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Simplifying Chickpea Seed Sample Preparation: An Integrated Solution Using the Bead Ruptor™ 96 Bead Mill Homogenizer and chemagic™ 360 Nucleic Acid Extractor

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Bead Ruptor™ 96
Bead Mill Homogenizer

Summary

Seeds, nuts, and other plant matrices are inherently resistant to fast and consistent sample preparation for downstream analysis. Large seeds, specifically, have unique challenges that typically require time consuming pre-processing steps and/or intensive cleaning procedures to reduce cross carryover between samples. These traditional methods can hinder high throughput workflows. Additionally, standard protocols for DNA or other analyte purification methods require powdered matrices prior to mixing with an aqueous buffer that is compatible with chosen downstream extraction. This process can be labor intensive, inexact, and time consuming.

In this application note, we discuss a sample preparation method for large seeds, using the Bead Ruptor™ 96 Bead Mill Homogenizer, that allows samples to be processed in both buffered and dry conditions which results in high quality and quantity DNA. This method can prepare samples with a high degree of throughput and is compatible with automated DNA extraction using the chemagic™ 360 Nucleic Acid Extractor.

Materials and Methods

Equipment

- Bead Ruptor™ 96 Bead Mill Homogenizer (Cat # 27-0001)
- Bead Ruptor™ 96 50 mL Tube Holder (Cat # 27-1003)
- 50 mL Tubes with Screw Caps (Cat # 19-6650)
- Active Grinding Media (Cat # 19-900M)

Procedure

Exactly 6 chickpea seeds were chosen as a representative sample size for nucleic acid extraction. Each sample of chickpea seeds was loaded into 50 mL conical tubes (OMNI, Cat # 19-6650). Two processing parameters (dry grinding, and liquid homogenization) were evaluated for DNA extractions and are detailed below, along with downstream methodologies.

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Dry Grinding

A single active grinding media (OMNI, Cat # 19-900M) was placed inside each 50 mL conical tube containing chickpea seeds. These tubes were loaded into the Bead Ruptor™ 96 50 mL Tube Holder (OMNI, Cat # 27-1003) and placed into the Bead Ruptor™ 96 Bead Mill Homogenizer (OMNI, Cat # 27-0001). The unit was set to process samples at 25 hertz for 6 minutes. After dry grinding, 20 mg of the powdered seeds was transferred to a 96 deep well plate (PerkinElmer, Cat # CMG-555-15) and 400 µL of lysis buffer from the chemagic™ DNA Plant 20 Kit H96 (PerkinElmer, Cat # CMG-795) was added to each well containing the powdered homogenate. All steps for automated DNA extraction were then completed per manufacturer's instructions.

Liquid Homogenization

The mass of all samples in each tube was recorded using an analytical balance prior to addition to the 50 mL conical tube. A single active grinding media (OMNI, Cat # 19-900M) was placed inside each 50 mL conical tube containing chickpea seeds. Lysis buffer from the chemagic™ DNA Plant 20 Kit H96 (PerkinElmer, Cat # CMG-795) was added to each tube following the ratio seen in the required downstream extraction (20 mg of plant tissue: 400 µL of lysis buffer). The 50 mL conical tubes were loaded into the Bead Ruptor™ 96 50 mL tube holder (OMNI, Cat # 27-1003) and placed into the Bead Ruptor™ 96 Bead Mill Homogenizer (OMNI, Cat # 27-0001). Samples were processed at 25 hertz for 6 minutes. After homogenization, all 50 mL conical tubes were centrifuged at 5,000 x g for 5 minutes at 4 °C to pellet the homogenate. 420 µL of the supernatant was removed from each centrifuged 50 mL tube and were transferred into a 96 well deep well plate compatible with the chemagic™ 360 Nucleic Acid Extractor. All steps for DNA extraction using the chemagic™ 360 Nucleic Acid Extractor were then completed per manufacturer's instructions, with the exception of using samples from the liquid homogenate in steps 4,5, and 6.

DNA Quantification

For all samples, DNA concentration and purity was determined via A_{260}/A_{280} spectrophotometry followed by visualization using 2 % agarose gel electrophoresis. Then, eluted DNA was added to qPCR mix (BioRad, Cat # 1725122) along with 5 µM of forward and reverse 18S primers. The 18S rRNA gene was targeted with forward primer 5' - CAG CAG CCG CGG TAA TTC C – 3', reverse primer 5' - CCC GTG TTG AGT CAA ATT AAG C – 3'. An 18S-positive DNA extract was used as the positive control along with nuclease-free water for the negative control. Reactions were loaded into the BioRad CFX Connect Real Time Instrument (BioRad Cat. # 1855201), and amplified for 40 cycles.

Results

High quality and quantity DNA was extracted from chickpea seeds using either dry grinding or liquid homogenization as a sample preparation method. Both methods yielded a large amount of DNA, greater than 5 µg, but the liquid homogenization method yielded a higher amount of DNA. Liquid homogenization returned 193.3 ng/µL more DNA on average (Table 1). DNA from the dry grinding method had an average A_{260}/A_{280} ratio of 2.01, while DNA from the liquid homogenization method had an average A_{260}/A_{280} of 2.07 (Table 1) indicating a high-level of purity within extracted DNA from both methodologies. Upon visualization of separated DNA, the dry grinding method produces less DNA shearing than the liquid homogenization method (Figures 1-2). All samples, however, were suitable for qPCR amplification of the 18S rRNA gene (Figures 3-4)

	Average DNA Concentration (ng/µL)	Average A_{260}/A_{280} Absorbance Ratio
Dry Grinding	53.2	2.01
Liquid Homogenization	246.5	2.07

Table 1. Concentration and purity data from DNA extracted using dry grinding and liquid homogenization methods.

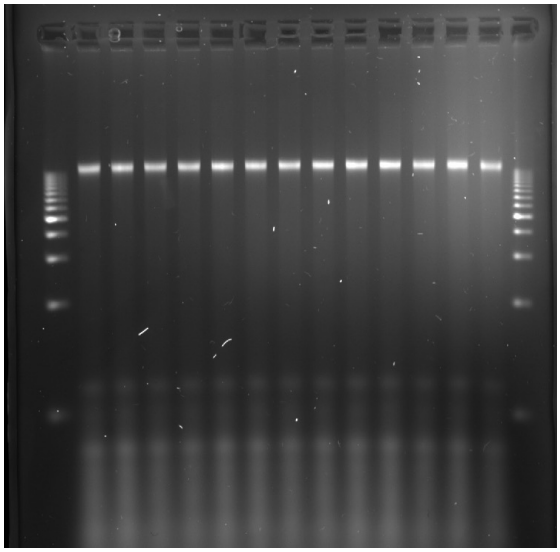

Figure 1. 2 % agarose gel from extracted DNA using dry grinding as a sample processing step.

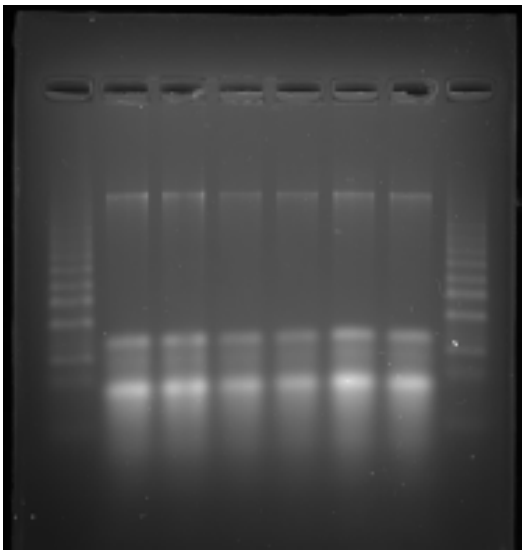

Figure 2. 2 % agarose gel from extracted DNA using liquid homogenization as a sample processing step.

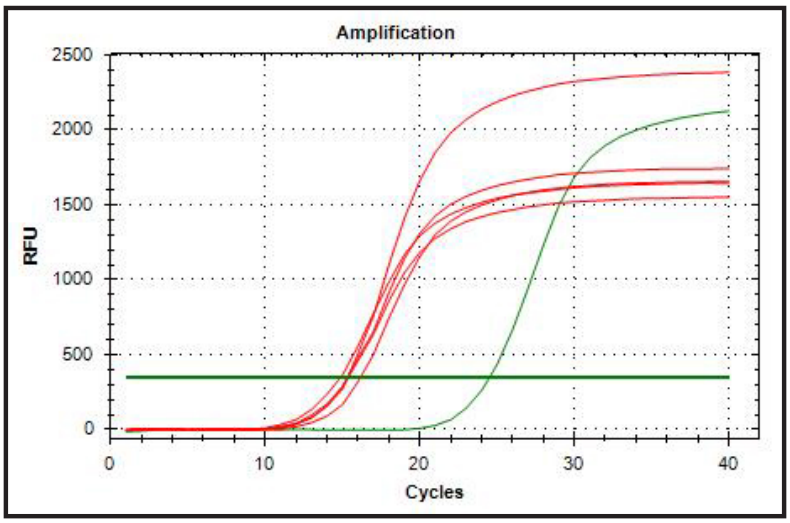

Figure 3. Resulting PCR amplification of extracted DNA from the dry ground sample. Samples are colored red, and the positive control is colored green.

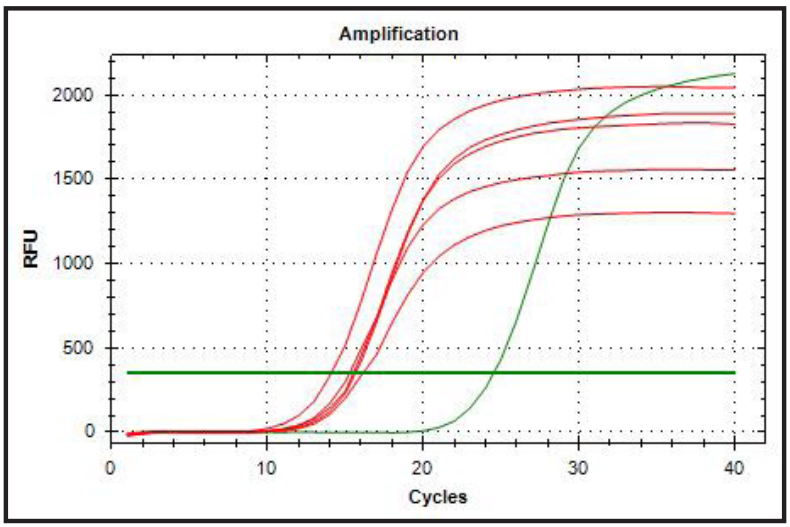

Figure 4. Resulting PCR amplification of extracted DNA from the liquid homogenized sample. Samples are colored red, and the positive control is colored green.

Conclusions

The Bead Ruptor™ 96 Bead Mill Homogenizer, in conjunction with active grinding media, and the chemagic™ 360 Nucleic Acid Extractor provides an efficient workflow solution, yielding nucleic acid that is compatible with the needs of downstream DNA-based applications, like qPCR. Dry grinding seeds with active grinding media produces a homogenate that is comparable with other grinding methods such as ball milling in stainless steel milling jars. However, the active grinding media approach allows for dry grinding in commonly available 50 mL conical tubes, along with the benefit of removing time consuming pre-processing steps. Sample preparation consistency is further streamlined when using the liquid homogenization method, because lysis buffer is added directly to the 50 mL conical tube, eliminating the need for a post-processing liquid addition step, as well as, several handling and weighing steps. In condensing these steps, the liquid homogenization method is compatible with automation post bead beating for centrifugation and liquid handling steps.

Both methods remove time consuming pre-processing steps such as manually crushing individual samples. Through the removal of pre-processing steps, crossover and carryover between replicates is also minimized and allows for more accurate and efficient processes. The demonstrated homogenization-based workflows for DNA extraction from chickpeas provide a blueprint for highly effective and reproducible workflows which can be incorporated into a variety of seed and nut based projects.

Ordering Information

Equipment	Catalog Number
Bead Ruptor™ 96 Bead Mill Homogenizer	27-0001
Bead Ruptor™ 96 50 mL Tube Holder	27-1003
50 mL Tubes with Screw Caps	19-6650
Active Grinding Media	19-900M

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