APPLICATION NOTE

DNA extraction from *Cannabis sativa* using the Omni Bead Ruptor 12 bead mill homogenizer for sample preparation.

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Introduction

Plant genetics has been studied for centuries dating back to Mendel and the crossbreeding of orchids to get different phenotypic flowers. As time continued, it was discovered that genes were responsible for these features. Scientists now look at the plant's DNA to discover genes for different characteristics like chlorophyll production, water absorption, seed formation, etc. As plants are a complex matrix including chlorophyll and starches, it's important to obtain pure DNA for genetic studies.

One plant in particular, hemp, has been a main focus in the market recently. Hemp, a strain of *Cannabis sativa*, is being studied not only for its industrial uses, but for its nutritional and medicinal uses too. Cannabis growing and cultivation is important for plant geneticists to look for what strains can produce different cannabinoids and terpenes to support the market.

Herein, we evaluated the OMNI Bead Ruptor 12 for homogenization of the plant sample prior to DNA purification.

Omni Bead Ruptor 12 bead mill homogenizer

Materials and methods

- Omni Bead Ruptor 12 bead mill homogenizer (Cat# 19-050A)
- Hard Tissue Homogenizing mix 2.8 mm Ceramic (2 mL) (Cat # 19-628)

Sample prep and DNA extraction

Hemp strain "Lifter" samples were obtained from North Carolina All-Natural Farms. Approximately, 30 mg samples were weighed out and placed in a 2 mL bead tube containing 2.8 mm ceramics. 500 µL of commercially available CTAB precipitation buffer and 10 µL of 2-mercaptoethanol was added to each tube. Samples were loaded to the Omni Bead Ruptor 12 bead mill homogenizer and processed at 3.7 m/s for 20 seconds. After homogenization, DNA was extracted using a commercially available plant DNA extraction kit. DNA was eluted in 100 µL of commercially available elution buffer. DNA concentration was determined on the NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific) as seen in table one.

Approximately 300 ng of DNA and 5 µL of TBE/Urea sample buffer (Bio-Rad) were loaded onto a 1% agarose gel. DNA was separated by electrophoresis at 140 V for about 50 minutes or until the samples travelled 3/4th of the way down the gel. The gel was stained with ethidium-bromide and then visualized on the Gel-Doc EZ system (Bio-Rad).

Results

Herein, the Omni Bead Ruptor 12 bead mill homogenizer was demonstrated as an efficient sample prep solution when preparing hemp samples for DNA extraction. Hemp, like many other plants, is an inherently challenging matrix due to the presence of polysaccharides and phenols that can interfere with the DNA purification process. Commercially available plant DNA extraction kits in conjunction with adequate steps to get rid of interfering substances allow for extraction of pure DNA, like seen in this application. Genomic DNA was quantified via spectrophotometry.

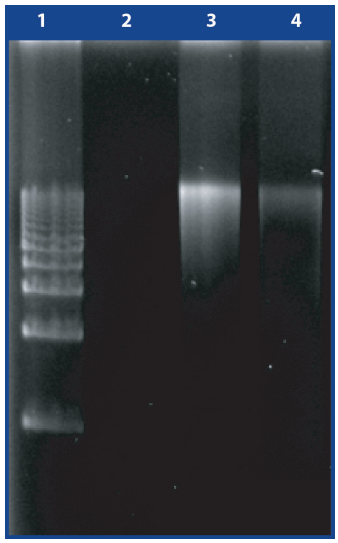
The DNA concentration averaged 396.5 ng/µL and 489.3 ng/µL respectively. Electrophoretic analysis showed bands of genomic DNA recovered of high quality with little DNA shearing.

Conclusion

The workflow showcased here was successful in preparing hemp for genomic DNA extraction using the Omni Bead Ruptor 12 bead mill homogenizer for sample preparation. The Omni Bead Ruptor 12 bead mill homogenizer was able to process the plant material in less than 30 seconds and high concentrations of DNA were recovered from downstream DNA extraction.

Table 1: Hemp processing parameters and average DNA concentrations

Sample	Weight	Speed	Time	DNA concentration	A_{260}/A_{280}
1	28 mg	3.7 m/s	20 sec	396.5 ng/uL	2.10
2	30 mg	3.7 m/s	20 sec	489.3 ng/uL	2.09



Lane 1: Ladder, Lane 3: Sample 1, Lane 4: Sample 2

Figure 1: Electrophoresis analysis of *C. sativa*

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