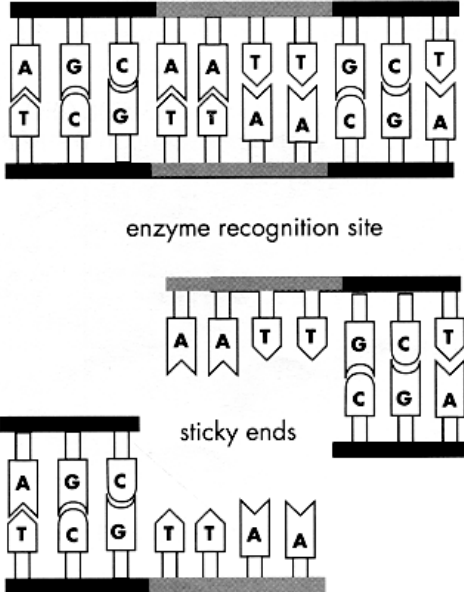


# Activity 6: Recombinant DNA Techniques

## Objective

Students will model the process of using restriction [enzymes](#) and [plasmids](#) to form [recombinant DNA](#).

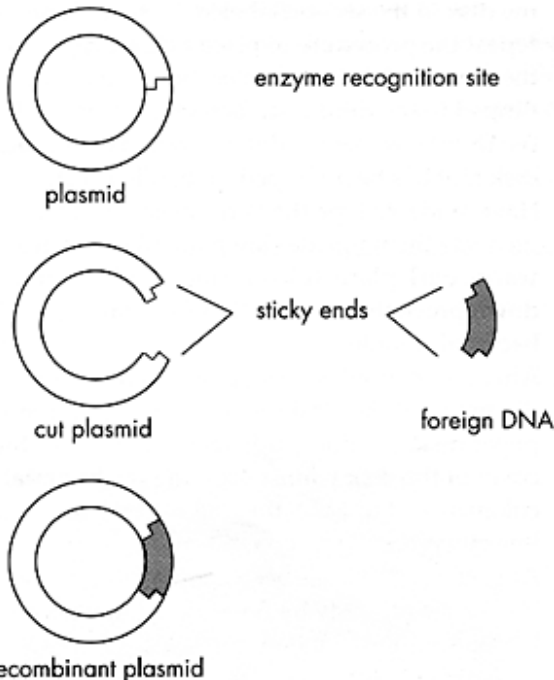


## Background Information

The major tools of recombinant DNA technology are bacterial enzymes called [restriction enzymes](#). Each enzyme recognizes a short, specific nucleotide sequence in DNA molecules, and cuts the backbones of the molecules at that sequence. The result is a set of double-stranded DNA fragments with single-stranded ends, called "sticky ends." Sticky ends are not really sticky; however, the bases on the sticky ends form base pairs with the complementary bases on other DNA molecules. Thus, the sticky ends of DNA fragments can be used to join DNA pieces originating from different sources.

## Cutting DNA strands

In order to be useful, the recombinant DNA molecules have to be made to replicate and function genetically within a cell. One method for doing this is to use plasmid DNA from bacteria. Small DNA fragments can be inserted into the plasmids, which are then introduced into bacterial cells. As the bacteria reproduce, so do the recombinant plasmids. The result is a bacterial colony in which the foreign gene has been cloned.



## Creating recombinant plasmids

## Materials for each group:

- Handout: [Plasmid Base Sequence Strips](#)
- Handout: [DNA Base Sequence Strips](#)
- Handout: [Restriction Enzyme Sequence Cards](#)
- Scissors
- Tape
- Pencil
- Paper

## Preparation

Duplicate Handouts [Plasmid Base Sequence Strips](#), [DNA Base Sequence Strips](#), and [Restriction Enzyme Sequence Cards](#) to distribute to students. You may want to duplicate each Handout on a different color page.

## Recombinant DNA Techniques: Instructions

1. Have students cut out the plasmid strips along the dotted lines. They should then connect the strips and tape them together to form a single long strip. Remind students that the letters should all be in the same direction when the strips are taped. The two ends of the strip should then be taped together with the genetic code facing out to form a circular plasmid.
2. Have students cut out the DNA base sequence strips, and tape them together to form one long strip. The pieces must be taped together in the order indicated at the bottom of each strip.
3. Next, have students cut out the restriction enzyme cards. Point out that the enzyme cards illustrate a short DNA sequence that shows the sequence that each particular enzyme cuts.
4. Have students compare the sequence of base pairs on an enzyme card with the sequences of the plasmid base pairs. If they find the same sequence of pairs on both the enzyme card and the plasmid strip, they should mark the location on the plasmid with a pencil, and write the enzyme number in the marked area. They should do this for each enzyme card. You may wish to point out that some enzyme sequences may not have a corresponding sequence on the plasmid, and that some enzyme sequences may have more than one corresponding sequence on the plasmid.
5. Once students have identified all corresponding enzyme sequences on the plasmid, have them identify those enzymes which cut the plasmid **once and only once**. They should discard any enzymes that cut the plasmid in the shaded plasmid replication sequence. They should record their findings on a separate piece of paper.
6. Next, have students compare the enzymes they listed against the cell DNA strip. Ask them to find any enzymes that will make two cuts in the DNA, one above the shaded insulin gene sequence and one below the shaded insulin gene sequence. Have students mark the areas on the DNA strip that each enzyme will cut.
7. After students have compared each enzyme with the DNA strip, have them select one enzyme to use to make the cuts. Point out that the goal is to **cut the DNA strand as closely as possible to the insulin gene sequence without cutting into the gene sequence**. Have the students make cuts on both the plasmid and the DNA strips. They should make the cuts in the staggered fashion indicated by the black line on the enzyme card.
8. Have students tape the sticky ends of the plasmid to the sticky ends of the insulin gene to create their recombinant DNA.

## Discussion Questions

1. Why was it important to find an enzyme that would cut the plasmid at only one site? What could happen if the plasmid were cut at more than one site?
2. Why was it important to discard any enzymes that cut the plasmid at the replication site?
3. Why might it be important to cut the DNA strand as closely to the desired gene as possible?
4. In this activity, you incorporated an insulin gene into the plasmid. How will the new plasmid DNA be used to produce insulin?