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RESEARCH ARTICLE

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USE OF BEVERAGES FOR ANALYSIS OF PLASMID AND GENOMIC DNA AMONG WILD AND MUTANT DH5A HOST SYSTEM

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Manuscript Info

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DH5α, DNA, EcoRI, HindIII, BamH1, Plasmid

Abstract

A broad stretch of display queries needed a multiple level approach with totally different experimental settings. An adequate approach for analysis of plasmid was necessary to answer the queries regarding the biological aspects of plasmid function, whereas totally different analytical techniques were required to further justify the role of beverages in plasmid and genomic DNA analysis. Beverages are nothing but various liquids used for drinking. Some of the beverages are Pepsi, Coke, Mountain dew, Coffee, Limca, Thumpsup charge, Thumps up, etc. Here in this project we used these beverages for analysis of plasmid and genomic DNA among wild and mutant DH5α host system.

Introduction:-

Most vectors are hybrids between bacterial and yeast sequences, possessing an origin of replication for E. coli and selection markers[1]. The plasmids also contain a multiple cloning site (MCS)[2]. To successfully perform molecular genetic techniques, it is essential to have a full understanding of the properties of the various *Escherichia coli* host strains commonly used for the propagation and manipulation of recombinant DNA[3]. *E. coli* is an enteric rod-shaped Gram-negative bacterium with a circular genome of 4.6 Mb[4]. It was originally chosen as a model system because of its ability to grow on chemically defined media and its rapid growth rate[5]. In rich media, during the exponential phase of its growth, *E. coli* doubles every 20–30 min; thus, during an overnight incubation period, single selected organisms will double enough times to yield a colony on an agar plate or 1–2 billion cells per milliliter of liquid media[6-8]. any useful strains are available through the American Type Culture Collection (ATCC) and the *E. coli* Genetic Stock Center at Yale, as well as from commercial suppliers such as Stratagene, Fermentas, Promega, Novagen, Invitrogen and New England Biolabs. *E. coli* is a popular host for the overexpression of recombinant proteins[9]. There are a number of factors that can influence protein yields and careful strain choice can greatly improve the chance of successful expression[10].

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Materials & Methods:-

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[11]. 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[12]. Plasmids were eluted in 40 µL 1 mM Tris/HCl pH 8, or sterile ddH2O.

Restriction Analysis

Analytical restriction digestions with specific restriction endonucleases (supplied by Fermentas or New England BioLabs) were performed for verifying the expected plasmid by means of the DNA-fragments analyzed with gel electrophoresis[13-14]. After visulaizing 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[15].

Results And Discussions:-

Restriction Analysis

In this gel plasmid and genomic DNA is seen effected by the different types of beverages. By doing this we observed that only the Pepsi and coke was affected so coke and Pepsi was under gone restriction digestion for specific identity [figure-1A]. In this gel genomic and genomic DNA is seen effected by the different types of beverages. By doing this we observed that only the Pepsi and coke was affected so coke and Pepsi was under gone restriction digestion for specific identity [figure-1B]. We have observed only digestion in the Pepsi EcoRI and BamHI enzymes [figure-1C]

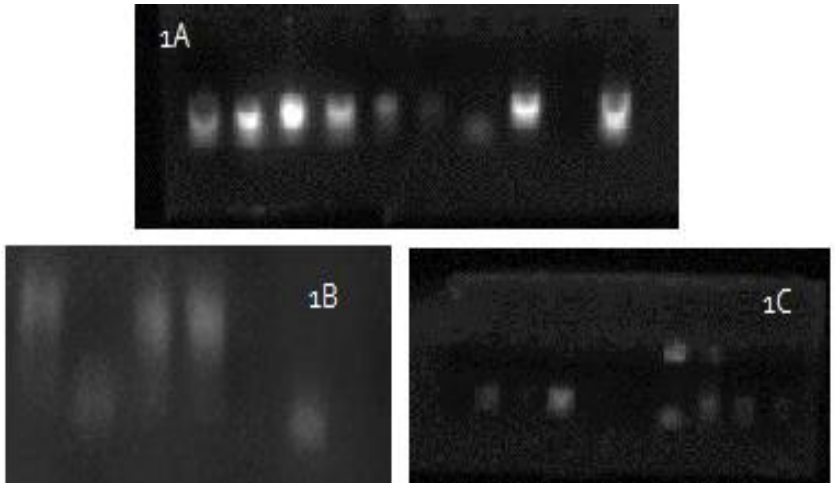

Fig 1:- Gel electrophoresis of restriction digestion and bands of genomic DNA observed under UV-trans illuminator and photographed using gel-doc.

Quantification of gel band

Analysis of ECL image was performed using the public domain Image J program (developed at the National Institutes of Health and available at http://rsb.info. nih.gov/ij/), using the "measure" functions. Results of the analysis is a value for each band which is proportional to the Integrated Density Value (IDV) of the band. All images were treated in a way that avoids loss of information.

Table 1:- Quantification of genomic DNA band intensity using ImageJ.

Sl.no	Label	Area	Major	Minor	Int Den
1	pepsi	627	169	115	150.3
2	coke	520	189	135	164
3	Mountain dew	476	217	132	174
4	coffee	390	187	134	166
5	limca	486	166	118	144.4
6	Thumbsup charge	420	164	113	141

7	**Thumbsup**	**493**	**211**	**125**	**167**
8	**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	**pepsi**	**375**	**150**	**105**	**130**
2	**coke**	**450**	**148**	**99**	**129.3**
3	**Mountain dew**	**361**	**194**	**94**	**126.9**
4	**coffee**	**--**	**--**	**--**	**--**
5	**Limca**	**450**	**149**	**92**	**123.1**
6	**Thumsup charge**	**486**	**150**	**104**	**130.3**
7	**Thumsup**	**567**	**140**	**93**	**122.2**
8	**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	**Pepsi (EcoRI)**	**2520**	**141**	**79**	**111.3**
5	**Pepsi (HindII1)**	**--**	**--**	**--**	**---**
6	**Pepsi (BamH1)**	**1564**	**125**	**84**	**106.1**
7	**Coke (EcoRI)**	---	---	---	---
8	**Coke (HindII1)**	---	---	---	---
9	**Coke (BamH1)**	--	--	--	--

Conclusion:-

With the choice of several beverages in the isolation of genomic and plasmid DNA of bacterial cells, the choice of Pepsi as 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.

Conflict of interest

The authors declare that there are no conflicts of interest exist among them regarding the publication of this paper.

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