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Investigation to Determine Lengths of DNA Fragments Digested by Various Restriction Enzymes

Introduction

In this investigation we isolated the plasmid component of cells. Plasmids are extrachromosomal DNAs that are circular and smaller than chromosomes in the cell. Plasmids are used to change, add, or remove sequences of bacteria by polymerase chain reaction in order to amplify DNA or change DNA expression. In this experiment we are studying the plasmid, pRS305. We prepared pRS305 out of E. coli by using a minipreparation technique to isolate the plasmids away from other cellular components to be purified. We digested our plasmids with various restriction enzymes that cut the plasmids at specific palindromic sequences. The restriction enzymes we used in this lab were EcoRI, EcoRV and Kpnl. These restriction enzymes cut the plasmids into different sized, linear fragments of DNA. DNA gel electrophoresis was then used to separate the fragments of DNA by size. In DNA gel electrophoresis a current causes the DNA to be pulled through the gel matrix. Since DNA is negative it is attracted to the positive lead of the electrophoresis chamber and migrates different distances based on the fragments length in base pairs. These distances can then be compared to DNA of known lengths. We subjected our digested plasmids to this technique by melting agarose gel with a buffer in order to pour it into a tray with a comb inserted, forming wells when the comb is removed. We could then inject our DNA into the wells so that it could be drawn towards the lead of the electrophoresis chamber. Since DNA is not visible to the naked eye, we stained it with GelRed making it visible under UV light. A GelDoc system containing UV light was then used to produce photographs of the glowing bands of DNA. The bands of DNA represent segments of DNA of specific lengths that could then be measured and compared to DNA of known lengths. Overall, the purpose of this investigation was to determine the lengths of DNA fragments cut by various restriction enzymes.

Discussion

In this experiment five digestions of plasmid DNA were carried out by restriction enzymes. When digested by EcoRI alone, two bands were predicted to be produced. In our photograph of the gel, we detected both bands. By looking at a restriction map indicating where the restriction enzymes will cut, we expected the size of the bands to be 3440 and 2064 base pairs. The experimental sizes that we calculated were 3500 and 2500 base pairs with a percent error of 1.7% and 21.1%. When digested by EcoRV alone, two bands were predicted to be produced. In our photograph of the gel, we detected both bands. By looking at a restriction map we expected the size of the bands to be 3324 and 2180 base pairs. The experimental sizes that we calculated were 3100 and 2500 base pairs with a percent error of 6.7% and 14.7%. When digested by Kpnl alone, two bands were predicted to be produced. In our photograph of the gel, we detected both bands. By looking at a restriction map we expected the size of the bands to be 3784 and 1720 base pairs. The experimental sizes that we calculated were 3500 and 1900 base pairs with a percent error of 7.5% and 10.5%. When digested by EcoRI and EcoRV, four bands were predicted to be produced. In our photograph of the gel, we detected only two of the bands. By looking at a restriction map we expected the size of the bands to be 3324, 2064, 111, and 5 base pairs. The experimental sizes that we calculated were 3100 and 2100 base pairs with a percent error of 6.7% and 1.7%. When digested by EcoRI and Kpnl, four bands were predicted to be produced. In our photograph of the gel, we detected only three of the bands. By looking at a restriction map we expected the size of the bands to be 3395, 1675, 389, and 45 base pairs. The experimental sizes that we calculated were 3100, 1750, and 400 base pairs with a percent error of 8.7%, 4.5%, and 2.8%. Our average percent error for fragments that were larger than 2000 base pairs was 8.6% and our average percent error for fragments that were smaller than 2000 base pairs was 5.93%. According to our data, 1% agarose gel is better at accurately separating small fragments of DNA.

Conclusion

In this experiment we were successful at isolating plasmid DNA away from other cellular components and the plasmid DNA was successfully cut in all five of our restriction enzyme digestions. For the restriction enzyme digests that were successful, some of our percent errors for experimental fragment sizes compared to expected fragment sizes were within 10% and some were greater than 10%. We discovered that larger fragments of DNA typically had a larger percent error. We concluded that the increased amount of base pairs in larger fragments inhibits its ability to move through the gel causing the larger sized experimental fragments to migrate shorter distances than we predicted resulting in percent errors that were greater than 10%.

Acknowledgments

I would like to thank Suho Lee and Bara Nabhani for helping conduct this experiment.