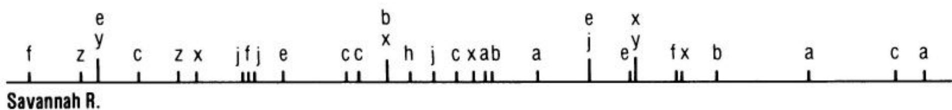
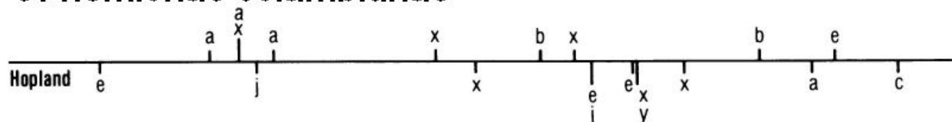
Laboratory 5
Restriction Endonuclease Mapping of DNA

Type-II restriction endonucleases are **nucleases** (DNA- cutting enzymes) that recognize particular four or six base-pair sequences in DNA [called **recognition** or **restriction sites**] and introduce double-stranded cuts into the molecule at that point. Any particular endonuclease may cut any region of DNA once, several or many times, or not at all, depending on its exact nucleotide sequence. **Restriction endonuclease mapping** is a method of obtaining an accurate physical map of a gene or cluster of genes, without the necessity of determining the complete nucleotide sequence of the region. The method involves digesting a piece of DNA with a series of **endonucleases**, singly and in pairwise combinations, and working out the logical patterns, in a manner that is very similar to solving a crossword puzzle. By determining the relative order and absolute distances among a series of restriction sites from a number of enzymes, it is possible to draw a general map of a large genetic region. Restriction mapping can be thought of as the molecular equivalent of a three-point test cross: each of the restriction sites can be ordered with respect to the others, and the distances (in base pairs) among them can be determined. Restriction maps may include a dozen or more endonuclease types that produce several dozen sites that will include several percent of the total sequence. Applications of restriction maps include identification of appropriate sites for molecular cloning, rapid screening of individuals for genetic disease markers, and studies of naturally-occurring variation within populations.

O. virginianus



O. hemionus columbianus



1 kb a, *Eco*RI; b, *Hind*III; c, *Hpa* II; e, *Xba* I; f, *Bam*HI; h, *Pvu* II; j, *Sst* I; x, *Bcl* I; y, *Cla* I; z, *Sst* II

The restriction map of a white-tailed deer (*Odocoileus virginianus*) from South Carolina, includes 33 restriction sites from 10 different **restriction endonucleases** (letters **a** - **z**). Comparison with the restriction map of a black-tailed deer (*O. hemionus columbianus*) from California shows 20 **restriction site polymorphisms**. Of these, nine are additional sites (letters above the line) and 11 are sites absent (letters below the line) in comparison with white-tailed deer. Comparison of the fraction of sites shared between maps therefore permits an estimate of the **DNA** sequence divergence between the two species. [Carr *et al.* 1986. Proc Natl Acad Aci 83:9576]

This laboratory will give you practical experience with the estimation of DNA fragment sizes from a standard curve, constructing a restriction map and will serve as an introduction to the use of restriction endonucleases in biotechnology.

Estimating Sizes of DNA Fragments.

In this exercise, you will estimate the sizes of DNA fragments using a standard curve**.** The diagram used for this exercise is a scanned image of a restriction mapping experiment with *AluI*, *HhaI*, & *HaeIII* digests of the mitochondrial cytochrome *b* gene of a black-tailed deer (*Odocoileus hemionus columbianus*).

a. **Measure the mobility of the molecular size markers** in the ΦX174 / *HaeIII* standard [ΦX174 is a bacterial plasmid whose DNA sequence and restriction sites are known]. Measure from the baseline to the middle of the band to the closest 0.5mm; be as accurate as possible.

b. **Construct a calibration curve** on three-cycle semi-log graph paper. The bottom, middle, and top lines are 10, 100, & 1000 bp, respectively. The sizes of the molecular markers should be drawn on the log (vertical) scale.

c. **Measure the mobilities of the restriction fragments** in the six restriction digests. From the standard curve, estimate the restriction fragment sizes. **Construct a table to record your results.** Smaller fragment sizes (< 600 bp) can be estimated more accurately than larger; rely more on your size estimates of the smaller fragments. (***Hint***: if you know the sizes of the smaller fragments, and that the complete gene is 1200bp, you can infer the size of the larger fragments).

Constructing a restriction map.

You will be given data to use and the **lab instructor will discuss this procedure in lab**. The following are hints on one way to construct them, however:

1. Do the single restriction enzyme maps first. This is to get a feel for how big the fragments are that the single enzymes cut and how many pieces they cut.

2. Next, do the double enzymes. The first thing you have to do is to figure out the "end pieces" though. These are fragments that are "conserved" from the singles. Any pieces from the double enzymes that are the same as the singles are the end pieces. The other pieces are in the middle and must be arranged so that they correspond to what the singles cut.

3. Check the double restriction map with the fragment sizes that the single enzymes cut!!! The numbers must correspond to what the singles cut!!

4. After you do the last double enzyme map, (and have checked it against the singles), redo it. Now, place the enzyme that's missing from it on it for your final restriction map (the final map must have ALL the enzymes on it). To do this, look back at the doubles which have the missing enzyme on it. Put it in the same location, readjusting the fragment you place it in (I.e. readjust the size of the fragments on either side of the added enzyme).

5. Check the final restriction map with both the singles AND the doubles. If everything works out, it should be correct!!

For additional practice: Work the restriction mapping problems in your text and on the homepage for the course!!

Expect a restriction mapping problem on the Final Exam!