

Genomic DNA Isolation from Fungi, Algae, Plant, Bacteria and Human Blood using CTAB

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Abstract: DNA isolation with good intensity and quality is always been a necessity, whether you need for PCR or any other analysis. Cetyltrimethylammonium bromide (CTAB) buffer is used for DNA extraction from plants with various other extraction buffers. CTAB is mainly used in extraction of DNA from plants species. Here we have used CTAB for DNA extraction not only from plants but various other samples like FUNGI, ALGAE, BACTERIA, and HUMAN BLOOD. In this paper, not only isolation is performed from various samples but also an optimized protocol is introduced. Same protocol is used for all the samples for extraction only the concentration used varies. This method is basically introduced for Universities where there is time limit, Research centre's where DNA extraction has to be quick enough with good quality and intensity in just one go and also can be used in High schools where students can easily handle with no risk of injury and extraction can be done quickly. This method is cheap enough to handle expenses in any organization as well. We have not used any other extraction buffer. CTAB buffer with beta-mercaptoethanol is used that helps in degradation of polysaccharides and proteins to get pure form of DNA.

Keywords: CTAB, Isolation of DNA from CTAB, DNA isolation, DNA extraction, Genomic DNA.

1. Introduction

The very first step in any research laboratory is to isolate DNA with good efficiency, yield and intensity. DNA purity matters a lot because contaminants like tannins, polysaccharides, and some secondary metabolites inhibits enzymes used for further analysis of DNA in PCR, AFLP, RFLP etc (Chen Niu¹, Hirut Kebede, et. al, 2008 ; article: R.I.H. Ibrahim, 2011). CTAB buffer is a type of detergent that helps in lysis of cell membrane. CTAB is the best method for problematic samples and helps in separating polysaccharides and rescue of nucleic acids. CTAB contains EDTA that prevents DNA from degradation by chelating magnesium ions needed for enzymes that degrade DNA (K.L Tiwari, S.K. Jhadav, 2012). Beta-mercaptoethanol used along with CTAB helps in protein degradation which is the immediate step after polysaccharides of cell membrane. CTAB inhibits some of the proteins and also denatures till some extend (Article: Loroto Lekgari, 2010). CTAB is associated with high salts that act as reducing agents and some selective precipitants of nucleic acids (R.I.H Ibrahim, 2011).

CTAB has proven to be a universal buffer to help in DNA isolation. CTAB with CIA (chloroform: isoamyl alcohol) is also used for extraction of DNA from previously soaked bones, buried bones and burned bones (Jian Ye ; Anquan Ji et.al , 2004). Basically the motive of this study is to develop an easy, harmless and quick method of DNA extraction with CTAB from five various organisms with one protocol. The resulting DNA is a high quality intense DNA that can be subjected to PCR, RAPD, RFLP etc. techniques with low cost beneficial for Universities, Non- funded laboratories, High schools.

2. Materials and Methods

2.1 Solutions

- a) CTAB BUFFER: (for 100ml)
 - TRIS-HCL - 100Mm
 - EDTA - 25Mm
 - NaCL - 1.5M
 - C-TAB - 2%
 - DOUBLE DISTILLED WATER – make up upto the volume
- b) CIA: chloroform:isoamylalcohol (24:1)
- c) β- mercaptoethanol
- d) 70% ethanol
- e) TE (1:10)
- f) RBC LYSIS BUFFER (in case of blood sample)
- g) PVP (in case of pant sample)

2.2 DNA Isolation Protocol

- In case of plant sample, crush leaves with help of mortar-pestle using PVP to remove phenolic contamination, add adequate amount of CTAB into leaves to make fine slurry, take 1.5ml of sample into 2ml eppendorf tube.
- Add 10µl of β-mercaptoethanol into each eppendorf tube, shake well to mix properly. Incubate at 65°C for 1 hour in water bath.
- Centrifuge at 13000rpm for 15mins, collect supernatant in new eppendorf tube and add equal amount of CIA to the supernatant.
- Three layers appear, collect the upper layer in a fresh eppendorf tube then add equal volume of chilled isopropanol.
- Incubate for 20mins at 20°C so as to precipitate the DNA.
- Centrifuge at 13000 rpm for 15 mins.

- Discard the supernatant and add 500 μ l of 70% ethanol to the pellet obtained for washing.
- Mix well and centrifuge at 8000 rpm for 5 mins.
- Discard the supernatant and add 70 μ l of TE buffer and proceed for gel electrophoresis and quantification.

NOTE: The above protocol will be used for DNA isolation from FUNGI, ALGAE, PLANT, BLOOD & BACTERIA. In case of bacterial DNA isolation, the incubation after adding CTAB will be at room temperature for 30 minutes. In case of blood CTAB buffer is used in place of WBC buffer.

2.3 Purity of DNA

The purity of DNA was carried out by determining absorbance ratio between λ 260 and λ 280nm by spectrophotometer. O.D recorded has given at maximum of 1.8 and at minimum of 1.56 of all samples.

2.4 Agar Gel Electrophoresis

1% of agarose gel was prepared (5 μ l of 10mg/ml concentration of ethidium bromide was added). Gel was submerged in running buffer. 3 μ l of loading dye and 7 μ l of sample was loaded in each well. A voltage of 90 was applied to electrophoresis apparatus. DNA band were observed under UV transilluminator.

3. Results And Discussions

It was observed that the DNA isolated from all five different samples all with good intensity and purity. Purity was checked by spectrophotometer and quality and quantity was checked by running in 1% agarose gel and observing under UV transilluminator. Hence, the protocol optimized for DNA isolation from all different samples from CTAB is successfully effective.



Figure 1.1: Results of agar gel electrophoresis performed. First well shows bacterial DNA, second well shows algae DNA, the third well shows plant DNA, the fourth well shows fungal DNA and the fifth well shows human blood DNA.

Table 1.1: Results of DNA Quantification by Spectrophotometric Analysis

Samples	Absorbance(260/280)
Fungi	1.74
Algae	1.67
Plant	1.80
Human Blood	1.56
Bacteria	1.72

4. Conclusion

We conclude that DNA isolation can be done by single protocol and chemical. So such unification is quiet beneficial and advantageous.

5. Future Scope

The future scope to our research is that isolation of DNA from all the above samples will be quick enough for any student or researchers who need their results quick to perform further experiments and continue with their research. It is also inexpensive due to use of fewer chemicals. Moreover the most important the quality of the DNA isolated is very high and with a very high intensity. This is our main motive so as to reduce the cost and save time to make the most out of it as we all know that research is not a few days work it takes ages to work on a single gene.

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