Restriction and gel electrophoresis of plasmid dna - lab report example

Science, Biology

Restriction and Gel Electrophoresis of Plasmid DNA

Known fragment sizes (Kb) Linear regression analysis: Y= mx + b X= -0.

8358 Calculation of sizes of unknown samples: Example: EcoR1 + plasmid Y:

Distance migrated (measured from gel) = 18. 1 mm

Corresponding Log (10) MW (read from graph) = . 613

Molecular weight

(Kb) = antilog . 613= 4. 1

RESTRICTION AND GEL ELECTROPHORESIS OF PLASMID DNA

The practical report

Introduction

The purpose of this laboratory exercise was to acquire an understanding of the principles and practice of DNA gel electrophoresis. The DNA used in this experimental protocol was obtained by culturing bacteria (E. coli) that contain plasmid DNA. Plasmids are small circular double-stranded DNA molecules that often contain antibiotic resistance genes. Many also contain a unique origin of replication and are autonomously replicated to high copy number within bacterial cells. Plasmids are frequently used for gene cloning purposes. Restriction enzymes are used to map plasmid DNAs and also to prepare plasmids for DNA insertion as part of gene cloning protocols. DNAs that are prepared in this way may then be analysed by a method called gel electrophoresis. This involves the use of electric currents to facilitate the separation of linear DNA molecules through a gel support, usually consisting of the polymer agarose or polyacrylamide. These polymers form a molecular sieve that permit the DNA to pass through at a rate that is approximately inversely proportional to the log of the molecular weight as measured in

kilobase pairs (Kb). The current initiates the movement of DNA from the site of application to the positively charged cathode as the negatively charged phosphate groups in the DNA molecule are drawn to the cathode by electrostatic attraction. If DNA fragments of Known molecular weight are electrophoresed simultaneously thre molecular weight of the DNA fragments generated by restriction enzyme digestion may be ascertained by comparing their rate of mobility with that of the standards of known molecular weight. This is usually calculated by preparing a graph representing the log of the molecular weight of DNA standards versus the measured distance traveled beach band in millimeters (mm). The distances of the unknown fragments is measured and their molecular weights are determined by locating the position these measured distances are located on the graph. Restriction enzyme digestion of DNA followed by gel electrophoresis is a commonly used method for preparing DNA maps and determining the molecular weights of unknown DNA samples.

Methods

The DNA used in this experimental protocol was obtained by culturing bacteria (E. coli) that contain plasmid DNA. Two types of plasmids were prepared from E. coli, designated plasmid X and Y. After the plasmid DNA was extracted from the bacterial cells, it was then digested with restriction enzymes, which are capable of making double stranded cuts in DNA molecules at specific recognition sequences. The enzymes used in this procedure were BamH1 and EcoR1. The DNA samples were incubated with the enzymes at 37C, which is optimal for restriction enzyme activity. The DNA samples were then mixed with tracking dyes and loaded onto a 0. 8%

agarose gel. The gel running buffer used was TBE buffer, which stands for Tris-Cl, borate, EDTA. The borate ions provide the negatively charged ions needed for the flow of current. The Tris-Cl is a buffer used to maintain stable pH, and EDTA is a chelating agent. Once the DNA samples from plasmid X and Y were loaded into wells at the top of the gel, DNA comprised of known molecular fragments, produced by the digestion of lambda DNA with HindIII was loaded onto the gel to provide molecular weight standards. A voltage was applied and the DNA migrated through the agarose gel to the positively charged electrode. Once the gel run was complete, the gel was stained with ethidium bromide (using care!) with binds specifically to DNA by intercalating between the base pairs of the double-stranded molecule. The DNA bands on the gel fluoresce when exposed to ultraviolet light. A photograph of the gel was made and the results were analysed.

The analysis consisted of making measurements of each of the DNA bands on the gel in millimeters from the point of application to the middle of the band. The log of the molecular weights of the DNA standard was calculated and a graph was prepared indicating the relationship between distance traveled and the log of the molecular weight of known standards. The molecular weights of the plasmid DNA samples were then determined by locating the position of distance traveled for each band on the graph and the results were recorded.

Discussion

The results indicated that both plasmids X and Y each contained a single restriction enzyme recognition site for Bam H1 and Eco R1. The location of the sites relative to one another could not be determined as this would

require further restriction mapping using additional restriction enzymes. The uncut DNA produced several band representing the varying amount of supercoiling of the individual circular DNA molecules. The most tightly supercoiled DNA migrates the fastest as it is the most compact form of DNA. The supercoiled DNA cannot be used to estimate the molecular weight of the DNA molecules as its migratory properties are different from linear DNA molecules. The molecular weight of the plasmids was determined by comparing the wlectrophoretic mobility of the restriction digested linearised plasmid molecules with the molecular weights of DNA standards. The graph generated was used to make this calculation.

There was some error introduced by the fact that the gel was not run for a long enough time to allow the maximal separation of the DNA fragments. In addition, the analysis was limited by the fact the wells contained too much DNA. Procedural changes would correct these sources of error. Despite these difficulties, a reasonable determination of the molecular weight of the plasmid DNA was made.

Conclusions

In conclusion, restriction enzyme digestion of two different plasmids isolated from E. coli and subjected to agarose gel electrophoresis permitted a determination of the molecular weight of the two DNA plasmids and a determination that a single restriction enzyme recognition site is found in each of these plasmids for EcoR1 and BamH1.

References

Duke, T. and Viovy, J. L. (1994). Theory of dna electrophoresis in physical gels and entangled polymer solutions. Physical Review E, 49(3): 2408+.

Frank-Kamenetskii, M. D. (1997). Biophysics of the dna molecule. Physics Reports, 288(1-6): 13-60.

Viovy, J.-L. (2000). Electrophoresis of dna and other polyelectrolytes: Physical mechanisms. Reviews of Modern Physics, 72(3): 813+.

Voytas, D. (2001). Agarose gel electrophoresis. Current protocols in immunology / edited by John E. Coligan ... [et al.], Chapter 10.

WATSON, J. D. and CRICK, F. H. (1953). Molecular structure of nucleic acids; a structure for deoxyribose nucleic acid. Nature, 171(4356): 737-738.

RESTRICTION AND GEL ELECTROPHORESIS OF PLASMID DNA SAMPLES

QUESTIONS

1. Plot log10 size (in kb) of lambda fragments against distance migrated in mm.

From the graph, calculate the apparent size or sizes (in kbs) of the plasmid DNA

molecules before and after digestion by both enzymes based on the position of bands in the gel.

Plasmid X Plasmid Y

Calculated size(s) of undigested

PlasmidX= 2. 375 and 2. 1Y= 9. 4, 4. 1 and 3. 4 Kb

Calculated size(s) of plasmid digested

by BamHIX= 3. 47 KbY= 4. 1 Kb

Calculated size(s) of plasmid digested

by EcoRI (if applicable)

X= 3. 47 KbY= 4. 1 Kb

2. What is the true size of each of the plasmids? Explain your answer.

The true sizes of the plasmids are:

X= 3. 47 Kb

Y= 4. 1Kb

Each of these restriction enzymes (BamH1 and EcoR1 cuts the plasmids once, linearizing the supercoiled DNA. In the linear form the molecular weight can be measured by comparison to lambda HindIII molecular weight standars.

Uncut plasmid DNA is supercoiled which addects the migration rate of the DNA in the gel; therefore the bands cannot be used to determine the molecular weight of the plasmid.

3. Explain the affect, if any, of digestion on the migration rate of the plasmid.

The restriction enzyme digestion linearizes the supercoiled plasmid; the linear DNA molecules migrate in the agarose gel at a rate that is inversely proportional to the log of the molecular weight of the molecule,

4. How many restriction sites for the enzymes does each of the plasmids possess? One

Give reasons for your answer.

A single primary band is produced using each of the restriction enzymes; this corresponds to the full length linear DNA molecule. The results indicate that there is a single restriction enzyme recognition site for Bam H1 and HindIII in both plasmids X and Y.

5. If you used EcoRI in your experiment, what gel results would have been found if

BamHI and EcoRI had been used together (a double digest)? Explain answer.

Since these two enzymes have different recognition sites in DNA, two cut would produce 2 fragments of DNA for each plasmid. Double digests and multiple digests can be used to prepare a restriction map that is unique for each unique DNA sequence.

6. What is the purpose of adding the ribonuclease solution (Stage B5)?

The purpose of adding the ribonuclease is to hydrolyze any RNA that may be present in the plasmid DNA sample and could interfere with migration of DNA and also produce additional bands on the gel.

7. In some situations ethidium bromide can be added to the gel and the buffer in the tank before the electrophoresis run begins. This saves time because it

obviates the need to stain the gel at the end of the run. However, this would not be suitable for the samples used in this experiment. Explain why.

The presence of ethidium bromide can alter the migratory properties of DNA. The uncut DNA, in particular would not migrate uniformly as ethidium bromide intercalates between bases and will not do so consistently in tightly coiled circular DNA. This would make the gel results more difficult to interpret. Also, ethidium bromide cannot be used in preparative gels as it may alter the base sequence due to its insertinal properties.