Promega

Product Application

Automated DNA Purification from Hemp

Purify DNA from hemp samples using the Maxwell® RSC PureFood GMO and Authentication Kit with the Maxwell® RSC Instrument.

Kit: Maxwell® RSC PureFood GMO and Authentication Kit (Cat.# AS1600)

Analyses:

- UV Spectrophotometry
- Dye-based quantitation
- qPCR
- PACE™ genotyping sex determination assay
- Long-read DNA sequencing (Oxford Nanopore)

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

Input: Up to 200mg

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 TM473, available at: **www.promega.com/protocols**

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

Materials Required:

- Maxwell® RSC Instrument (Cat.# AS4500)
- Maxwell® RSC PureFood GMO and Authentication Kit (Cat.# AS1600)
- Heat block set to 65°C with appropriate tube adapter
- 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 extraction bag grinding:
  - Extraction bags Universal 12x15cm (Bioreba, Cat.# 430100) (or similar) and bag clamp
  - Homogenizer hand model (Bioreba, Cat.# 400010) (or similar)

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 1ml of CTAB Buffer per tube.
3. Bead beat at 4m/s for 4 cycles of 20 seconds, with a 20-second pause between each cycle.
4. Briefly centrifuge tubes to gather foam away from the lids if necessary.

Extraction Bag Grinding Protocol

1. Weigh leaf or flower tissue and add the tissue to an extraction bag along with 5ml of CTAB Buffer. **NOTE:** Tissue input and CTAB Buffer volume may require optimization depending on the size/dead volume of the extraction bag used. Do not exceed a concentration of 200mg tissue/ml CTAB Buffer.
2. Clamp the bag closed and grind tissue thoroughly using a handheld homogenization device.
3. Remove homogenate using a serological pipette and aliquot 1ml per tube into 2ml tubes.

After homogenizing tissue using one of the above protocols, proceed with the standard protocol outlined in the Maxwell® RSC PureFood GMO and Authentication Kit Technical Manual (TM473), starting at Section 4.A, step 2.

Results

High quality DNA was successfully purified from multiple samples and amounts of hemp leaves and flowers using the Maxwell® RSC PureFood GMO and Authentication Kit with the Maxwell® RSC Instrument. Purified DNA was successfully amplified via qPCR using hemp-specific primers, with no observable inhibition. Purifed DNA was also used to determine plant sex using a PACE™ genotyping assay and was successfully sequenced using Oxford Nanopore technology.

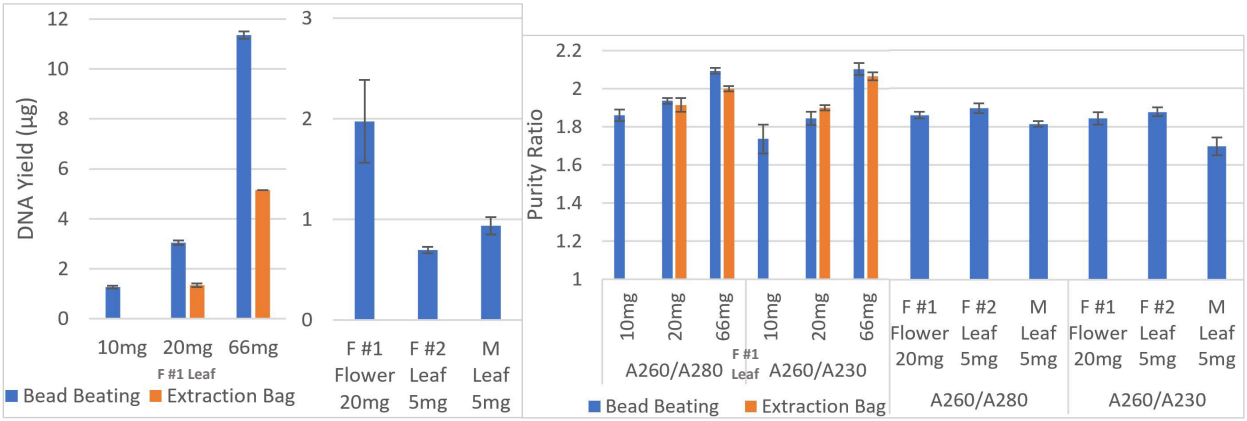

Figure 1. DNA yield and purity results. DNA was purified from multiple samples and amounts of hemp leaves and flowers using both the bead beating and extraction bag grinding protocols described here. For both graphs above, input is listed beneath each bar. F=female, M=male. N=3 for all samples except extraction bag samples (n=2). **Left:** Mean DNA yields ± standard deviation quantified using the QuantiFluor® ONE dsDNA System (Cat.# E4871). **Right:** Mean DNA purity ratios ± standard deviation analyzed via UV spectrophotometry.

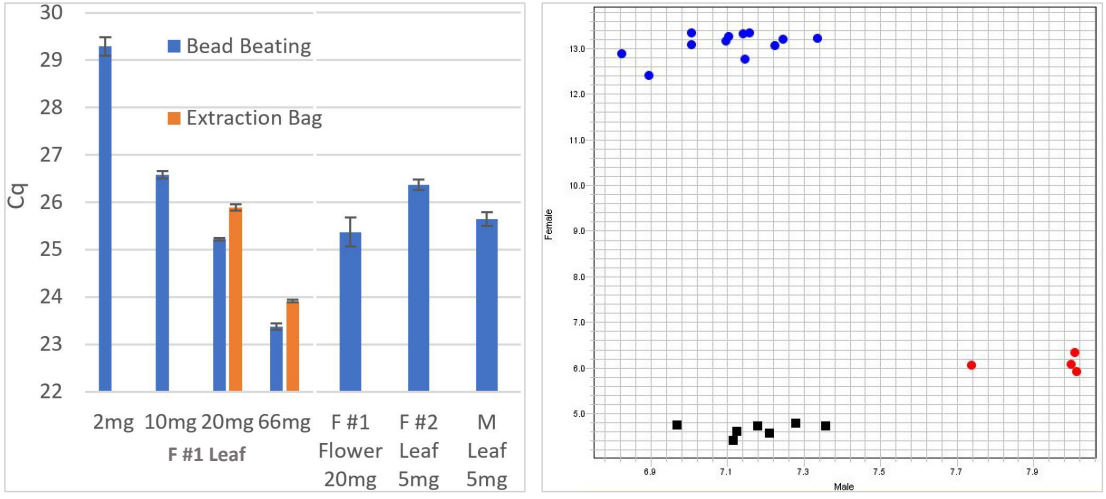

Figure 2. qPCR amplification and PACE™ genotyping sex determination assay results. DNA was purified from multiple samples and amounts of hemp leaves and flowers using both the bead beating and extraction bag grinding protocols described here. **Left:** Mean Cq values ± standard deviation resulting from qPCR amplification

using GoTaq® Probe qPCR Master Mix (Cat.# A6101) with hemp-specific primers. N=3 for all samples except extraction bag samples (n=2). Each sample was amplified in duplicate. Input is listed beneath each bar. F=female, M=male. **Right:** Allelic discrimination plot resulting from PACE™ genotyping sex determination assay[1,2]. Blue=female, red=male, black=NTC.

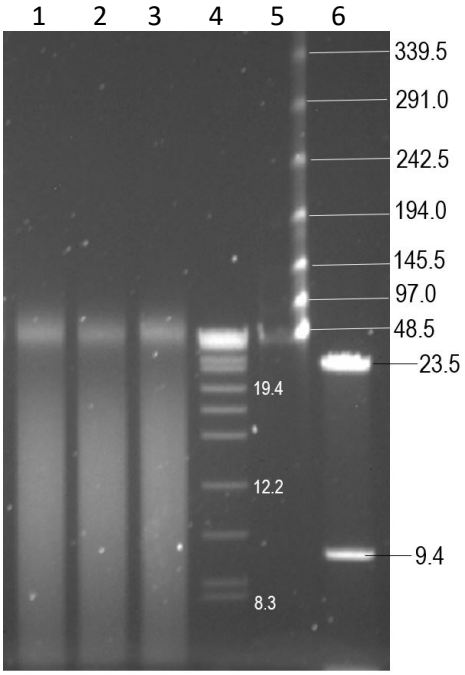

Figure 3. Gel image resulting from pulsed-field gel electrophoresis (PFGE) of purified hemp DNA. DNA was purified from 66mg of female hemp #1 leaf tissue using the bead beating protocol described here. 300ng of DNA per well was then analyzed using PFGE. The resulting gel image is displayed. Wells 1-3 contain hemp DNA. Wells 4-6 contain different DNA ladders. Ladder sizes are indicated (kB).

QC Metric	Value
Total Yield (GB)	18.53
Reads Analyzed	3,649,962
Mean Quality Score	9.9
Median Quality Score	9.9
Mean Seq Length	5077.6
Median Read Length	3911
Maximum Read Length	215,204
N50 read length	8,222

Table 1. QC metrics resulting from Oxford Nanopore sequencing of purified hemp DNA. DNA was purified from 66mg of female hemp #1 leaf tissue using the bead beating protocol described here. 1µg of DNA was then sequenced using Oxford Nanopore technology on a MinION sequencer[3]. The resuling QC metrics are displayed.

References

1. PACE™ 2.0 Genotyping Master Mix User Guide v1.0. 3CR Bioscience. https://3crbio.com/wp-content/uploads/2020/01/PACE-2.0-User-Guide-v1.0.pdf

2. Toth, JA, Stack, GM, Cala, AR, et al. Development and validation of genetic markers for sex and cannabinoid chemotype in *Cannabis sativa* L. *GCB Bioenergy*. 2020; 12: 213– 222.

3. Promega AppNote: Oxford Nanopore sequencing with Promega Purification Kits. 12/2019.