Promega

Product Application

Rice (*Oryza sativa*) Kernel DNA Purification

Isolate high quality, amplifiable DNA from brown or white rice kernels using the Maxwell® 16 System.

Kit: Maxwell® 16 FFS Nucleic Acid Extraction Kit (Cat. #X9431)

Analyses: GoTaq® qPCR, QuantiFluor® quantitation, gel

Sample Type(s): *Oryza sativa* kernels (brown or white medium grain or basmati rice)

Input: up to 200mg ground or whole kernels

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, please contact **techserv@promega.com**

Materials Required:

- Maxwell® 16 Instrument (Cat.# AS2000) with firmware version 4.97 or later
- Maxwell® 16 FFS Nucleic Acid Extraction Kit (custom Cat.# X9431)
- Optional: RNase A Solution (Cat.# A7973)
- 2.0mL screw-top tubes
- CTAB Buffer: 2% CTAB, 1.4M NaCl, 0.1M Tris 10mM EDTA pH 8.0
- Homogenization protocol only: Coffee grinder or other grinding apparatus
- Microcentrifuge
- Heat block - 65°C

Protocol:

Ground kernels (Recommended for brown rice): Grind kernels to a fine powder in liquid nitrogen with a mortar and pestle or in a coffee grinder with pulsing. Weigh up to 200mg of ground kernel into a 2ml tube.

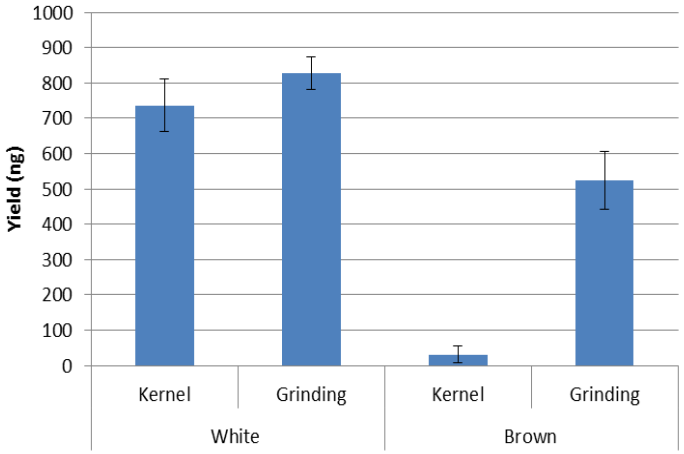
Whole kernels: Weigh up to 200mg of whole kernels (about 10 kernels) into a 2ml tube.

1. Add 1ml CTAB buffer spiked with 40µl Proteinase K and optionally 20µl RNase A Solution to prepared rice kernels.
2. Vortex vigorously and incubate 90 minutes at 65°C.
3. Centrifuge 10min at 16,000 x *g*.
4. Place the cartridge into the Maxwell® LEV cartridge rack and remove the seal.
5. Pipet 300µl sample supernatant and 300µl Lysis Buffer into well #1 of the Maxwell® 16 cartridge.
6. Place one of the supplied elution tubes into the sample rack and add 100µl of the supplied elution buffer.
7. Select LEV configuration of the Maxwell® Instrument and select method as follows: RUN, DNA: Plant. Start Run.

Results

Medium Grain Rice

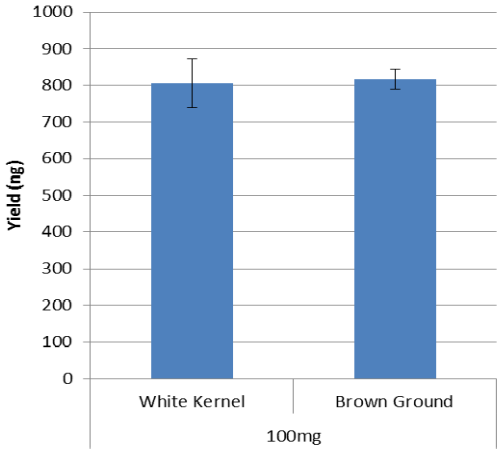
Rice Type	Input	[DNA] (ng/µl)
White	Kernel	35 ±10.4
	Ground	17.5 ±2.1
Brown	Kernel	2.1 ±0.7
	Ground	13 ±2.8



DNA concentration and yield. Left Panel: Concentration of DNA extracted from 100mg white and brown medium grain rice whole or ground kernels and eluted in 100µl. Concentration was calculated using the QuantiFluor® ONE dsDNA System (Cat. # E4871). **Right Panel:** Yield of amplifiable DNA determined by real-time PCR using GoTaq® qPCR Master Mix (Cat.# A6001), rice DNA specific primers, and a Rice Genomic DNA standard (Zyagen, Cat. #PLG-1004).

Basmati Rice

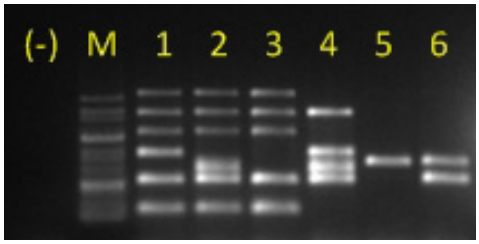
Rice Type	Input	[DNA] (ng/µl)
White whole kernel	100mg	28.3 ±5.1
Brown ground	100mg	23.7 ±11.5



DNA concentration and yield: Left Panel: Concentration of DNA extracted from white kernels or brown ground Basmati rice and eluted in 100µl. Concentration was calculated using the QuantiFluor® ONE dsDNA System (Cat.# # E4871). **Right Panel:** Yield of amplifiable DNA determined by real-time PCR using GoTaq® qPCR Master Mix (Cat.# A6001), rice DNA specific primers, and a Rice Genomic DNA standard (Zyagen, Cat. #PLG-1004).

Single Rice Kernel

To isolate DNA from single rice kernels, minor protocol modifications are suggested to maximize DNA concentration: Use half the CTAB and proteinase K volumes. After heating, add the entire sample (~520µl) to well #1, and elute in 50µl.



PCR amplification of extracted rice kernel DNA. The gel image shows PCR products from DNA extracted from a single rice kernel and amplified using the Rice Multiple-X™ PCR Kit (SolGent Co., Ltd); 5µl gDNA was added to a 25µl reaction. Six different types of rice were tested for SNP identification.