MagAttract® 96 DNA Plant Kits — for high-throughput isolation of DNA from plant tissue

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Introduction

A major limitation of plant genetics is the isolation of DNA from many plant samples in parallel. Often, plant researchers rely on laborious manual DNA purification techniques to obtain DNA for large plant screening projects. These techniques can involve centrifugation or organic phase separation steps, which are difficult to automate and additionally do not always remove plant metabolites, which can inhibit downstream enzyme applications.

The MagAttract 96 Plant Kit combines the speed and efficiency of silica based DNA purification with the convenience of magnetic particles, allowing high-throughput purification of plant genomic DNA (Figure 1). MagAttract technology provides high-purity DNA, which is suitable for use in most downstream applications. In addition a manual and automated protocol, for use on the BioRobot® Plant Science system, is available.

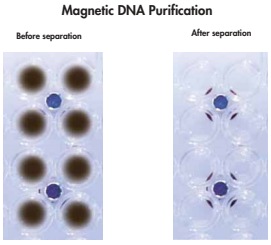


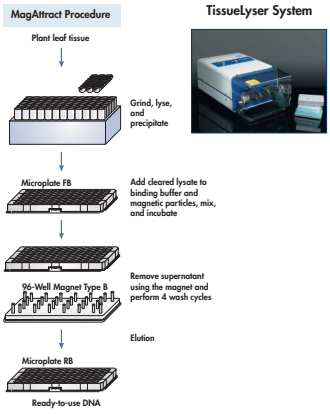
Figure 1 Genomic DNA selectively binds to the surface of the MagAttract particles and can then be further purified by washing the magnetic particles. Magnetic rods, which fit between the wells of a microplate or round-well block are used to attract all the particles to one side of the well, allowing the buffer to be removed.

Principle and Procedure

Fresh, frozen, or lyophilized starting material (10–100 mg) is mechanically disrupted to give a fine powder. The powder is resuspended in lysis buffer, thoroughly mixed, and then sedimented by a short centrifugation step. The lysates are then transferred to 96-well flat-bottom microplates. Genomic DNA selectively binds to the surface of MagAttract particles and is further purified by washing the magnetic particles with alcohol-containing buffers and ethanol. Pure genomic DNA is eluted from the particles with low-salt Buffer AE into a 96-Well Microplate and is ready for use in downstream applications.

Disruption of plant tissue

For disruption of plant tissue, optimal results are obtained using the TissueLyser together with the TissueLyser Adapter Set 2 x 96 and Tungsten Carbide Beads. The TissueLyser provides rapid and efficient disruption of 2 x 96 samples in 5 minutes. Plant tissue samples and a 3 mm bead are added to each of 192 collection microtubes in two racks. The racks are fixed into the clamps on the TissueLyser using adapter plates and disrupted by two 1-minute high-speed (30 Hz) shaking steps. Either fresh or lyophilized plant tissue samples can be processed using the TissueLyser.



Reproducible Yields

For consistent experimental data and the avoidance of false negative results, it is critical that DNA can be reproducibly purified. This is particularly important when undertaking large plant screening programs. In order to test the reproducibility of DNA yield using the MagAttract 96 DNA Plant Kit, 96 Maize samples were disrupted and homogenized using the QIAGEN® TissueLyser (Figure 2).

When stained with ethidium bromide and viewed under UV light, each sample showed a uniform intensity, thus providing evidence of the high reproducibility of sample yield using this system. Typical DNA yields are shown in Table 1.

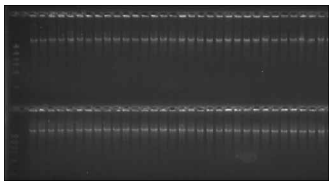


Figure 2 Maize lysate (30 ml) was generated and divided into aliquots. Each aliquot (containing DNA from 60 mg of wet plant material) was processed using the BioRobot Plant system, and 20 µl of each eluate (100 µl) was loaded onto a 0.8% agarose gel.

Table 1. Typical Yields Using the MagAttract 96 DNA Plant Kit

Sample*	Amount	Total yield (µg)
Arabidopsis leaf	100 mg	5.0
Barley leaf	30 mg	4.5
Corn leaf	60 mg	12.0
Flax	40 mg	5.5
Rape (Canola)	40 mg	5.7
Rye leaf	30 mg	5.1
Ryegrass (Lolium spp.)	30 mg	4.0
Sunflower leaf	30 mg	4.8
Tobacco leaf	40 mg	1.3
Tomato leaf	50 mg	7.5
Wheat leaf	30 mg	4.8

* DNA has been successfully purified from many species and tissues not listed.

High-Quality, Ready-to-Use DNA

Variation between different plant species often necessitates the use of different DNA isolation techniques, depending on the species being studied. For example, rapeseed has a high fat content, while some plant tissues (e.g., rose) contain high levels of secondary metabolites, which can inhibit enzyme activity in downstream applications. Removal of secondary metabolites can be a particular problem when using homemade DNA purification techniques.

To assess the suitability of the MagAttract 96 DNA Plant Kit for purifying DNA from a wide range of plant species, tissues from 6 different plant species were subjected to the manual MagAttract procedure. Subsequent PCR amplification of the purified DNA revealed high yield of specific product, suggesting that secondary metabolites had been effectively removed (Figure 3).

The MagAttract 96 DNA Plant Kit also provides DNA suitable for use in quantitative RT-PCR. Low SD and CV values indicate high uniformity and reliability between samples (Figure 4).

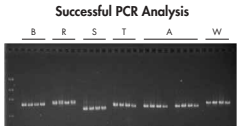


Figure 3 DNA from leaves of different plants was analyzed using the universal trnl PCR system (amplifying an intergenic region of a tRNA coding gene in the chloroplast genome). **B**: barley; **R**: rye; **S**: sunflower; **T**: tomato; **A**: arabidopsis; **W**: wheat.

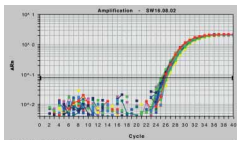


Figure 4 A lysate was made from 1.2 g of Maize leaves using the TissueLyser system. The lysate was divided into 48 samples and purified on a BioRobot using the MagAttract Plant Kit. The beta-amylase gene was amplified from each sample using the TaqMan® system. Average CT: 25.54; standard deviation: 0.32; CV: 1.24%.

Large Size DNA

Pulse Field Gel Electrophoresis (PFGE) was performed to determine the size of the DNA purified using the MagAttract procedure. DNA from 5 different plant species was purified from 60 mg of tissue and eluted in 100 µl Buffer AE. Twenty microliters of the eluate were run on a pulsed field gel (Figure 5). The average DNA size was approximately 23 kb, which is sufficiently large to perform restriction digest analysis of the DNA using frequently cutting enzymes.

DNA from rye, sunflower and tomato plants (60 mg) was purified using the MagAttract 96 Plant Kit. Two samples (20 µg) from each plant species were digested using the restriction enzyme *Bam*HI. Both digested samples and two undigested samples, from each plant, were run on a 0.8 % agarose gel (Figure 6).

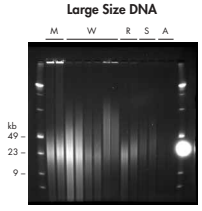


Figure 5 DNA (1 µg) from different plants, purified using the MagAttract DNA Plant Kit, was subjected to PFGE (6 V/cm, 2–8 sec switch time, 20 h) on a 1 % agarose gel using 0.5x TBE buffer. **M**: maize; **W**: wheat; **R**: rye; **S**: sunflower; **A**: arabidopsis.

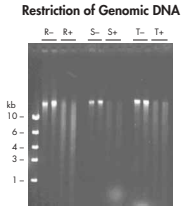


Figure 6 DNA (20 µl of 100 µl eluate) from different plants was digested (60 min at 37°C) with the restriction enzyme *Bam*HI and run on a 0.8% agarose gel. **R**: rye; **S**: sunflower; **T**: tomato; – = undigested; + = digested.

Conclusions

- The data presented demonstrate that the MagAttract 96 DNA Plant system provides reproducible yields of pure, ready-to-use DNA.
- Both manual and automated (using the BioRobot Plant Science system) procedures enable rapid and reliable DNA purification.
- DNA purified using the MagAttract 96 DNA Plant system is suitable for most downstream applications including: PCR, real-time PCR, microsatellite analysis, SNP genotyping and restriction digestion.

BioRobot Plant Science System

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