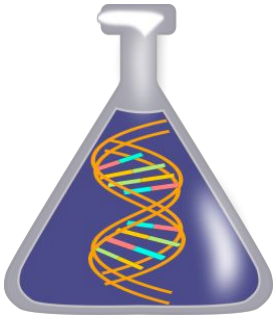

Background Information on Recombinant DNA

As you know, DNA is the material within a cell that determines what an organism looks like and how it functions. DNA does this by coding for proteins.

Today, scientists are able to insert pieces of "foreign" DNA into an organism's DNA (usually a bacteria) so that the organism will express a certain characteristic or produce a certain protein. This moving of DNA pieces between unrelated organisms is called **recombinant DNA technology.**

A **plasmid** is a circular ring of DNA found in some bacteria. It is used in genetic engineering. So how do you "recombine" DNA using this technology? Once you have found the DNA that contains the gene you want, you must isolate or remove this specific DNA section.

Restriction enzymes are special molecules that cut the DNA in specific places so that the section you are looking for can be removed. They are like special scissors. Once the DNA fragment is cut, it needs to be inserted into the vector DNA (the plasmid). You must first isolate the plasmid and then expose the plasmid to the restriction enzyme so that a gap in this circular DNA opens to combine with the new piece of DNA.

The restriction enzymes must be selected carefully so that 1) it cuts the right gene, and 2) it cuts the plasmid but leaves the rest of the plasmid intact!

Now you must "recombine" the plasmid with the DNA fragment coding for the specific characteristic you want. Once the plasmid and the new DNA piece are mixed together they must be joined. **Ligase** is an enzyme that joins the plasmid with the new DNA piece. Ligase acts like tape, binding the pieces together.

The new plasmid is put back into the bacteria. When the bacteria replicates, the new DNA will too.

Procedures:

In this experiment, we will be modeling how genes are inserted into bacteria.

Removing the desired gene from the DNA:

a) Beginning on the top of your DNA strand at the end that indicates "start" (the 5' end) read the bases of the strand until you reach an **A G C T** sequence all in a row in that order. (See example below).

b) Use your pencil to draw a line after the **A.** Do this all the way down that side of your DNA.

Start 5'	**ATGCTCGGCAAGCTTATTGAGGTAGCTGGCTACCGCT**	**3' STOP**
Stop 3'	**TACGAGCCGTTCGAATAACTCCATCGACCGATGGCGA**	**5' START**

c) Now begin reading the DNA on the bottom strand of your DNA ladder. Start reading from the end that indicates "start" and look for an **A G C T** sequence all in a row in that order.
d) As before, draw a line after the **A** in every four-base **A G C T** sequence.
e) One line on the top DNA strand should be two bases away from one line on the bottom DNA strand. Draw through the middle of the DNA strand to connect the two closest lines.
f) Have a teacher check your lines and initial here _____
g) When you are sure they are correct, cut the DNA gene out. You should have two exposed bases and a gene. This is the gene you want to duplicate.
h) Put this DNA aside and move on to the plasmid.

Getting the plasmid ready for insertion of the gene

a) Cut your circular plasmid out so that it looks like a doughnut.
b) Beginning on the outside, start reading clockwise along the plasmid until you find an **A G C T** sequence.
c) Draw a line after the **A** in every **A G C T** sequence. Make sure your line only goes to the middle of the DNA strand.
d) Going in the opposite direction, read counterclockwise along the inside loop of the plasmid, reading until you come
e) across the **A G C T** sequence on the inside DNA strand.
f) Draw a line after the **A** in every **A G C T** sequence. Make sure your line only goes to the middle of the DNA strand.
g) Draw a line to connect your lines, through the middle of the DNA.
h) Get your lines checked _____
i) With your restriction enzyme (scissors), cut along your line.

Creating Recombinant DNA

a) Open the loop and look closely at the two exposed rungs. Compare this DNA with the DNA gene you removed.
b) Match the shapes as well as the bases (**A** goes with **T** and **C** goes with **G**).
c) Take out your ligase (tape) and attach the gene into the plasmid.