

Homework #9 Key

There are several approaches to this assignment. Below one possible approach is discussed.

You have isolated DNA from your favorite organism and digest 10 μl of the DNA with *EcoRI* (GAATTC). A 1:50 dilution of the DNA has an A_{260} of 0.13.

1. A good first step is to calculate amount of digested DNA:

$$(0.13)(50 \mu\text{g/ml})(50 \text{ fold-dilution})(0.01 \text{ ml}) = 3.25 \mu\text{g}$$

2. To μg convert to a molar concentration you need to determine the average fragment size. The average size can be approximated from the expected frequency of the restriction site. For example, *Plasmodium berghei* has an AT% of approximately 80% and an estimated size of 2.5×10^7 bp. The expected frequency of GAATTC is $(0.1)(0.4)(0.4)(0.4)(0.4)(0.1) = 2.56 \times 10^{-4} \text{ bp}^{-1}$. The reciprocal of the frequency (i.e., 3.9×10^3 bp) is equal to the average fragment size.

[Why does the reciprocal of the frequency = average fragment size? Multiplying the genome size by the frequency equals the number of restriction fragments produced, or $(2.5 \times 10^7 \text{ bp})(2.56 \times 10^{-4} \text{ bp}^{-1}) = 6400$ fragments. Divide the genome size by the number of fragments to determine the average fragment size, or $2.5 \times 10^7 \text{ bp}/6400 = 3.9 \times 10^3 \text{ bp}$.]

3. The molecular weight of this average fragment size is $(3.9 \times 10^3 \text{ bp})(660 \text{ daltons/bp}) = 2.6 \times 10^6 \text{ g/mole}$. Calculate the molar amount of DNA by dividing the amount by the molecular weight: $(3.25 \times 10^{-6} \text{ g})/(2.6 \times 10^6 \text{ g/mole}) = 1.25 \times 10^{-12} \text{ moles} = 1.25 \text{ pmoles}$. [All organisms would result in approximately this same answer.]

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

4. The molecular weight of the plasmid is $(3.3 \times 10^3 \text{ bp})(660 \text{ daltons/bp}) = 2.2 \times 10^6 \text{ g/mole}$. Multiply the number of moles needed by the molecular weight, or $(2)(1.25 \times 10^{-12} \text{ moles})(2.2 \times 10^6 \text{ g/mole}) = 5.5 \times 10^{-6} \text{ g} = 5.5 \mu\text{g}$.

[Shortcut (skip steps 3 and 4): Use the ratio of the size difference between vector and average size of the digested DNA to determine the amount needed, or $(3.3 \times 10^3 \text{ bp})/(3.9 \times 10^3 \text{ bp}) = 0.85$. Multiply the amount of digested DNA by two and this ratio, or $(3.25 \mu\text{g})(2)(0.85) = 5.5 \mu\text{g}$ of plasmid.]

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?

This is equal to the number of restriction fragments produced as calculated in step 2 above, or 6400. However, the library would contain proportionately more of the smaller restriction fragments than the larger fragments and fragments above a certain size would be lost.

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?

- From Southern blotting experiments the size of the restriction fragment of interest could be determined. DNA of this size could be isolated and then used in the ligation instead of the total digested DNA.
- If the DNA of interest is expressed at significant levels as compared to total mRNA expression, then a cDNA library could be made.
- PCR could be used to specifically amplify the region of interest.