

# DNA Restriction Analysis

The analysis of DNA by restriction analysis is a fundamental technique used by the biotechnology industry. Since the completion of the human genome sequence it has been absolutely critical that students understand the basic tools used in this ever-growing segment of the research industry. There are multiple ways in which to accomplish this task in the secondary and college classrooms. The main difference is the amount of convenience that a teacher or professor is in need of. The advantages of a kit are that it takes much less time to prepare and less time organizing the materials to be used by students. The method described here is recommended for the more experienced teacher/professor or someone who does not have the funding necessary to implement the kit approach.

This instruction sheet has been designed in order for the teacher/professor to setup a DNA restriction analysis lab in their classroom by ordering components and putting them together to do the lab. In order to do this one would need to order: Lambda DNA (quantity to be explained later), restriction enzymes, restriction buffer, sterile water or TE buffer, Loading Dye, Agarose, TAE or TBE running Buffer, DNA stain.

## **Determining How Much Lambda DNA to Order**

To determine the amount of DNA to order one must keep some factors in mind. One is that to get really good staining results it is best to have approximately 2 ug of DNA in a lane. So if you ordered the DNA freeze dried, and it came in 10 ug quantities, you could hydrate it in 50 ul of sterile water and the concentration of DNA in a ul of water would be 0.2 ug/ul. This would enable a student to use 10 ul of 0.2 ug/ul DNA to have a total of 2 ug of DNA.

If you had 32 students and wanted them to load an eight-well gel with their samples, you would have lane 1 be loaded with a control marker. Student samples could be loaded in lanes 2-7. If you had every two students set up their own reaction you would need 16 digestions for the class. Since each one needs 2 ug, you would need 32 ug of DNA per class. So the teacher

would order three 10ug tubes of freeze dried DNA. It would give 8 ug extra. This could be multiplied by the number of class periods or lab sections that the instructor had.

## Enzymes

Since enzyme concentration is generally given in units (the amount of enzyme that it takes to digest a microgram of lambda in one hour in optimal conditions), it is generally more concentrated than you would need to have it. Many enzyme manufacturers have programs to provide enzymes for classes free of charge if they write-up how they are using it, it is possible to get these for free. If you purchase them, there is a wide variance in the price per enzyme. The most common and cheapest enzymes are HindIII, Eco R1, and Bam H1. Along with the enzyme one needs to order Restriction buffer. This usually comes in 10x concentration. Most enzymes come with the proper buffer when they are ordered. I usually use enzymes at 2 units per microliter concentration. I usually use 2 ul of that in my reactions so there are 4 units of enzyme per reaction. Since the enzyme usually has about 1000 units it is easy to have enough to cover all the DNA you have ordered.

## Setting up the Reaction

To setup a restriction digest one needs to determine the amount of DNA to be digested (see above). Once that is done, the amount of enzyme is determined (see above). The restriction buffer quantity is the next thing to determine. If the buffer is 10x concentration, the buffer must be diluted to 1x concentration. That just means that it must be one part of every 10 parts of the restriction digest:

| Quantity      | Substance                        |
|---------------|----------------------------------|
| 10 ul         | Lambda DNA                       |
| 2 ul          | Hind III enzyme (2 units per ul) |
| 2 ul          | 10x buffer                       |
| 6 ul          | Sterile Water                    |
| Total = 20 ul |                                  |

### **To check quantity of DNA:**

In the sample above it is important to review each part of the reaction and check to see if it is what you had desired. Since you wished to have a total of 2 ug of DNA digested, if you used DNA that had a concentration of 0.2 ug/ul and you had 10 ul of it you would have 2 ug of DNA.

$$0.2 \text{ ug/}\mu\text{l} \times 10 \mu\text{l} = 2 \text{ ug}$$

### **To check the quantity of enzyme used:**

In the sample above to review the amount of enzyme it is important to ensure that you have enough enzyme to digest the DNA in the time frame you have set aside. It is advisable to do it at 37 degrees centigrade for one hour or at room temperature overnight. Since the concentration of enzyme was diluted to 2 units per ul, and you used 2 ul of it then you had a total of 4 units of enzyme.

$$2 \text{ units/}\mu\text{l} \times 2 \mu\text{l} = 4 \text{ units}$$

### **To check the amount of Restriction Buffer:**

In the sample above it is important to review the total amount of 10x buffer used. The final concentration needs to be 1X. That means that one part of the restriction digest needs to be 10X buffer for every 9 parts of the rest. It is important to keep in mind that you still have to add loading dye(6x) to the digest after you have finished right before you load the gel. So the total amount of the digest should be as small as possible. So if 2ul of 10X buffer is used then the total ul of the reaction must be 20 ul. That would ensure that you get one part of 10X buffer for every 9 parts of reaction.

Since you already have 10 ul of DNA and 2 ul of enzyme and 2 ul of 10 buffer in the reaction, to make the total 20ul you just need to add water to make up the difference. That is 6 ul.

All Checked out!

Putting it all together:

To have the students set this up it is important to teach them the proper order of adding the reagents. It is important to start by adding the water, then the buffer, then the DNA and finally the enzyme. The enzyme should be kept on ice the entire time.

Running the gel:

In order to load the cut DNA into a gel it must be made heavier than the running buffer that the gel is under. To do that a nucleic acid loading buffer is added to the sample. The loading Buffer (loading dye) can come in different concentrations. Most common are from 10x to 5x. If you had 6x loading dye and you added 4 ul of it you would have to have 24 ul of total. Since you had a reaction of 20 ul, adding 4 ul of loading dye would make the final concentration just right of 24 ul.

Most common concentrations of agar are between 0.8% agarose to 1% agarose. This is made by mixing either 0.8 grams agarose or 1 gram agarose with 100 ml of 1x TAE buffer. Once you have done that you can place it into the microwave and heat on high for about a minute. Watching to make sure that it doesn't overflow. Once it is completely transparent, let it cool so you can hold it comfortably (55-60 degrees centigrade). Then pour into the gel tray with comb.

After it has solidified, remove the tape and comb gently. Make sure to keep it level so the gel doesn't slide out on the floor. Lower it into the gel box and pour 1x TAE buffer over the top of the gel.

Load samples into the wells. Replace the lid and run at 100 volts for approximately 30-45 minutes.

Place into the large weigh boat and pour stain enough to cover the gel.