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Isolation Of Genomic DNA From *E. Coli*

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Abstract- Isolation of nucleic acid, especially DNA, is very common and basic steps in molecular biology. For the isolation of these can be done from different biological materials for the different purpose like study, research or medical and etc. As per the objective "Isolation of genomic DNA from Escherichia coli (E. coli)" the easiest, best, high quality DNA isolation and cost-effective method has prominently four steps like cell lysis, enzymatic treatment, differential solubility and precipitation. For performing these crucial steps there are many old conventional protocols as well as modern and easy protocol are also there. But for the high precision and accuracy there are kits like GeNie also used in which there are less chances of contamination as well as time saviour and ready to use reagents and enzymes are present. The successful isolation and visualization of DNA are confirmed by the appearance of clear, well-defined bands, indicating high-quality genomic DNA. This method provides a reliable and straightforward means of isolating genomic DNA from E. coli for further molecular applications.

Keyword: DNA Extraction, Escherichia Coli, Electrophoresis, Genie Kit

I. INTRODUCTION

Escherichia coli is a gram negative, rod-shaped, coliform bacterium of genus Escherichia size range between 0.5-2.0µm long and 0.25-1.0µm in diameter which is facultative aerobic and anaerobic bacteria and generally found in lower intestine of warm-blooded animals, sewage sample, contaminated water/food, soil and etc.

On the basis of Gram staining the bacteria is divided into gram positive and gram-negative bacteria and E. coli is gram negative bacteria because it has thin cell wall made of peptidoglycan which is very fragile and easily disrupted as compared to gram positive bacteria (Barnard, et al.2011; Eusébio et al., 2016) DNA of E. coli it is not very organized, defined or packed in nucleus as compared to eukaryotic cell but it is circular double stranded and very highly condensed present in cytosol of the cell as well as it has some extra DNA in form of plasmid.

For the very first time the DNA isolation was done by a Swiss physician, Friedrich Miescher in 1869 from lymph node cells but later on he shifted to leucocytes because it was hard to obtain DNA in sufficient quantity (Siun Chee Tan, 2009).

For DNA isolation the procedure has four different steps. The first step is the Cell Lysis in which the cell wall is disrupted by the help of detergent like Sodium Dodecyl Sulphate (SDS). It reacts at hydrophobic site of protein, and binds to all proteins (i.e. lipoproteins, etc.) forming the SDS-polypeptide micelles complex, hence denaturation occurs causing the dissociation of nucleic acid. SDS also stop or inhibit the activity of nuclease enzyme which doesn't interact with hydrophilic nucleic acid. Some protein requires certain temperature to form SDS complex with reacts with reagents (e.g. mercaptoethanol) to cleave disulphide bond between proteins (Moore et al. 2004).

Cell lysis is followed by the secondary step which is Enzymatic Treatment done with two types of enzymes e.g. Proteinase K which is a serine protease isolated from Tritirachium album, which digests peptide bond of the adjacent to the carboxyl group of aliphatic and aromatic amino acid. RNase A is an endonuclease that cleaves 3' C and U residue.

which degrade the RNA which helps in easily separation of DNA from DNA-RNA hybrid (Moore et al. 2004).

Later on, in third step differential solubility the absolute alcohol, phenol which is an organic, non-

polar solvent that selectively eliminate the other cell disruption from DNA and hence DNA is extracted.

Absolute alcohol or phenols react with the hydrophobic protein and denature it and when Purified Phenol is used then result is more efficient (Moore et al. 2004).

And in fourth step, precipitation is done with the help of absolute alcohol and sodium acetate, here DNA is precipitated from dilute solution with ethanol or iso-propanol in the presence of sodium or potassium acetate, pH5.0-5.5. when added to fine concentration of 0.3 molar (m), sodium and acidic pH will neutralize the highly charged phosphate backbone and promote hydrophobic interaction.

The precipitated DNA is then collected by centrifugation or is spooled out with a pasture pipette. The DNA pellet is rinsed with 70% ethanol to remove any excess salt and dried as well as dissolved in appropriate buffer. Hence the excess or unwanted substances are removed and purified DNA is obtained (Moore et al. 2004).

II. METHODOLOGY

Sample Preparation

For accuracy and simplifying the procedure we have break open the lyophilized vial from GeNie DNA isolation kit and resuspend the host by adding 0.1 ml of sterile Luria-Bertani (LB) broth Streak a loop full of this suspension on to the Luria-Bertani (LB) plates and inoculate the remaining suspension in 5ml of sterile Luria-Bertani (LB) Broth.

Incubate the plates at 37°C for 20 min. Pick a single colony from LB plate to inoculate into three plates of sterile broth. Incubate in a shaker incubator set at 37°C for 16 hours.

Cell lysis

For this step Sodium Dodecyl Sulfate (SDS) which is an anionic detergent, which effectively reacts when used in low concentration. For simplifying this cell lysis, centrifuge 1.5ml of culture at 6000 RPM at 4°C for 8 minutes to get the bacterial pellet.

Remove the culture supernatant and carefully discard it. Resuspend the cells in 700µl of solution A from GeNie kit and incubate at room temperature for 5 minutes and spin at 10,000 RPM for 10 minutes.

Enzymatic Treatment, Differential Solubility and Precipitation

To perform this step, collect 5000µl of the supernatant in a fresh vial, avoid taking the pellet. Add 1 ml of absolute alcohol to 500µl of supernatant and mix well by inverting the tube till while strands of DNA precipitate out are seen. Centrifuge at 1000 RPM for 30 minutes and discard the supernatant or spool the precipitated DNA with the help of a tip and transfer into the fresh vial. Add 0.5ml of 70% alcohol to the DNA pellet and give a short spin 10000 RPM for 10 min.

decant the alcohol solution taking care not to dislodge the pellet repeat this step once again. Add 100µl of solution B from GeNie kit and incubate at 55°C-60°C followed by 20-30 minutes at room temperature to engage the solubility of genomic DNA. By performing these steps unwanted substance like cell debris and etc. are removed after cell lysis and enzymatic treatment.

The Precipitate of denatured cell material remains with organic phase and finally it is separated out by centrifugation. Here two phases are then separated in which upper aqueous phase that contains the nucleic acid is retained. Proteins are seen as flocculent material at the interphase. And finally separated easily.

Visualization of DNA by Gel Electrophoresis

Centrifuge at 6000 RPM for 2 minutes. Pipette out 25µl of supernatant (DNA sample) into fresh vial and add 2.5µl of gel loading buffer. Load the DNA sample along with 10µl of Controlled DNA on a 1% agarose gel. Note down the order in which the sample has been loaded. Electrophoresis runs at 50-100 volts for 1-2 hour. Purified DNA can be easily visualized under the UV Transilluminator.

III. OBSERVATION

The DNA was observed as a white flocculent material in micro-centrifuge tube, after the

precipitation step. It was further carefully isolated using micropipette without collecting any extra fluid, and was stored in fresh and dry micro-centrifuge tube in 4°C till running electrophoresis.

IV. RESULT

After the Isolation of Genomic DNA from E. coli with the help of GeNie kit, the integrity of the isolated genomic DNA was further evaluated by agarose gel electrophoresis (1% agarose) for the fine resolution of bands were visualized. High molecular weight band with no significant degradation was observed.

V. DISCUSSION

Genomic DNA isolation of Escherichia coli using the GeNie kit proved to be efficient, yielding high-quality DNA suitable for downstream molecular applications.

The integrity of the DNA was confirmed by the appearance of sharp, high-molecular-weight bands on agarose gel electrophoresis, suggesting minimal degradation and effective removal of contaminants.

Similar findings were reported by Oslaene et al. (2022), who emphasized the reliability of commercial extraction kits for obtaining pure DNA with consistent yield compared to conventional phenol-chloroform methods.

The use of SDS during the lysis step effectively disrupted the bacterial cell membrane and denatured proteins, while enzymatic treatments with Proteinase K and RNase A facilitated the removal of proteins and RNA.

These steps are consistent with standard bacterial DNA extraction protocols described by Moore et al. (2004) and Miranda et al. (2011).

The alcohol precipitation step efficiently concentrated and purified the DNA, producing visible precipitates that confirmed successful extraction.

Compared to traditional methods, the GeNie kit demonstrated a faster workflow, reduced handling errors, and minimized contamination risk.

This aligns with the observations of Bonatto et al. (2014), who reported that kit-based extractions offer better reproducibility and are time-saving, especially for teaching and diagnostic laboratories. The successful isolation of intact DNA underscores the suitability of this method for molecular techniques such as PCR, restriction digestion, and sequencing.

These findings are also consistent with those reported by De Sousa Alves et al. (2021), who demonstrated that optimized DNA extraction protocols improve DNA purity and produce well-resolved bands during agarose gel electrophoresis.

CONCLUSION

Overall, the results validate the efficiency of the GeNie kit as a reliable and cost-effective tool for genomic DNA extraction from E. coli. Future work could focus on comparing yield and purity across different bacterial strains or testing kit performance with environmental and clinical samples to further evaluate its versatility.

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