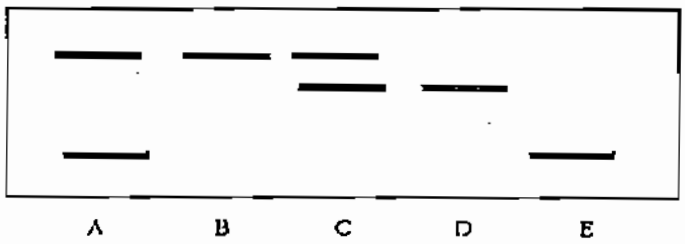
BEL 722 Genomics and Proteomics

Major Test

Total Marks – 50 Duration – 2 hours November 28, 2006

1. From in situ hybridizations, five different YACs containing genomic fragments were known to hybridize to one speeific chromosome band of the human genome. Genomic DNA was digested with a long-cutter restriction enzyme, and radioactively labeled YACs were cach hybridized to blots of the digest. The autoradio-gram was as follows:



(a) Use these results to order the three hybridized restriction fragments.

(b) Show the locations of the YACs in relation to the three genomic restriction fragments in part (a) **(2X2.5)**

2. Seven human-rodent radiation hybrids were obtained and tested for six different human genome molecular markers A through F. The results are shown here, where a plus sign indicates the presence of a marker.

	RADIATION HYBRIDS						
Markers	1	2	3	4	5	6	7
A	–	+	–	–	+	+	–
B	+	–	+	–	–	–	–
C	+	–	+	+	–	+	–
D	–	+	–	+	+	+	–
E	+	–	–	+	+	–	+
F	+	–	–	+	+	–	+

(a) What marker linkages are suggested by these results?

(b) Is there any evidence of markers being on separate chromosomes?. **(2X2.5)**

3. What is RFLP? A YAC clone of a human chromosome containing a 985 kb insert generates on digestion with *Not*I the fragments (in kb) 350, 225, 200, 100, 75, 25 and 10 ~~as shown below~~. Draw the RFLP pattern. The insert eontains a gene 'X' (250 kb) and an element 'B' (55 kb). Using 'X' as probe, the Southern blot of the

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digest gives positive hybridization with three large fragments. Another YAC clone from this region showing positive hybridization with probe 'X' generates *Not*I fragments 350, 255, 125 and 80 kb.. Southern blot of this digest gives positive signal with all the fragments except with 80 kb. Southern blots of the two RFLPs with probe 'B' gives positive signal with 100 and 75 kb with the first RFPL and none with the latter. Draw the RFLP pattern and restriction map of the second digest and give explanation for the observed results. 5

4. From a YAC library of 34,560 clones, you are required to identify at least one clone containing two STSs separated by 540 bp, Using 96 well (8 rows X 12 columns) microtiter plates for growth of the clones it is possible to screen the library by PCR. What is the minimum number of PCR reactions needed to be done to get at least one positive hit? Show schematically the strategy to be employed. 5

5. (a) How can you monitor the denaturation and renaturation process of a protein molecule using intrinsic and extrinsic fluorescence measurements?. **4**

(b). Let's consider that two recombinant proteins are of close molecular weight range and their PI's are pretty closer. How would you separate them?

2

6. (a) How a protein denaturation and renaturation process can be monitored by Circular Dichroism spectroscopy? 3

(b) What will be your approach to identify the transient intermediate species forming during the folding or unfolding pathway? 3

(c) How can a chemical reaction be monitored through IR speetroscopy? Explain with the physical basis of the process. 3

7. Describe the process of RAGE technique with diagrams **5**

8. What is Real Time-PCR, what is its advantage over normal PCR in gene expression studies? 3

9. What is a protein array? Why is it required and what are the major areas where it is used? 3

10. What are the differences between MALDI, electrospray and quadrupole mass spectrometry methods? **4**
