Performance Comparison of SYNERGY™ MNP DNA Extraction with Major Competitor MNP Kit

Written by Jessica Chicola

INTRODUCTION

Synergy™ homogenization chemistry for efficient cell lysis was coupled with silica coated magnetic nanoparticles (MNPs) to isolate DNA from plant samples. MNP based isolations are increasingly popular for nucleic acid isolations as processing can be automated, saving time and expense. The use of automation is especially helpful in high throughput laboratories. This experiment was conducted to compare the performance of a hybrid Synergy™ DNA extraction using MNPs to a market leader of MNP DNA isolation kit for plant DNA. Wheat tissues of the roots, stems, and leaves were used as models to compare the extraction chemistries and processes. For leaves and stems, the Synergy™/MNP hybrid protocol was able to isolate DNA with comparable performance to its competitor, while it was more efficient in isolating DNA from wheat root than its competitor.

METHODS

The extraction process was divided into three parts for review: homogenization, isolation, and analysis.

HOMOGENIZATION

Young wheat plants, near a week old, were removed from a planter with the roots intact. The plants were washed with water to remove residual soil. The root, stem, and leaf samples were cut and weighed (mg), in triplicate for each processing method. The average mass for each sample is recorded in Table 1.

Table 1. Average sample mass for MNP process comparison.

	Average Mass (mg)	
Sample	**SYNERGY™**	**Competitor**
Root	2.23	5.9
Stem	5.1	5.07
Leaf	5.37	5.33

Synergy™ Protocol: The Synergy™ samples were homogenized according to the Synergy™ 2.0 Plant DNA Extraction protocol. The samples were added to 2 ml Synergy™ homogenization tubes with 500 µl of Plant Homogenization Buffer.The tubes were homogenized for 1 minute in the HT 24™ at 4260 rpm and then centrifuged at 15,000 x g for 5 minutes. The clear lysate was used for the subsequent isolation steps.

info@opsdx.com 908-253-3444 PO Box 348, Lebanon, NJ 08833

Competitor Protocol: The competitor samples were homogenized in 2 ml disruption tubes with 2.8 mm stainless steel grinding balls (PFSS 2800-50-20U) in a HT 24™ according to the protocol provided with the kit. Each homogenization tube contained plant sample, 500 µl Lysis Buffer A, 70 µl Lysis Buffer B, and 20 µl RNase A. Tubes were vortexed prior to bead beating for 1 minute at 4260 rpm. The homogenate was incubated in a thermomixer for 10 minutes at 65°C while shaking at 700 rpm. Precipitation Solution (130 µl) was added to each tube, inverted to mix, and then centrifuged at 16,000 x g for 10 minutes. The lysate was used for the subsequent isolation step.

ISOLATION

Instrumentation: Following homogenization, all samples were extracted with magnetic nanoparticles using an IsoPure™ Mini automated extraction system. The IsoPure™ Mini is designed to function like larger magnetic bead extraction systems, such as the KingFisher, but on a smaller scale. It can process two columns, or 16 samples, at a time. It is efficient for lower throughput MNP sample processing. Samples were loaded in columns 1 and 7 of a 96 well plate, while the other columns contained the necessary MNPs, binding and wash solutions for the isolation. Magnets with plastic sleeves move the MNPs in parallel between wells 1-6 and 7-12 for the purification process. The IsoPure™ Mini is also temperature controlled to aid in stringency washes of the beads.

Synergy™ and competitor samples were loaded onto separate plates to avoid cross contamination. Both processes used the same IsoPure™ Mini purification program.

Synergy™ Protocol: Plate set up occurred in two stages. In columns 1 and 7 of a deep well plate, 5 µl of RNase A was added, followed by 180 µl lysate. The wells were sealed, and samples incubated at 37°C for 15 min.

The remaining reagents were added to the plate as follows: 400 µl ethanol (EtOH) is added to the lysate in columns 1 and 7 columns, 25 µl of OPSD MNPs to the 2nd and 8th columns, 500 µl of Wash 1 to the 3rd and 9th columns, 500 µl of 70% ethanol to columns 4 and 10, the 5th and 11th columns were left empty, and the 6th and 12th columns contained 100 µl of molecular biology grade water (MBW) for the elution. The layout of each row is represented by Table 2.

Table 2. Displays one row of the preloaded plate for the automated MNP isolation. Each sample has its own row reflecting what is seen in the table.

Lysate 5 µl RNase A 400 µl EtOH	25 µl OPSD MNPs	500 µl Wash 1	500 µl 70% EtOH	Empty	100 µl MBW

The plate is placed in the IsoPure™ Mini automated extraction system which is programmed to isolate the DNA with the MNPs. The automated protocol is described in detail in Figure 1.

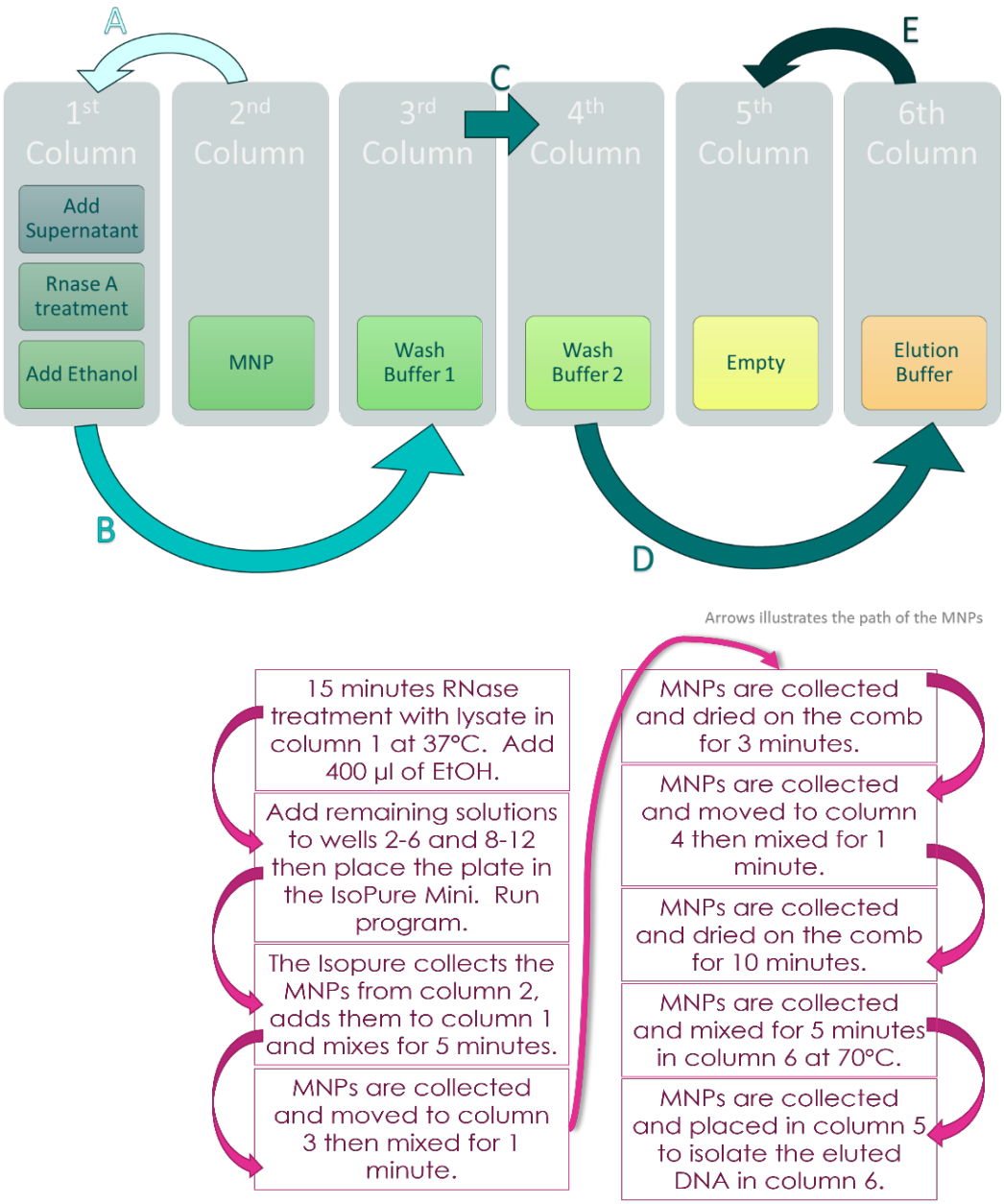


Figure 1. Detailed overview of the automated process for DNA extraction with the IsoPure Mini automated extraction system.

Competitor Protocol: All solutions were provided in the competitor kit, except ethanol. Columns 1 and 7 were preloaded with 400 µl of EtOH. The 2nd and 8th columns contained 25 µl of MNPs. The 3rd and 9th columns contained 800 µl of Wash 1 while the 4th and 10th columns have 800 µl of Wash 2. The 5th and 11th columns remain empty, and the 6th and 12th columns contain 100 µl of Elution Buffer. Table 3 summarizes the sample and reagent loading of the deep well plate.

Table 3. Sample and reagent loading for competitor deep well plate.

Lysate 400 µl EtOH	25 µl Competitor MNPs	800 µl Wash 1	800 µl Wash 2	Empty	100 µl Elution Buffer

Centrifuged lysate from the homogenization step (400 µl) was added to the 1st and 7th columns. The plate was placed in the IsoPure Mini, and the automated protocol was started. The protocol is identical to the one used for the Synergy™ samples (Figure 1).

ANALYSIS

The samples were analyzed by agarose gel electrophoresis, fluorescence, absorbance, and polymerase chain reaction (qPCR). Each method was aimed to determine the quantity and quality of the isolated DNA samples.

Agarose gels were 1% GTG agarose with Ethidium Bromide in 1X TAE buffer. The gel ran for 25 minutes at 104 V with 5 µl of DNA per well.

Samples were run on a DNA chip on a Bioanalyzer (Agilent). The chip uses fluorescence to quantify the DNA sample while producing a size analysis of the DNA.

A DeNovix spectrophotometer was used to test DNA purity using the 260/280 ratio.

QPCR was performed on a QuantStudio 5 (ThermoFisher) using SYBRGreen Universal Mastermix (BioRad). The PCR reaction targeted the *Triticum aestivum* isolate N7A-2-2 waxy gene (GenBank: JF682716.1). The primer set used was as follows:

Forward primer: 5'-CGAGCGGGCGAGTACAAATA-3'
Reverse primer: 5'-GCTCGTGAGTGAGTGAGTGT-3'

The reaction cocktail included 10 µl SYBRGreen, 8.6 µl MBW, 0.2 µl of the forward primer, and 0.2 µl of the reverse primer. Each reaction had a total volume of 20 µl; every tube received 19 µl of the cocktail and 1 µl of sample. The reaction protocol was the holding stage ran for 180 seconds at 95°C; the reaction then cycled through 10 seconds at 95°C, 30 seconds at 51.8°C and 40 seconds at 72°C for 40 cycles.

RESULTS

All bead beating shears DNA, thus the agarose gel of electrophoresed samples displayed broad bands in the 2-7 Kb range rather than genomic smears (Fig. 2). Both Synergy and the competitor processes yielded DNA. The Synergy process yielded more DNA from roots especially when considering the mass of the starting material was about half.

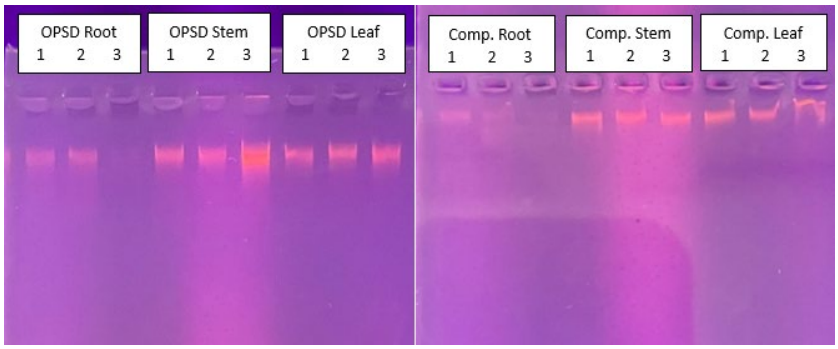


Figure 2. Agarose gels showing the bands of MNP isolated DNA.

Collaberating the agarose gel, the electropherograms from the Bioanalyzer yielded similar results. In Fig. 3, the size of the sheared DNA is more noticeable and is a consequence of the bead beating. The competitor root samples appear as faint bands while the OPSD root sample are faint but visible.

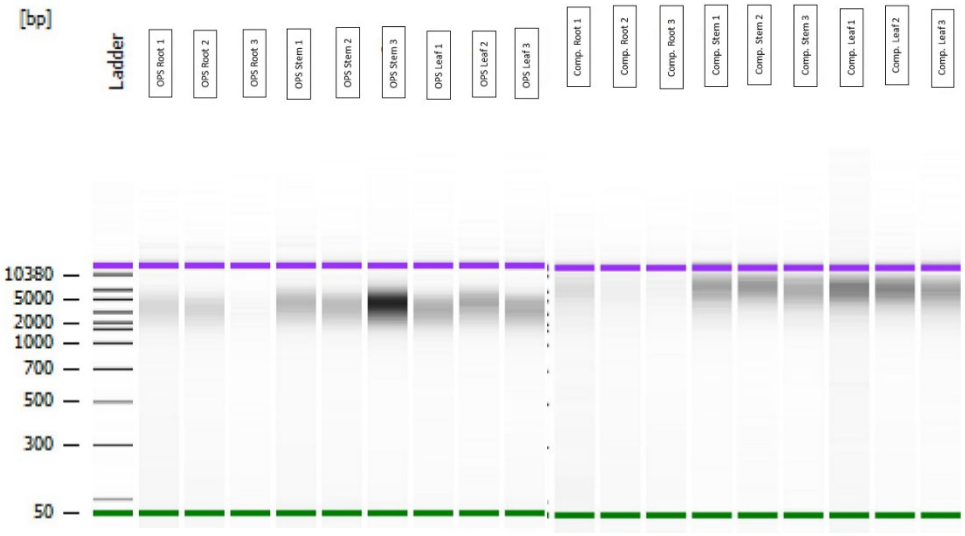


Figure 3. Bioanalyzer generated bands of the DNA samples isolated using MNPs.

info@opsdx.com 908-253-3444 PO Box 348, Lebanon, NJ 08833

Average concentrations of DNA were comparable between Synergy and the competitor kit, the root samples being the exception (Fig. 4). As noted above, sample mass for the Synergy root was half of the competitor kit. The concentration of root DNA from the competitor kit was undetectable even though a 260/280 ratio was measured below (Fig. 5). It is possible that the grinding matrix used on the competitor sample (2.8 mm grinding balls in a disruption tube) inadequately homogenized the roots and that the DNA liberated was larger than 15 Kb. Large DNA fragments are not measured on the Bioanalyzer while a sum of the smaller fragments is measured.

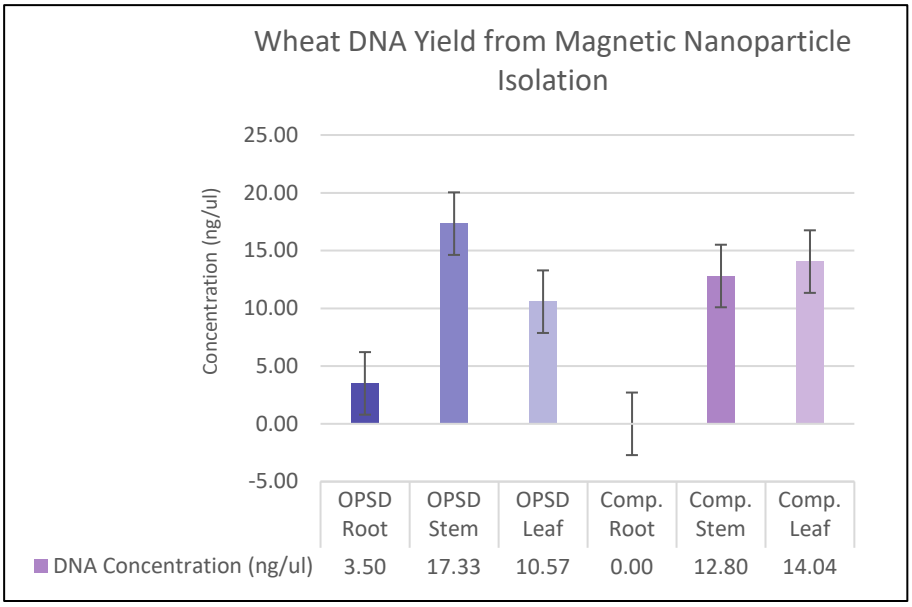
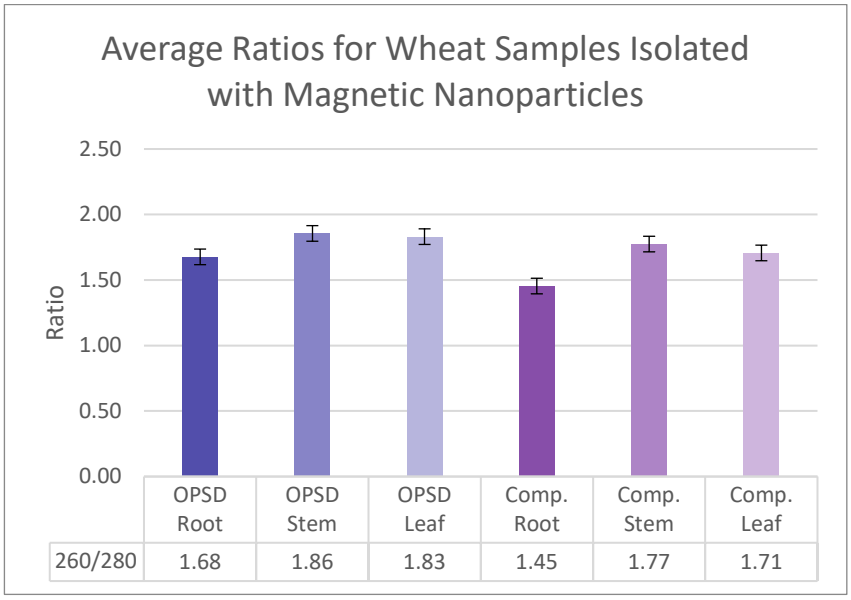


Figure 4. Concentration of each sample in nanograms per microliter.

Both Synergy™ and the competitor samples have good purity as measured by the 260/280 ratios, the one exception is the competitor root samples. The competitor root samples have a significantly lower 260/280 ratio than the Synergy™ root samples.



info@opsdx.com
908-253-3444
PO Box 348, Lebanon, NJ 08833

Figure 5: Display of the average 260/280 ratios for each sample.

QPCR was performed on the samples, the resulting cycle threshold (C_t) averages are presented in Fig 6. The Synergy™ root samples had a significantly lower C_t than the competitor indicating a higher copy number for the selected gene. The stem and leaf samples C_t's are comparable between the Synergy™ and competitor samples.

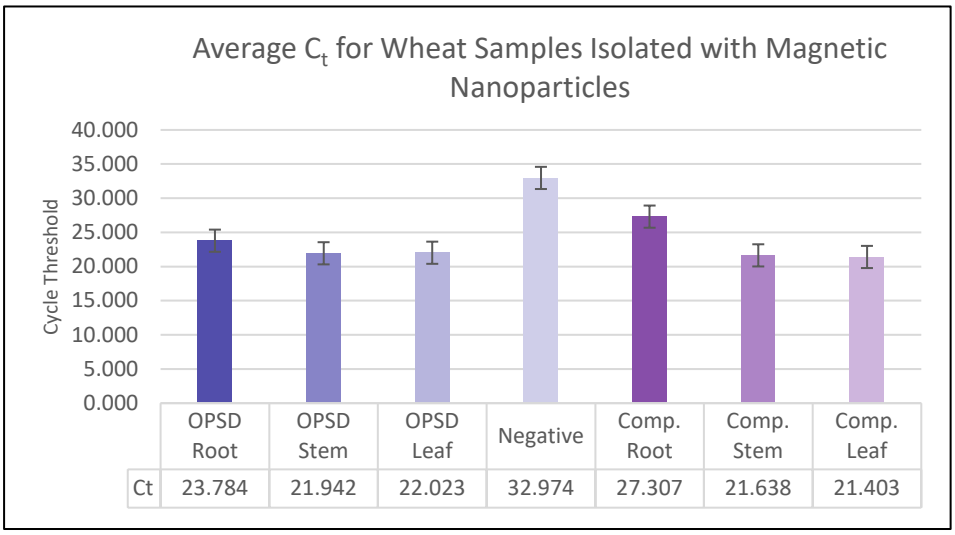


Figure 6: Cycle thresholds (C_ts) for each sample.

DISCUSSION

Overall, the Synergy™/MNP hybrid isolation was highly comparable to the leading competitor MNP kit, with the exception of price. The results of the experiment show that the Synergy™ extraction is more efficient than the competitor when extracting DNA from root samples. There is no major difference between Synergy™ and its competitor when extracting DNA from stem and leaf samples. Table 4 summarizes the comparison of the two isolation processes.

Table 4: Summary of the analysis including the values for the concentration, ratios, and cycle thresholds. The better results are bolded.

	Synergy			Competitor		
Sample	C_t (N7A)	260/280	Concentration (ng/µl)	C_t (N7A)	260/280	Concentration (ng/µl)
Root	**23.784**	**1.68**	**3.50**	27.307	1.45	0.00
Stem	21.942	**1.86**	**17.33**	**21.638**	1.77	12.80
Leaf	22.023	**1.83**	**10.57**	**21.403**	1.71	14.04

DNA isolation kits have become very popular, and such, are now offered by an array of manufacturers. A cost analysis of products from companies like ThermoFisher, Zymo Research, MagBio Genomics, Omega-Biotek, and Machery-Nagel show a wide range from about $200 to $500 per 96 preparations. Only the higher end kit includes a homogenization plate like Synergy™. Kits that do not include homogenization tubes or plates will require additional time or funds to homogenize the samples. Currently the Synergy™ 96 Well kit sells for around $550, which is for 2 x 96 preparations. It is estimated that a Synergy™/MNP kits, which is in development and currently being tested (summer 2023), will be approximately the same cost as the conventional 96 well kit. This will make the Synergy™/MNP product not only suitable for plant DNA isolations, but also highly competitive in price to other kits that do not include the homogenization materials.

info@opsdx.com 908-253-3444 PO Box 348, Lebanon, NJ 08833