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Artigo

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Um Protocolo Simples para Isolamento, Quantificação e Avaliação da Qualidade do DNA e RNA

Resumo: Este artigo apresenta um protocolo simples para isolamento de DNA plasmídeo de *Escherichia coli*. usando eletroforese em gel de agarose, espectrofotometria de UV e método de placa de agarose, foi possível determinar concentrações de 2170 $\mu g.mL^{-1}$ e 2455 $\mu g.mL^{-1}$ para amostras de DNA plasmídico em TE e RNase, respectivamente. Esses resultados confirmam que as amostras consistiram essencialmente de RNA. Um segundo experimento foi efetuado na tentativa de isolar e avaliar o DNA genómico de células epiteliais orais humanos, mas os métodos utilizados revelaram-se ineficiente para quantificação e qualificação de DNA.

Palavras-chave: Lise alcalina; Gel de agarose; Eletroforese; Extração de DNA; DNA genómico; DNA plasmídeo.

Abstract

This article presents a simple protocol for the isolation of plasmid DNA of *Escherichia coli*. using agarose gel electrophoresis, UV spectrophotometry and the plaque assay agarose, the concentrations of 2170 $\mu g.mL^{-1}$ and 2455 $\mu g.mL^{-1}$ were verified in nucleic acids for plasmid DNA samples with TE and TE + RNase, respectively. These results confirm that the samples mainly consisted of RNA. A second experiment was efectuated to isolate and assess genomic DNA of human oral epithelial cells, but the methods used proved inefficient for qualification and quantification of DNA.

Keywords: Alkaline lysis; Assay agarose; Electrophoresis; DNA extraction; Genomic DNA; Plasmid DNA.

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A Simple Protocol for the Isolation, Quantification and Quality Assessment of DNA and RNA

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1. Introduction

The caracterization of the structural integrity of DNA (plasmid and genomic) is very important in a wide variety of biological applications. Therefore, a simple protocol to assess DNA quality, such as this one, may have important clinical and molecular research applications. For example, it can assess the DNA samples before analyzing tissue specimens for various human diseases, as DNA quality is frequently poor due to tissue fixation and processing. Nonetheless, before it is necessary to use efficient methods for DNA isolation.

To isolate the plasmid DNA (circular and smaller than the chromosomal DNA) from *Escherichia coli*, the alkaline lysis method can be used.[1,2] This method is based on the denaturation of chromosomal DNA, as plasmid DNA does not denature, or refolds more rapidly than chromosomal DNA, with this process basically being divided into three phases. In the first phase, at pH 8, glucose (maintains osmotic equilibrium) and ethylenediamine tetraacetic acid (EDTA) are used (this combination sequesters ambivalent ions which destabilize the cell membrane,

carrying the DNase out of the cells). After that, NaOH is used, which increases the pH of the system, leading to denaturation of nucleic acids, along with sodium dodecyl sulfate (SDS), which destabilizes the cell membrane, releasing the cellular components. Lastly, potassium acetate is used to lower the pH, enabling the plasmid DNA to refold. As a result, it stays diluted, while the chromosomal DNA is precipitated.

For the isolation of genomic DNA from oral epithelial cells, a lysis buffer is used which, besides keeping the solution at pH 8 so that the DNA remains stable, opens the cell membranes, releasing the DNA. Following that, protease is used to destroy the proteins bound to the DNA, increasing the amount of intact DNA. The NaCl neutralizes the DNA by binding to its phosphate group so that the DNA molecules will not repel, facilitating its precipitation when alcohol is added. [4,5]

Agarose gel electrophoresis is used to estimate the plasmid DNA concentration and allows verification of whether the sample is contaminated with chromosomal DNA or with DNA, separating molecules in different dimensions, shapes and greatness of the total charge through the migration of the molecules an electric field. The DNA, if it has a negative charge, migrates to the positive pole.[6-10] In order to quantify nucleic acid, the UV (ultraviolet radiation) spectrophotometry is used. In solution, the nucleic acid is strongly absorbed by the UV radiation in 220 nm < λ <320 nm.[11] The nucleic acid concentration in the sample can be calculated by the lambert-Beer law. The maximum absorbance of nucleic acids lies at approximately 260 nm. The quotients A_{260}/A_{230} (EDTA, polysaccharides and ethanol) and A_{260}/A_{280} (proteins) provide an estimate of the nucleic acid purity. If the sample is pure, the ratio should be between 1.8 and 2 and between 1.8 and 2.2, respectively.[3]

The agarose plate method loosely measures the amount of DNA in the sample by comparing the emitted fluorescence with the fluorescence intensity of standards of known concentrations. [12-15]

2. Material and Methods

2.1. *Escherichia coli* plasmid DNA extraction

Biological material*: E. coli DH5 α [pBR322]*

Growth medium and solution

•Growth medium LB with ampicillin (tryptone 1 % w/v, yeast extract 0.5 % (w/v), NaCl 1 % w/v, adjust pH 7.5 with NaOH aqueous, add solution 100 µl.mL^{-1} of ampicillin in sterile ultra-pure water)

•Solution I (Glucose 50mM, Tris HCl 25 mM - pH 8.0, EDTA 10 mM - pH 8.0)

•Solution II (NaOH 0.2 N, SDS 1 %)

•Solution III (60 mL of potassium acetate 5M, 12 ml glacial acetic acid, 29 mL of H_2O ultra-pure)

•Buffer TE (Tris HCl 10 mM- pH 8.0, EDTA 1mM - pH 8.0)

In this work, the plasmid DNA extraction involved cell lysis by the alkaline lysis method and plasmid DNA purification method. O medium LB (2 mL), containing the antibiotic ampicillin, was incubated with a bacterial strain colony at 37 °C with strong agitation (~200 rpm) for 24h. In a microtube, 1.5 mL of the growth culture e was transferred and centrifuged at 12,000 rpm (revolutions per minute) for 30 seconds at 4 °C. Then, the supernatant was removed and resuspended in 100 µL of solution I at 4 °C. Then 200 µL of solution II was added, which was mixed by rapid inversion, and the mixture was left on ice. Into this mixture, 150 µL of solution III was added and stirred slowly. The mixture was centrifuged at 12,000 rpm for 5 minutes at 4 °C and the supernatant was transferred to a new microtube. DNA precipitation was performed with the addition of absolute ethanol at room temperature and incubated for 2 minutes before being centrifuged using the above conditions. The supernatant DNA pellet was removed from the microtube and

washed with 1 mL of 70 % ethanol at 4 °C and allowed to dry at room temperature. The DNA was then dissolved in 50 µL of buffer TE (containing RNase at 20 µg.mL^{-1}) and stored at 4 °C.

2.2. Isolation of genomic DNA from human oral epithelial cells

For the isolation of genomic DNA, the alkaline lysis method was used. The cell sample was removed from the inner cheek of the mouth with a cytology brush and immersed in lysis buffer to remove the epithelial cells (Figure 1). This process was repeated a number of times to remove the greatest number of cells possible. The buffer containing epithelial cells was treated with the addition of 20 µL of protease solution. The mixture was incubated at 50 °C in a water bath for 10 minutes and after that 40 µL of 5M NaCl was added. To this mixture, 500µl of cold isopropanol was added and centrifuged for 5 minutes at 12,000 rpm, the supernatant was decanted and the sediment was resuspended in 20 µL of buffer TE (stored at 4 °C).

Solutions

- Lysis Buffer (50mM Tris, 1 % SDS)
- Buffer TE (10mM Tris HCl - pH 8.0, 0,1 mM EDTA - pH 8.0)
- 5 N NaCl
- Proteinase K (100 mg/mL in TE)
- Isopropyl alcohol (99.5 %)

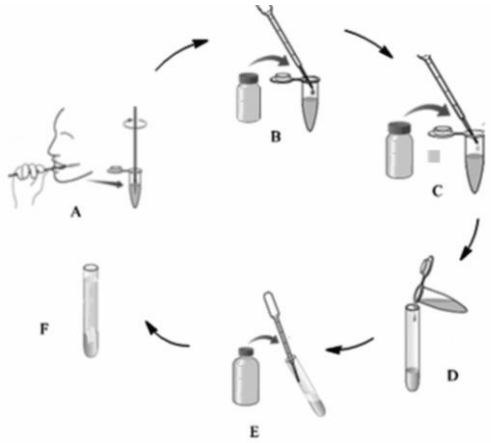

Figure 1. Procedures for the isolation of genomic DNA from oral epithelial cells

2.3. Electrophoresis of DNA on an agarose gel

Agarose gel

The agarose solution was prepared in 0.8 % w/v TAE buffer solution. The agarose solution was poured into the electrophoresis tray and covered with TAE.

Samples

The samples were prepared by adding 4 µL of buffer to the sample and 20 µL of ultra-pure water in 100-500 ng of DNA in a microtube. After that, the samples were applied to the wells of the electrophoresis cuvette and, after migration, were colored in a bath in an ethidium bromide solution 0.5 µg.mL^{-1} for 30 minutes at room temperature.

Solutions

•Buffer TAE (242 g Tris base, 58 mL glacial acetic acid, 100 mL EDTA 0.5M - pH8, 1000 mL ultra-pure water)

•Sample buffer (Bromophenol blue 0.25 % w/v, xylene cyanol FF 0.25 % w/v, glycerol 30 % w/v)

2.4. Determination of DNA concentration and purity grade of DNA

The concentration and purity of DNA were determined by a spectroscopic method, using a Cary 50 UV spectroscope (Varian). In the case of contaminated samples, the DNA concentration was evaluated by fluorescence intensity emitted by ethidium bromide when this was added to the samples. The fluorescence was evaluated in a UV transilluminator coupled with a Canon® digital camera. In the sample preparation for the application on agarose gel, 5 µL of plasmid DNA was pipetted into an *Eppendorf* tube and 5 µL of the genomic DNA mixture was added to the other *Eppendorf* tube.

3. Results and Discussion

In Figure 2, different samples (A-F) are loaded in sets of three, where the first and second wells contain plasmid DNA in TE and TE+RNase, respectively, and the third contains genomic DNA.

Sample E is shown in wells 13 to 15. As can be seen (Figure 2), little plasmid DNA exists and the quantity of RNA seems to be approximately equal. This can be explained because the RNase was not properly introduced in the corresponding sample or the conditions used were not optimal compared to other samples.

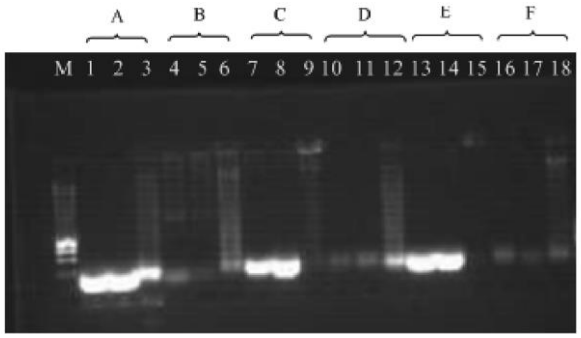

Figure 2. Electrophoresis on agarose gel. Well 1 – Marker M-hyperladder IV (Bioline)

Even so, in sample B, it is possible to see that there is a little plasmid DNA, as three isoforms of DNA can be observed. The relaxed plasmid DNA migrates more and super coiled plasmid DNA migrates lower (because the difficult pass through agarose pores). linear migrates between the two. Furthermore, there is less RNA, as expected, in the sample containing RNase (RNase is a type of nuclease that catalyze the degradation of RNA into minor components[16]). Regarding sample E, for genomic DNA, this did not migrate, possibly due to contamination with high molecular weight proteins, such as hemoglobin. Already in the genomic DNA well for sample F, it is possible to visualize a corresponding trace of intact DNA with large entrainment. On the other hand, in the well corresponding to genomic DNA of sample B, it was found that there is significant entrainment, revealing degraded DNA.

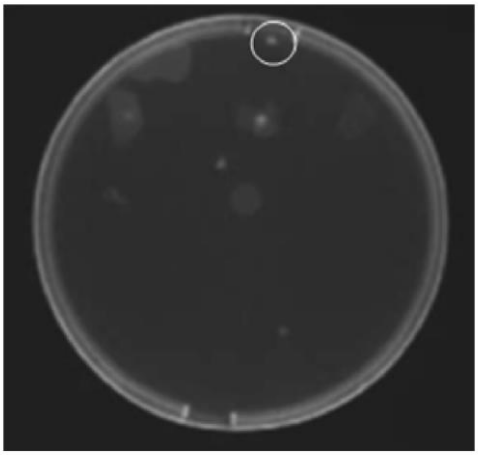

Figure 3. Agarose plate

Comparing the intensity of the fluorescence of the known concentration of marker with the samples (Figure 3), it was verified that the samples in positions 14 and 20 had similar concentrations to the marker, while those at positions 11 and 45 had lower DNA concentrations. In cases where fluorescence was not detected, this was probably a consequence of the lower concentrations of DNA that limit of eye observation. it was not possible to estimate, the amount of DNA in this sample using this method. On the other hand, it may be that the DNA extraction efficiency was low.

		1	2	M	4		
	5	6	7	8	9	10	
	11	12	13	14	15	16	
17	18	19	20	21	22	23	24
25	26	27	28	29	30	31	32
33	34	35	36	37	38	39	40
	41	42	43	44	45	46	
		47	48	49	50		

Figure 4. Relative position of the samples in an agarose plate. M marker

3.1. Quantification and determination of purity grade of plasmid DNA by UV spectroscopic method

The absorption spectra of the DNA are shown in Figure 5. Relevant absorption data are summarized in Table 1. The DNA concentration in the samples was calculated using the Lambert-Beer law.

Table 1. Relevant data from absorption spectra of the plasmid DNA in TE and TE+RNase solution

Absorbance	TE	TE+RNase
A_{260}	0.494	0.511
A_{280}	0.270	0.256
A_{320}	0.060	0.022

As the plasmid DNA solution was prepared in a dilution of 1:100, the concentration was 2170 µg.mL^{-1} for the sample with TE and 2455 µg.mL^{-1} for the sample with TE+RNase. Through the A_{260}/A_{280} ratio, it is possible to estimate the nucleic acid purity. as these ratios, when between 1.8 and 2.2 can be considered to indicate high quality of DNA. Although, it is possible to verify that RNA is present by the electrophoresis results. As RNA has a higher ε_{260} value (0.025 µg.mL^{-1}.cm^{-1}), this will have a greater significance on the absorbance peak.

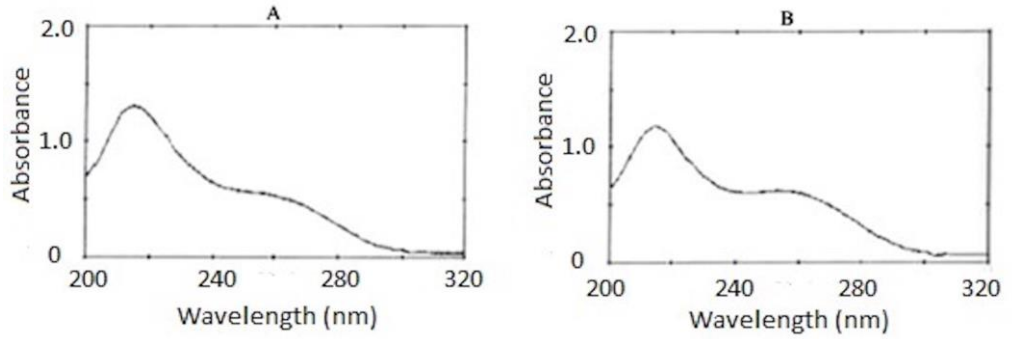

Figure 5. Spectrum of the DNA sample + TE (A) and DNA+ TE + RNase (B)

4. Conclusion

Considering the results obtained, it is possible to conclude that the plasmid DNA isolation was not performed successfully because of the large amount of RNA in the sample. However, it is not possible to say that there is no DNA present. By using UV spectrophotometry, it was possible to quantify the plasmid DNA in TE and plasmid DNA in TE and RNase as 2170 µg.mL^{-1} and 2455 µg.mL^{-1}, respectively, indicating that the samples presented high concentration of RNA. Moreover, nothing can be concluded about the genomic DNA because the methods used to quantify and assess their quality were unsuccessful.

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