Spring 2003 Molecular Biology Exam #1 - Learning the Tools

There is no time limit on this test, though I have tried to design one that you should be able to complete within 4 hours, except for typing. You are not allowed to use your notes, any books, any electronic sources except those specified in the exam, nor are you allowed to discuss the test with anyone until Wednesday Feb. 5, 2003. **EXAMS ARE DUE AT 11:30 ON WEDNESDAY, FEBRUARY 5**. You may use a calculator and/or ruler. The answers to the questions must be typed on a separate sheet of paper unless the question specifically says to write the answer in the space provided. If you do not write your answers on the appropriate pages, I may not find them unless you have indicated where the answers are.
There are three pages for this exam, including this cover sheet.
When you are ready to take the exam, send me an email with the subject line of Molecular Test. This will generate an automated email telling you how to download the exam.

-3 Pts if you do not follow this direction:
Please do not write or type your name on any page other than this cover page. Staple all your pages (INCLUDING THE TEST PAGES) together when finished with the exam.

Name (please print here):

Average score = 75.5
range = 48 – 100%
points added = 10

Write out the full pledge and sign:
"On my honor I have neither given nor received unauthorized information regarding this work, I have followed and will continue to observe all regulations regarding it, and I am unaware of any violation of the Honor Code by others."

How long did this exam take you to complete (excluding typing)?

average time = 4.3 hours

16 pts.

1. A new cult has indicated its intentions to clone a human. However, they have a few questions before they begin and they need your help to get started.

a) The wanna-be cloners borrowed a cup of DNA from another cult that believes all humans are derived from game show hosts on another planet. The cloners measured the absorption of 260 nm wavelength light and obtained a reading of 0.666. However, the DNA they measured had been diluted by taking 2 μL of DNA and mixing it with 148 μL of water. Remember that 1 OD_{260} = 50 μg/mL.

i) What was the concentration of the diluted DNA? **33.3 μg/mL**

ii) What was the concentration of the original cup of DNA borrowed from the game-show-hosteans? **2,497 μg/mL**

b) To be sure they had obtained human DNA and not game-show-host DNA, the cloners want to digest 3 μL of pure DNA in a final volume of 125 μL. Please tell them how to mix the ingredients for this digestion using both Eco RI and Bam HI. Luckily for them, the digestion buffer came as a 20X stock solution.

Water	**103.25 μL**
DNA	**3.00 μL**
Buffer	**6.25 μL**
EcoRI	**6.25 μL (max)**
Bam HI	**6.25 μL (max)**
Total	**125.00 μL**

c) Now that the DNA was digested, the clone-aterians wanted to run the DNA on a gel. How should they make a 90 mL gel that is 0.4% agarose w/v, 0.5X TBE, and contain 1 μL per 30 mL of an ethidium bromide stock solution?

0.36 g agarose
4.5 mL 10X TBE
85.5 g Water
microwave, cool some and then add 3 μL EtBr

d) It turns out they need a solution for washing nuclei. They need a solution that is 10 mM Tris-HCl (pH 7.8), 0.001 M EDTA, and 10% SDS v/v. Tell them how to make 500 mL of this washing solution.

about 200 mL water
0.605 g Tris
0.208 g EDTA
250 mL 20% SDS
pH with HCl to 7.8 and add water to 500 mL

FWs: NaCl = 58.5; EcoRI = 125; BamHI = 415; EtBr = 394; EDTA = 416; Tris = 121; HCl = 36.5; agarose = 204. Other raw materials include SDS = stock solution of 20%; TBE = stock solution that is 10X;

12 pts.

2. Using an outline format, tell me how to purify a cDNA for a protein found in human milk. Be sure to include an experiment to demonstrate you truly have cloned a correct cDNA.

Isolate lactating mammary gland tissue from biopsy
extract mRNA
produce cDNA using oligo-dT primer
produce cDNA library

Purify milk protein, get amino acid sequence (at least 7 amino acids) and deduce encoding DNA
Make probe from sequence above
probe library and isolate cDNA

to be sure you have the right one, sequence cDNA and compare with protein sequence

12 pts.

3. Design an experiment that detects every tissue in a human body that expresses the cDNA you cloned in the question above.

Inject protein into mouse and produce monoclonal antibody (mAb)
get biopsy from every major tissue
perform immunofloursence or western blot with these tissues
make sure you have + control tissue (lactating mammary glands or milk itself) and negative control (male breast tissue)
You could also have chosen to do a Northern blot using cDNA as probe.

12 pts.

4. Using outline format, tell me how you would clone and sequence the promoter responsible for the tissue-specific expression of the cDNA you isolated in question 2 and measured in question 3.

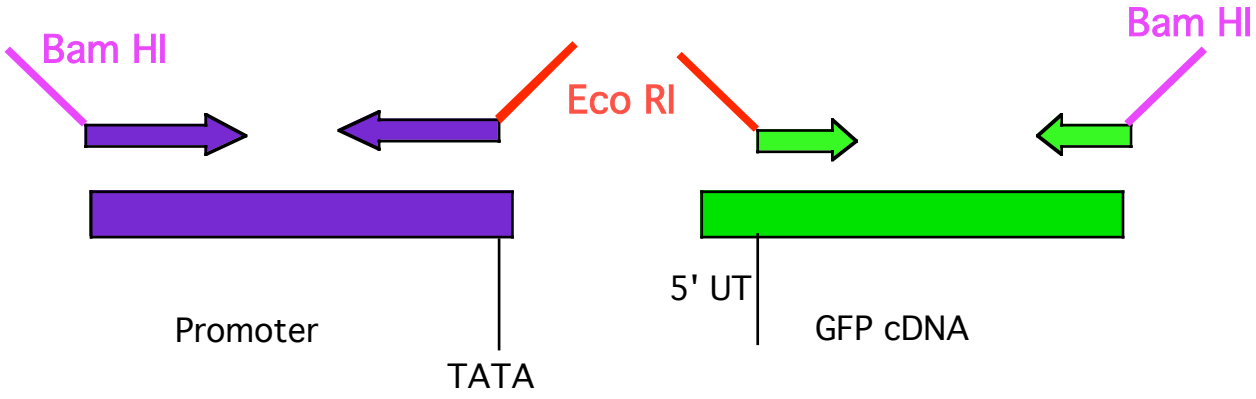
Make human genomic library from any cell type (white blood cells)
probe with cDNA from #2.
isolate gene fragment and sequence it. Sequence upstream of cDNA should be promoter. Look for TATA box to be sure.

10 pts.

5. How could you use PCR to isolate the promoter you identified in question 4 and connect the promoter to GFP cDNA so that GFP would be expressed in the same tissues as the original cDNA from question #3.

Design promoter primers so they have the indicated restriction sites (see figure below) and the downstream primer includes the TATA box.
Design the GFP cDNA primers to include the indicated restriction sites and make sure the upstream primer does not include all of the 5'UT region. This will facilitate spacing between promoter and start transcription site.

Perform PCR and then ligate the two products together via Eco RI sites and then ligate this product into plasmid via Bam HI sites.
You'd need to get this into every cell, but that is another question.



10 pts.

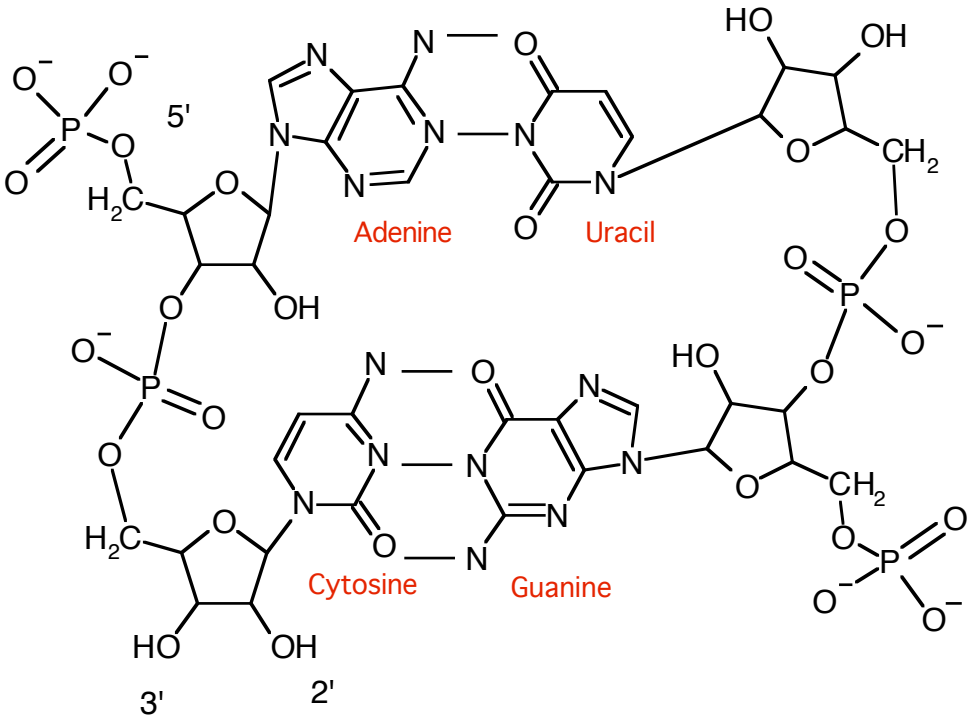
6. Design an experiment to compare the amount of a new mouse protein called earwaxase that is produced in the left ear v. the right ear of adult mice (male and female separately).

First, make a mAb to the earwaxase. You cannot use mouse so make it in chicken or goat, etc.
Obtain left and right ear tissue from male and female mice, keep them in separate tubes.
Run the proteins on SDS-PAGE and then transfer all proteins to nitrocellulose.
You would want to include purified earwaxase as + control and maybe tail tissue as – control. These would be in separate lanes too.
Probe with anti-earwaxase mAb. Also probe with anti-actin mAb to verify loading and allow you to compare expression amounts of earwaxase.

14 pts.

7. Draw a picture of double-stranded RNA composed of 4 nucleotides total (2 nucleotides on each strand). Your drawing must be chemically correct with no short hand notations for any parts except the bases. For bases, you may draw the correct number of simplistic rings, rather than full chemically correct structures. However, you must label all four bases to show which base you have drawn at each location.

SEE NEXT PAGE



10 pts.

8. In the same order as shown here, name the amino acids abbreviated:

PEACE IS THE DREAM

Proline	**Isoleucine**	**Threonine**	**Aspartate**
Glutamate	**Serine**	**Histidine**	**arginine**
Alanine		**Glutamate**	**Glutamate**
Cysteine			**Alanine**
Glutamate			**Methionine**