**AP Biology 12**

Biotechnology

*You must know:*

* The terminology of biotechnology
* The steps in gene cloning with special attention to the biotechnology tools that make cloning possible
* The key ideas that make PCR possible
* How gel electrophoresis can be used to separate DNA fragments or protein molecule



**Using Restriction Enzymes to Make Recombinant DNA**

Cloning and genetic engineering rely on \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_.

Hundreds of different restriction enzymes have been identified and isolated.

Each is very \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ to a particular short DNA sequence,

or \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_and cutting both DNA strands at precise points

within the restriction site.

DNA of bacterial cells is protected from the cells own restriction enzymes by the

addition of \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ to adenines or cytosines within the sequences

recognized by the enzyme.

Most restriction enzymes recognizes sequences containing four to eight

nucleotides, because this is short is usually occurs \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_in a

DNA molecule and a restriction enzyme will make many cuts, giving us \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_.

The most useful restriction enzymes cleave the sugar-phosphate backbone in the two DNA strands in a staggered manner, theses produce a \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_.



 The sticky ends will form \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_base pairs with complementary sticky ends on any other DNA molecules cut with the same enzyme.  They matches then becomes permanent with the help of \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_.

**Application**

Create a restriction map for the plasmid pGEN 101 (total length is 20kb).  When digested with EcoRI (a restriction enzyme), one linear fragment results.  Digestion with BamHI results in 3 fragments of the following sizes: 12kb, 2kb, 6kb.  A combination digest with EcoRI and BamHI results in 4 fragments: 8kb, 4kb, 2kb and 6kb.

Digest performed          Size of fragments obtained

EcoRI                               \_\_\_\_\_\_\_\_\_\_

BamHI                             \_\_\_\_\_\_\_\_\_\_

EcoRI + BamHI               \_\_\_\_\_\_\_\_\_\_

**Gel Electrophoresis**

Often, in order to study DNA, we need to use a gel electrophoresis. The technique uses a gel to \_\_\_\_\_\_\_\_\_\_\_\_\_ nucleic acids or proteins on the basis of \_\_\_\_\_\_\_\_\_\_ and \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_.

DNA carries a \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ charge on their phosphate group so they travel toward the positive pole in an electric field.   The gel impedes the \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ molecules more than the shorter ones, separating them by length.



**Restriction fragment analysis**

DNA fragments produced by restriction enzyme digestion are are sorted

 by gel electrophoresis.  Useful for \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ two different

DNA molecules ie. two alleles of a gene.

Example: Sickle cell destroys one of the DdeI restriction sites in the gene.

As a result, digestion with the DdeI enzyme generate \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

from the normal and sickle-cell alleles.

**Practice**

Below is a plasmid with restriction sites for BamHI and EcoRI.  Several restriction digests were done using these two enzymes either alone or in combination.

*HINT:* Begin by determining the number and size of the fragments produced with each enzyme.



Which lane shows a digest with *Bam*HI only?

Which land shows a digest with both *Bam*HI and *Eco*RI?

**Polymerase chain reaction (PCR)**

PCR is a method used to \_\_\_\_\_\_\_\_\_\_\_\_\_a particular piece of DNA without the use of cells.  PCR is used when the source is impure (as it would be in a crime scene).

1. Heat is used to\_\_\_\_\_\_\_\_\_ and separate the DNA
2. Cooling allows primers to form hydrogen bond with ends of \_\_\_\_\_\_\_\_sequences
3. DNA\_\_\_\_\_\_adds nucleotides to the 3’ end of each primer

This results in thousands or millions of \_\_\_\_\_\_\_\_\_\_ of DNA sequence for diagnosis and monitoring of genetic diseases

