Chemically Triggered Degradation of Polyacrylamide Gels for DNA Recovery

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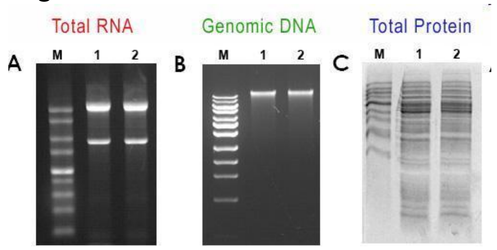
INTRODUCTION

PAGE, or polyacrylamide gel electrophoresis, has been utilized in the analysis, separation, and purification of DNAs or proteins. Acrylamide combined with a cross-linker is used to create a gel network which, via electrophoresis, entraps proteins or DNAs based on their length.



After the completion of electrophoresis, the DNA sometimes needs to be recovered. While there are many different ways to free the DNA from its gel network confines, these methods can take days to finish or result in sample loss.

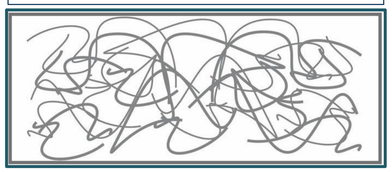
Through the use of degradable gels the sample can be recovered with a fast recovery rate and yield.

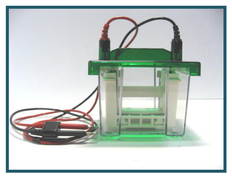
METHOD

Two polyacrylamide gels were created utilizing a 12 %T/0.3 %C composition for methylene bisacrylamide (Bis) cross-linked gel and 12 %T/0.5 %C composition for EG2 cross-linked gel.* Each gel was loaded with Low Molecular Weight DNA Ladder and oligodeoxynucleotide attached to 5'-fluorescein (FAM-ODN). Electrophoresis was then preformed for two hours at 120V. The section containing FAM-ODN was removed and placed in 10 mM TCEP buffer and placed in a 37 degree water bath for FAM-ODN recovery. The absorbance of the samples were collected over a period of four and half hours. The remaining gel was stained with ethium bromide and imaged.

*%T denotes the total percentage concentration of both monomers utilized. %C denotes the percentage concentration of the cross-linker relative to %T.

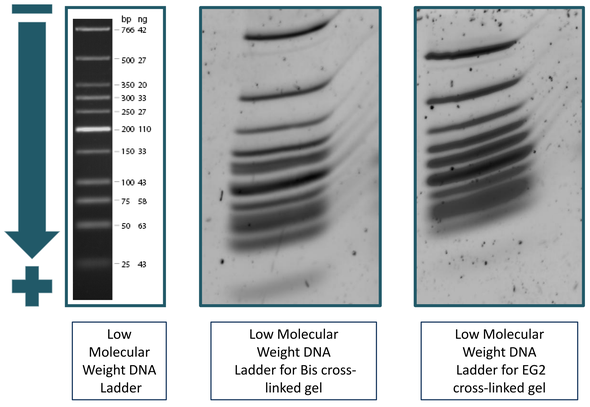
Polymerization of polyacrylamide gel with EG2 and Bis crosslinkers





Electrophoresis

2 hours
120 V



Low Molecular Weight DNA Ladder

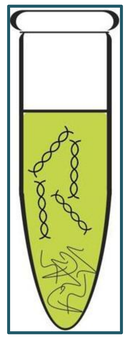
Low Molecular Weight DNA Ladder for Bis cross-linked gel

Low Molecular Weight DNA Ladder for EG2 cross-linked gel

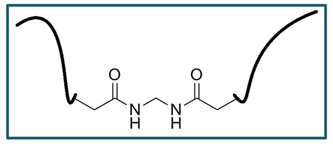
No degradation of the Bis gel

TCEP buffer induced degradation of EG2 gel

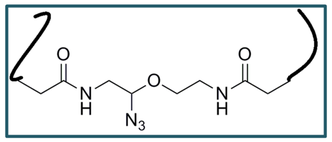
The Bis gel sample does not change, while the EG2 gel degrades and releases the DNA sample



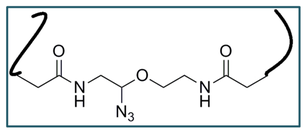
Bis Cross-linker



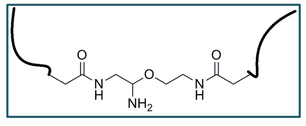
EG2 Cross-linker



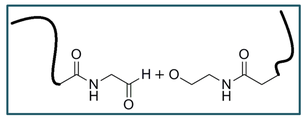
The chemical mechanism for the degradation of the EG2 gels can be seen below.



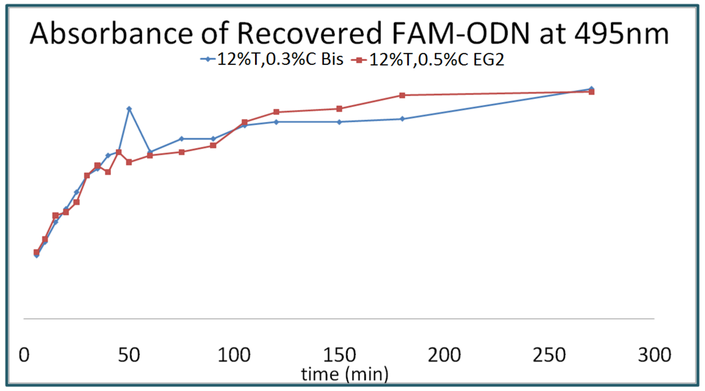
TCEP Buffer (reduction)



H_2O



FAM-ODN Recovery



RESULTS

The FAM-ODN that was utilized was too small for the pore size of the gels. This caused the pre-diffusion of FAM-ODN before the gel was able to degrade.

The Low Molecular DNA Ladder showed similar results in both gels and is comparable to the ladder for the product itself.

CONCLUSION

The EG2 gel had comparable separation of the low molecular weight DNA ladder which makes it a suitable candidate for electrophoresis. While the EG2 gel degraded in two hours time, it cannot be said whether or not the EG2 gel can be used for OFN-FAM recovery of short strands as the ODN-FAM utilized was too small and diffused out of both gels before any degradation could occur. EG2 gel would be a good alternative for longer strands of DNA.

FUTURE WORK

With the knowledge that EG2 can lead to degradation, further work can be preformed to monitor concentration of DNA recovered in EG2 versus Bis. Larger strands of DNA could also be utilized in addition to other nucleic acids, such as RNA, and proteins.

ACKNOWLEDGEMENTS

The Taton Group

UROP
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REFERENCES
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