

Plasmids

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The Griffith experiment was important, because it provided scientific evidence for a particle (now known to be DNA) that is responsible for giving an individual its traits. In the experiment, two strains of bacteria were used. The S strain was virulent (meaning that it was dangerous to hosts invaded by the bacterium), and the R strain was nonvirulent (harmless). When Griffith injected mice with heat-killed virulent bacteria, the mice were unharmed, just as when mice were injected with the nonvirulent strain. But when heat-killed virulent bacteria were mixed with living nonvirulent bacteria, and the mixture was injected into mice, the mice died, and living virulent bacteria were found in the blood of the dead mice. This demonstrated that a substance (DNA) was transferred from the dead cells to the living cells, changing them into virulent bacteria. The process in which bacteria take up DNA from the environment and express the genes in that DNA is called transformation.

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A plasmid is a small, circular bit of DNA containing some small number of genes. It is much like the circular chromosome of a bacterium, only much smaller. The bacterial chromosome contains all the genes that code for proteins that are necessary for survival under normal circumstances. Plasmids represent DNA that has been taken up by the bacterium from the surroundings (or from a different bacterium). Plasmids therefore contain extra genes (beyond the genes in the chromosome) that allow the bacterium to produce other kinds of proteins. This gives the bacterium extra abilities. A plasmid can be replicated just like the chromosome itself, so plasmids can be passed on to daughter cells during cell division. In some circumstances, a plasmid can insert itself into the chromosome, becoming part of that chromosome. This process is called integration.

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Plasmids occur naturally, but they can also be used in biotechnological applications. With modern technology, plasmids can be engineered to contain any gene of interest. Genes can be inserted into a plasmid with the help of a special type of enzyme called a restriction endonuclease. The engineered plasmids can then be used to transform bacteria for further study of the gene or for practical purposes (like creating oil-eating bacteria for cleaning up oil spills, for example). When working with an engineered plasmid, it is important to have a way of knowing whether the plasmid has successfully been taken up by the bacteria. Thus, in addition to the gene or genes of interest, a plasmid is engineered also to include some sort of marker gene that will give scientists a way of knowing that the plasmid is present in a cell. A commonly used marker gene is one that codes for a protein that gives the bacterium resistance to an antibiotic. An antibiotic is a substance that kills bacteria, but a bacterium with a resistance gene can survive the antibiotic. The resistance gene serves as a marker, because the antibiotic can then be used to separate non-transformed bacteria from transformed bacteria.

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In a typical transformation experiment, a sample of solution containing copies of the engineered plasmid is added to a suspension containing bacteria. The bacterial suspension is then exposed to a transformation solution (typically containing calcium chloride, CaCl_2) and to heating and cooling. This treatment maximizes the likelihood of bacteria in the suspension taking up the plasmid and becoming transformed. Even under ideal circumstances, just a small fraction of the bacteria become transformed. If a sample of the suspension is grown on a plate that does not contain antibiotic, both transformed and non-transformed bacteria will form colonies, and there is no way to tell them apart. However, if a sample of the suspension is grown on a plate that contains the antibiotic, only the transformed bacteria will survive and form colonies, because only transformed bacteria have the marker gene for resistance to the antibiotic.

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The ability of a bacterial cell to take up DNA via transformation is called its competence. Competence is maximized experimentally by using transformation solution, heating, and cooling. In this example, a pGLO plasmid is taken up by a competent *E. coli* bacterium. Since the pGLO plasmid also contains a gene for resistance to the antibiotic, ampicillin, a transformed cell will survive on a plate containing ampicillin, but a non-transformed cell will not survive. The pGLO plasmid is so named, because the gene of interest that the plasmid also contains is a gene that codes for green fluorescence protein (GFP), which allows any cell containing GFP to glow green when exposed to ultraviolet (UV) light.

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While bacteria are able to undergo transformation, eukaryotic cells are not, so plasmids cannot be used to change the DNA of eukaryotic cells. However, a similar biotechnological mechanism can be used to insert genes into eukaryotic cells. Instead of inserting the gene of interest into a plasmid, scientists can insert the gene into the DNA of a virus that is able to invade the recipient cell. When the virus invades the cell, the viral DNA (containing the engineered gene of interest) is injected into the cell.