

Restriction Enzymes

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Slide 1

Bacteria are vulnerable to attack by bacteriophages (viri that attack bacteria). A virus consists of a DNA molecule packaged in a protein capsule. When a virus attacks a bacterium, it injects its DNA into the bacterium. The viral DNA is then replicated within the bacterium (making copies for more viri), and genes in the viral DNA are expressed to produce the proteins required for new copies of the virus. The bacterium is eventually destroyed, and copies of the virus are released. Bacteria have evolved a defense against viral attack. They are able to produce proteins called restriction endonucleases, which are enzymes that are able to cut DNA strands into smaller pieces. If a viral DNA molecule is cut by a bacterium's restriction endonuclease, the DNA will not function, and the virus will not destroy the bacterium. Bacteria protect their own DNA from being cut by the endonucleases by methylating their DNA.

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A DNA molecule consists of two polynucleotide strands. Each strand is a long sequence of nucleotides held together by phosphodiester bonds between sugar and phosphate components of adjacent nucleotides within the strand. These phosphodiester bonds form the sugar-phosphate backbone that holds a strand together along its length. The nitrogenous bases (A, C, G, & T) are not involved in the sugar-phosphate backbone, but they form hydrogen bonds with the nitrogenous bases in the other strand to hold the two strands together. The sequence of nitrogenous bases serves as the code contained in DNA. Each restriction endonuclease is able to recognize a specific sequence of nitrogenous bases and cut phosphodiester bonds between nucleotides within that sequence.

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A replicating DNA strand is shown. The two strands are arranged in an antiparallel orientation, with the 3' end of one strand matched up with the 5' end of the other strand, and vice versa. Whether DNA is being replicated (shown here) or transcribed, the two strands must be separated, which is why it is important that the hydrogen bonds holding the two strands together are weak.

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The table shows just a sample of the many restriction endonucleases that have been discovered (and the bacterial species in which they were discovered). While restriction endonucleases are naturally used by bacteria to defend themselves against viri, biotechnologists exploit restriction endonucleases for many uses, including the engineering of plasmids. Each restriction endonuclease is able to recognize one specific and short sequence of nucleotides called a recognition sequence. Recognition sequences are palindromes, because the sequence in one strand is the same as the sequence in the other strand when read in reverse. When a restriction endonuclease recognizes a sequence, one cut is made in each of the two strands, breaking one piece of DNA into two smaller lengths. If both strands are cut at the same position, the resulting pieces will have blunt ends. If the two strands are cut at different positions, the resulting pieces will have sticky ends. Sticky ends are useful in engineering plasmids, because this gives biotechnologists a way to snip open a circular plasmid and insert a gene of interest into that plasmid.

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The pGLO plasmid is engineered to include two genes. The bla gene codes for a protein that gives the bacterium resistance to ampicillin. That gene is always turned on. The GFP gene codes for a protein that allows the bacterium to glow when exposed to ultraviolet light. The GFP gene includes a promoter region called araC, which controls whether the GFP gene is turned on. The GFP gene is tuned on only when the sugar, arabinose, is present. In the absence of arabinose, the GFP gene is not expressed, no green fluorescence protein is produced, and the bacterium does not glow.

Slide 6

A bacterium is shown taking up a pGLO plasmid via transformation. The transformed bacterium will have continuous resistance to ampicillin, but it will glow only if arabinose is present.