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Date _____ Per _____

Electrophoresis and Gel Quantitation of DNA

Introduction

In this exercise you will be able to run a gel and determine the approximate amounts of DNA and the size of each DNA fragment. It is very important for researchers to not only determine the size of the DNA fragments that are generated by cutting the DNA with restriction endonucleases but also the amount of each of the fragments. There are multiple mechanisms for determining this information. One method is to check the DNA with a spectrophotometer. This allows very accurate determination of DNA quantity. Another method that is equally effective though not as accurate is to use a known quantity of a DNA marker like Lambda cut with the restriction endonuclease Hind III. This generates known fragment sizes and if you start with a known quantity of the marker in your well you can calculate the amount of DNA in each band on the gel. After the gel is stained photographed you can compare the known marker DNA bands to the unknown bands and estimate the amount of DNA and the size of each. It sounds complex but it is relatively easy.

Objectives

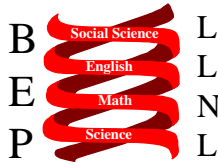
- The student will correctly estimate the amount of DNA in a unknown band of DNA on a gel.
- The student will develop an experiment that would utilize gel quantitation as part of the laboratory.

Materials

- Pictures of various gels with a Lambda HindIII Marker lane.

Protocol to Practice this technique:

1. To be able to predict the amount of DNA in a particular DNA band on a gel there needs to be a control that is known. One common control is the Lambda viral DNA cut with the restriction endonuclease Hind III. It generates known bands. Below is an example of Lambda cut with Hind III.



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2. By knowing the size of each fragment you can calculate the percentage of the total Lambda virus DNA.

$$23,130 / 48502 = 48\%$$

$$9416 / 48502 = 19\%$$

$$6557 / 48502 = 13\%$$

$$4361 / 48502 = 9\%$$

$$2322 / 48502 = 5\%$$

$$2027 / 48502 = 4\%$$

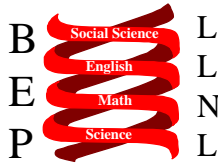
$$564 / 48502 = 1\%$$

$$125 / 48502 = .2\%$$

3. By knowing how much DNA was placed into the well you can calculate the percentage of the total amount of DNA in each band.

If the concentration of DNA you were using was 0.1 ug/ul and you loaded 10 ul of the sample into the well how much DNA did you load all together?

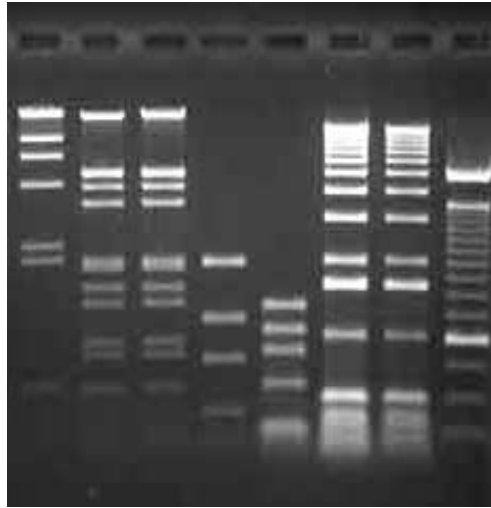
How much DNA in each band? Show all work



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4. Below is a picture of a gel that uses Lambda Hind III markers. Lane 1 has Lambda Hind III marker. There was 1 ug of DNA in the marker lanes to start. Estimate the amount of DNA in the various other bands.



Extensions

1. Since exploring the concept of gel quantitation you now understand how to estimate the amount of DNA in a given band of DNA. How could you use this technique to help in an experiment? Design an experiment that would utilize this technique.