



# The *E. coli* Insulin Factory

## BACKGROUND

Bacteria have not only their normal DNA, they also have pieces of circular DNA called plasmids. Plasmids are a wonderfully ally for biologists who desire to get bacteria to produce very specific proteins. The plasmids conveniently can be cut, fused with other DNA and then reabsorbed by bacteria. The bacteria easily incorporate the new DNA information into their metabolism. This “recombining” of DNA is called RECOMBINANT DNA.

Extracting a gene from one DNA molecule and inserting it into another requires precise “cutting and pasting.” To carry out this procedure, a piece of DNA containing the gene of interest must be cut out of a chromosome and “pasted” into a bacterial plasmid.

The cutting tools for making recombinant DNA in a test tube are bacterial enzymes called *restriction enzymes*, which were first discovered in the late 1960s. In nature, these enzymes protect bacteria against intruding DNA from other organisms and phages. They work by chopping up foreign DNA, a process called restriction because it restricts foreign DNA from surviving in the cell. Other enzymes chemically modify the cell’s own DNA in a way that protects it from the restriction enzymes.

Most restriction enzymes recognize short nucleotide sequences in DNA molecules and cut at specific points within these recognition sequences. Several hundred restriction enzymes and about a hundred different recognition sequences are known.

## GOALS

In this activity, a make-believe DNA message for the protein insulin is marked on the cell DNA. Your task will be to find an enzyme that cuts the plasmid once (and only once) and the cell DNA as close as possible on both ends of the insulin gene so that the insulin code can be fused into the circle of the plasmid DNA.

To do this you will need to:

1. Determine which restriction enzyme to use to cut your DNA strand.
2. Determine which antibiotic you would use to determine if your finalized recombined DNA was absorbed by the bacteria or not.

## SUMMARY OF PAGES

*White* = instructions

*Pink* = plasmid

*Goldenrod* = cell DNA

*Yellow* = record keeping/answer sheets

*Green* = restriction enzymes

 Ampicillin resistance

 Tetracycline resistance

 Kanamycin resistance

 Origin of Replication

 Insulin protein gene

# E Coli Insulin Factory Answers Bestappsore

**RS Peters**



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