Isolation Kits

Wheat leaf	Pawpaw leaf	Chicory
Maize leaf	Earth pea	Sorghum
Paddy leaf	Sunflower	Pea
Sweet Potato leaf	Barley	Cabbage
Soybean	Maize Seed	Onion
Tomato leaf	Rice flour	Cucumber
Ginger leaf	Wheat Flour	Milkweed
Garlic	Maize flour	Lettuce
Potato leaf	Tobacco	Squash
Pepper leaf	Cotton	Chive
Carrot	Chickpea	Strawberry
Caraway	Cole	Pine
Apple	Lichee	Mango
Pear	Redbud	Glass
Bamboo	Grape	Lemon
Arabidopsis thaliana (L.) Heynh		

E.Z.N.A.™ SP Plant DNA Kit

The SP Plant DNA kit provided a rapid and simplified procedure for isolating total DNA from most common plant samples with a fast



- | | |
|-----------------|-----------------------------|
| 1. Pawpaw Leaf | 8. Sweet Potato Leaf |
| 2. Maize Seed | 9. Pine Leaf |
| 3. Cassava Leaf | 10. Sunflower leaf |
| 4. Ginger Leaf | 11. Glass |
| 5. Caraway Leaf | 12. Allium sativum L (seed) |
| 6. Cabbage Leaf | 13. Garlic |
| 7. Rice Flour | 14. Apple |

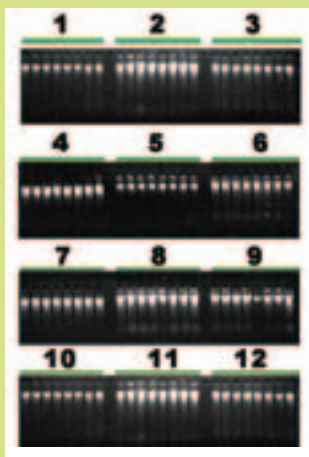
Figure 1. Genomic DNA was isolated from 14 different Plant species (50 mg) using E.Z.N.A.™ SP Plant DNA Kit. 1/20 of total DNA yield was analyzed on a 0,7% agarose gel.

Materials and Methods

Samples were collected from fields or obtained from commercial sources. Qitec Biotech Co. Ltd supplied all the reagents except reagents from kits.

DNA extraction

DNA extractions were performed by following instructions in each kit.



- 1. Pawpaw Leaf
- 2. Maize Seed
- 3. Cassava Leaf
- 4. Ginger Leaf
- 5. Caraway Leaf
- 6. Cabbage Leaf
- 7. Rice Flour
- 8. Sweet Potato Leaf
- 9. Pine Leaf
- 10. Sunflower leaf
- 11. Glass
- 12. Allium sativum L (seed)

Figure 2. Genomic DNA was isolated from 12 different plant species (30 mg) with E.Z.N.A. Mag-bind Plant DNA Kit. 1/10 of total DNA Yield was analyzed on 0,7% agarose gels.

Table 2. Typical DNA yield from Different Plant Species Using E.Z.N.A.™ SP Plant DNA Kit

Plant Specimens (50mg)	Total DNA Yield
Tomato Leaves	25-40 µg
Paddle Leaves	10-15 µg
Maize Leaves	20-35 µg
Maize Seed	20-32 µg
Potato leaves	5-6 µg

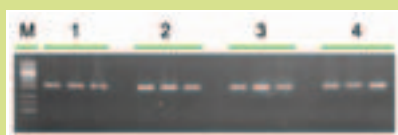


Figure 3. Amplification of purified DNA with PCR. DNA was purified from Tomato (1), Rice (2), Maize (3), Sweet Potato (4), using E.Z.N.A. Mag-Bind Plant DNA. The purified DNA was amplified using PCR by GAPDH primer, and PCR products were separated on 1,5% agarose gel.

Product	Size	Cat. No.
Plant DNA Mini Kit	50	733-0823
Plant DNA Mini Kit	200	733-0824
E-Z 96 Plant DNA Kit	1 x 96	733-0819
E-Z 96 Plant DNA Kit	4 x 96	733-0820
SP Plant DNA Kit	50	733-0825
SP Plant DNA Kit	200	733-0826
HP Plant DNA Kit	50	733-0821
HP Plant DNA Kit	200	733-0822
E-Z 96 Mag-Bind Plant DNA Kit	2 x 96	733-0827
E-Z 96 Mag-Bind Plant DNA Kit	8 x 96	733-0828

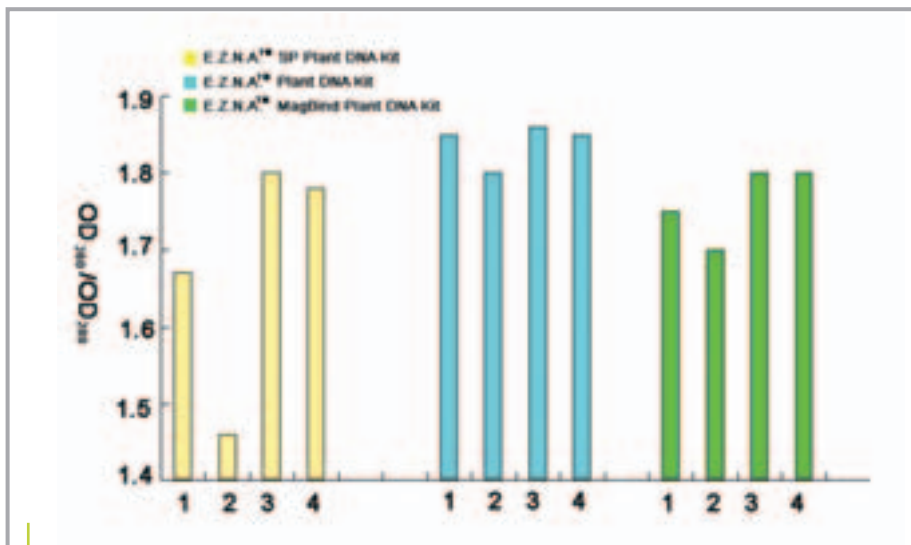


Figure 4. The Ratio of OD260 and OD280. Four difficult Plant specimens (1: Potato leaves, 2: rice powder, 3: tobacco, 4: cotton) were used to isolate genomic DNA by these three kits. The purified DNA was analyzed by UV DU640 (Beckman) at OD260 and OD280. The OD260/OD280 show the quality of extracted DNA. Yellow is for SP Plant DNA, Blue is for Plant DNA Kit, and Green is for Mag-Bind Plant DNA.

and convenient protocol. Figure 1 demonstrates that good quality of DNA can be successfully isolated from 14 selected plant species. Due to the space limit, typical yield from five selected samples is shown in Table 2.

E-Z 96® Mag-Bind Plant DNA Isolation Kit

By using the E-Z 96 Mag-Bind Plant DNA Kit, high quality DNA was successfully isolated from 30 mg of 12 different Plant samples within 1 hour (not including samples dispense and preparation). DNA quality was analysed through gel electrophoresis and PCR. Figure 2 shows the result of the 1/20 extracted DNA run on a 0,8% agarose gel. Figure 3 shows the results of PCR product run on a 1,8% agarose gel.

E.Z.N.A.™ Plant DNA kit

As Omega Bio-Tek's original plant DNA isolation system, the E.Z.N.A.™ Plant DNA system is very reliable and suitable for a wide variety of plant samples including some samples containing high level flavones, long chain sugars, polyphenolic material and other

secondary metabolites. Traditional methods and many other commercial kits normally have difficulty with those samples. To show the benefit of this system, four plant specimens (potato leaves, rice powder, tobacco leaf, cotton leaf) were used to isolate DNA corresponding by three systems. DNA quality was analysed and compared by spectrophotometric analysis (Figure 4).

Conclusion

DNA purification from plant samples has become the bottleneck in sample processing from plant sample to PCR results. By combining silica membrane based HiBind® technology and paramagnetic beads based Mag-Bind® technology with our proprietary buffer system, Omega Bio-Tek offers a complete solution for plant DNA isolation from widely diverse plant materials and samples types.

References

1. Varadarajan G,S and Parakash C,S (1991) A rapid and efficient method for the extraction of total DNA from the sweet potato and its related ration. *Plant Mol, Biol. Reporter* 9, 6.
2. Do N. and Adam RP. A simple technique for removing plant polysaccharide contaminants from DNA. *Biotechniques*. 10: 162-166.
3. Pandey RN, Adams RP and Flourmoy LE (1996) Inhibition of random amplified polymorphic DNAs (RAPDs) by plant polysaccharides. *Plant Mol Biol Rept* 14: 17-22.

