Promega

Product Application

Automated RNA Purification from Hemp

Purify RNA from hemp samples using the Maxwell® RSC Plant RNA Kit with the Maxwell® RSC Instrument.

Kit: Maxwell® RSC Plant RNA Kit (Cat.# AS1500)

Analyses:

- UV Spectrophotometry
- Dye-based quantitation
- RT-qPCR
- TapeStation

Sample Type(s): Hemp (*Cannabis sativa* L.), leaf and flower

Input: Up to 100mg

This protocol was developed by Promega Applications Scientists and is intended for research use only.

Users are responsible for determining suitability of the protocol for their application.

For further information, see Technical Manual TM459, available at: **www.promega.com/protocols**

or contact Technical Services at: **techserv@promega.com**

Materials Required:

- Maxwell® RSC Plant RNA Kit (Cat.# AS1500)
- Maxwell® RSC Instrument (Cat.# AS4500)
- For bead beating:
  - Tissue punch device
  - Lysing Matrix SS, 2ml Tubes (MP Biomedicals, Cat.# 116921100)
  - Mechanical bead beating device such as the FastPrep-24™ 5G System (MP Biomedicals, Cat.# 116005500)
- For liquid nitrogen grinding:
  - Mortar and pestle
  - Liquid Nitrogen
  - Tissue Homogenizer (e.g., Tissue-Tearor™ homogenizer)

Protocol:

Prepare the required solutions as indicated in the Maxwell® RSC Plant RNA Kit Technical Manual (TM459) Section 3.B. Keep both solutions on ice.

Bead Beating Protocol

1. Take leaf punches using a clean tissue punch device, or roughly chop flower tissue, and add the required amount(s) to 2ml Lysing Matrix SS tubes (containing a single steel bead, 1/4'' diameter).
2. Add 400µl of chilled 1-Thioglycerol/Homogenization solution per tube.
3. Add 200µl of Lysis Buffer per tube.
4. Vortex tubes for 15 seconds.
5. Bead beat at 4m/s for 4 cycles of 20 seconds, with a 20-second pause between each cycle.
6. Centrifuge samples at maximum speed (≥16,000 x *g*) for 4 minutes.

Liquid Nitrogen Grinding Protocol

Follow the protocol outlined in TM459, Section 3.C "Preparation of Samples".
After homogenizing tissue using either of the above protocols, proceed with the standard protocol outlined in TM459, Section 3.D. "Maxwell® RSC Plant RNA Cartridge Preparation".

Results

High quality RNA was successfully purified from multiple samples of hemp leaves and flowers using the Maxwell® RSC Plant RNA Kit with the Maxwell® RSC Instrument. Purified RNA was successfully amplified via RT-qPCR using hemp-specific primers.

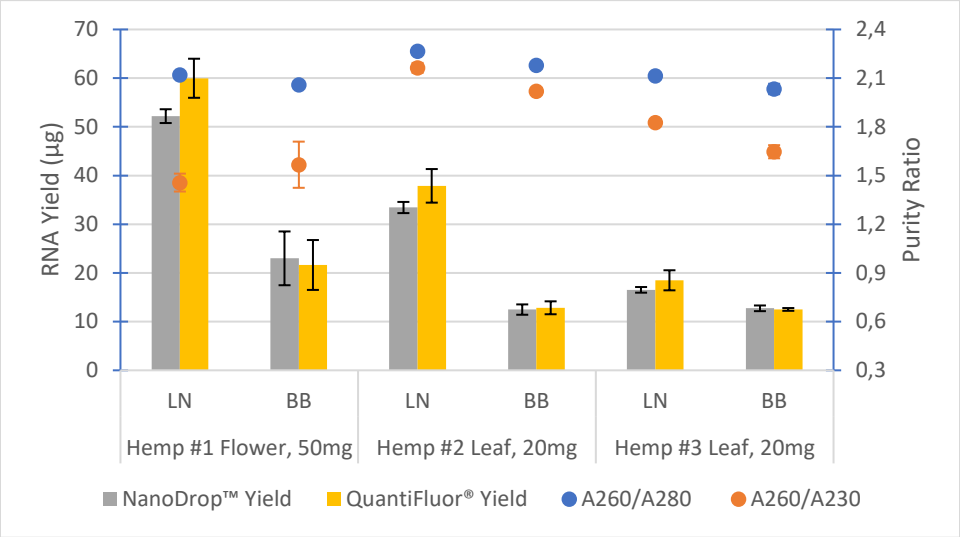

Figure 1. RNA yield and purity results. RNA was purified from multiple samples of hemp leaves and flowers using the Maxwell® RSC Plant RNA Kit. Both bead beating (BB) and liquid nitrogen grinding (LN) were used to homogenize tissue. Purified RNA was quantified spectrophotometrically (NanoDrop™) and by using an RNA-binding fluorescent dye (QuantiFluor® RNA System, Cat.# E3310). Mean DNA yields and purity ratios ± standard deviation are displayed (n=3).

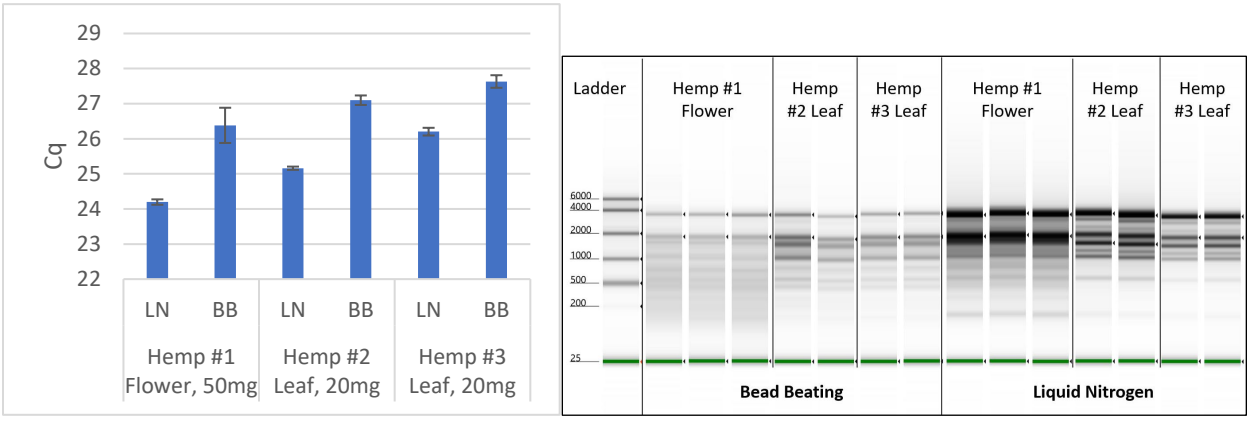

Figure 2. RT-qPCR amplification and TapeStation results. RNA was purified from multiple samples of hemp leaves and flowers using the Maxwell® RSC Plant RNA Kit. Both bead beating (BB) and liquid nitrogen grinding (LN) were used to homogenize tissue. Purified RNA was diluted 1:100 and amplified via RT-qPCR using hemp-specific primers[1]. Diluted RNA was also analyzed using the High Sensitivity RNA ScreenTape Assay on the 4200 TapeStation System. **Left:** Mean Cq values ± standard deviation for triplicate purification replicates amplified in duplicate. **Right:** TapeStation Gel Image.

References

1. Guo, Hongyan Guo, Qingying Zhang, Mengbi Guo, Yanping Xu, Min Zeng, Pin Lv, Xuan Chen & Ming Yang (2018) Evaluation of reference genes for RT-qPCR analysis in wild and cultivated *Cannabis*, Bioscience, Biotechnology, and Biochemistry, 82:11, 1902-1910, DOI: 10.1080/09168451.2018.1506253