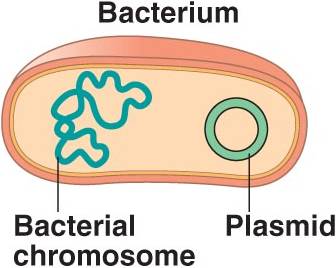
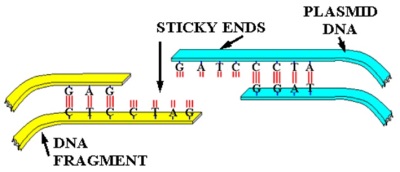
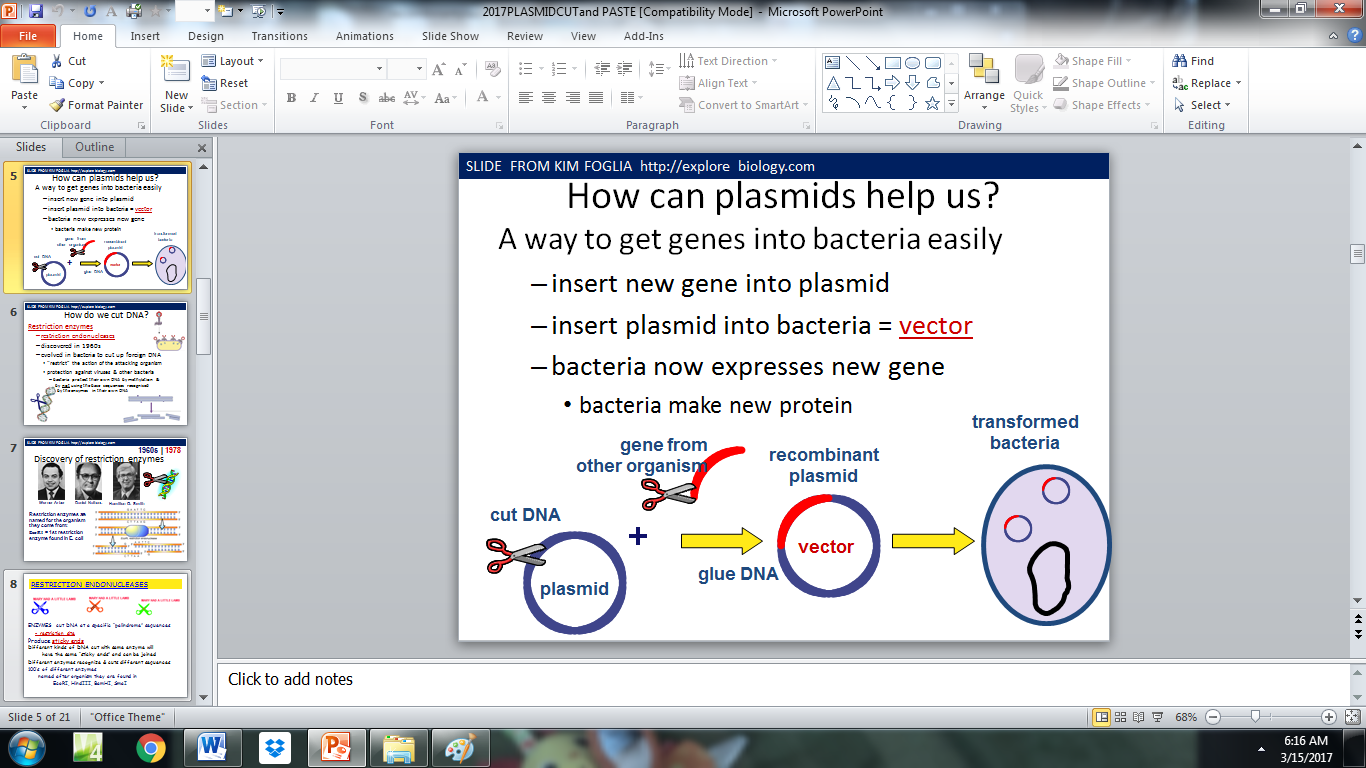
NAME \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

RECOMBINANT PLASMID ACTIVITY  
In this activity you will be making a recombinant bacterial plasmid that contains the human insulin gene.

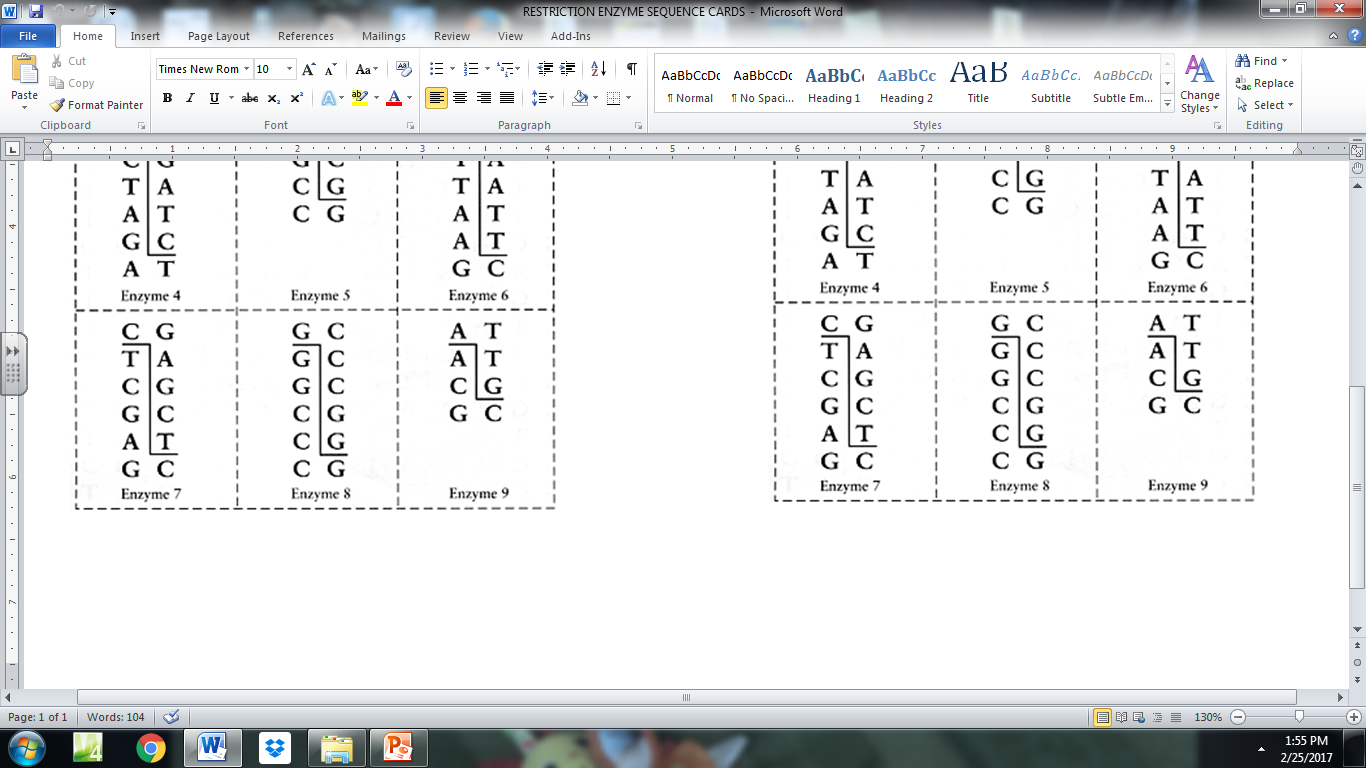
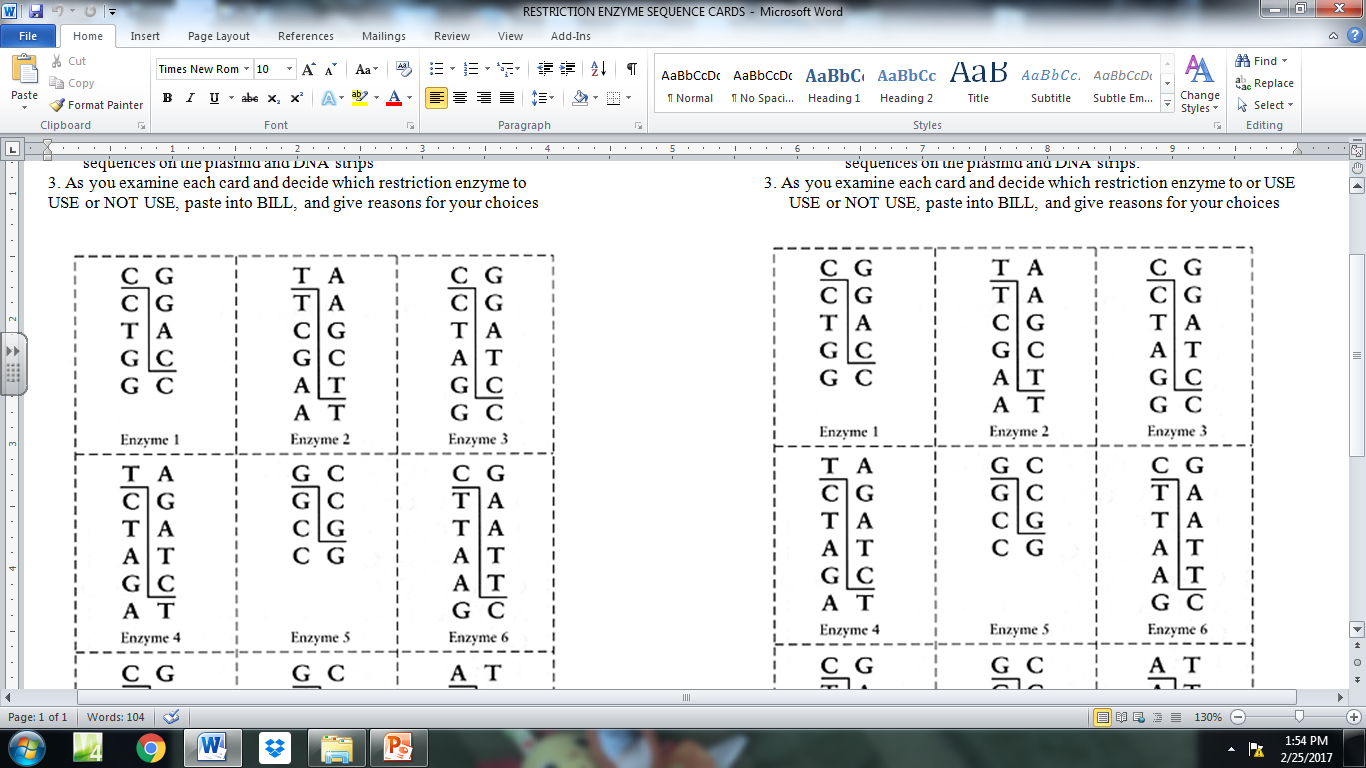
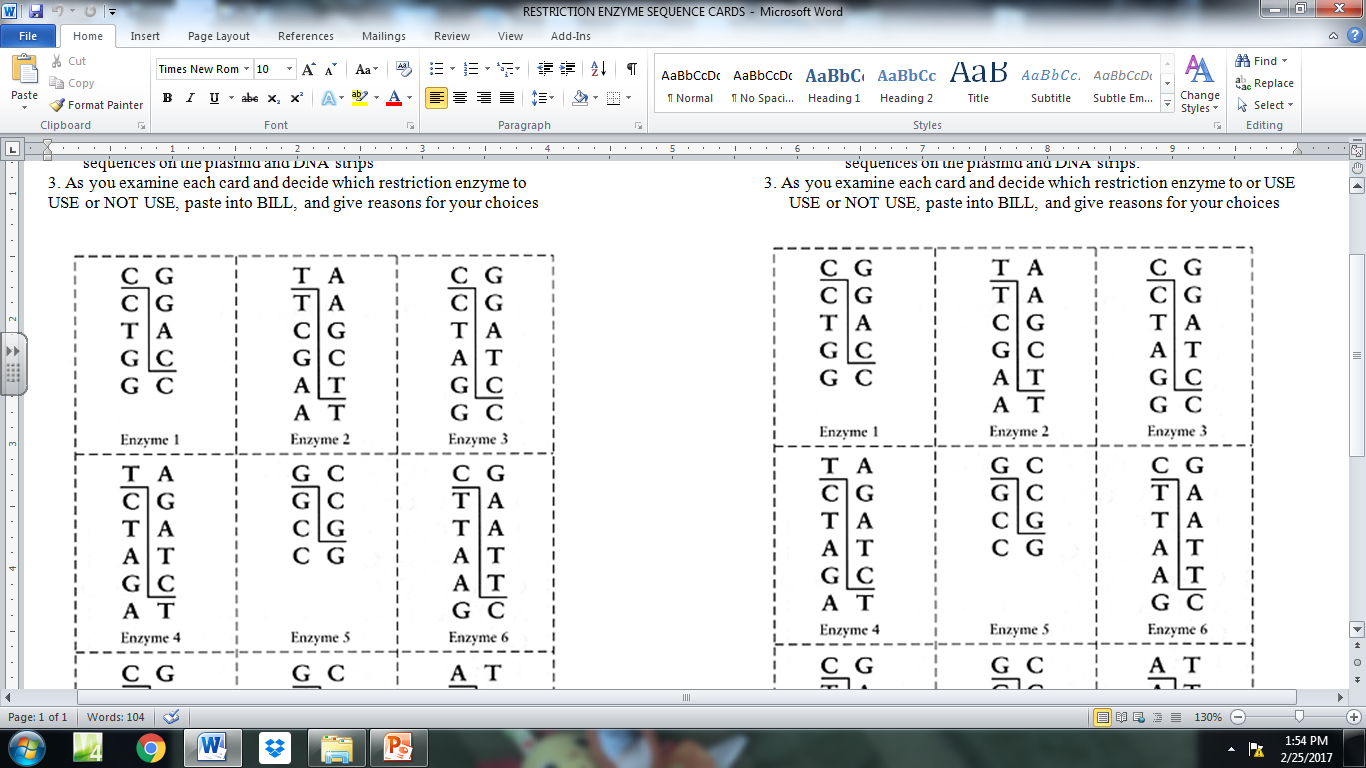
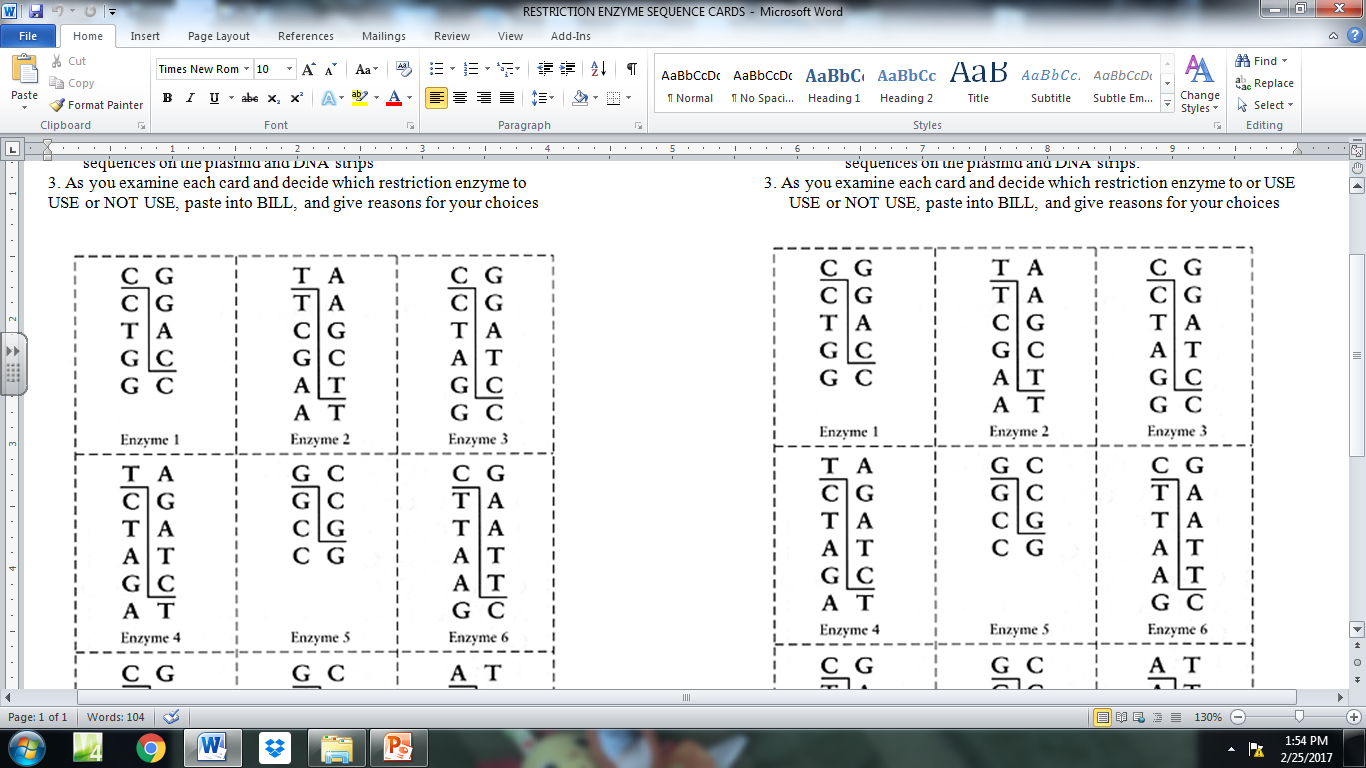
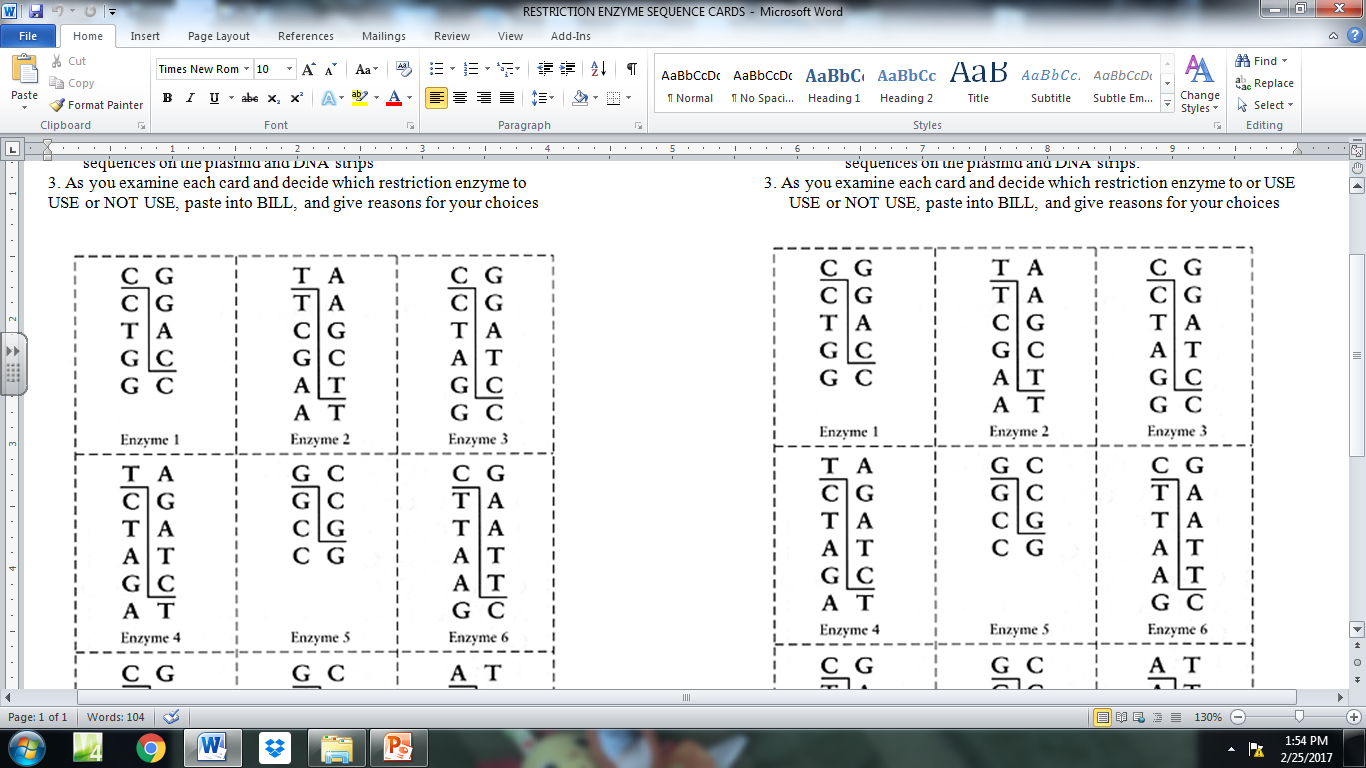
GENETICALLY MODIFIED ORGANISMS (GMO’s)  
• contain DNA from combined from different organisms using restriction enzymes  
• bacterial plasmids can act as vectors to move genes from one organism into another  
 EX: Bacteria that produce human products like insulin, human growth hormone, etc.  
 Golden rice” has vitamin A gene added (increased nutrition)  
 BT corn produces a bacterial toxin that kills corn borer (caterpillar pest of corn)  
 Strawberries with antifreeze gene from fish (prevents frost damage)

PLASMIDS  
  
• single loop of double stranded DNA  
  
• Extra-chromosomal  
  
• self-replicating (have origin of replication)  
  
• can be passed from one bacterium to another via transformation  
  
• often contain genes for antibiotic resistance and/or fertility (ability to make sex pili)  
• can be used to create recombinant DNA  
• can be used as vectors to move recombinant DNA into another organism  
  
RESTRICTION ENDONUCLEASE ENZYMES (Restriction enzymes)  
• Naturally found in bacteria  
• used for protection against foreign DNA from invading viruses  
• recognize specific sequences (restriction sites) in DNA and make a cut  
• many restriction sites are “palindromes”  
• many restriction enzymes cut in zig-zag pattern resulting in “sticky ends”  
 - enzymes that cut with “blunt ends” can’t be joined/nothing to hold pieces together  
• any DNA with same “sticky ends” can be joined together  
• Used to create recombinant DNA

MAKING A RECOMBINANT PLASMID CONTAINING A HUMAN GENE (insulin):  
• SAME restriction enzyme must be used on BOTH plasmid and human  
 DNA so that they have the same “sticky ends” which can be joined  
• Restriction enzyme MUST NOT cut ORIGIN OF REPLICATION   
 site on plasmid  
 - Plasmid needs this in order to copy itself (along with human gene)  
• Restriction enzyme should cut plasmid in ONLY ONE location  
 - Multiple cuts produce some plasmid fragments without the ORIGIN OF REPLICATION site  
 - If these combine with human gene, plasmid won’t be able to replicate when placed in bacteria  
• Restriction enzyme must cut human DNA TWICE (once on either side of and close to desired gene)  
• Restriction enzyme MUST NOT cut the human insulin gene  
 - This would make the gene non-functional

PROBLEM WITH EXPRESSING HUMAN INSULIN DNA (and other genes) IN A BACTERIAL CELL  
• Human DNA contains introns that must be edited out of mRNA before translation  
• Prokaryotes don’t have editing enzymes (no introns) Can’t make human proteins if human gene is used directly  
• SOLUTION = “borrow” reverse transcription enzyme used by retroviruses to make DNA from RNA  
• Reverse transcriptase can be used to make DNA from edited human mRNA for insulin  
• This cDNA (complementary DNA) has no introns and can be inserted and expressed in bacterial cells  
• Violates Central Dogma = DNA→ RNA → proteins by passing info from RNA → DNA

RESTRICTION ENDONUCLEASE ENZYMES  
COLOR CODE THE RESTRICTION SITE PATTERN SHOWN BELOW and use the SAME COLOR to mark the corresponding sequence on your HUMAN DNA and PLASMID DNA strands.   
Mark the number and location of cuts the restriction enzymes below cut plasmid/human DNA.  
Circle the restriction enzyme you chose to create your recombinant plasmid



What would happen if you chose an enzyme that cut the plasmid in more than one place? BE SPECIFIC.

Why is it important to use the SAME ENZYME to cut the plasmid DNA AND human DNA?

Why was it important to NOT use a restriction enzyme that cut the plasmid in the ORIGIN OF REPLICATION site?

Is ENZYME 9 a good choice to use to make your recombinant plasmid? EXPLAIN YOUR ANSWER.

Is Enzyme 6 a good choice to use to make your recombinant plasmid? EXPLAIN YOUR ANSWER.

Which restriction enzyme did you choose to create your plasmid containing the human insulin gene sequence in this activity? What criteria did you use to decide which was the “best” enzyme to use?

Where are RESTRICTION ENZYMES found in nature? What is their function?

There are some RESTRICTION ENZYMES that make straight NOT “zig zag” cuts on DNA. Why would restriction enzymes that create “blunt” ends not be as useful in creating recombinant plasmids as those that create “sticky ends”?

What did each of the following represent in this simulation activity,?  
 SCISSORS \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_  
 TAPE \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_  
  
Once the plasmid is created bacterial cells must pick up the recombinant plasmid DNA.  
This is an example of which of the three types of HORIZONTAL GENE TRANSFER you learned about?  
  
  
THINK ABOUT IT:  
In real life there is another step involved before human DNA can be used in recombinant plasmids.  
Explain what happens to eukaryotic mRNA before it leaves the nucleus and is translated into a protein.

How is this different from what happens in bacteria?

What problem does this create for scientists using human genes that have not been processed to create recombinant plasmids to be used to make human products like insulin in bacteria?

What “trick” have scientists borrowed from retroviruses that gets around this problem?

What is the CENTRAL DOGMA OF BIOLOGY? How does this “trick” violate the Central Dogma?

What is this reversely transcribed DNA called? How does it compare to the original DNA strand?

Modified from: <http://www.biologycorner.com/worksheets/DNA_analysis.html>