CHEM-342 Introduction to Biochemistry
Final Examination - Group (Part II)
Friday, 25 May 2007
5:30 – 6:30 PM
H. B. White - Instructor

Group Members

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Important - Please read this before you turn the page.

- You must sign your name on this page to receive the group grade. In case of lack of consensus, you may hand in your answer separately from your group for individual grading.
- You may refer to your notes, course reader, handouts, or graded homework assignments. Textbooks and reference books are not permitted.
- In CHEM-342, hemoglobin is a vehicle for learning how to learn by asking questions and pursuing answers to those questions. Undoubtedly you have learned a lot about hemoglobin in the process but you also should be developing habits of mind that will enable you to solve problems in other courses and throughout your life. This part of the final examination provides an opportunity for you and the other members of your group to display problem-solving skills as a team. It is extremely unlikely that anyone in your group or in the class has encountered the information on the following pages. Your answers should display your collective:
    - breadth of knowledge (not limited to hemoglobin or biochemistry)
    - ability to analyze, make connections, and ask probing questions
    - sense of logic and organization
    - skill at generating models (testable hypotheses)

1. Restriction Mapping of the Human Gamma Globin Genes

The hemoglobin present in a human fetus is different than adult human hemoglobin. Fetal hemoglobin (HbF) has a subunit composition of $\alpha_2\gamma_2$, while that of adult hemoglobin (HbA) is $\alpha_2\beta_2$. The gamma (γ) subunit is actually the product of two very similar, closely linked genes. The Gγ gene produces a globin chain with glycine at position 136, while the Aγ gene product is identical except for an alanine at position 136 of the 146 residue chain. A 500 nucleotide base pair (0.5 Kb) cDNA corresponding to the coding part of the Gγ gene has been cloned. This short piece of DNA corresponding to γ-globin mRNA and lacking introns, hybridizes strongly and specifically to the two γ-globin genes. When labeled with ^{32}P, this cDNA can be used as a probe to detect DNA fragments containing all or part of one or both γ-globin genes.

DNA isolated from human placenta was cleaved with various restriction endonucleases (singly or in pairs). Restriction endonucleases are to DNA as trypsin is to proteins in that they cleave a linear DNA polymer at sequence-specific sites to generate restriction fragments that are analogous to tryptic peptides. The restriction fragments were separated by electrophoresis, transferred to nitrocellulose sheets, and hybridized with the ^{32}P-labeled γ-gene cDNA probe. Autoradiography of the sheets revealed the positions of restriction fragments containing the γ-gene sequences. Using standard DNA fragments of known size, the sizes of the hybridizing fragments could be estimated by the distance they migrated on electrophoresis. The table below represents the results of this analysis. For instance, only one *Bgl*II fragment 13 Kb long was observed to hybridize with the labeled probe. (Restriction enzymes are named after the bacterial from which they were isolated, e.g. *Eco*RI is short for *Escherichia coli* restriction enzyme I)

Restriction Enzyme	***Bgl*II**	***Bam*HI**	***Xba*I**	***Eco*RI**	***Pst*I**
Fragments generated (Kb)	**13**	**15.0 5.0 2.6**	**7.5 5.0 3.7**	**6.5 2.5 1.65 0.65**	**10.0 5.0 4.0 0.9**

Restriction Enzymes	***Bgl*II *Bam*HI**	***Bgl*II *Eco*RI**	***Bgl*II *Pst*I**	***Bgl*II *Xba*I**	***Bam*HI *Eco*RI**	***Bam*HI *Xba*I**	***Eco*RI *Pst*I**	***Pst*I *Xba*I**
Fragments generated (Kb)	**6.0 5.0 2.1**	**3.0 2.5 1.65 0.65**	**5.0 2.2 0.9**	**6.0 5.0 2.25**	**2.6 1.65 1.05 0.65**	**7.5 5.0 2.6**	**4.0 1.8 1.6 0.8 ~0.4**	**6.5 5.0 3.7 0.9**

In addition to the size of the restriction fragments, the following information is known about restriction cleavage sites within the **coding** portion of each γ gene.

	Aγ	**Gγ**
***Bam*HI**	**99-100**	**99-100**
***Eco*RI**	**121-122**	**121-122**
***Pst*I**	**135-136-137**	**None**
***Xba*I**	**None**	**None**
***Bgl*II**	**None**	**None**

The numbers correspond to the amino acid codon position at the restriction enzymes recognition site.

Using the information provided, your group must complete the restriction map on the next page and then make several conclusions. The incomplete map contains the locations of *Bgl*II, *Bam*HI, *Xba*I, and one of the *Eco*RI cleavage sites.

Note: The closely-linked γ genes are the result of a relatively recent gene duplication. The two genes are thus closely related and might be expected to have some corresponding restriction sites. Secondly, the estimation of restriction fragment size may contain errors of a few percent and restriction fragments of similar size may not be resolved. Small restriction fragments (<0.4 Kb) will not be observed. Finally, you do not need to know the specificity of the various restriction endonucleases.

A. (10 points) On the worksheet, please locate the *Pst*I and remaining *Eco*RI sites on the *Bgl*II restriction fragment.

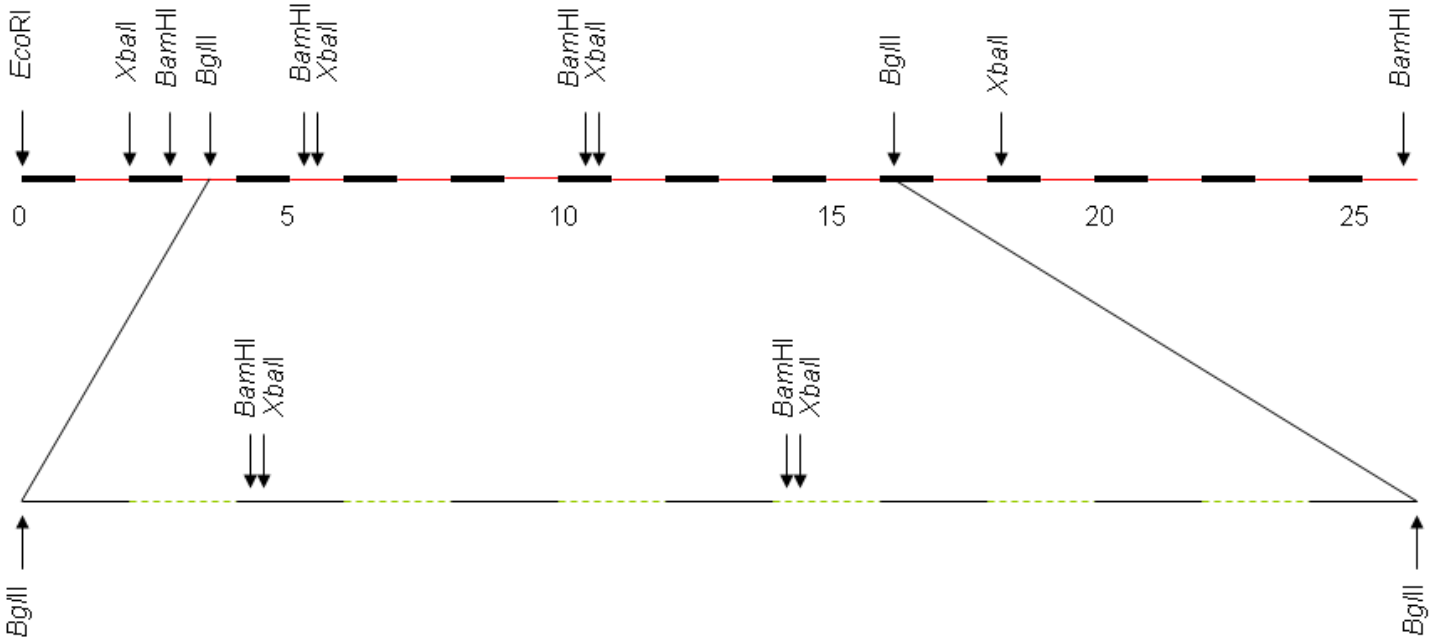
B. (4 points) Define as closely as you can the location of the γ-genes on the *Bgl*II restriction fragment.

C. (4 points) Orient the two genes relative to the N and C termini of the encoded γ globin subunits.

D. (2 Points) Approximately how large was the duplication event that generated the second γ-gene?

E. (5 Points) Do the γ-genes contain introns (non-coding regions within the genes)? Explain.

Restriction Enzyme Map of the Human Fetal Hemoglobin Genes



Adapted from: Little et al. (1979) Structure of the human fetal globin gene locus, *Nature* **278**, 227-231.