UNIVERSAL PROTOCOL FOR NUCLEIC ACID PURIFICATION FOR PLANT TAXA

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Abstract

Good quality DNA is the prerequisite for any type of biotechnological research. Several protocols are described by many researchers as well as quite a few dozen commercial DNA isolation kits are available in the market. Most of the protocols are very specific for typical taxonomic group and may not have similar quality of DNA with different taxa. Under this study we have developed a protocol which is universal for all plant taxa and can be used in isolation of DNA from live, dried and alcohol preservedtissues of variety of plants taxa.This protocol is relatively economical as it does not involve the RNAse-A and Proteinase-K use. It is environmental friendly as no use of phenol and non-hazardous since no use of liquid nitrogen. The protocol is proved to be less time consuming with better DNA yield from small tissue.

Key words: DNA; plant taxa; tissue; CTAB; PVP; rbcL; sequence.

Introduction

In molecular biology or biotechnological research, obtaining good quality DNA is the prerequisite and important step (Kumari, et al. 2012).Recently, vascular plant systematics has begun to include studies of DNA variation as one of the common methods of analysis (Chase and Hills, 1991).Obtaining DNA from plant resources is cumbersome and becomes complicated due to presence of many active principles. It includes the polysaccharides, polyphenolic compounds and other secondary metabolites whichcoprecipitate with DNA in the extraction procedure and inhibit DNA digestion and PCR (Zhange andMcStewart, 2000). The renowned use of CTAB for purification of DNA from plants is historic but is applicable specifically in angiosperms(Murray and Thompson, 1980).The present protocol is the developed by modifying the CTAB protocol (Murray and Thompson, 1980) and tested fordifferent plant taxa and prove to be better in DNA quality, quantity, quicker as well as cost effective as compared to options available to date.

Material methods

Plant material collectionThe fresh plant material was collected and preserved in 95% ethanol (Pyle and Adams 1989)during June, 2011 to October, 2012. It includes, dry leaves, bark, stem and hard wood. The representative plants used for the present study were *Spirulinarobusta* (Algae), *Ricciahimalensis* (Bryophyte), *Nephrolepisexaltata* (Pteridophyte), *Thujaorientalis* (Gymnosperm), *Bambusavulgaris*(Monocot) and*Acalyphahispida* (Dicot).

Reagents and chemicals

Extraction buffer:1M Tris-HCl5ml (pH 8.0);0.5M EDTA, 2ml (pH 8.0);5M NaCl, 17.5ml;10% CTAB (Cetyl-trimethly-ammonium-bromide)10ml, (5gm in 50ml);double distilled water, 15.5ml to make the volume 50ml and adjust the pH to 7.5to 8.0. Autoclave the whole content before use.

PolyVinylPyrrolidone (PVP)(molecular weight 30-40kd) 20g/l of CTAB buffer added just before use.

βmercapto ethanol (2 mercapto ethanol).

20% SDS (Sodium-dodacyl-sulphate)v/v in water.

24:1 Chloroform: Isoamyl alcohol made by mixing the two solutions together v/v.

TE buffer: 10mM Tris and 1mM EDTA mixed at pH: 8.4.

Protocol details

Take small quantity of plant tissue (approximately 100mg, 0.5-1cm^2) and dry it on clean tissue paper, while dealing with angiosperms, mid rib region of the leaves must be removed; transfer it to microcentrifuge tubes (1.5 ml).Add to it 600µl of pre-warmed (60^{0}C) CTAB buffer (extraction

buffer)+PVP mixture and 5µl ofβ-Mercapto-ethanol.

Crush the plant tissue in the mixture with sterile scissor in fume hood chamber.Incubate the mixture at 55^0C for one hour with gentle vortexfor every 20 minutes.Add to it 10 µl of 20% SDS solution and vortex mixture vigorously. Incubate the lysate for 2 hours at 55^0Cwith gentle vortex every 20 minutes.

Cool the mixture (lysate)at room temperature and add to it 600µl of Chloroform:Isoamyl alcohol mixture (24:1) and mix the solution on vortex until the solution turns milky white.Centrifuge the mixture at 14000 rpm for 10minutes at room temperature.

Take the supernatant in fresh micro centrifuge tube and pallet is discarded.Add chilled 400µl of isopropyl alcohol and mix slowly until white threads of DNA become visible.Keep the content at -20^0C for one hour.Centrifuge at 14,000 rpm for 10 minutes at 4^0C.Decant the supernatant carefully and add 500µl of 70% chilled ethanol and invert several time gently.Centrifuge at 10000 rpm for 10 minutes at 4^0C.Decant the ethanol carefully and dry the pellet at room temperature under vacuum.Dissolve the DNA pellet in 50µl of TE for further use. Usually for dissolving DNA in TE solution can take 3-4 hours. The DNA was quantified by a nanodrop spectrophotometer (ND-1000, Thermo Corporation),quality was then checked on 1% agarose gel and gel image was documented using gel documentation system (BioRad Corp., USA). Extracted DNA was tested for its PCR and DNA sequencing compatibility using various primers. Results were compared and documented.

Result and discussion

A total of – plants were used for testing the DNA isolation protocol capability. Different plant tissues like, plant leaves, bark, hard wood, fruit, flower, stem etc. were used for DNA isolation. Following the above mentioned protocol we have isolated DNA. The quantity of DNA tested and found to be in the range of 60-100 ng/µl. The quality of DNA was tested on agarose gel (figure 1) having the first band of genomic DNA followed by second band of mitochondrial DNA with 260/280 ratio of around 1.75 for each of the sample. The protocol suggested by Doyle and Doyle (1990) and Khanuja et al. (1999) is restricted for isolation DNA from angiosperms only and some critical steps in this protocol were eliminated from present protocol were like, use of liquid nitrogen (Doyle and Doyle, 1990; Khanuja et al., 1999; Porebski et al., 1997; Jobes et al., 1995), proteinase-K (Porebski et al., 1997; Jobes et al., 1995) and RNAse (Porebski et al., 1997; Jobes et al., 1995) and activated charcoal (Zhang and McStewart 2000, Zidani et al. 2005).

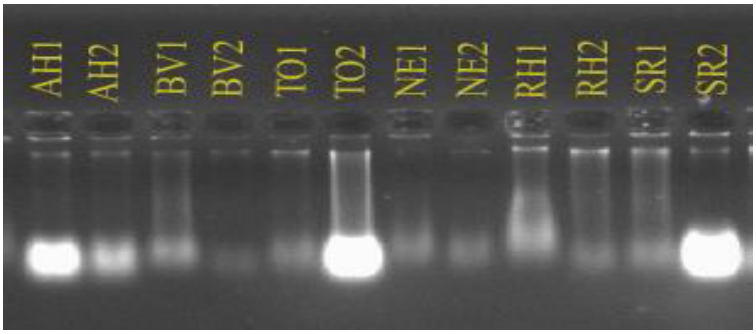


Figure 1. The agarose gel picture of isolated DNA from samples: *Acalyphahispida* (AH1 and 2), *Bambusa vulgaris*(BV1 and 2), *Thujaorientalis* (TO1 and 2), *Nephrolepisexaltata* (NE1 and 2),*Ricciahimalensis* (RH1 and 2), *Spirulinarobusta* (SR1 and 2).

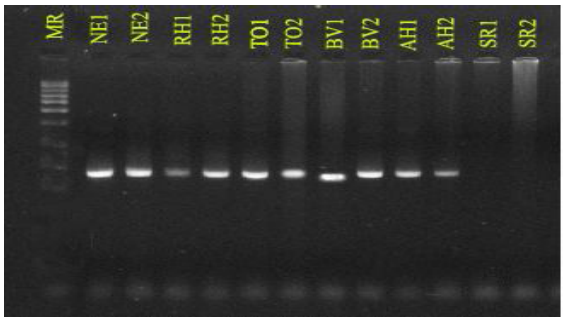


Figure 2. The agarose gel picture of PCR amplified DNA for rbcL gene from samples: Marker DNA (MR), *Nephrolepisexaltata* (NE1 and NE2), *Ricciahimalensis* (RH1 and RH2), *Thujaorientalis* (TO1 and TO2), *Bambusavulgaris*(BV1 and BV2), *Acalyphahispida* (AH1 and AH2), *Spirulinarobusta* (SR1 and SR2) taken under Bio-Rad Gel Documentation System.

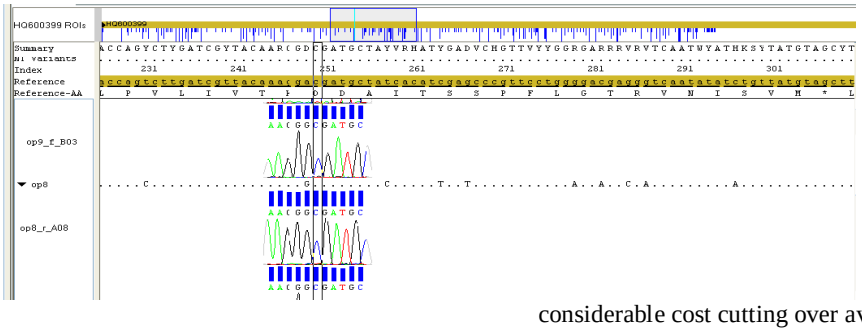
From the gel image it is evident that there is a presence of RNA which is glowing at bottom, but without treating with RNAse enzyme, we had good PCR amplification using primers rbcla-F: sequence:
ATGTCACCACAAACAGAGACTAAAGC
(Levin et al., 2003)and rbcla-R: with sequence: GTAAAATCAAGTCCACCRCG (Kress & Erickson, 2007) for all samples except sample no. SR1 & 2 (figure 2) since we do not have appropriate primers for this taxon (Algae). From the gel image we can conclude that there are very less primer dimers (figure 2) which can be possibly eliminated by lowering the primer and templet DNA concentration which we have not tried. This can even help in eliminating the PCR clean-up step for sequencing (BigDye® Terminator v3.1 Cycle Sequencing Kit Protocol, 2010). In many protocol the DNA quality is responsible for bad or no PCR amplification (BigDye® Terminator v3.1 Cycle Sequencing Kit Protocol, 2010). Very common PCR inhibitors include presence phenol, salts and sugars getting into the reaction from DNA isolation protocol itself (Zhange and McStewart, 2000). In our protocol we have not used phenol which already declared hazardous (Shih et al., 1996) and to avoid presence of polyphenolic compounds we have used PVP (Doyle and Doyle, 1990) and for eliminating salts and sugars a very careful steps were suggested in the protocol.

The PCR product was further tested for its sequencing quality using ABI 3130 genetic analyser and we found that as per expected product size we had excellent sequence quality (figure 3) tested with SeqScape2.7ver. The sequencing chemistry used was ABI BigDye terminator v3.1 (BigDye® Terminator v3.1 Cycle Sequencing Kit Protocol, 2010). If the DNA obtained is having salt, sugar or phenolic residues can either interfere during PCR or during sequencing (BigDye® Terminator v3.1 Cycle Sequencing Kit Protocol, 2010) but in our experiment we had good PCR as well as excellent sequence quality which shows that our protocol is not having any contamination issues mentioned above.

Figure 3. Electrophorogram of obtained sequences

This protocol is relatively inexpensive with available commercial kits or protocols due to the avoidance of most expensive consumables like Proteinase K, RNAse, phenol and liquid nitrogen.

ConclusionFrom the results obtained it can be conclude that theexperimental protocol for DNA isolation is



advantageous over some of the existing protocols as it avoids usage of proteinaseK and RNAse, skips hectic crushing methods,use of liquid nitrogen. The isolation methods also skips use of activated charcoal and phenol which is hazardous. The quality and quantity of DNA is comparable with any existing methods and can be compatible for PCR amplification as well as high quality sequencing. Additional benefit associated is the considerable cost cutting over available options for DNA isolation.

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