Chapter 4-14: Recombinant DNA

A new technology was developed in the 1970s that allowed scientists to splice DNA fragments from one organism into another and then clone the new molecules. This technology came to be known as recombinant DNA technology. It is also known as genetic engineering.

Recombinant DNA technology has broad applications in the pharmaceutical, agricultural, and breeding industries. This plate discusses the series of steps by which recombinant DNA molecules are synthesized. The recombined DNA encodes for various proteins, as we will explain in the next plate.

Looking over this plate, you will notice that it consists of a progression of four diagrams that show the steps involved in the formation of a recombinant DNA molecule. The objective of the process is to synthesize a single molecule of DNA from two different DNA molecules.

Basically, recombinant DNA is formed by the introduction of foreign DNA into a DNA molecule. In diagram 1, we display **DNA molecule #1 (A)**. You should focus on its middle portion, and a pale color should be used to color the brackets outlining the **recognition sites (B)**.

At these recognition sites, enzymes called **restriction enzymes (C)** specifically cleave the strand of DNA. A medium color may be used to designate the enzyme in our diagram. Restriction enzymes are so-named because they operate on restricted points on the DNA molecule. In diagram 1 you can see the restriction enzyme cutting double-stranded DNA. The enzyme displayed is called EcoRI, it is the restriction enzyme that's derived from the colon bacterium *Escherichia coli*.

In diagram 2 we see that EcoRI has located two recognition sites and cut the DNA molecule. The result is a **DNA fragment (E)** that is left with single-stranded tails at each end. These tails can bond with DNA strands that have complementary tails, and for this reason the unpaired base sequences are called **sticky ends (F)**. The bracket indicating the sticky ends should be colored with a dark color to highlight its presence.

We have now isolated a single DNA fragment. The remaining section of the DNA molecule will now be set aside as **discard DNA (D)**, as the plate indicates.

We have now isolated a DNA fragment from the first DNA molecule. This DNA molecule has exposed bases at its sticky ends. Any DNA molecule with complementary bases can bind to these sticky ends. We continue this process by introducing new DNA, in the next diagram. Continue your coloring as you read below.

Now examine diagram 3, which shows a second DNA molecule, designated **DNA #2 (G)**. This molecule has also been treated with EcoRI; it also has a sticky end (F).

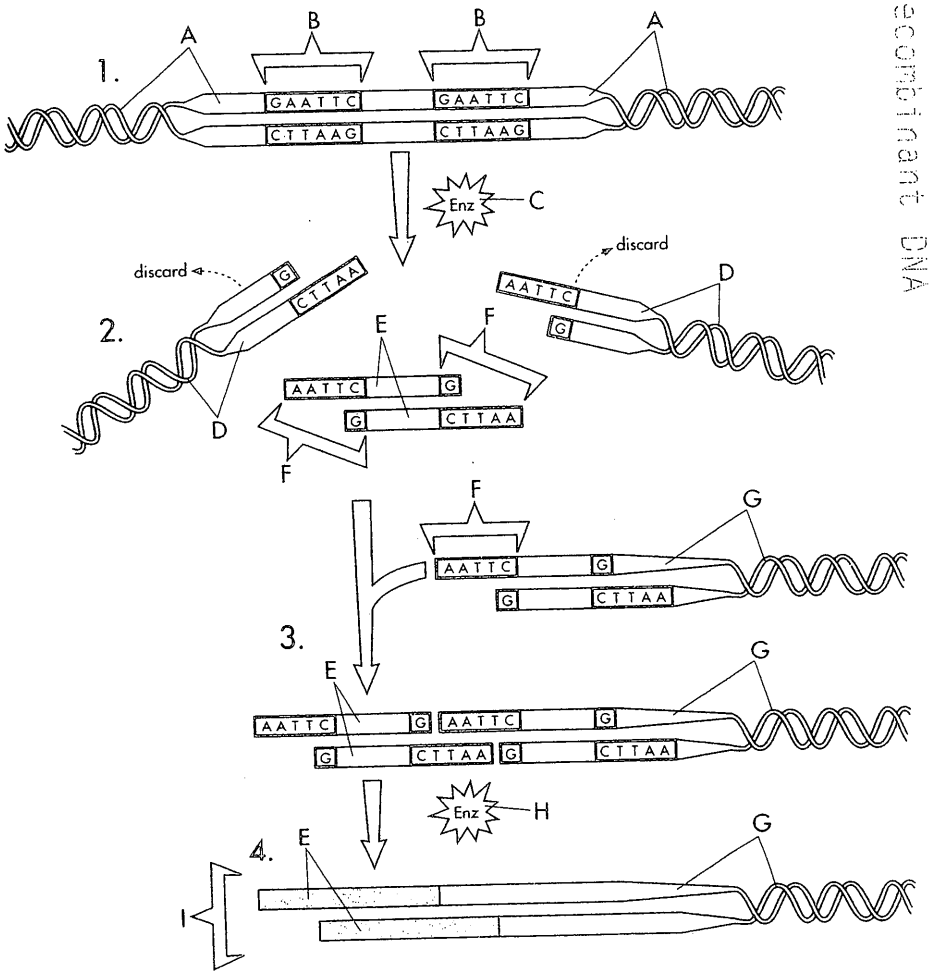
We now bring the two DNA fragments together. The sticky end of DNA #2 overlaps with the sticky end of the original DNA fragment. The bases are complementary, that is, adenine (A) matches with thymine (T) and T matches with A. The cytosine and guanine bases are not involved in the matching.

We now move to diagram 4. A new enzyme called the **ligase enzyme (H)** is used at this point. Ligase is responsible for sealing any breaks in a DNA molecule in living cells. Biochemists use this enzyme to seal DNA #2 to the DNA fragment; the ligase splices the DNA molecule to the DNA fragment.

The result of the ligase activity is shown in diagram 4. We see DNA #2 (G), which has combined with the DNA fragment (E); the result is called **recombinant DNA (I)**. The bracket should be colored in a dark color.

The technology of recombinant DNA permits scientists to attach one or more genes to a carrier molecule of DNA. This recombined molecule can be placed in an environment in which the DNA fragment will be expressed. For example, the recombinant DNA molecule can be placed in a bacterium where the foreign DNA fragment will encode a protein that is not normally produced by that bacteria. If the DNA fragment included the gene responsible for the production of the hormone insulin, then the bacteria would produce insulin.

Products of recombinant DNA technology include vaccines, research chemicals, medical proteins, and genetically-altered organisms used for such purposes as resolving pollution problems, increasing soil fertility, and killing insect pests.



Recombinant DNA

○ DNA #1 .....A	○ Discard DNA .....D	○ DNA #2 .....G
○ Recognition Sites.....B	○ DNA Fragment .....E	○ Ligase Enzyme .....H
○ Restriction Enzyme C	○ Sticky Ends.....F	○ Recombinant DNA I

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a. Technology developed in the 1970's that allowed scientists to splice DNA from one organism into another & then clone the new molecules:

b. In what industries does recombinant DNA technology have applications?

c. Basically, recombinant DNA is formed by the introduction of:

d. What specifically cleaves the DNA at the recognition sites?

e. Describe the ends of the DNA fragment resulting from the cleaving of restriction enzymes:

f. What are the ends of the DNA fragment called?

g. Does the second DNA fragment with which fragment #1 will combine also have sticky ends?

h. What happens the sticky ends of each DNA fragment?

i. What enzyme splices the DNA to the DNA fragment?