GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

A high-throughput, high-quality plant genomic DNA extraction protocol

H. Li[1,2], J. Li[1,2], X.H. Cong[2], Y.B. Duan[2,3], L. Li[2], P.C. Wei[2], X.Z. Lu[2] and J.B. Yang[2]

[1]Key Laboratory of Ion Beam Bioengineering,
Institute of Technical Biology and Agriculture Engineering,
Chinese Academy of Sciences, Hefei, China
[2]Key Laboratory of Rice Genetics Breeding of Anhui Province,
Rice Research Institute, Anhui Academy of Agricultural Sciences, Hefei, China
[3]College of Life Sciences, Anhui Agricultural University, Hefei, China

Corresponding author: J.B. Yang
E-mail: yjianbo@263.net

Genet. Mol. Res. 12 (4): 4526-4539 (2013)
Received July 27, 2013
Accepted October 8, 2013
Published October 15, 2013
DOI http://dx.doi.org/10.4238/2013.October.15.1

ABSTRACT. The isolation of high-quality genomic DNA (gDNA) is a crucial technique in plant molecular biology. The quality of gDNA determines the reliability of real-time polymerase chain reaction (PCR) analysis. In this paper, we reported a high-quality gDNA extraction protocol optimized for real-time PCR in a variety of plant species. Performed in a 96-well block, our protocol provides high throughput. Without the need for phenol-chloroform and liquid nitrogen or dry ice, our protocol is safer and more cost-efficient than traditional DNA extraction methods. The method takes 10 mg leaf tissue to yield 5-10 µg high-quality gDNA. Spectral measurement and electrophoresis were used to demonstrate gDNA purity. The extracted DNA was qualified in a restriction enzyme digestion assay and conventional PCR. The real-time PCR amplification was sufficiently sensitive to detect gDNA at very low concentrations (3 pg/µL). The standard curve of gDNA dilutions from our phenol-chloroform-free protocol showed better linearity (R^2 = 0.9967) than the phenol-chloroform protocol (R^2 =

0.9876). The results indicate that the gDNA was of high quality and fit for real-time PCR. This safe, high-throughput plant gDNA extraction protocol could be used to isolate high-quality gDNA for real-time PCR and other downstream molecular applications.

Key words: Genomic DNA extraction; Liquid nitrogen-free protocol; High-throughput protocol; Phenol-chloroform-free protocol; High-quality protocol; Real-time PCR

