Biology 324 Restriction Digestion: Laboratory 1 Moyer
Winter 2008

Restriction Digestion of Plasmid DNA

The first way that a DNA molecule is characterized is by mapping restriction sites. This requires a detailed understanding of the distance between restriction sites. To determine the size of a restriction fragment, DNA is resolved by electrophoresis and it's migration is compared to known samples. In this lab, you will cut plasmid DNA with *Eco*R I and then determine the size of the resulting fragments with DNA standard size markers by agarose gel electrophoresis. Please note that you should be familiar with how to set up restriction digest tables before starting this lab.

Experimental Protocol

1) Set up restriction digestion
2) Pouring agarose gel
3) Preparing DNA samples
4) Gel electrophoresis
5) Photographing gel under UV light
6) Analyzing gel with gel doc system
7) Graphing mobility versus size of restriction fragments

Each person will set up a restriction digest of plasmid DNA cut with *Eco*R I.
Each group of 2 can share a gel and a gel box.

Reagents:

Sterile water - in bottle at bench
10X Restriction buffer - in MF tube on ice
1.0 mg/ml BSA (10X) - in MF tube on ice (optional)
Restriction enzymes; *Eco*RI - on ice or in -20°C freezer
1X TAE gel buffer
6X Loading Buffer

Equipment

Clean MF tubes
Clean pipette tips
Pipettemen, various sizes
Power Supplies
Submarine mini-gel boxes

Setting up restriction digest table:

(1) Calculate the amount of the following stock reagents for the restriction digestion of 0.5 μg of plasmid DNA in a total volume of 30 μl. **Before you start your reaction, you must have your restriction digest table approved!**

Sterile/Best Water	
DNA (100 ng/μl)	need ~0.5 to 1 μg
10X RE buffer	final concentration is 1X
1.0 mg/ml BSA	final concentration is 100 μg/ml (same as ng/μl)
*Eco*RI (10 U/μl)	want 2-5X what is theoretically needed

Initial your tubes!

Restriction Digestion:

(1) Add the appropriate amount of these in reagents in the above order into a marked MF tube. We will help you in getting the restriction enzymes. ALWAYS KEEP RESTRICTION ENZYMES ON ICE.

(2) After adding the reagents to the MF tube, spin the tube a few seconds in the microfuge to get all the solutions to the bottom of the tube. Then flick the tube gently to mix and place it in a floater in a water bath on the side bench.

(3) Let the restriction digest go for 1 hour at 37°C in a water bath (or incubator).

Pouring agarose gels:

(1) Place the comb into the plastic gel tray. The comb has teeth which will form the wells where the DNA samples are loaded. **The bottom of the teeth should be very close but not touching the bottom of the plastic gel tray.**

(2) Make an 1% agarose solution in 50 ml of 1X TAE. Melt the agarose solution in a glass beaker by microwaving 1 to 2 minutes at 30% power. Let the agarose solution cool to ~65°C.

(3) The instructor/TA will show you how to add 5 μl of 10 mg/ml EtBr OR how to stain your gel post-electrophoresis. **ETBR IS A MUTAGEN AND MAY CAUSE CANCER. DISPOSE OF ALL ETBR CONTAMINATED MATERIALS IN THE DESIGNATED CONTAINER IN HOOD.**

(4) Pour in the molten agarose. **The agarose should fill a little less than 1/2 of the gel tray.**

(5) Let agarose cool to room temperature and harden. As it does so, it will turn cloudy. After 15-20 minutes at room temperature, the gel will be ready for electrophoresis.

Preparing the digested plasmid DNA and loading samples onto gel:

(1) Determine what the concentration of restriction fragments are in the restriction digestion.

Use the table below to determine the appropriate amount of DNA, water and 6X LB. (The **6X loading buffer** is dense solution with a dye. The dye helps track how far the DNA has migrated over time).

Reagent	Concentration	Final conc. or amt.	Volume
Plasmid DNA		150 ng	
6X LB	6X	1X	
water			
TOTAL VOLUME			12 µl

(2) Spin the MF tubes few seconds in the microfuge to centrifuge the solution to the bottom of the tube. Your instructor/TA will help you with the microfuge.

(3) Before loading the DNA samples into the 1.0 % gel, each person should practice loading 1X sample buffer into the "practice" gel. After the practice gel has set, place the gel in a horizontal gel box and pour the gel buffer (1X TAE) to barely cover the gel. Each person should practice loading 12-15 µl of a 1X sample buffer into the gel ~2 times.

(4) After this practice gel loading, add your DNA samples into the wells of the 1.0% agarose gel. Record & keep track of where the samples were loaded on the gel.

Electrophoresis:

(1) After loading, hook up the red electrode to the "bottom" of the gel box, and the black electrode to "top" of the gel box.

(2) Turn on the power supply. **Set the voltage for ~75 - 100 V.** You should see that the blue dye (bromphenol blue) migrating out of the well towards the "bottom" of the gel. (towards the positive electrode). Rem: ~5 V / cm between electrodes.

(3) Let the gel electrophorese until bromphenol blue is about 2/3 down the length of the gel, towards the anode, about 45-60 minutes.

Turn off the power supply, disconnect electrodes from the power supply, and view the gel under UV light box.

REMEMBER THAT THE GEL AND GEL BUFFER ARE CONTAMINATED WITH ETBR! Use a plastic dish or saran wrap to transfer gel to trans-illuminator without drips.

Visualizing the DNA under UV light:

In order to see the DNA, the gel will be placed on the trans-illuminator gel doc system. The gel will be printed out and archived in your notebook. You will be shown how to use the hardware and software in lab by instructor or TA.

The trans-illuminator has a bank of UV lights underneath a quartz glass plate. When it is on, UV light goes through translucent gel, DNA fluoresces orangish-pink. Because the amount of EtBr bound determines the degree of staining, a larger fragment will stain more intensely than a smaller fragment.

Always wear a face shield or safety glasses when using the UV light.

CLEANING UP

POUR ETBR-CONTAMINATED GEL BUFFER INTO THE LARGE BOTTLE MARKED "ETBR CONTAMINATED LIQUID WASTE"

RINSE OUT ELECTROPHORESIS CHAMBERS (with tap water)

PUT AGAROSE GELS IN BUCKET MARKED "ETBR CONTAMINATED SOLID WASTE"

WIPE DOWN YOUR LAB BENCH

QUESTIONS:

(1) What size are the DNA fragments?
(2) Is the standard curve linear for all sizes of DNA fragments?
(3) What sizes of DNA may resolve poorly on this gel system?
(4) How could the resolution of these poorly resolved DNA fragments be improved?

POTENTIAL TEST QUESTIONS:

(1) Dilution and solution calculations
(2) Anything covered in this laboratory exercise
(3) See pp. 39 to 50 from Handout "Basic Tools and Techniques of DNA Science"