High Throughput DNA Extraction from Staple Crops Utilizing the Bead Ruptor™ 96 Bead Mill Homogenizer and chemagic™ 360 Nucleic Acid Extractor

Madelyn Carlson, Caleb Proctor M.S.; Gabriella Ryan M.S.; Rodney Nash Ph.D.

Summary

Plants are a crucial part of our diet and the entire ecosystem we inhabit. In fact, plants are such a vital aspect of our planet that they account for over a quarter of new species discovered yearly [1]. With this rapid level of discovery, researchers are in constant need to understand the genetic makeup and diversity of plant species. Lysing plant material to expose the genetic material contained within samples during preparation, however, can be quite difficult due to a variety of factors with the thick polysaccharide cell wall being among the most obstructive.

Enzymatic lysis is often used to break down these cell walls and expose the genetic material within; however, these methods can be time consuming and ensuring complete breakdown of the cell wall is very difficult. In addition to the challenging nature of the plant cell; every variety of plant type will provide their own obstacles due to their unique genomic makeup and could require optimization tailored to each species.

Enzymatic lysis however, is not the only form of lysis that can be utilized. Physical disruption is a frequently utilized alternative with some common implementations including manual methods such as mortar and pestle as well as vortexing. While these manual methods are suitable and are often cited in protocols or publications, they require manual effort on the part of the researcher which can introduce variability, as well as limiting throughput. Introducing semi-automated sample preparation can help solve this problem by delivering a consistent and repeatable processing step. One such method can be found in physical bead-based lysis.

Bead mill homogenization can lyse difficult samples with consistency that manual methods struggle to achieve. Consistency is not the only value provided by homogenization however, in addition to this consistency, semi-automated bead beating offers a significant time savings compared to manual methods allowing the user to process far more samples than would be possible with other lysing methods. OMNI International offers a wide range of sample prep solutions to meet the researchers needs, with the Bead Ruptor™ 96 bead mill homogenizer offering a high throughput solution. Utilizing this instrument, users can process up to 192 samples simultaneously with enough force to homogenize their toughest samples prior to downstream processes.

Plant tissue is utilized in numerous downstream applications including PCR, sequencing, and genotyping among others. These nucleic acid-based applications require the genetic material contained within the plant tissue to be purified prior to analysis. As with lysis, several nucleic acid isolation techniques are available to the researcher; with manual spin column-based, or magnetic bead-based extractions being common manual extraction methods for the researcher. These methods, however, are limited in a variety of ways with the inherent variability of manual handling and limited throughput being of the most concern in plant genomic applications. Automating the nucleic acid extraction process not only ensures consistency from sample to sample, but also allows the researcher to purify a larger number of samples in only a fraction of the time that would be required otherwise. The chemagic™ 360 instrument enables automated nucleic acid extraction and utilizes patented magnetic bead technology and automated reagent filling to carry out automated extraction of nucleic acid from as many as 96 samples in one run.

As plants require greater amounts of genetic data to fuel the needs of next generation data analysis, higher sample volumes will be required to meet the demands of the rigorous research requirements ahead. Modern techniques such as next generation sequencing or long read sequencing are not only allowing for a deeper analysis of a sample's genetic material; but also allow for an exponentially higher number of samples to be examined at once. As such, high throughput workflows are becoming a necessity in today's research laboratories. In this work, we demonstrate a high throughput plant DNA workflow utilizing the Bead Ruptor™ 96 bead mill homogenizer and chemagic™ 360 automated nucleic acid extractor for end-to-end sample preparation and DNA isolation from a variety of staple crops yielding high quality DNA suitable for next generation sequencing.

Materials and Methods

Equipment

- Pre-filled 2 mL Deep Well Plates with 1.4 mm Ceramic Beads (OMNI, Cat # 27-6009)
- Bead Ruptor™ 96 Bead Mill Homogenizer (OMNI, Cat # 27-0001)
- Bead Ruptor™ 96 Well Plate Adapters (OMNI, Cat # 27-101)
- chemagic™ 360 Instrument (Revvity, Cat # 2024-0020)
- chemagic™ DNA Plant 20 Kit H96 (Revvity, Cat # CMG-795)
- chemagic™ 96 Deep Well Plate (Revvity, Cat # CMG 555-15)

Procedure

To demonstrate this protocol's applicability across different plant species, crops that are ubiquitous in agriculture were utilized. A variety of seeds were obtained from a commercial seed store including corn, wheat and rice plants. The seeds were then sprouted via pre-soaking seeds in water then wrapping them in a wet paper towel and placing them in the dark for 5 days. Once sprouted, radicles were obtained from the sprouted seeds and aliquoted into the appropriate sizes of 10 mg with a tolerance of +/- 1 mg. To assist in removal of inhibitors from the lysate, a CTAB buffer was created to aid in the break down polysaccharides. This buffer was created following protocol posted by the Soltis lab [2, 3]. A total of 60 corn, 96 rice and 12 wheat radicles were obtained for processing.

Each plant tissue sample was placed into a 2 mL 96 deep well plate pre-filled with 1.4 mm ceramic beads (OMNI, Cat # 27-6009) along with 200 µL of pre-made CTAB buffer. The well plate was then homogenized on the Bead Ruptor 96 bead mill homogenizer (OMNI, Cat # 27-001) at 30 Hz for 2 minutes. Once processing was complete, the plate was then centrifuged at 4700 rpm for 15 minutes to pellet cellular debris and eliminate foam from CTAB buffer. After centrifugation was completed, 3 µL of RNase was added to each well, and the plates were sealed with PCR adhesive (BioRad, Cat # MSB1001) then incubated at 55° C for 1 hour. Once incubation was completed, the plates were centrifuged again at the above settings to settle evaporate.

When completed, 100 µL of lysate was transferred into a Riplate (CMG 555-15) containing 300 µL of lysis buffer provided in the chemagic DNA Plant 20 H96 Kit (Revvity, Cat # CMG-795). This filled well plate was then placed onto the chemagic™ 360 nucleic acid extractor. The standard chemagic DNA Plant 20 H96 protocol was executed with the only modification being a reduction in elution buffer volume to 75 µL. Following extraction, the resulting eluted DNA was then quantified via spectrophotometry to determine concentration and quality.

Results

Sixty corn radicals were extracted using the above method which resulted in an average total DNA yield of 1531.25 ng. An additional 96 rice samples as well as 12 wheat samples were also processed in the same manner and resulted in an average total DNA yield of 997.97 ng and 965.25 ng, respectively. In addition to concentrations, quality was measured in the form of both A_{260}/A_{280} and A_{260}/A_{230} ratios. Utilizing the Bead Ruptor™ 96 homogenizer with the chemagic™ 360 instrument, all samples were processed and extracted, resulting in the average concentrations and ratios listed below in Table 1. Average nucleic acid concentration from the three sample types had standard deviations of 11.70 ng/µL, 12.98 ng/µL and 3.67 ng/µL for corn, rice and wheat respectively. The DNA concentration spreads for all three sample types are displayed in Figures 1-3.

Sample Type	Concentration (ng/µL)	A_{260}/A_{280}	A_{260}/A_{230}	Total Yield (ng)
Corn	20.42	1.99	1.32	1531.25
Rice	13.31	1.96	1.34	997.97
Wheat	12.87	1.78	1.26	965.25

Table 1. Average Nucleic Acid Concentrations, A_{260}/A_{280} Ratios, A_{260}/A_{230} Ratios, and Total Yields for Each Crop

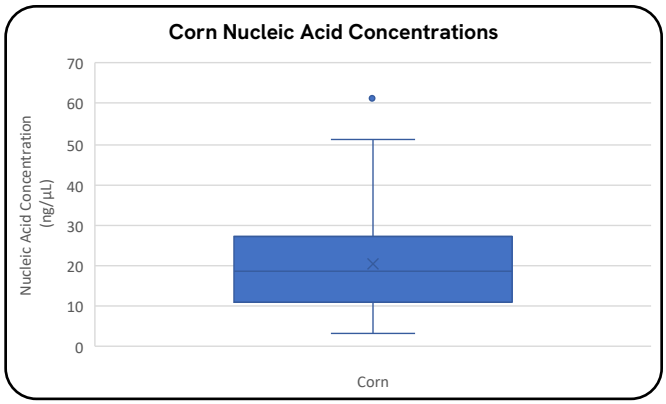

Figure 1. Spread of Corn Radicle DNA Concentrations (ng/µL)

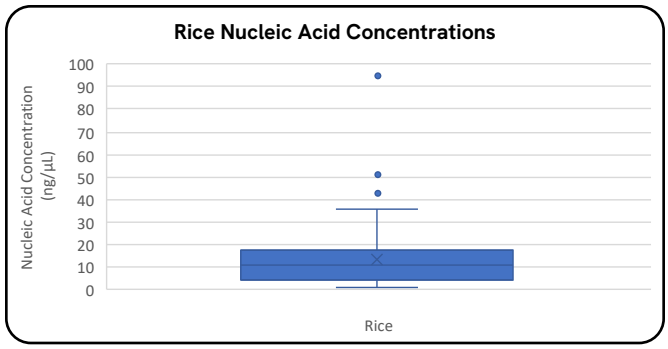

Figure 2. Spread of Rice Radicle DNA Concentrations (ng/µL)

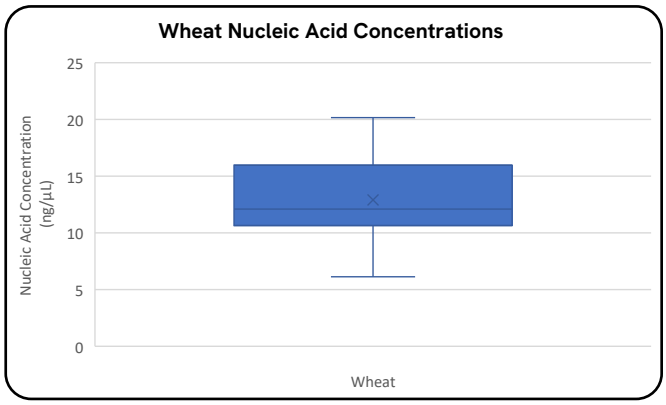

Figure 3. Spread of Wheat Radicle DNA Concentrations (ng/µL)

Conclusions

Based upon the results shown in the above application note, we have demonstrated a high throughput workflow for plant applications that results in a high quality and quantity product. By introducing high throughput sample preparation and nucleic acid extraction with the Bead Ruptor™ 96 bead mill homogenizer and chemagic™ 360 nucleic acid extractor, researchers can process multiple samples in a more robust manner compared to alternative methods. The Bead Ruptor™ 96 bead mill homogenizer provides a simple and efficient method for simultaneously processing up to two 96 well plates worth of samples resulting in a consistent and quality homogenate, as well as being capable of processing a researcher's toughest samples with excellent quality and time savings. By adding further automation in the form of extraction via the chemagic™ 360 instrument, the end user can be confident in the extracted nucleic acid quantity and quality. Utilizing these methods, the researcher is able to implement a high throughput workload of samples with a far greater efficiency than if a similar workload was attempted by manual homogenization and extraction methods. These methods will not only save the researcher time but result in a consistent product they can reliably refer to for future downstream processes.

References

1. Christenhusz, M. J., & Byng, J. W. (2016). The number of known plants species in the world and its annual increase. Phytotaxa, 261(3), 201-217.
2. Doyle, Jeff J., and Jane L. Doyle. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. No.RESEARCH. 1987.
3. Cullings, K. W. (1992). Design and testing of a plant-specific PCR primer for ecological and evolutionary studies. Molecular ecology, 1(4), 233-240.

Revvity
940 Winter Street
Waltham, MA 02451 USA
(800) 762-4000
www.revvity.com

OMNI International
935 Cobb Place Blvd NW
Kennesaw, GA 30144 USA
(800) 776-4431
www.omni-inc.com

For a complete listing of our global offices, visit www.revvity.com