Promega

Product Application

Automated Purification of Fungal DNA

Purify DNA from fungal fruiting bodies and mycelia using the Maxwell® RSC PureFood GMO and Authentication Kit on a Maxwell® RSC Instrument.

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

Analyses:

- UV absorbance
- Dye-based quantitation
- qPCR
- TapeStation Analysis
- Oxford Nanopore Technologies® MinION Sequencing

Sample Type(s): Fungal fruiting body (*Ganoderma*, *Hericium*)
Fungal mycelium (*Ganoderma*)

Input: ≤ 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 PureFood GMO and Authentication Kit (Cat.# AS1600)
- Maxwell® RSC Instrument (Cat.# AS4500) or Maxwell® RSC 48 Instrument (Cat.# AS8500)
- Liquid nitrogen, mortar, and pestle *OR*
- Bead beating supplies
  - Lysing Matrix A – MP Biomedicals, Cat.# 6910050
  - Digital Vortex Genie II – Scientific Industries or similar
  - Horizontal Vortex Adaptor for 1.5-2.0ml tubes – Qiagen, Cat.# 13000-V1 or similar
- Heat block set to 65°C, preferably with shaking (e.g. Thermomixer R)

Protocol:

1. If using a mortar and pestle for sample disruption
   a. Transfer tissue to a mortar pre-cooled with liquid nitrogen.
   b. Grind to a fine powder using a pre-cooled pestle.
   c. Transfer ≤ 200mg of ground tissue to a 1.5ml tube.
   d. Move tubes to room temperature and add 500µl of CTAB, 20µl of RNase A, and 40µl of Proteinase K.
2. If using bead beating for sample disruption
   a. Transfer ≤ 200mg of tissue to a Lysing Matrix A tube.
   b. Add 500µl of CTAB.

   c. Secure sample tubes on a digital vortex outfitted with a horizontal tube adaptor. Vortexes may require balancing of tubes and/or limited tube numbers to maintain vortex speed.
   d. Vortex for 10 minutes at 2600-3000rpm.
   e. Remove tubes from the vortex and add 20µl of RNase A and 40µl of Proteinase K.
3. Place samples in a heat block at 65°C for 1.5 – 2 hours with shaking at 600rpm.
4. During incubation, prepare RSC cartridges as described in the Maxwell® RSC PureFood GMO and Authentication Kit Technical Manual.
   a. Add 300µl of Lysis Buffer to Well #1.
   b. Place a plunger in Well #8.
   c. Add 100µl of Elution Buffer to each elution tube.
5. After incubation, centrifuge samples for 10 minutes at ≥16,000 × g.
6. Transfer entire clear supernatant (up to 500µl) to Well #1 of the reagent cartridge. Avoid pipetting any solid material.
7. Place the prepared cartridges in the Maxwell® RSC Instrument and run the Maxwell® RSC PureFood GMO and Authentication Kit method.

Results:

DNA was purified from fungal fruiting bodies and mycelium using the Maxwell® RSC PureFood GMO and Authentication Kit (Figure 1). Eluates may contain PCR inhibitors, but are amplifiable in qPCR when diluted (Figure 2). Sample disruption by grinding under liquid nitrogen may recover higher molecular weight DNA (Figure 2), while bead beating with Lysing Matrix A tubes on a vortex adaptor may allow for higher throughput purification. DNA is compatible with sequencing on an Oxford Nanopore Technologies® MinION Sequencing Device (Table 1, Figure 3).

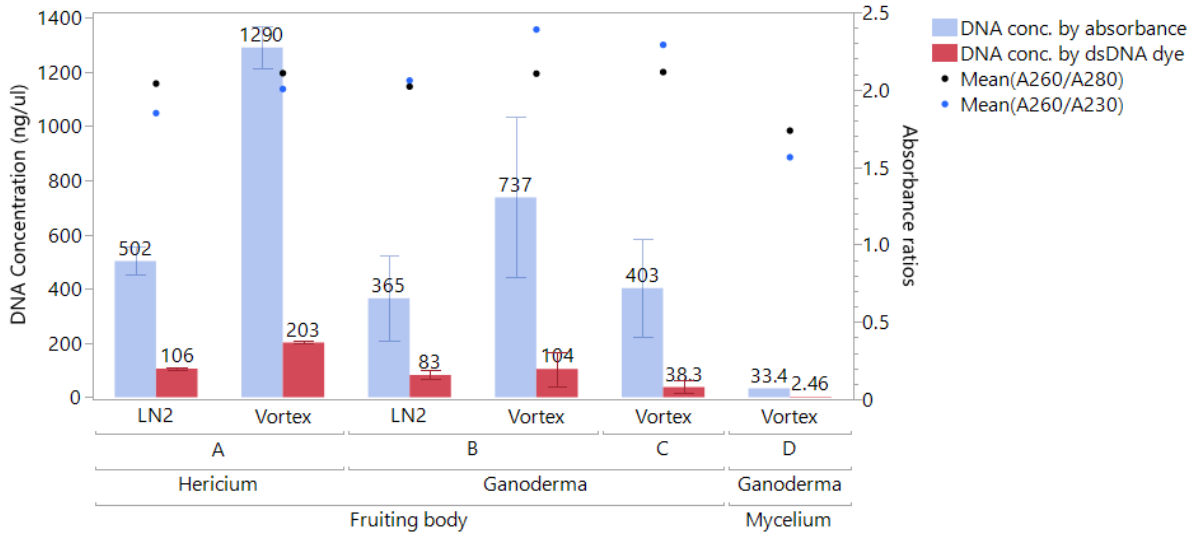

Figure 1. Concentration and absorbance ratios of DNA purified from *Hericium* or *Ganoderma* fungi using the Maxwell® RSC PureFood GMO and Authentication Kit. DNA was purified from fungal fruiting bodies (A,B,C) or mycelium (D) using either disruption by grinding under liquid nitrogen (LN2) or vortexing in Lysing Matrix A tubes as described in the protocol above. DNA concentration and A260/A280 and A260/A230 ratios were measured by absorbance using a NanoDrop™ One Spectrophotometer. DNA concentration was also measured using QuantiFluor® ONE dsDNA System (Cat.# E4871) on a Quantus™ Fluorometer (Cat.# E6150). Mean values ± stdev are shown for n=2 (A) or n=3 (B,C); n=1 for mycelium (D).

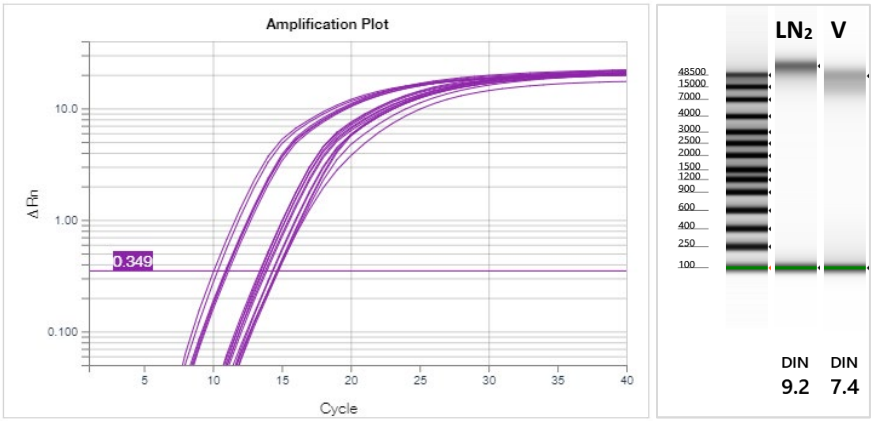

Figure 2. DNA amplification and integrity. (Left) *Ganoderma* DNA eluates shown in Figure 1 were diluted 1/100 (or 1/10 for lower concentration mycelium DNA) in Nuclease-Free Water (Cat.# P119E) and 2µl amplified in duplicate 20µl reactions with GoTaq® qPCR Master Mix (Cat.# A6001) and universal fungal 18S rRNA gene primers[1] on a QuantStudio™ 6 Pro Real-Time PCR Instrument (Applied Biosystems™). Semi-log plot shown. (Right) DNA integrity was evaluated for a subset of *Hericium* DNA eluates disrupted by grinding under liquid nitrogen (LN_2) or by vortexing in Lysing Matrix A tubes (V) using Genomic DNA ScreenTape reagents (Agilent, Cat.# 5067-5365) on a 4200 TapeStation (Agilent). DNA Integrity Numbers (DIN) are indicated for each sample.

Table 1. Sequencing metrics for *Hericium* DNA sequenced using Oxford Nanopore Technologies® Ligation Sequencing Kit on a MinION Sequencing Device. The *Hericium* fruiting body sample disrupted by vortexing in Lysing Matrix A tubes that is shown in Figure 2 was used as input for sequencing. Sequencing was performed as recommended by the manufacturer[2], using the Long Fragment Buffer for final library selection and substituting the ProNex® Size-Selective Purification System (Cat.# NG2001) for both library clean-up steps[3]. Fast5 sequences were basecalled using the high accuracy option (HAC) of the Guppy basecaller (v5.0.14). Fastq files were analyzed and plotted with NanoPlot (v1.29.1).

Sample	*Hericium* DNA
Sequencing time	~51 hrs
Total Yield (GB)	10.68
Reads Analyzed	1462148
Avg Quality Score	11.5
Median Quality Score	11.5
Mean Seq Length	7306
Median Read Length	5439
Maximum Read Length	192543
N50 read length	11707
Number of reads > 100kb	13

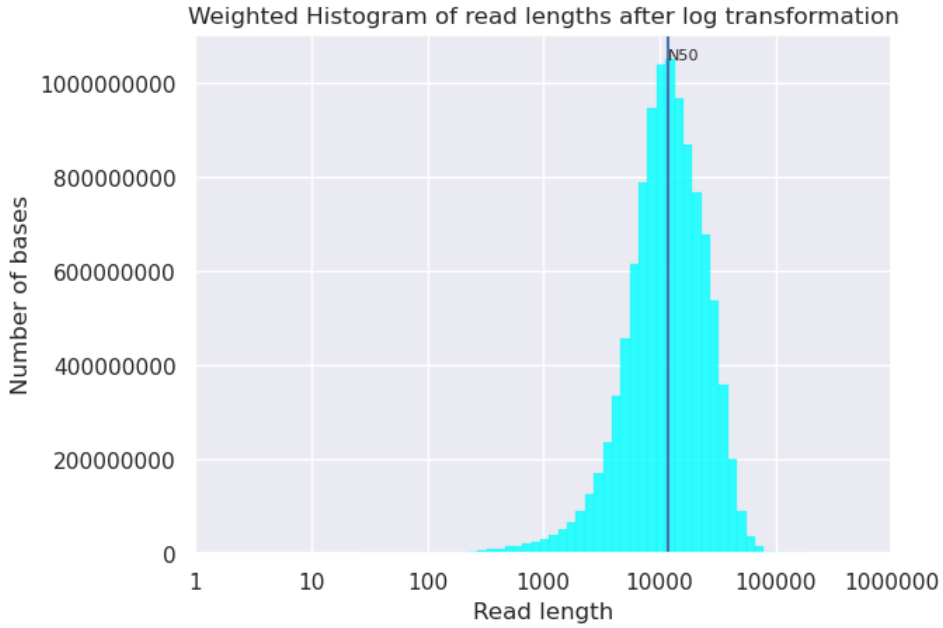

Figure 3. Distribution of bases sequenced versus read lengths for *Hericium* DNA sequenced using Oxford Nanopore Technologies® Ligation Sequencing Kit on a MinION Sequencing Device. Fast5 sequences were basecalled using the high accuracy option (HAC) of the Guppy basecaller (v5.0.14). Fastq files were analyzed and plotted with NanoPlot (v1.29.1). Data shown are weighted for the number of bases sequenced, with read length shown on a log scale. N50 is indicated by a vertical line.

References:

1. Embong, Z, Hitam, W., Yean, C., et. al. (2008) Specific detection of fungal pathogens by 18S rRNA gene PCR in microbial keratitis. BMC Opthalmol. 8:7.
2. Nanopore Protocol: Genomic DNA by Ligation (SQK-LSK110). DE_9108_v110_revI_10Nov2020
3. Application Note: ProNex® Chemistry Based Clean-Up in the Oxford Nanopore Ligation Sequencing Kit. PA411 08/21.