APPLICATION NOTE

High throughput RNA extraction from corn utilizing the Omni Bead Ruptor Elite bead mill homogenizer and chemagic 360 nucleic acid extractor.

Authors

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Summary

Staple crop transcriptomics holds the key to deciphering the intricate language of gene expression in plants, shaping the future of agricultural science and sustainable food production. In a world grappling with the ever-increasing challenges of a growing 8 billion person population, climate change, and diminishing arable land, understanding the molecular mechanisms that underlie crop growth, development, and response to environmental stressors has become paramount. The elucidation of transcriptomes opens new avenues for advancing crop breeding, improving crop yield, and enhancing resilience to environmental fluctuations or disease.

The advent of high-throughput sequencing technologies has revolutionized the field of plant transcriptomics, enabling researchers to comprehensively analyze the transcriptome with unprecedented precision and depth. These methods allow us to not only quantify gene expression levels but also provide insights into alternative splicing, post-transcriptional regulation, and non-coding RNA-mediated gene regulation. As a result, it has become increasingly evident that the transcriptome of crops is far more intricate and dynamic than previously envisioned. This ultimately results in the need for more transcriptomes to be analyzed.

However, the transcriptome cannot be analyzed without high quality RNA to sequence. Part of the difficulty in performing RNA purifications from plant tissue comes from the challenging nature of the tissue itself. Unlike animal tissue, plants are surrounded by thick polysaccharide cell walls that

Omni Bead Ruptor Elite™ bead mill homogenizer

makes exposing the genetic material contained within more challenging. Typical methods to resolve this problem involve lengthy and manual steps utilizing techniques such as enzymatic lysis and manual disruption. While effective, these methods can impose considerable time and labor cost to the researcher, as well as increasing the risk for RNA degradation due to exposure to nucleases and heat.

A solution to this problem can be found by introducing semi-automated homogenization techniques which resolve issues with reproducibility and scalability of common nucleic acid extractions. Bead mills and other similar devices provide a high shear environment where physical lysis of the tough plant matrix allows for the soluble RNA to be released into a protective lysis buffer where denaturing of the molecule is minimized. Along with ensuring the integrity of the RNA, the lysis buffers provide a liquid medium conducive to maximally remove downstream inhibitors ensuring that both library preparations and other amplification techniques are not impeded when analysis is ultimately preformed.

Herein, we demonstrate integration of the Omni Bead Ruptor Elite bead mill homogenizer into a semi-automated total plant RNA purification workflow using the chemagic™ 360 instrument. This workflow solution not only provides a rapid nucleic acid extraction, but the resulting product is high quality, high quantity and compatible for many RNA analysis methods.

Materials and methods

Equipment

- Omni Bead Ruptor Elite bead mill homogenizer (Cat # 19-042E)
- Omni Bead Ruptor Elite 2mL Tube Carriage (Cat # 19-373)
- Hard Tissue Homogenizing Mix 2.8 mm Ceramic 2 mL reinforced tubes (Cat # 19-628)
- chemagic 360 (Cat # 2024-0020)
- chemagic DNA Plant 20 Kit H96 (Cat # CMG-795)

Procedure

Corn radicals were sprouted by placing corn seeds in an airtight bag containing a damp paper towel. The bag was then incubated at room temperature in a dark environment for 4 days. Once the corn radicals sprouted in the bag, 10 mg (+/- 1 mg) of plant tissue was recovered and placed inside of a reinforced 2 mL tube containing 2.8 mm ceramic beads (Cat # 19-628). Each 2 mL bead tube was filled with 200 µL of a CTAB buffer created using methods described by Cullings, and Doyle (1,2).

Samples were then placed into the Omni Bead Ruptor Elite and were homogenized at 5 m/s for two 30 second cycles with a 30 second dwell in between each cycle. The resulting homogenate was then centrifuged at 10,000 x g for 5 minutes to separate soluble and non-soluble components of the lysate and reduce foam from the CTAB buffer. The entire supernatant was transferred to a clean well plate followed by treatment with 3 µL of DNase (5 U/µL). The 96 well plate was then covered with a PCR film and incubated at 37 °C for 30 minutes. After incubation, 100 µL from each well was transferred into a chemagen deep well plate containing 300 µL of chemagen plant lysis buffer (Cat # CMG-795). This well plate was then placed into the chemagic 360 and the standard plant DNA extraction was performed with 75 µL of chemagen elution buffer.

After the extraction was completed, the elution plate containing the RNA was placed on ice, and each well was then quantified via spectrophotometry. The resulting A_{260}/A_{280} ratios and concentrations were recorded. After these values were obtained, the eluate was further examined for quality by obtaining a RIN value utilizing a Bioanalyzer (Agilent, Cat # G2939BA) and RNA 6000 Nano kit (Agilent, Cat # 5067-1511).

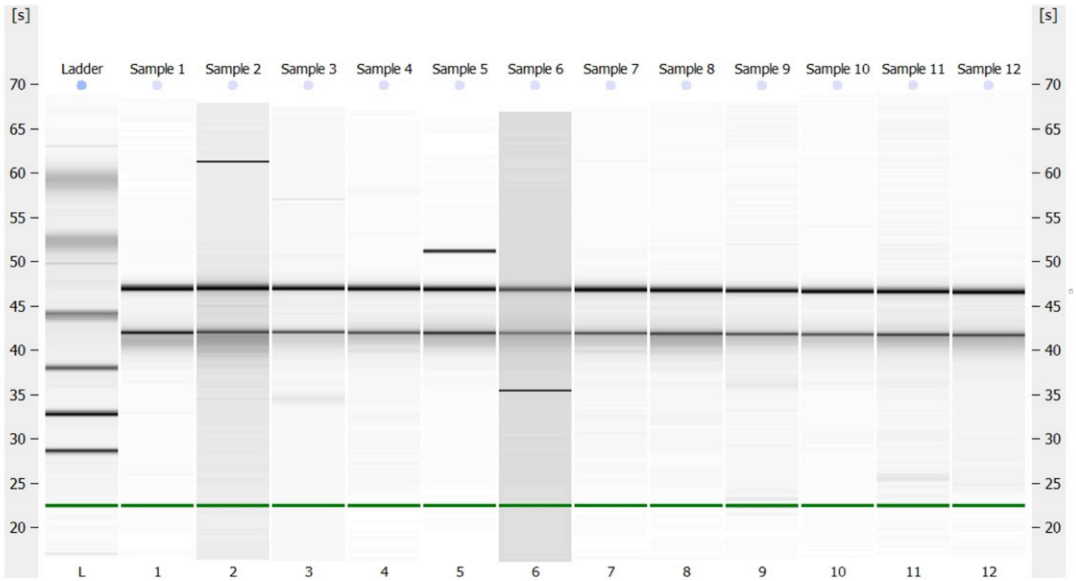
Results

Extracted RNA displayed both high quality and quantity metrics with minimal RNA degradation. Nanodrop analysis of the extractions showed an average nucleic acid concentration of 87.93 ng/µL and an average A_{260}/A_{280} ratio of 2.09 (Table 1). Each eluate produced a distinct 28S and 18S RNA band from all extractions when evaluating the products by bioanalyzer (Figure 1). Additionally, the average RIN value reported by bioanalyzer across each extraction was 9.1 (Table 1).

Table 1. Nucleic acid yield and quality results from 12 RNA extractions.

Sample number	Nucleic acid concentration (ng/µL)	Total yield (ng)	A_{260}/A_{280} ratio	RIN value
1	94.6	7,095	2.06	7.8
2	100.3	7,522.5	2.10	9.7
3	66.6	4,995	2.14	10
4	95.8	7,185	2.12	8.7
5	144.8	10,860	2.11	9.8
6	132.4	9,930	2.07	8.5
7	111.7	8,377.5	2.08	8.7
8	87.9	6,592.5	2.09	9.5
9	34.8	2,610	2.09	8.7
10	71.6	5,370	2.12	9.7
11	35.7	2,677.5	2.05	9.2
12	78.9	5,917.5	2.12	8.9

Figure 1. Bioanalyzer RNA electropherogram.



Conclusions

The above RNA extraction is an efficient and effective method of extracting total RNA from plant tissues. When utilizing the Omni Bead Ruptor Elite bead mill homogenizer, samples are rapidly homogenized, and the soluble nucleic acids are released into the liquid lysis buffer in minutes compared to alternative lysis techniques that take hours or days to fully disintegrate the sample using heat, proteases, or chemical lysis buffers. Additionally, the rapid homogenization allows a laboratory to scale their plant RNA extractions from individual, non-automated, preparations into scalable methods that return consistent results in each extraction. These extractions are completed using 96 well plates and are terminally compatible with additional automation like liquid handlers. This combined with high quality RNA metrics displays the resulting products are compatible with most current RNA sequencing methods for transcriptomics.

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.

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