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Effect of Matrix and Electrophoretic Conditions on Analysis of Linear pDNA

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Using AGE Analysis of Linearized pDNA as a Tool for Process Monitoring

Agarose gel electrophoresis (AGE) analysis is an important method for monitoring plasmid DNA (pDNA) quality, as it can separate supercoiled (sc), open circular (oc), linear (lin), and multimeric pDNA isoforms. Monitoring plasmid linearization aids in the production of the starting material for mRNA synthesis. Electrophoretic conditions and, more importantly, the matrix used for sample dilution before gel loading can affect analytical results. We have observed that purified linear pDNA shows an additional band in the AGE analysis of the sample in water, which can lead to misinterpretation of results (Figure 1).

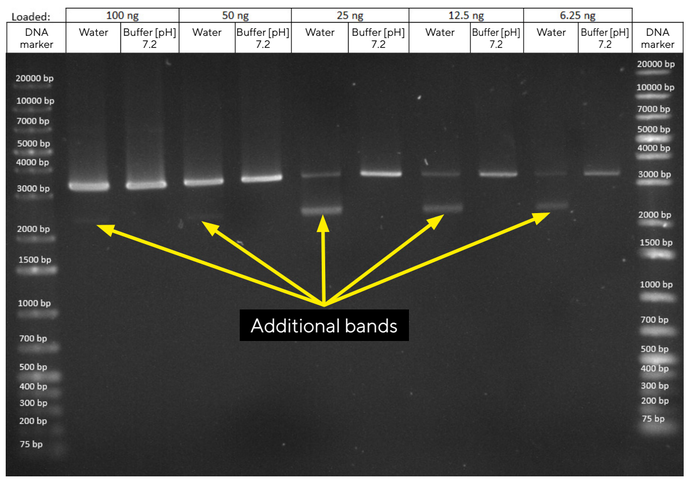

Figure 1:
AGE Analysis of Linear pDNA Diluted in Either Tris Buffer or ddH_2O Under Different Loading Conditions

Note. Electrophoretic conditions: 1% gel, voltage: 100 V, run time: 60 minutes, sample mass loaded: 100, 50, 25, 12.5 and 6.25 ng

Here, we present experimental findings on the appearance of additional bands following pDNA gel electrophoresis, a phenomenon influenced by electrophoretic conditions, media used for sample dilution, and sample preparation before gel loading. Additional experiments and analysis suggest that linear pDNA undergoes conformational changes in water. This results in the emergence of an additional band, which in our study, appears lower (Figure 2).

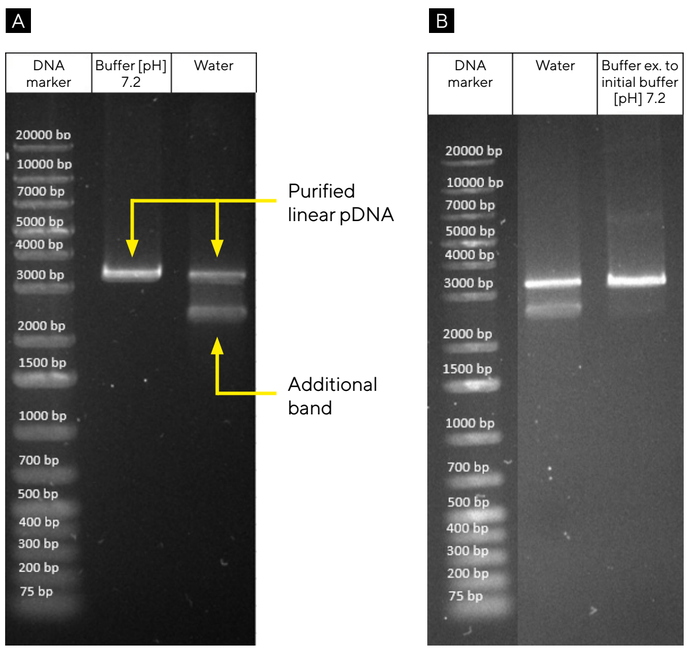

Figure 2:
(A) AGE Analysis of Linear pDNA for Sample in 50 mM Tris pH 7.2 Buffer (Left) and ddH_2O (Right)
(B) AGE Analysis of Sample in Water Medium (Left) And Same Sample Buffer Exchanged to 50 mM Tris pH 7.2 Buffer (Right)

Note. Electrophoretic conditions: 1% gel, voltage: 100 V, run time: 60 minutes, sample mass loaded: 50 ng

Our current hypothesis is that linear pDNA undergoes at least a partial transition from B to A conformation when the matrix is changed from buffer to water (Figure 3).

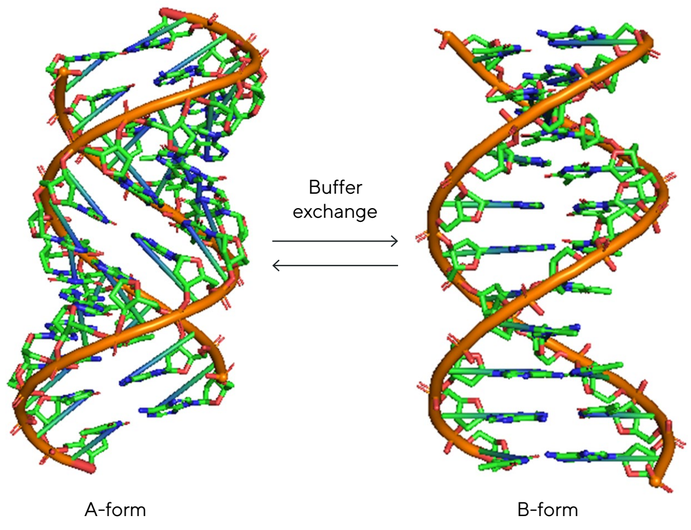

Figure 3:
Conformational Changes of Linear pDNA After Matrix Change

Note. Transition from A form (left) to B form (right) depending on matrix composition

Additional Bands in AGE Analysis of Linear pDNA

Experiments were done using two electrophoresis systems. One aliquot of the same sample batch was buffer exchanged from storage buffer (50 mM Tris, pH 7.2) to ddH_2O. Analysis was done simultaneously on the original and buffer-exchanged samples. Lower band migration depends on the voltage applied during the gel run. Lower band migration was faster at lower voltage conditions and slower at higher voltage conditions (Figure 4). Upper band migration does not change with voltage.

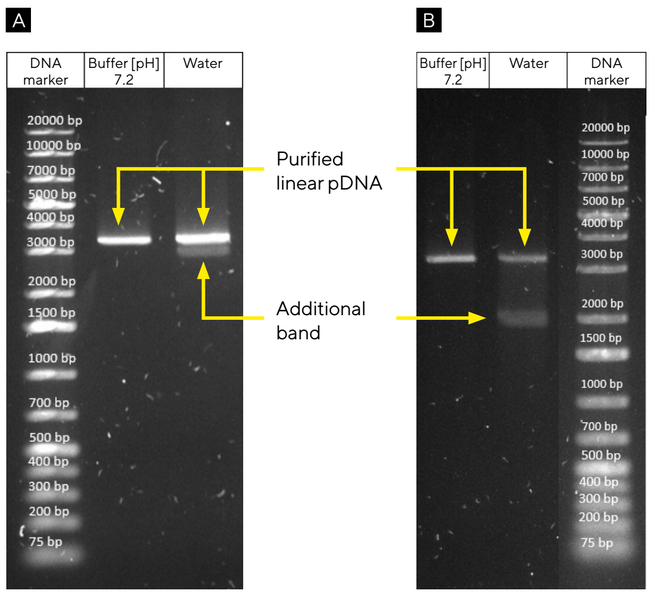

Figure 4:
Lower Band Migration Correlates With Applied Voltage During AGE Analysis

Note. Electrophoretic conditions: 1% gel; sample mass loaded: 50 ng; voltage (A) 135 V and (B) 50 V; run time: (A) 45 minutes and (B) 115 minutes

A similar phenomenon was observed for different sizes of linear pDNA ranging from 3 kbp to at least 8 kbp (see Figure 5A for 8 kbp). The experiment was also performed on a DNA ladder (GeneRuler 100 bp DNA Ladder): one sample was prepared according to the manufacturer's instructions, another was prepared by dilution in ddH_2O. Additional bands were observed in the DNA marker prepared by dilution in ddH_2O (Figure 5B).

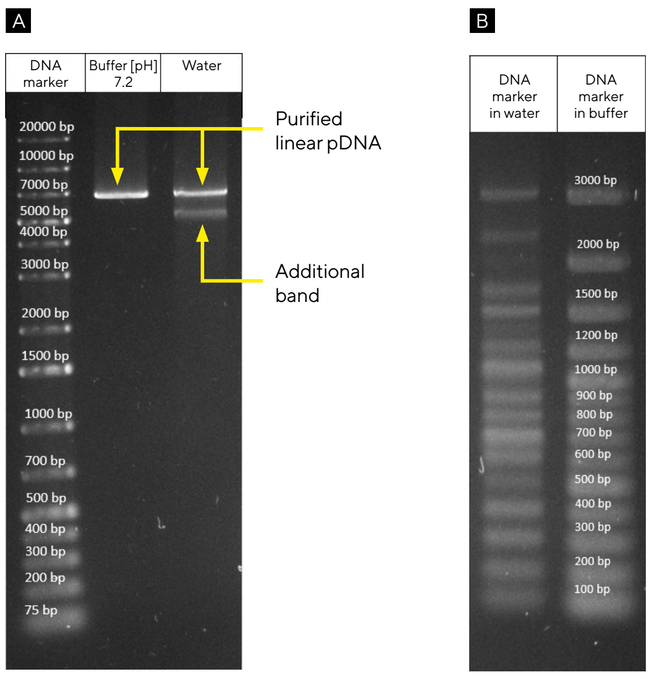

Figure 5:
AGE Analysis of Linear pDNA (A) And DNA Ladder GeneRuler 100 bp DNA Ladder (B) In Buffer (Per the Manufacturer's Instructions) and Water

Note. Electrophoretic conditions: 1% gel, voltage: 100 V, run time: 60 minutes, sample mass loaded: 50 ng

Conclusion

- AGE analysis of purified linear pDNA shows an additional band if the sample is diluted in water
- Only one linear pDNA conformation is present in the sample in buffered medium
- Additional band migration depends on electrophoresis conditions (applied voltage for constant voltage analysis mode). Additional bands were observed during the analysis of different sizes of linearized pDNA and DNA ladder prepared with sufficient sample dilution in water
- Experiments indicate the appearance of an additional band is a result of conformational changes caused by the medium, not by sample degradation

References

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