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AN EFFICIENT PROTOCOL FOR GENOMIC DNA ISOLATION FROM CULTIVABLE BACTERIA

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ABSTRACT

A standardized protocol can play a major role in isolation of DNA especially when dealing with bacterial cultures. The aim of the present study was to develop a standard protocol of ultrapure high yield bacterial genomic DNA extraction from bacterial culture suitable for use in molecular biology. Extraction procedure was optimized with series of steps, which involved an unpublished modification of an alkaline lyses procedure followed by equilibrium ultracentrifugation incubated in a lysozyme, buffer and treated with alkaline detergent. Detergent solubilizes proteins and membrane proteins are precipitated with sodium acetate, and the lysates was clear. The cleared lysate with further more steps likewise spooling, phenol: chloroform: isoamyl alcohol and ethanol purification of DNA will give a very high yield, purified bacterial DNA when compared to already existing methods. This method describes the recovery of highly purified nucleic acids that are well-suited for molecular purposes even though a new challenge concerns the recovery of large bacterial DNA essential for functional investigation of gene clusters and biosynthetic pathways.

KEY WORDS: **Genomic DNA, bacterial culture, DNA isolation.**

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INTRODUCTION

The rapidly growing field of molecular biology and biotechnology has a tremendous need for high quality, simple, robust and high-throughput procedures for extraction of DNA from diverse sources [1]. The process of genomic DNA isolation and purification has evolved considerably within the last decade. The new demands of high-throughput facilities have resulted in the development of new technologies for easier and faster DNA processing than ever before. Genomic DNA isolation from bacteria has became popular for the phylogenic and population genetic research owing to microbiology concerns. For these molecular genetic studies, it is important to isolate large fragments of genomic DNA [2]. The basic steps of DNA isolation are disruption of the cellular structure to create a lysate, separation of the soluble DNA from cell debris, other insoluble material and purification of the DNA of interest from soluble proteins and other nucleic acids. Historically, this was done using organic extraction (e.g., phenol: chloroform) followed by ethanol precipitation [3]. These methods were time consuming and used a variety of hazardous reagents. The rapid availability of genomic DNA (gDNA) from microorganisms is necessary for cloning genes and selecting recombinant constructs, for taxonomy and diagnostics[4] described the gDNA extraction from bacteria which has taken several hours to complete. These methods include using SDS/CTAB/proteinase K, SDS lysis, lysozyme/SDS, lysozyme/SDS/proteinase K, bead-vortexing/SDS lysis and mechanical lysis using high-speed cell disruptions. Although these methods are suitable for DNA extraction from bacteria, but the effect of the chemicals on DNA will be enormous and still have the drawbacks including laborious manipulations, such as changes of micro centrifuge tubes, incubation, and precipitation, elution, and washing, drying and even flocking with buffers. Release of DNA is often poor in previously described protocols due to the multiple manipulations. All these methods were using detergents such as SDS to lyse the cell wall and it often remains in the DNA solution and inhibits further purification steps of DNA. The main objective of this protocol is to overcome all drawbacks and to design a highly efficient standard method for the isolation of extra pure, high yield gDNA from bacterial cultures.

MATERIALS AND METHODS

DNA isolation from bacterial culture

According to this method a colony from a freshly streaked plate was inoculated in 10ml of LB medium and incubated for overnight ensue, Feel necessity for large quantity of DNA 2ml of overnight grown cultures were dispensed in 500ml LB media followed by 12 hours incubation at optimal growth temperature. As with smaller cultures, to achieve a highly reproducible yield, the cell density was determined used in a typical experiment and grown cultures to this density in each subsequent experiment. Overnight incubated cell suspension was taken and spin at 6000rpm for 15 min, taken the pellet down and dissolved in 50ml 1x STE buffer (PH-8.0) and added 10mg/ml lysozyme [5,6] for disrupting the gram positive bacterial cell wall, kept this reaction mixture for 1 hr, at room temperature. The efficiency of lysis was determined by direct cell count [7] with phase contrast microscope and by adding acridine orange direct cell count with fluorescence microscopy [2]. After incubation 3.5ml of 10% SDS and 13.5ml of 5M NaCl were added continuously. Following this, the mixture was cooled in ice and extracted with equal volume of chloroform: Isoamyl alcohol mixture (24:1), swirl the flask for 15min and centrifuged at 7000 rpm for 15 minutes. The supernatant was separated with care and equal volume of 2% ammonium acetate and 1/3 volumes off ice cold Isopropanol were added. Finally Spool the DNA on to the glass rod and simultaneously transferred in to test tube and added 5ml TE buffer.

Precipitation of DNA

After spool the DNA with TE buffer this was again centrifuged at 6000 rpm for 15 minutes. Supernatant was decanted and the pellet was washed with 70% ethanol, followed by sterile milli-Q water and dried by desiccator. Finally the dried pellet of genomic DNA was dissolved in 1ml of TE buffer and stored at 4 °C until next use.

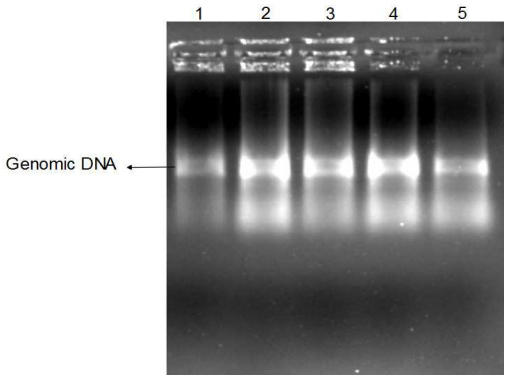
Purification of DNA

For the sake of extra purified DNA, 10 µl of RNAase was added and kept at room temperature for 1hr, further purification from protein contamination 15µl of proteinase K (100mg w/v) were added .Following this, the mixture was cooled in ice and extracted with equal volume of phenol: chloroform: Isoamyl alcohol mixture (25:24:1) and centrifuged for15min at 6000rpm collected the supernatant and given above the treatment for couple of times. Later ultrapure high yield bacterial genomic DNA was dissolved in 1ml of TE buffer and preserve at 4 ºC for further using.

RESULTS AND DISCUSSION

Successful isolation of quality genomic DNA begins with the culture preparation. A number of factors can influence the growth of gram negative (*E.coli, Rhizobium, Rheinheimera,* and *Azospirillum*) and gram positive (*Bacillus palamurensis*) bacterial cells. Bacterial growth in liquid culture occurs in three phases. Depending on inoculation size and the size of the culture, stationary phase will be reached in 6–8hours, in current researches ideal overnight incubation (8hrs) was required [8, 1] for reaching stationary phase. Cell lysis is generally done by treatments with detergent and lytic enzymes. The most widely used detergent is sodium dodecyl sulphate (SDS) [9, 10 11]. 10% (W/V) SDS is strong anionic detergent [9, 10], high quantity of SDS (above 15%) affects the DNA efficiency [9, 2]. It removes the negative ions from the protein and destroys its confirmation [3]. The proteins of the cell membrane get damaged and the cell gets broken. Mucopeptide is a polysaccharide component of the gram positive bacterial (*Bacillus palamurensis*) cell wall that gives rigidity and osmotic protection to the cell [2]. 10mg/ml lysozyme is enough to lyse large quantity of gram positive bacterial culture. Proteinase K will help to free nucleicacids. Chemical lysis occurs by the enzymatic action of 1x STE buffer (pH-8.0). EDTA is a chelating agent and has great affinity for metal ions and Mg-ion present in DNase and acts as a cofactor and is responsible for DNase action that degrade the DNA. Here EDTA binds with Mg-ion and nullifies the action of DNase and protects the DNA from shearing [4, 12].

Organic extraction is a conventional technique that uses organic solvents to extract contaminants from cell lysates. The Correct salt concentration (5M NaCl) and pH.8 must be used during extraction to ensurethe contaminants are separated into the organic phase and that DNA remains in the aqueous phase. It was found that pH 8.0 of the DNA extraction buffer was more efficient than earlier reports. Alcohol (70% ethanol) precipitation was commonly used for concentrating, desalting, and recovering nucleic acids. Precipitation is mediated by high concentrations of salt and the addition of either isopropanol or ethanol. Since less alcohol is required for isopropanol precipitation, this is the preferred method for precipitating DNA from large volumes. In addition, isopropanol precipitation can be performed at room temperature which minimizes co-precipitation of salt that interferes with downstream applications. To allow visualization of the DNA samples, agarose gels were stained most commonly with intercalating fluorescent dye ethidium bromide (10µg/1ml of ethanol), which can be added before pouring the gel. Extracted DNA samples (10 µl) were electrophoretically analyzed on a 0.8% molecular grade agarose gel containing 12µl of 0.5µg/ml ethidium bromide. Using 1x Tris acetate ethylene diamine tetra acetic acid (TAE) electrophoresis buffer, the gel was run for one hour at 90 volts to confirm the presence of DNA.



Genomic DNA isolated from* Rheinheimera *(lane 1), Bacillus Palamurensis (lane 2), Azospirillum (lane 3), Rhizobium (lane 4) and E.coli (lane 5)

CONCLUSION

Present protocol for DNA extraction from bacterial cultures has three requirements: extraction of high molecular weight DNA; extraction of ultrapure DNA for subsequent molecular biological manipulations to be performed; and representative lysis of microorganisms within the sample. In conclusion, an efficient protocol for Genomic DNA Isolation from cultivable bacteria has been reported. The protocol of DNA extraction was tested for its ability to fulfill these requirements.

CONFLICT OF INTEREST

Conflict of interest declared none

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