A Simple and Rapid System for DNA and RNA Isolation from Diverse Plants Using Handmade Kit

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Farhad Masoomi-Aladizgeh masoomi.farhads2030@gmail.com
1:Department of Tissue Culture and Gene Transformation, Agricultural Biotechnology Research Institute of Iran (ABRII), AREEO, 3135933151 Karaj, Iran
Corresponding Author

Leila Jabbari
1:Department of Tissue Culture and Gene Transformation, Agricultural Biotechnology Research Institute of Iran (ABRII), AREEO, 3135933151 Karaj, Iran

Reza Khayam Nekouei
2:Department of Agronomy and Plant Breeding, Faculty of Agricultural Sciences, University of Guilan, Rasht, Iran.

Ali Aalami
2:Department of Agronomy and Plant Breeding, Faculty of Agricultural Sciences, University of Guilan, Rasht, Iran.

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Abstract

This protocol describes a rapid DNA and RNA extraction method for plant tissues. Hexadecyltrimethylammonium bromide (CTAB), sodium chloride (NaCl), tris base, and ethylenediaminetetraacetic acid (EDTA) are the main components of the extraction buffer. In contrast to all previously reported protocols, this extraction method does not require any stock solutions. This isolation buffer is potential of extracting both DNA and RNA simultaneously. Depending on the purpose of the project, the corresponding steps can be slightly altered to obtain either DNA or RNA. The big advantage of this method is to use general laboratory chemicals to make a powerful extraction buffer, resulting in high quality and quantity nucleic acid. Also, CTAB in this buffer is capable of isolating nucleic acid from recalcitrant plants enriched in secondary metabolites. Importantly, this method is recommended for the projects at which isolating nucleic acid in a short time is of crucial importance. This method probably is usable for all plant tissues and takes about an hour.

Introduction

Nucleic acid extraction is the purification of DNA and RNA from biological samples which is an important step for further investigations. Studying on nucleic acid has been started by Johannes Friedrich Miescher in 1869, leading to the discovery of nucleic acid[1]. He tested a variety of experiments, and finally detected a substance with unexpected properties that was called nuclein (today is called DNA). Until now, many protocols have been devised for extracting nucleic acid from diverse samples. Chomczynski and Sacchi (2006) developed an extraction method based on acid guanidinium thiocyanate phenol chloroform which is universally used[2]. A modified cetyltrimethylammonium bromide (CTAB) protocol was introduced for DNA isolation[3]. Ghawana et al. (2011) described an RNA isolation system free of guanidinium salt for plant tissues that have high secondary metabolites[4], and

other protocols which are usable for specific samples sources. Each of them has its own advantages and disadvantages, and is used for a specific range of tissues and organs. Among this several protocols, the CTAB protocol, which was developed by Murray and Thompson has wide applications[5]. This method is appropriate for the isolation of nucleic acid from plants and is particularly suitable for the elimination of polysaccharide and polyphenolic compounds which reduce the quality of nucleic acids. Now, CTAB as an important plant DNA and RNA isolation system is routinely used for many purposes.

In this study, a new system is developed to shorten the nucleic acid extraction steps and strengthen their quality. It is applicable to isolate nucleic acid from most plants as well as recalcitrant ones from which the isolation of nucleic acid is difficult. Thus, it provides a high throughput protocol which can be employed for a widespread range of plant tissues. This protocol has been tested on different samples, including apple, pear, potato, thyme, wheat, rice, and many other samples. This method is simple and cost-efficient in comparison to other methods.

Reagents

• Hexadecyltrimethylammonium bromide (CTAB; Sigma-Aldrich, cat. no. H5882)

• Tris (hydroxymethyl) aminomethane (Tris base; Sigma-Aldrich, cat. no. 77-86-1)

• Ethylenediaminetetraacetic acid disodium dehydrate (EDTA; Duchefa Biochemie, cat. no. 6381-92-6)

• Sodium chloride (NaCl; Duchefa Biochemie, cat. no. 7647-14-5)

• 2-mercaptoethanol (βME; Sigma-Aldrich, cat. no. 60-24-2)

! CAUTION the βME is caustic, avoid contact by using it under a fume hood and wear protective gloves, lab coat and eye protection.

• Polyvinylpyrrolidone (PVPP; Sigma-Aldrich, cat. no. 9003-39-8)

• Isopropanol (ROMIL Pure Chemistry; cat. no. 67-63-0)

• Chloroform (ROMIL Pure Chemistry, cat. no. 67-66-3)

! CAUTION the chloroform is toxic and produces fumes. Use it under a fume hood and wear protective gloves, lab coat and eye protection

• Isoamyl alchohol (Acros Organics, cat. no. 30899-19-5)

! CAUTION The isoamylalchohol is caustic. Use it under a fume hood and wear protective gloves, lab coat and eye protection

• Ribonuclease A (RNase A; Thermo Fisher Scientific, cat. no. 12091-039) • Ethanol absolute (EtOH; Bidestan Co, cat. no.64-17-5)

Equipment

• NanoDrop ND-2000 spectrophotometer (Thermo Scientific, cat. no. ND-2000)

• Mortar and pestle

• Water bath

• Fume hood

• PH meter (Brinkmann Metrohm 744 pH Meter 1.744.0010)

• Refrigerating benchtop centrifuge (Heraeus Biofuge Fresco, # 1502)

• Micropipetts (Eppendorf Research 2100, 0.5-10 µl, 10-100 µl, 100-1000 µl)

• RNase-free tubes, 1.5 and 2 ml (FarayandDanesh Arian Co)

Procedure

Extraction buffer preparation

Add CTAB (0.5 g), EDTA (1 g), Tris base (2.5 g), and NaCl (5 g) as tetrad components of the extraction buffer to 100 ml autoclaved water and then gradually dissolve it by shaking at room temperature.

CRITICAL Add 10 µl βME to 1 ml of the extraction buffer before using to decrease the

possible oxidation only for tissues with high polysaccharides and secondary metabolites.

CRITICAL Add 15 mg PVPP per 1 ml of extraction buffer only for tissues with high polyphenolic pollution.

Homogenization of Tissues (TIMING 10 min for 5 samples)

1 Ground samples (leaf, shoot, root, and recalcitrant samples, approximately 0.5-1 g) using 1 ml of the extraction buffer with or without liquid nitrogen in mortar and pestle that are sterilized.

CRITICAL STEP The procedure are carried out at room temperature except the centrifugation steps (at 4℃) as well as the time of precipitating of the nucleic acid using the isopropanol (on ice).

CRITICAL STEP Severely disrupt the tissues to create the glaze mode of samples.

2 Transfer the resulting solution to a sterile centrifuge tube (size=2 ml), and then mix sample by briefly vortexing until the sample is thoroughly resuspended.

Triple Phase Separation (TIMING 25 min)

3 In this step, incubate samples at 65℃ for 10 min for lysing cells completely.

4 Add 600 µl of chloroform: isoamyl alcohols (24:1) to each tube, homogenize them by vortexing.

5 Centrifuge at 13700 g at 4℃ for 10 min.

CRITICAL STEP The PH of the extraction buffer is about 8-9, resulting in DNA and RNA precipitation, however, it could result in lower DNA and higher RNA concentrations in case of reducing the PH to around 6-7.

Precipitation of Nucleic Acid (TIMING 15 min)

6 Transfer the upper aqueous phase to a new tube (size= 1.5 ml).

7 Add 700 µl of cold isopropanol to precipitate RNA or DNA and then invert tubes 3-4 times to mix the solution.

8 Centrifuge tubes at 13700 g at 4℃ for 10 min. The white pellet will be visible on the bottom of the tubes.

CRITICAL STEP Do not disturb bottom phases of the solution when you pipet the upper phase.

Purification of Nucleic Acid (TIMING 5 min)

9 Discard the supernatant and wash the precipitate nucleic acid gently with 70% EtOH.

10 After centrifuging at 5400 g at 4˚C for 5 min, remove the supernatant and then air-dry the pellet.

Dissolving and Storage Condition (TIMING 5 min)

11 Dissolve the pellet in 20-30 µl of RNase free water (commercial) or autoclaved water.

12 After incubating at room temperature for a few minutes, keep the solubilized nucleic acid in −20˚C for a short time storage or in −80˚C for a longtime storage. Figure 1 shows a schematic model of all the DNA and RNA isolation steps of this procedure.

Timing

TIMING

Tissue grinding, Steps 1-2: 10 min

Isolation of nucleic acid, Steps 3-5: 25 min

Nucleic acid precipitation, Steps 6-8: 15 min

Preparation and dissolving the nucleic acid, Steps 9-12: 10 min

Troubleshooting

?TROUBLESHOOTING

Troubleshooting advice can be found in Figure 2.

Anticipated Results

The main purpose of this research was to access a simple protocol by which nucleic acid

isolation would be possible for all plants, and relevant projects can be performed at each laboratory with having access to general chemical resources. Dual applications of the extraction buffer is resulted in both DNA and RNA (shown in Figure 3). DNA and RNA can be purified by adding RNase and DNase enzymes, respectively, or by adjusting the PH of the extraction buffer. It is considerable that all the buffer components consist certain amount of the chemicals that is one of the big advantages of this protocol. PCR, RT-PCR, real time-PCR and southern blot analysis have been conducted to approve the quality of the extracted DNA and RNA which are acceptable (shown in Figure 4).

References

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Figures

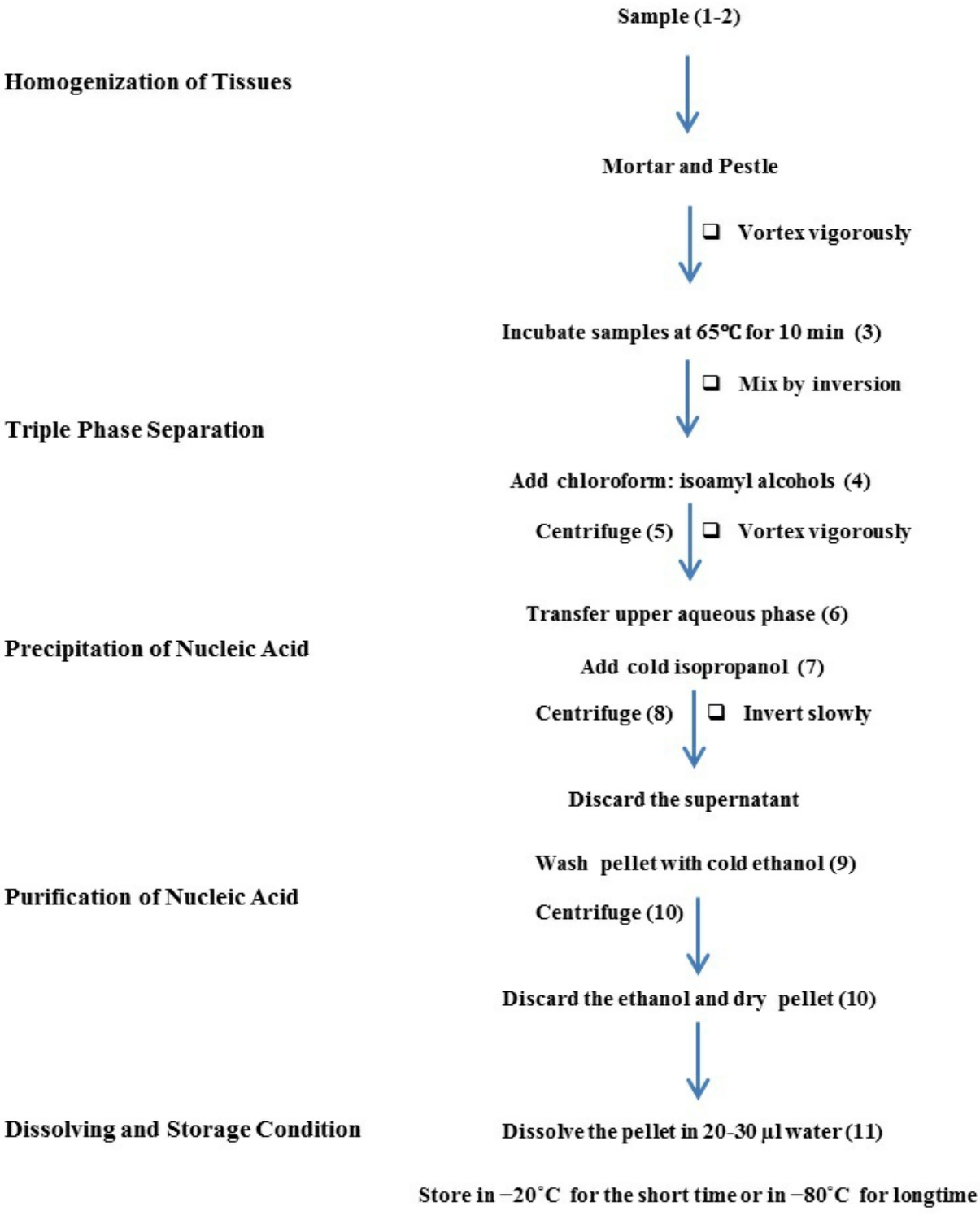

Figure 1

Figure 1 Schematic model of all the DNA and RNA isolation steps

Problems	Possible reason	Solution
Low A_{260}/A_{280} ratio	Phenol contamination	Do not carry the organic phase with upper phase
	Incomplete homogenization or disruption of samples	Use 1 ml extraction buffer for lysing 0.1 g tissue and disrupt severely
	DNA or RNA pellet have not been completely dissolved	Incubate at room temperature for 5 min or 50°C for a minute
Low DNA and RNA yields	Incomplete homogenization or disruption of samples	Use 1 ml extraction buffer for lysing 0.1 g tissue and disrupt severely
	DNA or RNA pellet have not been completely dissolved	Incubate at room temperature for 5 min or 50°C for a minute
RNA degradation	RNA diluted with RNase contamination water	Sure use the RNase free water
	RNase contamination during extraction processing	βME break the disulphide bonds of RNases and help to maintaining the RNA during extraction.
	RNase contamination of electrophoresis tank and running buffer	Check the running buffer and electrophoresis tools

Figure 2

Table 1 ?TROUBLESHOOTING

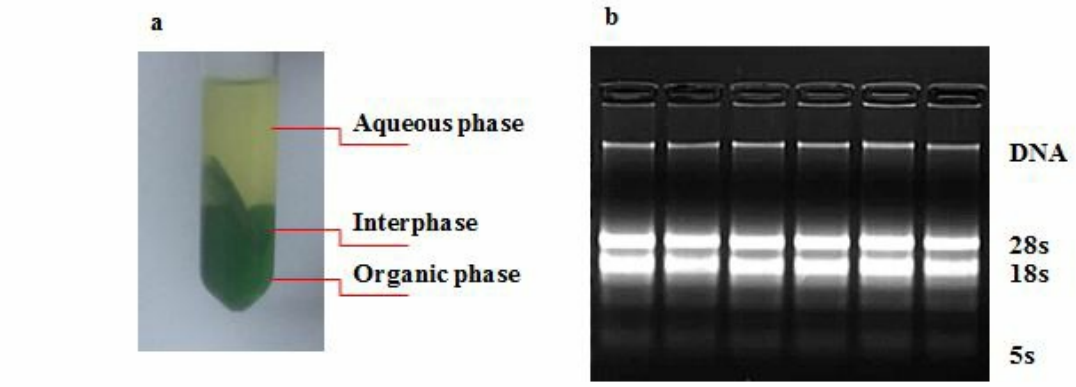

Figure 2 Examples of DNA and RNA quality from isolated samples (a) Triple phase separation (b) Isolation of both DNA and RNA

Figure 3

Figure 2 DNA and RNA quality from isolated samples

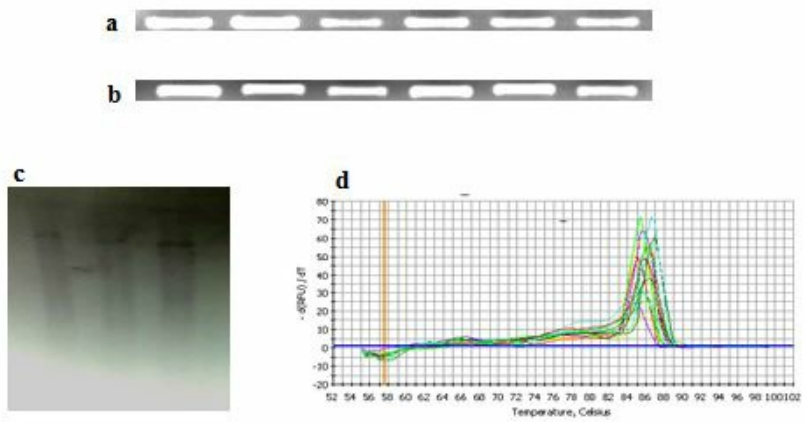

Figure 3 PCR, RT-PCR, real time-PCR and southern blot analysis. (a) PCR product of isolated DNA (b) cDNA synthesis of extracted RNA (c) Southern blotting was performed accurately (d) Expressed single peak of real time-PCR analysis

Figure 4

Figure 3 PCR, RT-PCR, real time-PCR and southern blot analysis