Cut, Copy, and Mutate: *Eco*RI and its function in Genetic Engineering

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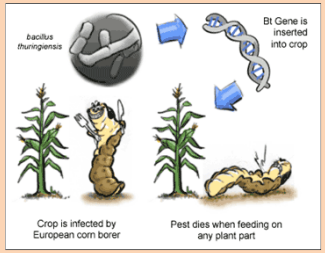
-Abstract-
While farmers plant insect resistant corn, millions with diabetes inject themselves with the hormone, insulin. Despite the differences between these practices, they have a common root: genetic engineering. Genetic engineering allows genes of interest to be moved from one species to another to create a desired protein or trait. This is accomplished through using restriction enzymes to cut DNA at a specific recognized sequence. Bacteria naturally use restriction enzymes to destroy viral DNA. One of these restriction enzymes, EcoR1 endonuclease, is commonly used to genetically engineer insulin. In the early 1900s, insulin extraction and purification from a cow's pancreas was a time consuming and expensive process that yielded only a small amount of the hormone. More efficient production of insulin occurred in the 1950s when EcoR1 was used to cut the insulin gene from the human genome. Insertion of the gene into the genome of the bacteria, *Escherichia coli* did not disrupt bacterial division but did reprogram the bacteria to produce human insulin which could be collected for use. To investigate the structure of EcoR1, the Milwaukee Academy of Science SMART (Students Modeling A Research Topic) Team used 3D printing technology to design a model of the protein. EcoR1 is a homodimer composed of two polypeptide chains. Amino acids Asp91, Glu111, and Lys113 bind to the DNA sequence, GAATTC and cut between the guanine and adenine, allowing gene insertion. Successfully engineered bacteria will now be able to use the inserted DNA sequence to create the desired protein. Using the protein EcoR1, limitless gene combinations such as insect resistant plants can be achieved.

Bt Corn

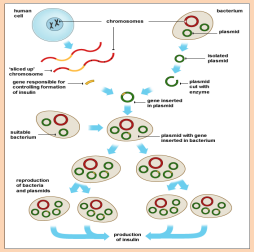
Corn has long been both a major agricultural product for humans and a target for many pests. Bt corn was created by inserting a gene from a microorganism called *Bacillus thuringiensis* into corn. When *Lepidoptera* (butterflies and moths) larva eat the corn, it causes the formation of pores in their digestive system. The pores allow enteric bacteria such as *E. coli* to enter the hemocoel and multiply and cause sepsis to happen, thereby killing the larva. Bt corn therefore reduces both the need for pesticides and the loss of crop yield due to insect consumption.



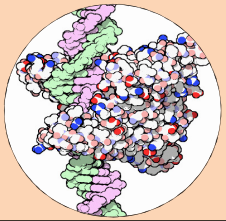
http://oregonstate.edu/orb/terms

Insulin

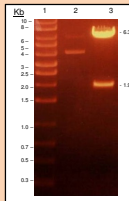
Insulin is a hormone that is used to control glucose concentration in the bloodstream. When a person eats a meal, the blood sugar level begins to rise sharply. The pancreas responds by releasing insulin, which triggers the absorption of glucose into body tissues and the liver for storage. Individuals with type 1 diabetes are either unable to produce insulin and must regulate their blood sugar by injecting themselves with insulin. This insulin used to be derived from the pancreases of cows, but is now produced by genetically modified bacteria.



http://www.bbc.co.uk/bitesize/



EcoRI bound to its restriction site on a DNA strand.
http://10.2210/rcsb_pdb/mom_2000_8



Lane 1. 1 kb Ladder (Axygen)

Lane 2. pEX18Tc/pJJJ undigested plasmid

Lane 3. pEX18TC/pJJJ *Eco*RI digested

Agarose Gel Electrophoresis after Digestion of a Plasmid with the Restriction Endonuclease *Eco*RI.

A 1.9 kb fragment which had two EcoRI sites flanking it was cut out from the vector after digestion with EcoRI for 2 hours at 37 ℃ with appropriate buffer. After digestion, DNA strands are compared to DNA ladder to confirm if EcoRI digested the plasmid DNA. First lane shows DNA ladder with its respective sizes, the second lane is the supercoiled DNA sequence as a whole, and the third is the potential two strands of DNA we expected cut by EcoR1.

Genetic Engineering

Genetic engineering is the process by which scientists take a gene from one organism and insert it into another organism's genome to make a new trait. The new organism is therefore a genetic hybrid known as a genetically modified organism (GMO). The first GMO was a bacteria created in 1973, but since many other GMOs have been created using special proteins called restriction enzymes.

Restriction Enzymes

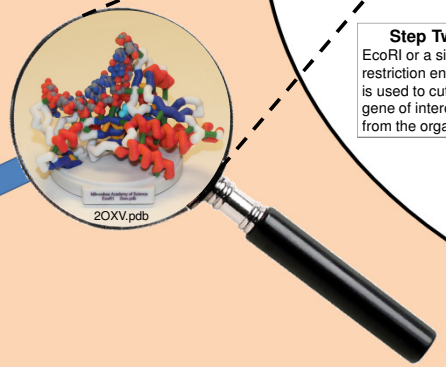
Restriction endonucleases are enzymes that cut DNA at specific sites called restriction sites. Restriction enzymes can be found in bacteria and archaea as a protection against bacteriophage DNA. These enzymes differ in the ways by the way they cut each strand. In our protein model (*Eco*R1), restriction enzymes search for and cut the palindromic pattern.

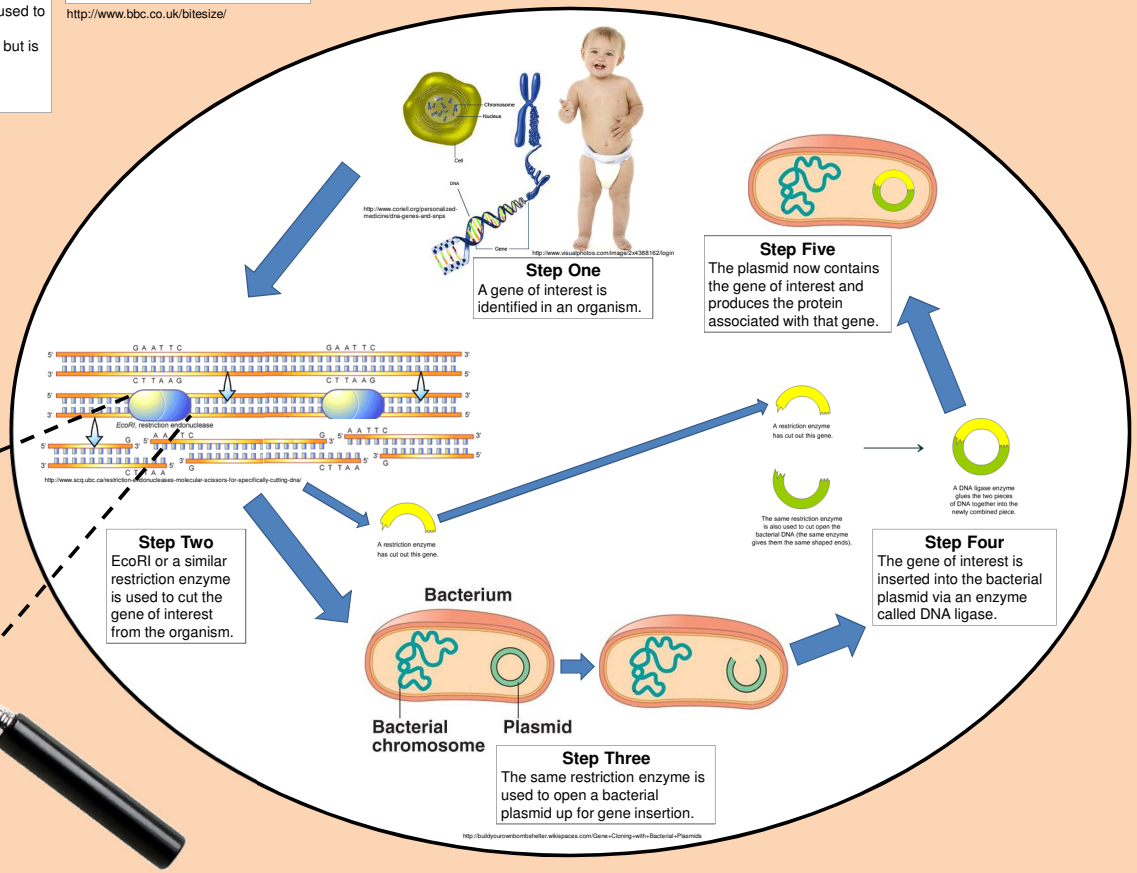
G|AATTC
CTTAA|G

These enzymes cut between guanine and adenine and produce two overhanging ends. These overhanging ends, or sticky ends, are able to join with other similar sticky ends.

EcoRI

*Eco*R1 is a endonuclease enzyme that is derived from the bacteria *Escherichia coli*. *Eco*R1 is a 277 amino acid homodimer, which is a macromolecule with a structure from formed two of the same protein chains together. This enzyme is used as a restriction enzyme. This enzyme has 3 specific amino acids that cut DNA strands at a specific recognition site. The amino acids, Glu111, Lys113 and Asp91, sever the bonds in the sequence, GAATTC. This enzyme cuts between the guanine and the first adenine nucleotides. When cut, *Eco*R1 leaves behind a "sticky" end on both the 3' and 5'. These sites can be used to join another DNA strand cut with *Eco*RI.





Examples of genetically modified organisms (GMOs)

Enviropig: Pig that's been genetically altered to better digest and process phosphorus, a common pollutant in pig feces.

Pollution-fighting plants: Poplar trees that can clean up contamination sites by absorbing groundwater pollutants through their roots. The plants then break the pollutants down into harmless products that are incorporated into their roots, stems and leaves or released into the air.

Web-spinning goats: Goat that produces spider web protein in its milk.

Fast-growing salmon: AquaBounty's genetically modified salmon grows twice as fast as the conventional variety.

Flavr Savr tomato: First commercially grown genetically engineered food to be granted a license for human consumption.

Less-flatulent cows: A cow that produces less methane, a harmful greenhouse gas.

Medicinal eggs: British scientists have created a breed of genetically modified hens that produce cancer-fighting medicines in their eggs.

Super carbon-capturing plants: Genetically engineered plants and trees that are optimized for capturing this excess carbon.

Grapple: The grapple is a relatively new fruit which is a genetic cross between an apple and a grape. It produces plenty of vitamin C for 3rd world countries.

Graisin: A variety of raisin which has been modified to grow to enormous proportions.

Dolion: A cross between a lion and a dog.

Fern Spider: The spider is a cross between a common Italian Wolf spider (Lycosa tarantula) and the ponga fern (Cyathea dealbata) to study the effects of camouflage on survival rate.

Cork Rubber Tree: Combines the porousness of cork with the permanence and flavorlessness of rubber to produce an ideal wine cork.

Summary

- Both synthetic insulin and Bt corn are produced through the technique known as genetic engineering.
- Genetic engineering is the process of transferring a gene from one organism to another organism to create a genome and combination of traits that would otherwise be impossible.
- Genetic engineering relies upon restriction enzymes to cut DNA at specific locations to both remove genes of interest and open chromosomes or plasmids for gene insertion.
- EcoRI is a homodimer restriction enzyme that binds to the palindromic nucleotide sequence GAATTC and cuts between the guanine and adenine.
- With the aid of restriction enzymes such as EcoRI, researchers have access to limitless genetic combinations between organisms.

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