

## ***Experiment 2***

# **Effect of Agarose Concentration on Migration of DNA Fragments**

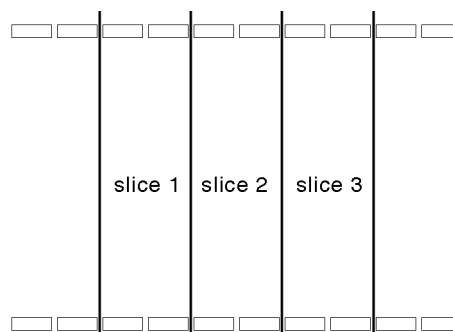
The relationship between molecular weight and distance migrated is logarithmic, not linear. Thus large fragments are packed close together and small fragments are spread far apart. Moreover, the small fragments produce broad faint bands. This is the result of two factors. First, the intensity of a band is dependent upon the amount of ethidium bromide that is intercalated into the DNA. Since small fragments do not have as many bases available for intercalation as big fragments, small fragments will be less intense. Second, the farther a fragment travels, the more the DNA tends to diffuse. Thus small fragments produce fuzzy bands. Your gels, which are 1% agarose thus only distinguish mid-size fragments. Often, however, you are working with fragments that are either very big or very small and a 1% gel is inadequate.

In Experiment 1 you saw that migration rate through a gel is a function of DNA size and conformation. But there is a third factor: concentration of the gel. An agarose gel is a meshwork of interlinked agarose strands that impede the migration of DNA. By increasing the size of the mesh, one can facilitate the passage of large fragments of DNA. Alternatively, by decreasing the mesh, one can retard the progress of small fragments, thereby minimizing diffusion. Thus one might examine a restriction digest by running it on a series of gels that optimize different size ranges.

In this experiment we will examine the effects of agarose concentration by running our molecular weight standards on three different concentrations: 0.5%, 1.0%, and 1.5%. The results of this experiment will be combined with Experiment 1 for your first lab report, to be prepared according to the format on page 4.

**Procedure**

1. You will need to collaborate in pouring gels with two other pairs. One pair should pour a 0.5% gel, the second should pour a 1.0% gel, and the third should pour a 1.5% gel. Remember to make the gel using TBE buffer. If you use water, your bands will streak and smear.
2. After you pour the gel insert two combs into the tray, one at each end and allow it to harden.
3. When the gels solidify, remove the combs and cut the gels into 3 strips, with each strip containing 2 usable lanes in the middle. Use a plastic ruler to cut the gel. Do not use a razor blade, as this will scratch the tray. When cutting, use the wells to line up your slices.



4. Exchange the strips so that each pair has one of each, lying side by side on the gel tray. Be very careful when handling the 0.5% gel. It is very soft and gooey.
5. Load each strip with standard. You need to load 2  $\mu\text{l}$  of DNA in each well. Since you are not running any 20  $\mu\text{l}$  restriction digests as well, the final sample volume is not important, although it is difficult to work with just 2  $\mu\text{l}$ . For convenience, make up 15  $\mu\text{l}$  samples:
  - 2  $\mu\text{l}$  standard
  - 8  $\mu\text{l}$  water
  - 5  $\mu\text{l}$  tracking dye
6. Run the gel at 125 V until the tracking dye reaches the end. Then stain for 10 minutes. 15 minutes of destaining in water, especially for the 0.5% gel will greatly help the visualization of these gels because these strips absorb lots of stain very quickly.
7. Measure the migration distance and plot your data on semi-long paper. The data for all three concentrations should be plotted on the same graph.