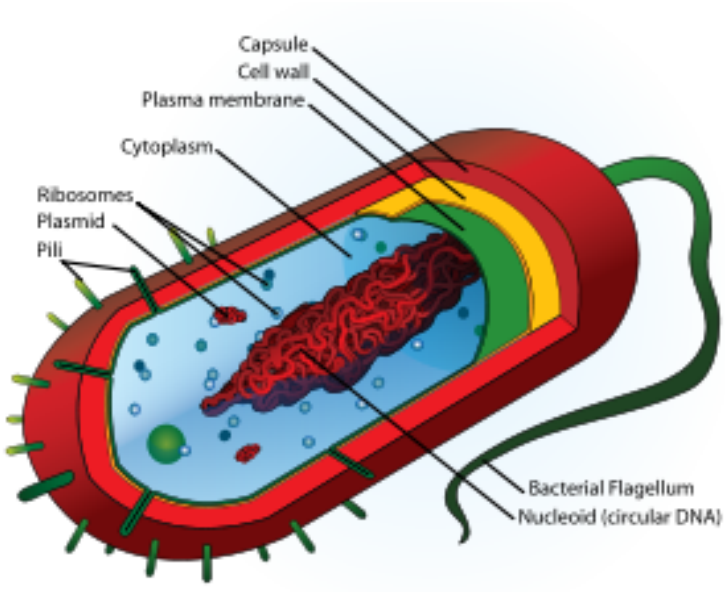
Restoring the American Chestnut

Modeling Bacterial Transformation:

Biotechnology is a process which uses living organisms or parts of organisms to produce goods and solve problems. Recombinant DNA technology genetically engineers organisms by recombining fragments of DNA from different organisms, and makes it possible to take virtually any gene and express it in any other living organism. The benefit of this type of technology has not only led to new and improved medicines, such as the production of human insulin by bacteria, but also agriculture processes such as genetic manipulation to allow plant strains to be disease resistant. An example of current research using this technology is the work being done to restore the American chestnut tree. As seen in previous lessons, the tree was devastated by a fungal disease. Researchers are trying to locate the genes in Chinese chestnut trees which are resistant to the fungus so that they can genetically engineer American Chestnut trees to contain these genes for resistance.

Bacteria are ideal organisms for recombinant DNA technology for a number of reasons. Their genome is much smaller than those of eukaryotes and the replicate asexually very quickly. This activity will model the steps used in transformation of a bacterial cell. First, we need to review the structure of bacteria.



Some bacteria, not all, are surrounded by a capsule which provides a layer of protection, followed by a cell wall and cell membrane. Bacteria may have flagella, one or many, to aid with movement. The pili on the surface provide a means for the bacteria to stick to different surfaces, and in some cases, provide a means of DNA transfer called conjugation. Inside the bacteria there are ribosomes for the production of proteins during translation as well as 2 forms of DNA. The larger mass of DNA is called the nucleoid; since it is not enclosed in a membrane it cannot be called a nucleus. The second type of DNA is a much smaller circular piece called a plasmid. Plasmids occur naturally in bacteria, but may also be artificially produced and inserted into bacteria. They are copied along with the nucleiod DNA during cell division so that each new bacterial cell produced receives both types of DNA.

Before you can model the process of transformation, you must first build a model of the bacterial that will be transformed and the artificial plasmid that you will be inserting.

Restoring the American Chestnut

Materials:

1 empty water bottle – this will be the bacterial cell wall
2 ft. yarn – this is the nucleoid DNA
small adhesive dots – these are the ribosomes
toothpick –for pili ,
plastic lacing – for flagella
1 sandwich size Ziploc bag – for the cell membrane
1 pair scissors
1 sharpie
3 different colors of Velcro cut in 4 in. strips

Stick the adhesive dots at various places inside of the Ziploc bag. Place the yarn inside the bag as well. Punch a small hole in the bottom of the water bottle with the scissors. Tie a piece of plastic lacing to a toothpick and insert the toothpick into the hole you created (the toothpick in this case is just to keep your flagellum in place). Put the Ziploc bag inside of the water bottle and blow air into the bag to push it against the sides of the bottle. Poke several toothpicks into the sides of the bottle in random areas for the pili. Use the sharpie to draw negative signs (-) on the surface of the bottle. This is due to the fact that the plasma membrane has a slightly negative charge. (You are drawing the symbols on the cell wall just to prevent making large holes in your bottle.) You have now created your model of a bacteria – lets build our plasmid.

List your 3 colors of Velcro:

1._____ = bacterial plasmid DNA

2._____= DNA for antibiotic resistance
3._____=DNA from a different organism to be expressed.

Plasmids used in transformation experiments will usually also contain a gene giving resistance to an antibiotic. Cells able to grow on media containing this antibiotic will have been transformed by the plasmid, as cells lacking the plasmid will be killed. This provides a phenotype (which can be easily seen) to tell that the procedure has worked. The DNA from the other organism may be a gene for producing human insulin, a gene for resistance to a disease, or even a gene to make the bacteria glow under black light. Remember, it is possible to take virtually any gene and express it in any other living organism. Also, in order to get the DNA from all of these sources to stick together, the DNA had to be cut with restriction enzymes where sticky ends were produced. For example, these two "sticky" ends are compatible:

5'-ATCTGACT GATGCGTATGCT-3'
3'-TAGACTGACTACG CATACGA-5'

If the DNA sources are cut with the same enzyme, they will automatically cut at the same nitrogen base sequence, leaving ends that can be joined together by an enzyme called ligase. Once the DNA in a plasmid has been joined, all of the DNA is inserted together and passed on to new cells together during mitosis.
Take your 2 Velcro strips and connect them so that they make a circle. We are now ready to transform, or put our created plasmid into our bacteria.

Restoring the American Chestnut

Transformation:

Materials:
Labels: Ice, Hot Water, Nutrient broth
1 bottle of white-out

1. The plasmid and the bacteria are placed in a tube containing $CaCl_2$ (calcium chloride) and then placed on ice for about 5 minutes. The ice acts to slow down the movement of the molecules that make up the cell wall and cell membrane. When they slow down, they move closer together, leaving small holes where the plasmid DNA could get through.

   Place the "Ice" label on the desk with the bacteria and plasmid, remove the cap from the bottle to represent the holes created.

   Recall that we drew negative signs on the outside of the bottle to represent the negative charge of the plasma membrane. DNA also has a negative charge. What happens between 2 like charges? The calcium chloride turns into Ca^+ and Cl^- in water. The Ca^+ will attach to the negative charges on the cell membrane, making it neutral and therefore easier for the DNA to get through.

   Take the white-out and cover up all of the negative charge symbols you had drawn on the water bottle.

2. The second step of transformation is called "heat shock." The bacteria and plasmid are put into a hot water bath (about 42 ^{0}C) for 50 seconds. Inside of the cell is very cold at this point; placing the cell and plasmid in this hot water creates a current and dramatically pushes the DNA into the cell.

   Watch as your teacher demonstrates how temperature difference can cause movement into an area. When the demonstration is complete, replace the "Ice" label with the "Hot Water" label and push your plasmid into the Ziploc bag which is inside of the bottle.

3. The final step of transformation is an incubation step. The bacteria is placed back into ice for 2 minutes. After this time the bacteria are given a nutrient broth. The ice is to help return the bacteria to a temperature necessary for survival, the broth acts as food for the bacteria. They have been put under a large amount of stress during this process and it helps them to recover.

   Replace the "Hot Water" label with the "Ice" label and then add the "Nutrient broth" label. Reseal the cap to the bottle.

The bacteria now contains the transformed plasmid and will be put in conditions where it can grow, reproduce, and make the protein from the gene of foreign DNA that was inserted. Again, they will be grown in a medium that contains antibiotics so that only the transformed bacteria will survive.

Concept check:

What are plasmids?
What is the function of restriction enzymes
How can transformed bacteria that carry genes of interest be identified and isolated from the non-transformed bacteria?
Describe the steps involved in transforming a bacterial cell.

Restoring the American Chestnut

Now that we know that we can alter cells, let's look at how this process can be applied. One application that has been discussed in earlier lessons is producing American Chestnut trees that have genes from Chinese Chestnut inserted so that they will be resistant to the Chestnut blight fungus. Another possibility is the production of engineered bananas that are resistant to Panama disease.

Circle one of the following topics and use the internet to research this area. Prepare to discuss your findings with the class:

Medical:	production of human insulin
	Production of vaccines
	Milk of transgenic pig which is used to treat hemophilia
	Production of human growth hormone to treat dwarfism
Agricultural:	Plants which have resistance to pesticides
	Plants which have resistance to herbicides
	Plants with enhanced nutrition
Environmental:	Bacteria which can clean up beaches after oil spills
	Bacteria which can remove pollutants from the soil

Be sure to describe what organisms are being combined to create the GMO. Tell how it is useful to humans and explain any controversies that may surround the production of this organism. While other groups are sharing their information, listen for the similarities and differences between your topic and theirs.

Notes:	Other group's similarities:	Differences: