AP Chapter 20 Study Guide: DNA Technology & Genomics

(Rob Hamilton)

Teacher's Note: Biotechnology is changing our world. The implications and application of this technology have medical, pharmaceutical, agricultural, social, economic, legal and ethical ramifications. In this chapter you will examine techniques for altering the genome of living things. No other undertaking of mankind offers greater promise or danger than biotechnology.

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Cloning a Human Gene in a Bacterial Plasmid

You are probably quite unaware of the fact that several otherwise small males in our school take weekly injections of human growth hormone synthesized by bacteria. Prior to this recombinant technology, only the wealthy could afford HGH injections because the only source of HGH was from the anterior pituitary gland of human corpses.

1. Locating the gene: Read *pgs 384-390 and fill in the missing information. You should know part 1 already*

a) Even if you could sort the HGH gene from all of the other human genes, it would be worthless because the gene would be filled with **intron** or **exon** DNA that cannot be processed by a prokaryotic cell. The way to solve the problem is to locate a molecule of **DNA** or **mRNA** that codes for HGH in an anterior pituitary gland.

b) Now expose the polynucleotide strand to _____ (made by retroviruses) which will convert the single stranded m-RNA to _____ which contains the complete complimentary sequence without intron. Then use DNA polymerase to make a double strand with the coding sequence.

2. Restriction Enzymes: *This stuff is on pg 386*

a) What kind of organism produces restriction enzymes? _____

b) Why would they do this? _____

c) Why is their own DNA unaffected? _____

d) What exactly do restriction enzymes do? _____

e) Where exactly do restriction enzymes function? _____

f) Why is DNA that is cut with certain restriction enzymes said to have "sticky ends?" _____

_____

g) How do the "sticky ends" of DNA fragments cut with the same restriction enzyme compare? _____

_____

h) How could this be useful? _____

_____

i) Why do you think restriction **enzymes** are:

1) stored on ice? _____

2) always used with a buffer? _____

3) incubated at 37^{o}C _____

3. Inserting the HGH gene into a cloning vector (plasmid): Examine Fig 20.3 & Fig 20.4

a) Put the HGH gene in an eppendorf tube with **linker DNA** that has the restriction site for EcoR1 (a restriction enzyme) Expose the gene and linkers to **DNA ligase** which will perform a blunt ligation (joining) of the linkers to the gene.

b) Select a plasmid that has a single recognition site for _____ within a Lac Z gene (gene that breaks down lactose) and containing a gene for the production of β-lactamase which will protect the host cell from the antibiotic ampicillin.

c) Expose the plasmid and the HGH gene/linkers to the restriction enzyme _____

d) The open plasmid will now have sticky ends that are the _____ as the sticky ends of the HGH/linker DNA strand.

e) Mix DNA and add DNA _____ to join the molecules.

f) Sometimes the plasmid will re-ligate (bummer), sometimes the HGH gene will ligate into a circle (bummer), sometimes the HGH gene will become a part of a plasmid creating a _____ DNA plasmid.

4. Inserting the recombinant plasmid into a bacterial host. Fig 20.4

a) Select a host bacterium that is not capable of growing in the presence of _____

b) Ice down the bacterium with a solution rich in Ca^{++} ions. This produces cells that are _____. (Cells capable of being transformed.)

c) Add the recombinant plasmid to the competent cells and _____ _____ the cells by exposing them to a 42^{o} C water bath for 90 seconds and placing them quickly back on ice. *(you've done this!)*

d) Most of the bacterial cells will not have been transformed and some of the transformed cells contain a plasmid that religated without the HGH gene, so to eliminate them, the cells should be plated out on a medium containing _____ and a sugar called _____. When this sugar is hydrolyzed by β-galactosidase (coded for by the Lac Z gene), it produces a blue product.

e) Only transformed cells will grow in the presence of the antibiotic and only cells containing the HGH gene will not be blue. These cells will produce human growth hormone which can now be harvested.

5. Gel Electrophoresis Fig 20.8

Instructions: *Read pages 392 & 396.*

a) What characteristics of the structure of DNA fragments allows them to be separated using a gel electrophoresis? _____

b) How do fragments separate in an agarose gel? _____

c) Why do they separate in that fashion? _____

d) What is the function of the power supply? _____

e) How would a change in the amount of current affect the separation of DNA fragments? _____

_____

f) What is the function of the dams and comb? _____

_____

g) Why won't the box open when the power is connected? _____

h) What is the function of the agarose gel? _____

i) How does the concentration of the gel affect the separation of DNA fragments? _____

_____

j) What is the function of the running buffer? _____

k) How would using water instead of running buffer affect gel electrophoresis? _____

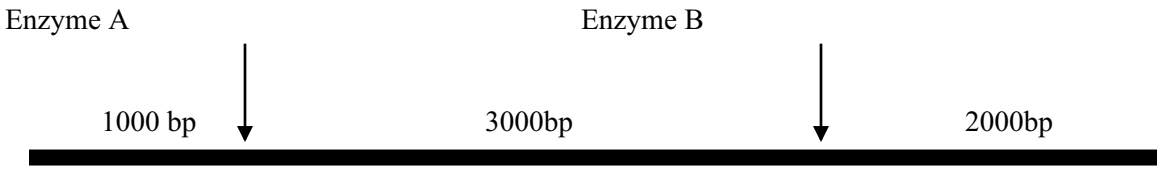
_____

l) What are the three functions of loading dye? _____

_____

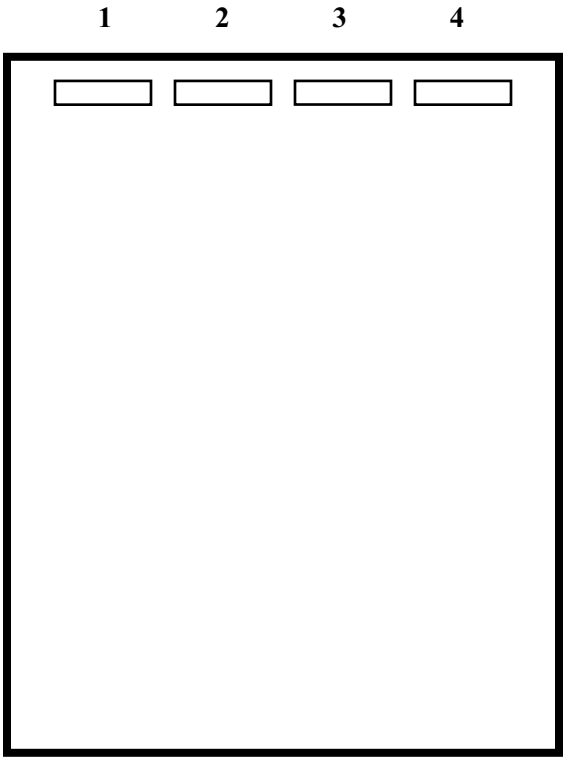
GEL ELECTROPHORESIS APPLICATION

Pictured below is a piece of DNA 6,000 base pairs in length with the indicated restriction sites



m) Draw how the DNA fragments would separate if the following were put in each well:

Well #1: DNA only
Well #2: DNA with Enzyme A
Well #3: DNA with Enzyme B
Well #4: DNA with Enzymes A & B



n) Why must DNA of unknown length always be run in a gel with DNA of known fragment length? _____

_____

o) What is the name of the graph that is constructed? _____ _____

6. The Polymerase Chain Reaction Fig 20.7

Instructions: *Read pages 391 & 392.*

a) What does PCR do? _____

b) What reagents (starting materials) are needed for PCR?_____ &_____,

& _____, & _____

c) Why is human DNA polymerase not used for PCR?_____

d) What is the source of DNA polymerase used in PCR? _____

e) Describe the three steps in a single PCR cycle:

1. _____
2. _____
3. _____

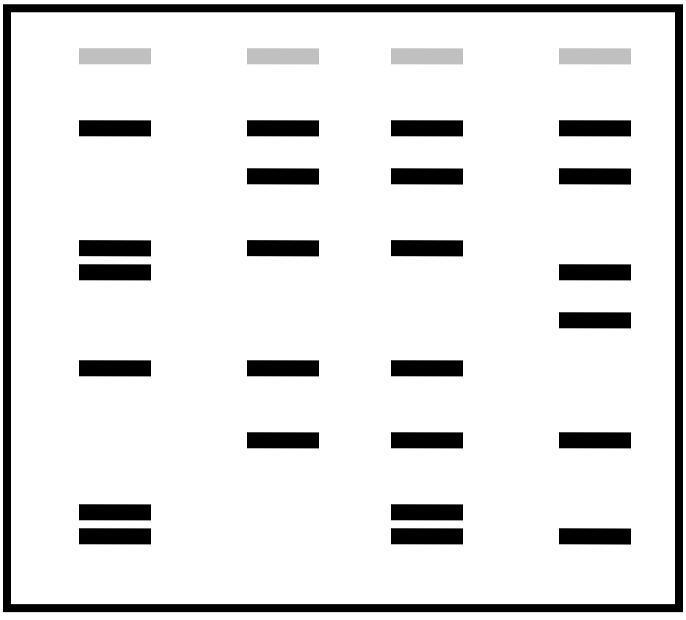
7. DNA Fingerprints

Instructions: *Read pgs 393-396 & 404-405 and answer the following questions.*

RFLP analysis using a southern blot can be used to produce bands of marker DNA that are unique to individuals. The gels produced by this technique can be used to settle paternity cases and identify suspects in a crime.

Examine the gel below:

A **B** **C** **D**



Which of the following statements is consistent with the results? (Circle your answer)

D is the child of B and C A is the child of C and D

A is the child of B and C B is the child of A and C

C is the child of A and B

To read this gel and answer the question:

1. Locate **informational markers** (DNA bands that are either present or absent in both parents). If one parent has the marker and the other does not, that marker is considered **non-informational**.
2. Examine the offspring's marker. (If both parents have the marker then the offspring would possess the marker. If neither parent has the marker then it will not show up in their offspring.)

8. DNA Sequencing Fig 20.12

Instructions: Read pgs 396-398 and answer the following questions

a) Why does the addition of a dideoxynucleotide end DNA replication? _____

_____

b) In order for the dideoxy chain-termination method to work, what are the reagents need to be added to the DNA fragments that will be sequenced? _____

c) After the DNA fragments have been replicated, how are the fragments separated? _____

_____

d) When the fluorescent detector senses the color of the fluorescent tags, the results are printed out as a spectrogram and the **coding sequence** or **complimentary sequence** of nucleotides can be read.

9. Applications of Biotechnology

Instructions: *Read pgs 402-407 and answer the following questions:*

Biotechnology is being used in many areas such as medicine, pharmacology, forensics, environmental clean up, and agriculture. Select two applications of biotechnology from any of these areas and discuss the following:

1) Name the technology

2) Provide a brief description of the procedure

3) Explain what benefit does mankind derive from the application of the technology?

Biotechnology #1

Biotechnology #2

10. Potential Problems with Biotechnology

Instructions: *Read pgs 407-408 and answer the following question:*

Briefly discuss a safety or ethical concern raised by biotechnology. Name the problem, explain how this problem might arise, and describe the danger or potential danger this problem causes.