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Modified Methods for Quick and Safe Extraction of DNA from Common Microbiological Samples

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Abstract

Isolating and purifying DNA are very important steps in DNA molecular techniques used in microbiological studies for the identification of genotypes, traits associated with genes of interest, and genetic diversity. Plant and soil materials are among the most difficult for high quality DNA extractions. While carrying out DNA extraction for over 2000 different sample types using 2 different kits (ZYMO Bacterial/Fungal extraction kit, ZYMO Soil extraction kit, ZYMO Plant extraction kit and E.Z.N.A. soil DNA Kit) and crude extraction CTAB method, several modifications were made to ensure extraction of good quality DNA. These modifications in the methods were documented; re used severally to confirm their accuracy and was done without the use of liquid Nitrogen. The extracted DNA obtained from CTAB extraction were extremely high and was diluted for use in PCR using a ratio 1:10 of DNA to water while those obtained from the kit were used directly (but in small quantities ~0.5 µL) or diluted in a ratio 50:50. The mean DNA yields obtained for CTAB extraction were between 500-4000 mg/µl while that obtained for the kits were between 70-400 mg/µl. The 260/280 nm absorbance ratio had a high level of purity between 1.8-2.0. These modified methods can be used for day to day extraction processes in the laboratory.

Keywords: DNA • Recovery • Extraction • Microbial • Efficient

Introduction

For over three decades microbiologists have done several analyses to extract DNA from environmental samples (eDNA) to enable them study the diverse microorganisms in their various communities [1]. The first isolation of Deoxyribonucleic Acid (DNA) was done in 1869 by Friedrich Miesche [2]. High quality DNA is a major necessity for all experiments that involves DNA manipulation and molecular techniques used molecular studies. Every DNA extraction protocols follow the same basic steps including the rupturing of the cell wall, cell membrane and nuclear membrane to let out the DNA into solution after which the DNA is precipitated out contaminate biomolecules (such as the proteins, polysaccharides, lipids, phenols and other secondary metabolites) are removed [3,4]. In the several recent microbiological researches the need for molecular studies has become of major importance hence the need for effective efficient, simple and reliable method for extraction of DNA [5,6]. DNA can be extracted from various sample types such as plant leaf, plant root, algea, soil samples, water samples, food samples, bacteria and fungi samples etc., the sample type and the DNA concentration needed determine the methodology of DNA extraction that will be followed by the researcher.

The need for more efficient methods to extract DNA with high qualities and yields have led to the development of several protocols, however the fundamentals of DNA extraction remains the same [7,8]. CTAB extraction protocol is one of the earliest and cheapest methods that have been used in the extraction of DNA for several sample types [9,10]. CTAB is a surfactant which has cationic nature and is required to break down cellular membrane to initiate cell lysis so that the genic materials can be extracted. It is therefore employed in DNA extraction/isolation and in manufacturing of novel Gene Therapies. It is sometimes takes a longer time and requires the use of a lot of harmful chemicals and may still give low yields of DNA with poor quality but it is very cost friendly especially for researchers in developing parts of the world and for commercial purposes [11].

Over time and in more recent years, DNA isolation and extraction kits have been invented and they have several advantages over crude methods (CTAB etc.). They are faster and simpler to use, and also rarely contain harmful chemicals such as phenol or chloroform and contact with samples and chemicals is highly reduced. They use spin columns or filters, which have silica-gel-based membranes that are able to bind to the DNA. The DNA when trapped in the membrane can be washed and cleaned free of contaminants and then eluted or released from the column (membrane) using nuclease

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free water. The DNA obtained using kits are normally purer and cleaner than DNA extracted using crude methods. However, kits are very expensive, with prices ranging between $250 and above for 50 reactions (samples) [12]. Thus making them unattractive for researchers in developing countries.

Plant and soil samples are among the most difficult for high quality DNA extractions. Proper preparation of the tissues before extraction is essential to achieve good results. In many cases liquid nitrogen flash freezing method is adopted followed by grinding the frozen tissue with a mortar and pestle [13-15]. Liquid nitrogen is very difficult and dangerous and harmful to use and therefore needs to be handled with care. We have therefore modified previous DNA extraction protocol for two commercial kits and CTAB to suit the facilities that we have to meet our target.

Materials

- Zymo extraction kit (bacterial/fungal, soil, plant, water)
- E.Z.N.A. soil DNA kit (Omega Bio-Tek, Norcross, GA, United States)
- Zymo extraction kit: protocols
- E.Z.N.A. soil DNA kit extraction: protocol

CTAB extraction protocol

CTAB buffer: 2% CTAB (hexadecyltrimethylammonium bromide), 100 mM TrisHCl (pH=8), 20 mM EDTA, 1.4 M NaCl, 0.2% β-mercaptoethanol, 0.1 mg/mL proteinase K

NB: Add 4.1 g NaCl in 80 mL of distilled water and add 10 g of CTAB slowly while heating (56˚C) and stir. It may take more than 3 hrs to dissolve CTAB completely. The final volume should be adjusted to 100 ml by adding distilled water and sterilized in an autoclave at 121˚C for 15 mins at 1 atm.

Methods

The methods for the kits were modified from the extraction protocols given from the manufacturer and the well-known Cetyltrimethylammonium Bromide (CTAB) method where CTAB is used for DNA extraction was also modified. The protocol for the ZYMO kits and the CTAB are simple and fast compared to other methods and the use of liquid nitrogen was not employed. The CTAB method was used for extracting bacteria, fungi, algea and plant samples, the EZNE was used for only soil and the zymo kit was used for extraction of DNA from water, soil, plant, algae etc. Each extraction was done using five replicates each. Plant samples, soil samples and water samples were processed as follows before extraction:

Plant samples: It should be noted that the leave and plant samples were allowed to dry in oven leaving them with little or no moisture in them. The use of Nitrogen was not required.

Soil samples: soil samples were allowed to dry to remove moisture from the samples.

Water samples: Water samples of about 500 mL were collected and each sample was passed through a sterilized Buchner funnel sterilized weighing paper (Fisher brand weighing paper cat No. 09-898-12 B made by fisher scientific Pittsburgh USA cut into the size of filter paper fitting the funnel) was used to filter the water sample. The forced pressure from the pump connected to the funnel was used to pull the water through the weighing paper. This too a longer time due to the small pore size of the weighing paper. The weighing paper was used because it was easier to fold into the eppendorf tube and too lesser space.

The modified protocols are as follows:

E.Z.N.A. soil DNA kit (Omega Bio-Tek, Norcross, GA, United States) modification to manufacturer's instructions

- Add 500-700 mg (instead of 100-250 mg) soil sample to a distruptor tube.
- Add 725 µL SLX-MLUS Buffer. Bead beat for 2 minutes mix manually for 2 minutes. Then vortex at maximum speed for 5 minutes to lyse sample.
- Spin at 500 xg for 5 seconds to remove drops of liquid from the lid.
- Add 72 µL DS buffer vortex at maximum speed for 4 minutes to mix thoroughly.
- Incubate at 70˚C for 10 mins. Briefly vortex the tube once during incubation.
- Centrifuge at 10,000 x g for 5 minutes at room temperature.
- Transfer 400 µL supernatant into a new 1.5 mL microcentrifuge tube
- Add 135 µL chilled p 2 Buffer. Vortex for 1 minute to mix thoroughly
- Let sit on ice for 5 mins
- Centrifuge at 13,000 x g for 3 minutes.
- Carefully transfer the supernatant to a new 1.5 µL microcentrifuge tube
- Add 200 µL HTR Reagent. Vortex to mix thoroughly. (HTR should be properly mixed).
- Let sit at room temperature for 3 minutes.
- Centrifuge at 13,000 x g for 5 minute.
- Transfer cleared supernatant (~500 µL) to a new 1.5 Ml microcentrifuge tube (step should be repeated if solution is still dark)
- Add an equal volume of XP 1 Buffer. Vortex for 1 minute to mix thoroughly.
- Insert a HiBand DNA Mini column into a 2 mL collection Tube.
- Transfer up to 700 µL sample from step 16 to the HiBind DNA Mini Column.
- Centrifuge at 13,000 x g for 5 minute at room temperature. Discard the filtrate and reuse the collection tube.
- Discard the filtrate and reuse the collection Tube
- Repeat steps 18-20 until all the lysate from step 16 has passed through the HiBind DNA mini column.
- Add 500 µL HBC Buffer (dilute with 32 mL 100% isopropanol before use).
- Centrifuge at 13,000 x g for 3 minute.
- Discard the filtrate and the collection tube.
- Centrifuge at 20,000 x g for 1 minute to get rid of excess HBC Buffer.

- Transfer the HiBaind DNA mini column into a new 2 mL collection tube.
- Add 700 µL DNA wash buffer (diluted with 100 Ml 100% ethanol).
- Centrifuge at 13,000 x g for 5 minute
- Discard the filtrate and reuse the collection tube.
- STEPS 26-28 should be repeated
- Centrifuge at 13,000 x g for 8 minutes
- Transfer HiBind DNA mini column into a clean 1.5 mL microcentrifuge tube
- Add 100 µL 70˚c pre heated elution buffer into the center of the HiBind matrix
- Let sit at room temperature for 1-2 minutes
- Centrifuge at 13,000 x g for 6 minutes
- Collect filtrate and place into the center of the same HiBind DNA Mini column used in the procedure.
- Let sit at room temperature for 30 mins
- Centrifuge at 13,000 x g for 5 minute
- Store eluted DNA at -20˚C.
- Load on gel or check quality using a nanodrop machine.
- Use for PCR and other molecular genetics studies.

DNA extraction procedure (uing Zymo kit)

- Add 250 (50-100) mg (wet weight) fungal or bacterial cells (can be centrifuged from broth repeatedly until ¼ of the tube is full of bacteria cells) that have been suspended in up to 100 µl of water or isotonic buffer (e.g., PBS) or up to 200 mg of tissue to a ZR BashingTM lysis tube. Add 750 ul lysis solutions to the tube (Figure 1).
- Secure in a bead fitted with 2 ml tube holder assembly and process at maximum speed for >5 minutes.
- Centrifuge the ZR BashingBeadTM lysis tube in a micro centrifuge at >10,000 x g for 1 minute.
- Transfer up to all the supernatant to a Zymo-SpinTM IV spin filter (orange top) in a collection tube and centrifuge at 7,000 x g for 1 minute.
- Add 700 µl of fungal/bacterial DNA binding buffer to the filtrate in the collection tube from step 4.
- Transfer 800 µl of the mixture from Step 5 to a Zymo-SpinTM IIC column in a collection tube and centrifuge at 10,000 x g for 1 minute.
- Discard the flow through from the collection tube and repeat Step 6.
- Add 150 µl DNA pre-wash buffers to the Zymo-Spin TM IIC column in new collection tube and centrifuge at 10,000 x g for 1 minute
- Add 350 µl fungal/bacterial DNA wash buffer to the Zymo-SpinTM IIC column and centrifuge at 10,000 x g for 1 minute
- Transfer the Zymo-SpinTM IIC column to a clean 1.5 ml micro centrifuge tube and add 70 ul DNA elution buffer directly to the column matrix. Centrifuge at 10,000 x g for 30 seconds to elute the DNA.
- Load on gel or check quality using a nanodrop machine.
- Use for PCR and other molecular genetics studies.

CTAB extraction for bacteria, soil and plant samples

- Add 250 mg bacteria and 500 mg soil or plant into an eppendorf tube
- Add bashing beads of almost equal amount to tubes for bacteria and plant samples while silver balls can be used for soil.
- Add 1 mL pre-heated CTAB DNA extraction buffer
- Add 1 µL Ribonuclease a solution (10 mg/Ml in glycerol, 10 Mm Tris-HCL pH 8.0)
- Mix in tissue lyser for 15 mins
- Incubate at 65˚C for 1 hour
- Centrifuge at 13,000 rpm for 8 mins
- Transfer clear supernatant to new 2 mL microcentrifuge tube
- Add equal volume of CIA (24:1 chloroform: Isoamyl alcohol mix)
- Mix for 5 minutes in a distruptor
- Centrifuge at 13,000 rpm for 2 mins.
- Transfer supernatant (clear) to new 2 mL eppendorf tubes
- Add equal volume of 2-propanol (100%) and
- Vortex using distruptor for 5 mins
- Centrifuge at 13,000 rpm for 5 minutes
- Discard supernatant
- Wash pellet by adding 1.8 mL 70% ethanol
- Vortex in distruptor for 10 Minutes
- Centrifuge at 13,00 rpm for 5 minutes
- Discard supernatant using micropipette or air dry by opening the tube till ethanol has completely evaporated
- Check for whitish pellets of DNA at the base of the tube.
- Add 150 µL TE buffer (1 m M Na_3 EDTA, 10 Mm Tris-HCl p^H 8.0)
- Place in water bath at 65˚C for 1 hour.
- Load on gel or check quality using a nanodrop machine.
- Use for PCR and other molecular genetics studies.

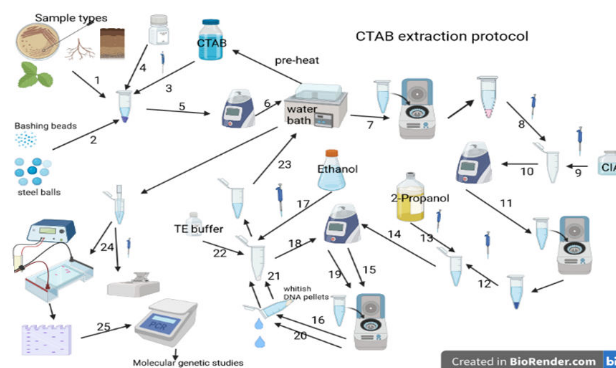

Figure 1. Pictural steps for DNA extraction using CTAB method.

Results and Discussion

For all the modified methods liquid nitrogen was not used unlike in most methods of extraction where use of liquid nitrogen to grind leave, plant sample and soil samples is required as in the methods. Mechanical beating using bashing beads enhance the distruption of the cells this was similar to the findings of where it was observed that use of bead beating gave DNA with good qualities [16]. Only inexpensive chemicals and ordinary laboratory equipments are

enough for DNA extraction. Weighing paper was used to filter water samples collecting more and tiny bacteria samples, differing from the method where filter paper was used as commonly practiced [17-19]. Following the modified methods, the ZYMO kit chemicals were used for 100 reactions instead of the 50 reactions recommended by the manufactures. DNA from CTAB extraction had higher yields while those from kits had higher purity this differs from the findings where kit extraction had higher yields [20].

The yield of DNA obtained was highly variable between the three different modified extraction methods and between samples and CTAB extracted DNA had the highest yield but the least quality this was similar to the findings of Djurhuus et al., 2017 and the extracted DNA were stable and applicable for marker assisted selection, DNA fingerprinting, quantitative traits loci analysis, screening of transformants and enzymatic digestion (Table 1) [21-23].

Average DNA Concentration and Purity

		Previous		Modified	
Extraction method	**Sample type**	**Conc. (ng/µl)**	**Purity**	**Conc. (ng/µl)**	**Purity**
ZYMO (kit)	Plant	27.27 ± 3.84	1.74 ± 0.15	137.9 ± 51.05	1.80 ± 0.08
	Soil	17 ± 0.95	1.89 ± 0.19	84.77 ± 5.79	1.87 ± 0.06
	Bacterial	33.16 ± 10.34	1.00 ± 0.10	146.1 ± 15.85	1.74 ± 0.09
	water	26.58 ± 7.68	1.35 ± 0.16	272.96 ± 30.66	1.91 ± 0.03
	Fungal	41.2 ± 11.43	1.71 ± 0.04	146.1 ± 15.85	1.89 ± 0.19
E.Z.N.A (kit)	Soil	27.47 ± 4.22	1.10± 0.12	172.46 ± 23.32	1.71 ± 0.04
CTAB	Plant	316.67 ± 74.08	1.60 ± 0.04	716.67± 104.08	1.8 ± 0.08
	Soil	616.67 ± 04.08	0.90 ± 0.15	616.67 ± 104.08	1.37 ± 0.28
	Bacterial	700.67 ± 00.08	0.92± 0.03	4100.00 ± 655.74	1.74 ± 0.15
	Fungal	172.47 ±23.37	0.78 ± 0.15	3505.35 ± 251.05	1.52±0.04

Table 1. Comparism of average DNA Concentration and Purity of extracted DNA using modified methods and previous methods.

Conclusion

In this study, three DNA extraction methods were used to extract high quality DNA that can be efficiently amplified using PCR and for other molecular analysis. It was observed that mechanical grinding of cells directly in the DNA isolation buffer and use of sufficient quantities of the sample was effective and efficient enough to avoid the use of liquid nitrogen for the modified protocols. The modified CTAB DNA extraction method used in this study was observed to be the more efficient in extracting high DNA yields with better quality from the samples used and could be used whole-genome sequencing, meta genomics, advanced sequencing technologies, and bioinformatic tools. Our results shows that CTAB–based extraction method can effectively extract DNA from most microbiological samples within short time frame and that DNA extraction efficiency was dependent on quantity of sample and the duration of beating the cells with bashing bead.

We are able to describes efficient modified protocols using established CTAB based extraction method and two commercial kits protocols for isolation and/or purification of high molecular weight genomic DNA from a range of fresh and difficult sources from plant, animal, fungi, bacteria and soil material without the use of liquid Nitrogen.

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