Homework #9

You have isolated DNA from your favorite organism and digest 10 µl of the DNA with *Eco*RI (GAATTC). You want to ligate this digested DNA with a plasmid and prepare a recombinant DNA library. A 1:50 dilution of the DNA solution has an A_{260} of 0.13.

1. How many µg of plasmid (size = 3.3 kb) already digested with *Eco*RI and dephosphorylated do you added to the digested DNA to obtain approximately a 2:1 molar ratio of vector to insert?

   Hint: To determine the molar amount of the digested DNA you will either need to determine the average size of the restriction fragments or estimate how many restriction fragments will be produced by the digestion. See http://www.cbs.dtu.dk/databases/DOGS/ for a listing of genome sizes or use a reasonable size for your favorite organism. Assume a GC composition of 50% unless you know better. (Conversion factors: 1 bp = 660 daltons (i.e., g/mole); 1 A_{260} unit of dsDNA = 50 µg/ml)

2. How many potentially different recombinants (i.e., independent inserts) would you expect in this library assuming that all genomic DNA fragments are equally likely to be incorporated into the plasmid?

3. What could be done to increase the percentage of recombinants which contain the restriction fragment of interest? What other information do you need to know in regards to carrying this out?