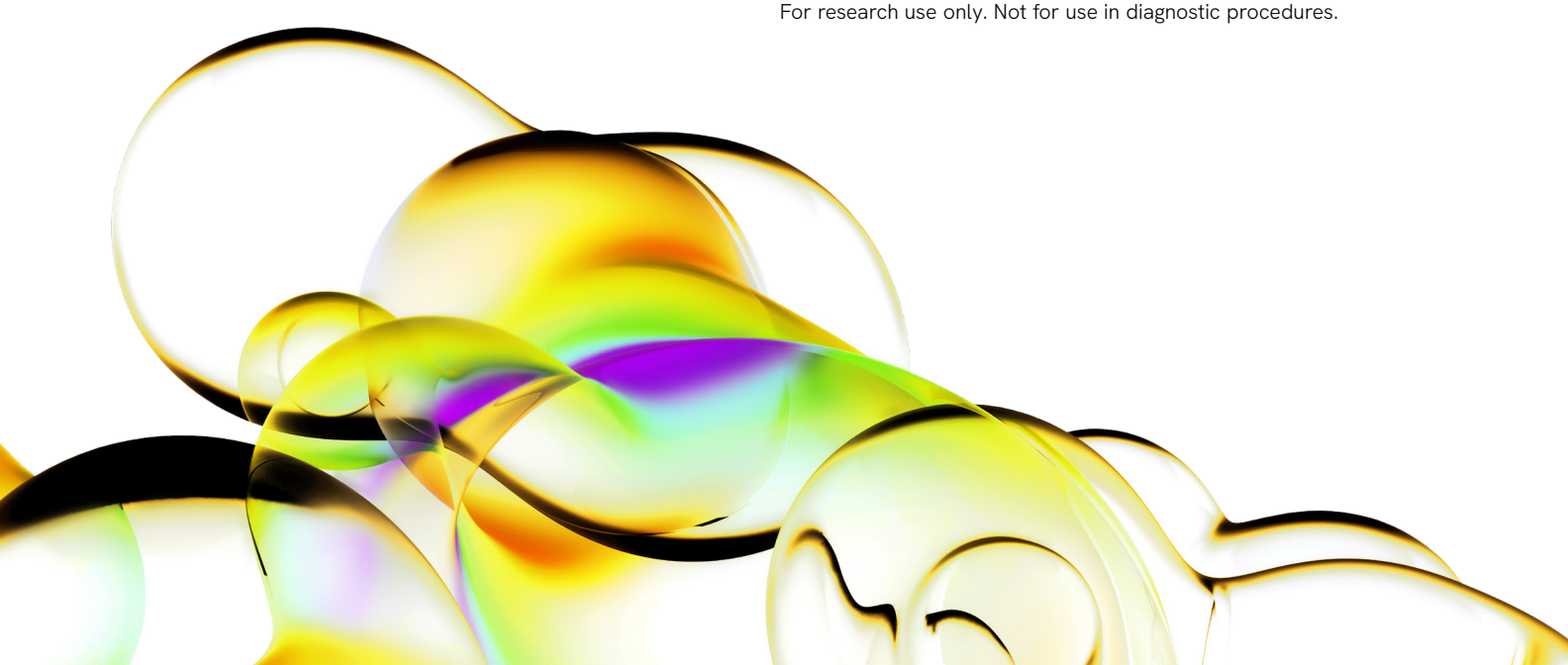
APPLICATION NOTE

Genomic DNA extraction from plant tissues on the Omni Bead Ruptor Elite bead mill homogenizer.

Genetically Modified crops (GM crops) are food products such as fruits and vegetables that have had specific changes introduced into their DNA. The DNA changes are designed to impart new traits into the crops such as resistance to pathogens, delayed ripening or in the case of hybrid soybeans produced by Pioneer Hi-Bred International, higher levels of oleic acid. Production of GM crops is a multistage process that ultimately involves integration of the target gene into the recipients' genome. However, uptake and integration of the gene into the recipients genome does not always guarantee that the gene will be expressed. Furthermore, it must be confirmed that the target gene is transferred at an acceptable level to progeny.

In order to confirm gene expression, genomic material (DNA/RNA) must first be extracted from the target sample. Bead mill homogenizers are one of the newest technologies for the disruption of tough plant material and are routinely used when multiple samples must be processed or when the target sample is particularly robust such as seeds and tough plant material. The Omni Bead Ruptor Elite™ bead mill homogenizer is capable of homogenizing samples in volumes of 0.5 mL to 50 mL through rapid oscillation of a tube containing dense bead media. The bead media is projected through the tube with sufficient energy to impact and dissociate the sample. Herein, we demonstrate DNA extraction from two common consumer crops, spinach and okra using the Omni Bead Ruptor Elite bead mill homogenizer. The extraction efficiency and analyte integrity were evaluated.



Materials and methods

Equipment

- **Omni Bead Ruptor Elite bead mill homogenizer** (Cat #19-042E)
- **Omni Bead Ruptor Elite 2 mL Tube Carriage** (Cat # 19-373)
- **Hard Tissue Homogenizing Mix 2.8 mm Ceramic (2 mL)** (Cat #19-628)

Sample preparation

Fresh spinach leaves and okra pods were obtained from a local grocer and frozen at 0 °C overnight. Approximately 50 mg of spinach and okra was harvested and cut into 10 mm pieces and placed in a 2 mL tube containing six 2.8 mm ceramic beads (Cat # 19-628). 500 µL plant tissue lysis buffer was added and the samples were homogenized on the Omni Bead Ruptor Elite bead mill homogenizer at 4 m/s for 1 minute. After processing, the lysates were transferred to a clean 1.5 mL microcentrifuge tube containing 10 µL of 2-mercaptoethanol and incubated at 65 °C for 15 minutes. The sample was inverted twice during incubation. DNA extraction was then carried out using a commercially available plant DNA extraction kit per manufacturer's instructions. 1 µL of the eluted DNA was quantified using a NanoDrop Spectrophotometer (Thermo Fisher) to determine DNA yields.

20 µL of each sample was then mixed with 10 µL of TBE/Urea sample buffer (Bio-Rad Cat#161-0768). The DNA was then separated on a 1.2 % TBE agarose gel at a constant 60 V and stained with ethidium bromide(Bio-Rad Cat#161-0433) for 30 minutes. The gel was washed with DD H_20 for 10 minutes and visualized at 254 nm on a GelDoc EZSystem (Bio-Rad).

Results

Optimizing the sample disruption process is a critical first step for nearly every downstream genomic analysis. Both analyte yields and integrity must be considered when selecting a sample disruption method. Bead milling has been demonstrated to be an effective method of rapid sample dissociation for extraction of nucleic acids from a wide range of crop samples including legumes, wheat and maize. In this study, we evaluated the performance of the Omni Bead Ruptor Elite bead mill homogenizer for the disruption of two common crops, spinach and okra. Both plant samples were disrupted through a one minute cycle at 4 m/s. Visual inspection confirmed that both samples were completely homogeneous after processing on the Omni Bead Ruptor Elite bead mill homogenizer.

Genomic DNA was then extracted and quantified by spectrophotometry (Table 1). DNA yields were 3.25 ng/µL and 9.83 ng/µL for the spinach and okra samples respectively. The DNA was then analyzed by gel electrophoresis to verify the quality of the extracted DNA. The gel image in figure 1 indicates that the extracted DNA was of good quality with relatively no shearing observed as demonstrated by the strong high molecular bands (Figure 1).

Table 1. Starting sample quantity and DNA yields as determined by spectrophotometry

Tissue type	Mass (mg)	Average concentration (ng/µL)
Spinach	45	3.25
Okra	50	9.83

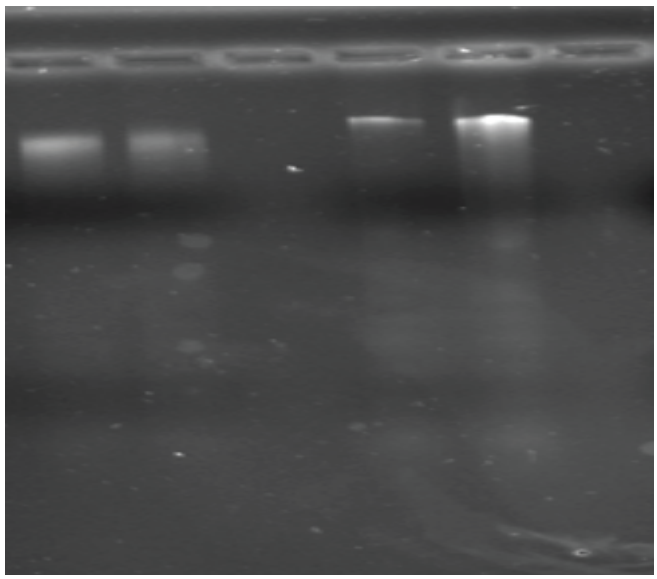


Figure 1. DNA agarose gel electrophoresis of pant lysates: Lane 1: *2.5kb ladder*, Lane 2-3: *Spinach* and Lane 5-6: *Okra*

Conclusion

The Omni Bead Ruptor Elite bead mill homogenizer is capable of disrupting both soft and fibrous plant tissue samples as demonstrated by the homogenization of spinach and okra samples in one minute. The bead mill disruption process was demonstrated to be sufficient for extraction of genomic DNA in quantities sufficient for analysis by 1D gel electrophoresis and traditional staining and visualization procedures. DNA integrity was maintained as well.

Omni Bead Ruptor Elite bead mill homogenizer

Revvity, Inc.
940 Winter Street
Waltham, MA 02451 USA
www.revvity.com

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