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Fungal and plant DNA extraction using cellulose based paper strip method

A.P.H. Somarathna[1*], T.T.D. Manilgama[1], N. Ambanpola[1], K.N. Seneviratne[1], and N. Jayathilaka[1]

[1]Department of Chemistry, Faculty of Science, University of Kelaniya, Sri Lanka

The PCR is a frequently used laboratory technique. It can amplify a minimal amount of DNA in the range of picograms and even in the presence of various contaminants. Extraction of pure DNA from fungi and plants for PCR and sequencing purposes is very difficult due to their compact cell wall structure. This study aimed to check whether the impurities can be removed from the recovered DNA using the cellulose-based paper strip method and obtain clean DNA for PCR and sequencing. Three DNA extraction methods (paper strip method after cell lysis, paper strip method after organic extraction with chloroform/isoamyl alcohol step and paper strip method following organic extraction with chloroform/isoamyl alcohol step after adding phenol/chloroform/isoamyl alcohol) were compared with the classical CTAB method in terms of DNA yield (ng/mg) and the purity of DNA based on A260/280. The absorbance was taken with thermo scientific μDrop plate using the spectrophotometer. The DNA amplification was analyzed using SYBR Green-based Real-Time quantitative PCR (Step One Real-Time PCR, Applied Bio Sciences). Data were analyzed by the Krsuskal Wallis test using SPSS software. The purity of DNA from all four methods for each species did not show a significant difference ($P<0.05$). However, better PCR amplification curves were given for all species with the paper strip method following organic extraction with chloroform/isoamyl alcohol after the phenol/chloroform/isoamyl alcohol step, though this method has a low yield and purity even compared to the classical CTAB method. According to the Cq values, there is abundant target nucleic acid in the samples except those purified with paper strip method following chloroform/isoamyl alcohol. Clean DNA is produced from *Aspergillus niger* (17 ± 0.7 ng/mg), *Candida albicans* (21 ± 6.0 ng/mg) and *Plumeria rubra* (33 ± 21 ng/mg) and *Orchidaceae* (31±21 ng/mg) with the paper strip method following organic extraction with chloroform/isoamyl alcohol step after adding phenol/chloroform/isoamyl alcohol for PCR. Therefore, this method can be used as a substitute for the classical CTAB method to obtain clean DNA for PCR and sequencing purposes.

Keywords: CTAB, DNA extraction, Paper strip method, PCR.

[*]pavithrahiru@gmail.com 0706703556
ORCiD ID: https://orcid.org/0009-0002-8799-8055