APPLICATION NOTE

Nucleic acid extraction from *Ocimum basilicum* using the Omni Bead Ruptor Elite bead mill homogenizer for sample preparation.

Summary

Isolation and purification of nucleic acids from plant tissues is a required step before proceeding to downstream assays like PCR or genome sequencing. Areas of study that require nucleic acids as an input, include genome modification analysis in the case of genetically modified plants or pesticide resistance, and molecular identification of plant species.

Sample preparation of plant tissues requires lysis to release analytes of interest from cells that comprise the plant tissue. Commonly used methods, including enzymatic digestion or mortar and pestle, can be time consuming and tedious processes. Implementing the Omni Bead Ruptor Elite™ bead mill homogenizer in front-end sample preparation is a robust solution to homogenize plant tissue.

Herein, we evaluate the Omni Bead Ruptor Elite bead mill homogenizer as a sample preparation solution in front-end processing of plant tissue for downstream purification and quantification of nucleic acids.

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Omni Bead Ruptor Elite bead mill homogenizer

For research use only. Not for use in diagnostic procedures.

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)

Procedure

Plant DNA extraction

50 mg of plant leaf tissue was added to a 2 mL Hard Tissue Homogenizing Mix tube (Cat # 19-628) containing 500 µL of commercially available plant tissue lysis buffer. The plant leaf samples were homogenized using the Omni Bead Ruptor Elite bead mill homogenizer (Cat # 19-042E) at 5.5 m/s for 3 cycles of 30 seconds with a 30 second dwell (Table 1). After this lysis step, 5 µL of RNase A was added to sample tube containing homogenized plant leaf samples. Samples were incubated at 65 °C for 15 minutes per guidelines set out in commercially available plant DNA extraction kit. After RNase incubation step, the remainder of the plant DNA extraction was carried out following manufacturer instructions from commercially available plant DNA extraction kit.

Samples were eluted with 100 µL of Elution Buffer supplied with the DNA extraction kit. DNA concentration and integrity were determined by A_{260} /A_{280} spectrophotometry.

After elution, approximately 100 ng of eluted DNA was electrophoresed on a 2.0 % agarose gel in TBE. After ethidium bromide staining, DNA bands were visualized on a gel documentation system following standard procedures.

Plant RNA extraction

50 mg of plant leaf tissue was added to aa 2 mL Hard Tissue Homogenizing Mix tube (Cat # 19-628) containing 500 µL of commercially available plant tissue lysis buffer. The plant leaf samples were homogenized using the Omni Bead Ruptor Elite bead mill homogenizer (Cat # 19-042E) at 5.5 m/s for 3 cycles of 30 seconds with a 30 second dwell (Table 1). After sample lysis, the remainder of the RNA extraction was carried out using commercially available plant RNA extraction kit following manufacturer's instructions.

Samples were eluted with 100 µL of Nuclease-free Water supplied with the RNA extraction kit. RNA concentration and integrity were determined by A_{260} /A_{280} spectrophotometry.

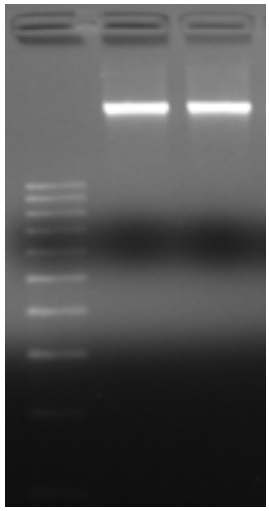
After elution, approximately 100 ng of eluted RNA was electrophoresed on a 2.0 % agarose gel in TBE. After ethidium bromide staining, RNA bands were visualized on a gel documentation system following standard procedures.

Sample type	Speed (m/s)	Time (sec)	Cycles	Dwell time (sec)
Basil, 50 mg	5.5	30	3	30

Table 1: Sample homogenization summary. All plant leaf samples were weighed out within ± 5 mg of the desired sample weight.

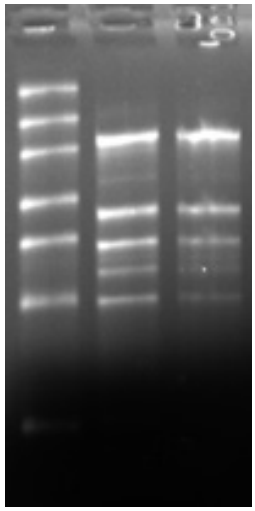
Results

Homogenization of 50 mg basil leaf using the Omni Bead Ruptor Elite bead mill homogenizer, yielded an average of 10.5 ng/µL of DNA (Table 2) and an average of 46.5 ng/µL of RNA (Table 3). Eluted DNA had an average A_{260}/A_{280} ratio of 1.85 (Table 2). Eluted RNA had an average A_{260}/A_{280} ratio of 1.95 (Table 3), indicating that the eluted nucleic acid is >90% pure and free of contaminants like phenols and polysaccharides. Separation of eluted DNA and RNA via gel electrophoresis (Figures 1-2) yielded distinct bands that demonstrated high molecular weight, when compared to the molecular ruler, and showed no signs of degradation.



Sample	DNA concentration	A_{260}/A_{280}
Basil #1	11 ng/µL	1.9
Basil #2	10 ng/µL	1.8

Figure 1, Table 2: Gel electrophoresis and photometric assessment of DNA quantity and quality purified from two basil leaf samples.



Sample	RNA concentration	A_{260}/A_{280}
Basil #1	41 ng/µL	1.9
Basil #2	52 ng/µL	2.0

Figure 2, Table 3: Gel electrophoresis and photometric assessment of RNA quantity and quality purified from two basil leaf samples.

Conclusions

The Omni Bead Ruptor Elite bead mill homogenizer and 2 mL Hard Tissue Homogenization tube is an efficient sample preparation solution to homogenize plant tissue. The resulting homogenate is suitable for isolation of intact and pure nucleic acid that is suitable for further downstream analysis.

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