Molecular Biology
Lab 4
February 9th , 2010
DNA electrophoresis / Analysis of pGLO DNA restriction fragments

Analysis of restriction digestion of any DNA molecule begins with **DNA electrophoresis**. DNA fragments are separated by **size** (molecular weight/ number of base pairs) and **charge** as they migrate through a semisolid matrix called **agarose**. Agarose resembles firm Jell-O and the procedure is often called agarose gel electrophoresis. Due to DNA's strong negative charge it will migrate toward a positive charge in an electrical field. The gel is actually porous allowing small DNA fragments to migrate fast relative to large fragments. Specifically, linear fragments migrate at rates inversely proportional to the $\log_{10}$ of their molecular weight (or number of base pairs) For example, a DNA fragment 100 basepairs (10^2 bp) in length will migrate twice as far as one that is 10000 bp (10^4 bp) in the same amount of time. After a period of electrophoresis, a mixture of DNA fragments will become separated, with fragments of the same size migrating the same distance. Fragments of the same size will collect at one position in the gel, forming a band. DNA bands can then be detected in a number of ways, and the number and sizes of DNA fragments can be analyzed.

Today we will continue with analysis of the pGLO plasmid DNA by separating restriction fragments with agarose electrophoresis. We have 4 goals.

1. Set up and perform DNA electrophoresis.
2. Visualize and photograph the results of the gel electrophoresis
3. Use DNA molecular weight standards to create a standard curve based on relative migration distance. Use the curve to determine the size of the pGLO DNA restriction fragments.
4. Create a restriction map of pGLO, showing the position (if any) of enzyme recognition sites relative to each other.

Materials

Digested pGLO DNA
Loading dye (kinds vary)
Molecular weight standards
(100 bp ladder and 1 kb ladder)
Gel casting tray
Gel comb
Agarose powder
Electrophoresis buffer (make from concentrate)
Electrophoresis chamber
Power supply
Ethidium bromide
Plastic trays
Gel documentation system

Procedure:

1. Gather your DNA samples: pGLO digested with 1) Hind III 2) EcoRV or 3) both enzymes Keep them on ice until we are ready to use them.

2. Prepare a casting tray for the gel as directed. **(This was done as a group, last week!)**

3. Make two, 1% agarose gel. (100 ml in 1X TAE buffer)
    a. Make 1x TAE from 50 X stock (2 liters for the whole class)
    b. Measure needed agarose powder
    c. Mix agarose and 50 ml 1X TAE in the flask provided (swirl briefly to mix)
    d. Microwave 30 seconds

e. Carefully remove and swirl (agarose can boil violently and burns like a molten wax). Use hot mitts/gloves and eye protection.
f. Return to microwave for 30 second intervals until all agarose looks dissolved; let cool for 5 minutes.
g. Add 1 μl Ethidium Bromide to the molten agarose. Caution EtBr is a powerful mutagen which binds DNA and fluoresces. Handle bottle with gloves and remember your gel now has the mutagen within it. **Handle gel with gloves from now on.**
h. Slowly pour the agarose into the prepared gel mold/casting tray and allow it to solidify. (10-15 minutes)

5. Prepare DNA samples
   a. Add 3 μl of loading dye solution to each tube of digested pGLO DNA (+ undigested control.

   b. Make a tube of each of the DNA standard fragments:
      1. 100- base-pair ladder
         a. pipette 20 ul sterile water into a microfuge tube
         b. add 1 ul of DNA from the 100 bp stock tube
         c. add 3 ul loading dye

      2. I kb ladder (fragments of increasing size by 1kb)
         (a, b, c as above for the 100 bp ladder)

6. Assemble the gel in the gel rig and load DNA samples as instructed. (practice with 5 μl loading dye in one lane of the gel).
7. Hook the gel rig up to the power supply as directed. Run the gel at 80-90 Volts and monitor for migration.
8. Photograph your gel with a ruler to note actual migration distances from the wells.

ANALYSIS

(In class)

1. Note the pattern of restriction fragments in each lane of your gel. Did one or both enzymes seem to cut the pGLO plasmid? How do you know?

2. Note the migration distances of fragments in different lanes. Can you note larger and smaller fragments within the gel?

After class, using the photograph of your gel)

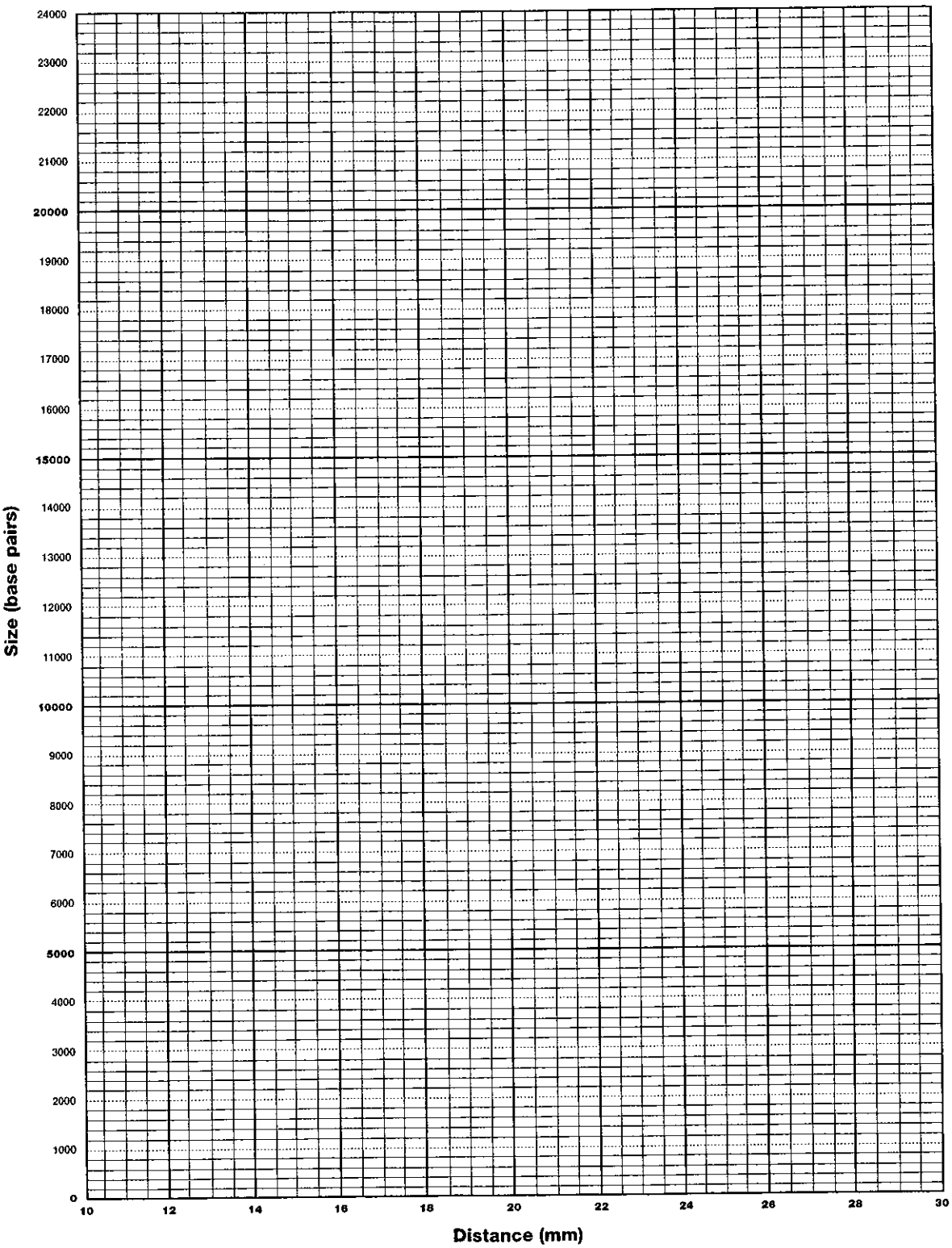
3. Create a standard curve for your electrophoresis experiment to use to determine the sizes of the restriction fragments of pGLO. (see attached handouts/instructions)

4. Use your data to try to determine the size in base pairs of pGLO. (Remember supercoiled circular DNA does not run true to its molecular weight).

5. Use your data to try to determine the position of the restriction enzyme recognition sites within pGLO. (relative to each other).

Sketch the pGLO circular molecule noting the positions of the Hind III and Eco RV cutting sites. This is called a restriction map.

Graph Paper


Size (base pairs)
24000
23000
22000
21000
20000
19000
18000
17000
16000
15000
14000
13000
12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
10
12
14
16
18
20
22
24
26
28
30
Distance (mm)

Semi-Log Graph Paper