Background Information:

PART I: Bacteria are the most common organisms modified by genetic engineers due to the simple structures of bacteria cells compared to those of eukaryotic cells. Engineers are able to add genes to bacteria using structures called recombinant plasmids, which enable the bacteria to produce any number of different desired beneficial proteins.

Plasmids are circular pieces of DNA; when placed near bacteria, the plasmid is absorbed and incorporated into the bacterial cell. Once inside the bacteria, the plasmid is treated the same as the bacteria’s original DNA. This means that the bacteria will use this new DNA from the plasmid to create proteins, and the plasmid will be replicated when the cell divides.

Part II: Genetically engineering a bacteria’s DNA is one of the most simple and common types of genetic manipulation used today. Insulin for diabetics is made in this manner. The steps are simple: First, a desired gene, such as the gene which produces insulin, is isolated and removed. Genes are removed using a restriction enzyme. These special enzymes search for specific nucleotide sequences in the DNA, called a recognition site, where it "cuts" the DNA by breaking specific bonds within the double helix. The bonds break in a staggered manner, creating “sticky ends.” Second, the same restriction enzyme is used to cut the DNA found in the plasmid, creating matching “sticky ends.” Third, the isolated gene and the plasmid a placed together and the plasmid takes up the foreign gene, smoothly adding it to its own DNA, creating a longer loop in its plasmid. It is now considered to be recombinant DNA. The fourth step is to get the bacteria to reabsorb the recombinant plasmid. The fifth, and final stage, is to place the bacteria in an ideal environment so that they reproduce quickly. Remember, prokaryotes reproduce asexually, which means that each new bacteria produced is a clone.

Key Vocabulary/Definitions:

DNA: Genetic information contained in the cells of living organisms; includes the nitrogen bases Cytosine, Guanine, Adenine, & Thymine.
GENE: A specific DNA sequence in an organism that contains information for a specific trait
GENOME: An entire set of EVERY gene contained within an organism/species.
LIGASE: An enzyme that performs the job of reattaching DNA bases to nearby DNA bases. It attaches CGAT’s to other CGAT’s.
MODEL: (noun) A representation of something for imitation, comparison or analysis, sometimes on a different scale. (verb) To make something to help learn about something else that cannot be directly observed or experimented on.
PLASMID: The circular DNA structure used by bacteria (a second set of DNA found in bacteria)
PROKARYOTE: A cell without a nucleus or any membrane-bound organelles. Bacteria = prokaryote.
RECOMBINANT DNA: DNA in which a section has been removed and replaced (recombined) with a new sequence of DNA (CGAT’s).
RESTRICTION ENZYME: An enzyme that “cuts” DNA at a specific nucleotide sequence. Ex. GAATTC it will cut between the G and the A.
TRANSFORMATION: A change in form, appearance, nature, or character. Bacterial Transformation occurs when a desired gene is recombined with an existing bacterial plasmid, thus transforming the bacteria’s genome.
Materials per Group (Groups of 2):

<table>
<thead>
<tr>
<th>Item</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 piece of Plasmid DNA</td>
<td>1</td>
</tr>
<tr>
<td>1 Insulin Gene</td>
<td>1</td>
</tr>
<tr>
<td>Scissors</td>
<td>1</td>
</tr>
<tr>
<td>Tape</td>
<td>1</td>
</tr>
<tr>
<td>1 or 2 highlighter(s)</td>
<td>1</td>
</tr>
<tr>
<td>1 pen/pencil</td>
<td>1</td>
</tr>
</tbody>
</table>

Procedure:

STEP 1: Inventory your group’s materials. Be sure you know which set of DNA is the Insulin gene and which set of DNA is the plasmid.

STEP 2: Tape the ends of the plasmid DNA together to form a circle (See Fig 1) so that the DNA segments are on the OUTSIDE of your plasmid. This is your initial plasmid which will be recombined with your insulin gene.

**YOU SHOULD NOW HAVE A FLAT PIECE OF DNA (THE INSULINE GENE) & A CIRCULAR PIECE OF DNA (THE PLASMID).**

STEP 3: Find the recognition site for the restriction enzyme to cut the DNA apart for both the plasmid and the insulin gene. The restriction enzyme is looking for a specific DNA sequence along which it will cut the DNA apart. The sequence is **5’ to 3’ AAGCTT**. Take your highlighters, and highlight every sequence that follows that DNA pattern on both the plasmid and the insulin gene (see Fig 2).

STEP 4: Take the pen/pencil and draw a dotted line between the two A’s, between the DNA and down the two A’s of the complementary strand (See Fig. 2).

STEP 5: Using the scissors (which represent the restriction enzyme), cut the DNA along the marked/dashed lines at every site on both the plasmid and the insulin gene. If cut correctly, you should have staggered cuts forming “sticky ends.” See Fig. 3.

STEP 6: Using another enzyme called ligase (the tape), rebuild the plasmid with the newly inserted insulin gene inside it. This will make the original plasmid quite a bit larger. Be sure to reform the plasmid loop (which is what the enzymes would actually do) with more ligase (tape). See Fig. 4.

STEP 7: Take your finished, circular Plasmid and attach it to your worksheet. Answer all of the Post-Lab Questions.
Bacteria Transformation
Applying Genetic Engineering

PRE-LAB:

1. What does the tape represent in the model transformation?

2. What do the scissors represent in the model transformation?

3. What is a bacterial transformation?

4. What are sticky ends?

5. What are restriction enzymes?

6. What are Ligase (enzymes)?

7. What is a plasmid?

8. What is a Recombinant Plasmid or Recombinant DNA?

DATA:

[Staple your Recombinant model Here]
POST-LAB/Assessment Questions:
1. Using the word choices provided in the boxes, fill in the numbered boxes with the steps of bacteria transformation and the lettered lines with the name of the structure next to it.

<table>
<thead>
<tr>
<th>Word Choices for Letters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Desired Gene</td>
</tr>
<tr>
<td>Plasmid</td>
</tr>
<tr>
<td>Recombinant DNA</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Word Choices for Numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transformed Bacteria</td>
</tr>
<tr>
<td>Plasmid cut w/Restriction Enzyme</td>
</tr>
<tr>
<td>“Sticky Ends” joined by Ligase to form Recombinant Plasmid</td>
</tr>
</tbody>
</table>

2. Why are sticky ends important?

3. Describe the role of Restriction Enzymes (RE) in the process of transformation.

4. Why are Ligase (enzymes) important?

5. Why is a plasmid important?

6. Why do scientists conduct bacterial transformations with the insulin gene? (What is the point)?

7. How might a bacterial transformation be similar or different from a transformation used on a mammal, such as a human?
8. Write the missing steps of bacterial transformation in order of occurrence. Use the background information from the lab for help.

**Step 1:** Bacteria encounters foreign DNA (or genes) from the environment.

**Final Step:** Foreign DNA (genes) is/are duplicated with bacterial DNA after the next round of Cellular division (reproduction).

9. The Restriction Enzyme, BamH1, cuts DNA between the two G’s when it encounters the base sequence.

\[
\begin{align*}
\text{GGATCC} \\
\text{CCTAGG}
\end{align*}
\]

Using a highlighter, mark all of the recognition sites that the following segment of DNA has that could be cut by the restriction enzyme BamH1. Be sure to highlight both complementary segments. Take a pen or pencil, and draw a dotted line to show where the restriction enzyme would cut the DNA apart.

\[
\begin{align*}
\text{TACGGATCCTAGGGCATAGCTCAGGATCCCGTCAATGGGGGATCCC} \\
\text{ATGCCTAGGATCCCGTATCGAGTCCTAGGGCAGTTACCCCTAGGG}
\end{align*}
\]

10. Do you think recombinant organisms could also pose a threat to a population or ecosystem? Explain.

11. What would be some specific genes that the scientists at CAY Station needed to isolate so that they could genetically engineer the Quaddies?