Bi1x Spring 2012
Assignment 3

Part 1: PCR Gels

1) Look at your PCR gels and describe what you see. Be sure to compare to the ladder. Try to explain the results using what you know about PCR.

Part 2: TPM

Biology of restriction enzymes

1) What organisms have restriction enzymes? What is their function in these organisms?

2) Most restriction enzymes have binding sites that are about 6 bp long. How many times would a particular 6-bp sequence appear in 500 kbp of random DNA? How does that relate to the function of restriction enzymes?

3) Restriction enzymes have been essential to modern molecular biology. Explain why.

Comparing bulk and single-molecule rates

4) a. Double-sided tape is about 100 μL thick. Estimate the volume of the chambers you used.

b. Using the first frame from your control movie, estimate the number of tethers in the entire chamber, and use that to estimate the concentration of DNA in the chamber at the start of the experiment.

c. Restriction enzyme concentrations are given in standardized units called "units" of activity, where one unit (U) is defined as the amount of enzyme required to cut 1 μg of DNA in 50 μL total volume in 1 hour, at 37 ˚C. The enzyme stock that we used was 20 U/μL. Use the amount of enzyme that the TA's told you they'd added to the 200 μL that you flowed onto the slide (between 0.5 μL and 6 μL) to calculate the concentration of enzyme in the flow chamber. Compare this to the concentration of enzyme in the restriction digests you did previously with HindIII.

d. How does the rate of HindIII activity you calculate from your single-molecule movies, relative to the amount of DNA in the chamber, compare to the rate of activity that you expect in a bulk assay, based on the definition of a unit?

e. List some reasons why the bulk and single molecule rates might differ. Think about reaction conditions as well as the geometry of the two settings.