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Study on the Plasmid and Genomic DNA Stability of DH5α Host System

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Short Research Article

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ABSTRACT

Plasmids are extremely valuable as a source of DNA and for use in biotechnology. Our research goal was to develop plasmids with extremely high copy numbers for lab-scale plasmid preparations. Using the beverage method simplifies the process of obtaining a large quantity of plasmid DNA for various applications. Diet Coke as a beverage demonstrated that DNA recovery is highly adequate. Diet Sting with EcoRI and BamHI enzymes was used to detect restriction digestion with high sensitivity. However, for lab-scale preparative work, our findings show that plasmid yield can be significantly increased by using standard growth procedures and commonly used growth media.

Keywords: DH5α; DNA; EcoRI; HindIII; BamH1; plasmid.

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1. INTRODUCTION

For more than a decade, geneticists have been interested in inducing mutations with chemicals and radiations with the goal of discovering mutagenic compounds through their specific activity, which can likely provide some understanding and knowledge of the chemical basis of mutation and gene structure [1]. Plasmids are extremely valuable as a source of DNA vaccines and in biotechnology applications. Increasing plasmid yield would benefit molecular biology research by lowering reagent costs and increasing experimental throughput [2]. Plasmids are used as vectors in many expression systems to produce recombinant proteins or non-proteinous recombinant components [3]. Plasmids have a significant impact on productivity, it is necessary to conduct research on plasmid stability and colony forming units. at both the industrial and research levels [4]. Plasmids can also perform additional functions, such as plasmid-encoded genes that can improve the host's survival by completely killing a different organism or synthesizing toxins that act as defensive mechanisms. [5]. A single organism may contain multiple plasmids, each with a distinct function [6]. Restriction digestion is a technique that involves cutting a specific region of genetic material and incubating the targeted sequence with a restriction enzyme that binds to specific DNA sequences and cleaves at specific nucleotides [7]. Restriction digestion allows for the ligation of genetic material fragments, which is required for biological research [8].

2. MATERIALS AND METHODS

2.1 Plasmid Preparation

Single-cell colonies were picked and streaked out on selective LB-agar plates and further transferred to 2 mL selective LB-medium for 24 h at 37°C. The master LB-agar plate was stored at 4°C, whereas the overnight culture was used for plasmid Mini-Prep (GeneJET Plasmid, Fermentas) as per the manufacturer's instructions. Plasmids were eluted in 40 µL 1 mM Tris/HCl pH 8, or sterile water [9].

2.2 Restriction Analysis

Analytical restriction digestions with specific restriction endonucleases (Fermentas or New England BioLabs) were performed for verifying the expected plasmid by means of the DNA fragments analyzed with gel electrophoresis. After visualizing the correct band pattern for the estimated plasmid size within one or more clones, the respective clone was used for inoculation of approximately 100 mL selective LB-medium and incubated overnight at 37°C under shaking [10].

3. RESULTS AND DISCUSSION

3.1 Restriction Analysis

In this gel plasmid and genomic DNA is seen affected by the different types of beverages. By doing this we observed that only Diet Coke and Sting were affected so Sting and Diet Coke was undergone restriction digestion for specific identity with EcoRI, BamHI and HindIII [Fig. 1A]. In this gel, genomic DNA is seen affected by the different types of beverages. By doing this we observed that only the Diet Sting and Sting were affected so Sting and Diet Sting was undergone restriction digestion for specific identity [Fig. 1B]. We have observed only digestion in the Diet Sting EcoRI, BamHI and HindIII enzymes [Fig. 1C].

3.2 Quantification of Gel Band

The public domain Image J tool was used to analyse the ECL image (developed at the National Institutes of Health and available at http://rsb.info. nih.gov/ij/), using the "measure" functions. The results of the analysis are a value for each band which is proportional to the Integrated Density Value (IDV) of the band. All images were processed in such a way that no information was lost.

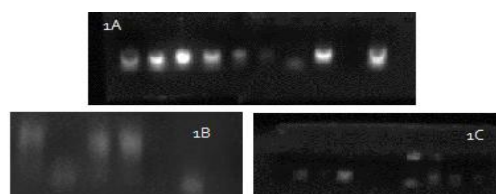

Fig. 1. Gel electrophoresis of restriction digestion and bands of genomic DNA observed under UV-trans illuminator and photographed using gel-doc

Table 1. Quantification of genomic DNA band intensity using ImageJ

Sl.no	Label	Area	Major	Minor	Int Den
1	Diet Coke	627	169	115	150.3
2	Coke	520	189	135	164
3	Fanta	476	217	132	174
4	Sprite	390	187	134	166
5	7up	486	166	118	144.4
6	Wild	512	188	103	158.6

Table 2. Quantification of plasmid DNA band intensity using ImageJ

Sl.no	Label	Area	Major	Minor	Int Den
1	Diet Sting	375	150	105	130
2	Sting	450	148	99	129.3
3	Fanta	361	194	94	126.9
4	Sprite	--	--	--	--
5	7up	450	149	92	123.1
6	Wild	391	133	99	118.3

Table 3. Quantification of restriction digestion DNA band intensity using ImageJ

Sl.no	Label	Area	Major	Minor	Int Den
1	Wild (EcoRI)	2448	135	82	108.8
2	Wild (HindII1)	2992	115	74	94.6
3	Wild (BamH1)	2100	132	84	111.1
4	Diet Coke (EcoRI)	2520	141	79	111.3
5	Diet Coke (HindII1)	--	--	--	---
6	Diet Coke (BamH1)	1564	125	84	106.1
7	Sting (EcoRI)	---	---	---	---
8	Sting (HindII1)	---	---	---	---
9	Sting (BamH1)	--	--	--	--

4. CONCLUSION

With the choice of several beverages in the isolation of genomic and plasmid DNA of bacterial cells, the choice of Diet Coke as a beverage proved that the recovery of DNA is highly adequate and however the role of these beverages to be further studied to understand the recovery process and any changes or modifications to be known if various other recombinant technology methods are carried out.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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