The Effect of TBE and TAE Buffers on DNA Ligation
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Introduction

Tris-acetate EDTA (TAE) and Tris-borate EDTA (TBE) are common buffers used in electrophoresis. Ion complex formations between ions in the buffer and the DNA being analyzed have been found to occur in both TBE and TAE buffer solutions. The effects of both of these buffers on various characteristics of electrophoresis, such as electrophoretic mobility, gel resolution, and buffer conductivity, have been documented in other studies.

This study focused on the effect of the DNA-ion complex formation on the efficiency of ligation after exposure of the DNA to the buffer solutions. The efficiency of ligation of each buffer treatment was compared to each other and to a non-treated control to determine if the buffer effected ligation and which had a greater impact.

Materials and Methods

The plasmid pSTBlue was cultured in *Escherichia coli* at 37°C and selected for growth on LB agar plates with 25μg/mL kanamycin. Overnight cultures were grown and plasmid DNA was extracted using organic extraction and ethanol precipitation procedures. The purified plasmid was digested using *Hind*III restriction enzyme at 37°C for 16 hours. The completion of digestion was verified using agarose gel electrophoresis.

Materials and Methods Cont’d

The digested product was separated into three samples. TAE from 50X concentrate or TBE from 10X concentrate were added to two of the samples to a final 1X concentration. The third sample was maintained as a no-buffer control. The digest-buffer mixtures were incubated for 90 minutes and then buffer solution was removed. Duplicate ligations of the samples were performed using the T4 DNA Ligase. Transformations were performed using NEB5α Competent *E. coli* (High Efficiency) cells. Each transformation was plated in triplicate.

Colony growth was counted after 16 hours incubation at 37°C and recorded in colony-forming units (CFU)/μg DNA. Data were analyzed using a one-way ANOVA.

Results

![Figure 1. The average CFU/μg DNA for each treatment method and its standard deviation.](image)

There was no significant difference in colony growth between the two buffers (p=0.94).

Both TAE and TBE were significantly different from the no-buffer control (p<0.01 for both buffers).

Discussion

There is a negative correlation between buffer treatment of DNA and DNA ligation efficiency. The plates that were treated with buffer had a lower CFU/μg DNA than the plates from the non-buffer control. This shows that the DNA-ion complexes formed in electrophoresis buffers have a residual effect on DNA ligation.

There is no difference in ligation efficiency between the two buffers, showing that TAE and TBE have a similar effect on ligation. With regards to ligation efficiency, one buffer is no less restrictive over the other.

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