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Science & Tech

recombinant DNA

genetic engineering



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Also known as: recombinant DNA technology

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Top Questions

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Recombinant DNA, [molecules](#) of [DNA](#) from two different [species](#) that are inserted into a host organism to produce new genetic combinations that are of [value](#) to [science](#), [medicine](#), agriculture, and industry. Since the focus of all [genetics](#) is the [gene](#), the fundamental goal of laboratory geneticists is to isolate, characterize, and manipulate genes. Although it is relatively easy to isolate a sample of DNA from a collection of [cells](#), finding a specific gene within this DNA sample can be compared to finding a needle in a haystack. Consider the fact that each human cell contains approximately 2 metres (6 feet) of DNA. Therefore, a small tissue sample will contain many kilometres of DNA. However, recombinant DNA [technology](#) has made it possible to isolate one gene or any other segment of DNA, enabling researchers to determine its [nucleotide](#) sequence, study its transcripts, mutate it in highly specific ways, and reinsert the modified sequence into a living organism.

DNA cloning

In [biology](#) a [clone](#) is a group of individual cells or organisms descended from one progenitor. This means that the members of a clone are genetically identical, because cell replication produces identical daughter cells each time. The use of the word *clone* has been extended to recombinant DNA technology, which has provided scientists with the ability to produce many copies of a single fragment of DNA, such as a gene, creating identical copies that [constitute](#) a DNA clone. In practice the procedure is carried out by inserting a DNA fragment into a small DNA molecule and then allowing this molecule to replicate inside a simple living cell such as a bacterium. The small replicating molecule is called a DNA vector (carrier). The most commonly used vectors are [plasmids](#) (circular DNA molecules that originated from [bacteria](#)), [viruses](#), and [yeast](#) cells. Plasmids are not a part of the main cellular genome, but they can carry genes that provide the host cell with useful properties, such as [drug resistance](#), mating ability, and [toxin](#) production. They are small enough to be conveniently [manipulated](#) experimentally, and, furthermore, they will carry extra DNA that is spliced into them.

Creating the clone

The steps in [cloning](#) are as follows. DNA is extracted from the organism under study and is cut into small fragments of a size suitable for cloning. Most often this is achieved by [cleaving](#) the DNA with a [restriction enzyme](#). Restriction enzymes are extracted from several different [species](#) and strains of bacteria, in which they act as defense mechanisms against viruses. They can be thought of as “molecular

defense mechanisms against viruses. They can be thought of as “molecular scissors,” cutting the DNA at specific target sequences. The most useful restriction enzymes make staggered cuts; that is, they leave a single-stranded overhang at the site of cleavage. These overhangs are very useful in cloning because the unpaired [nucleotides](#) will pair with other overhangs made using the same restriction enzyme. So, if the donor DNA and the vector DNA are both cut with the same enzyme, there is a strong possibility that the donor fragments and the cut vector will splice together because of the complementary overhangs. The resulting molecule is called recombinant DNA. It is recombinant in the sense that it is composed of DNA from two different sources. Thus, it is a type of DNA that would be impossible naturally and is an [artifact](#) created by DNA [technology](#).

The next step in the cloning process is to cut the vector with the same restriction enzyme used to cut the donor DNA. Vectors have target sites for many different restriction enzymes, but the most convenient ones are those that occur only once in the vector molecule. This is because the restriction enzyme then merely opens up the vector ring, creating a space for the insertion of the donor DNA segment. Cut vector DNA and donor DNA are mixed in a test tube, and the complementary ends of both types of DNA unite randomly. Of course, several types of unions are possible: donor fragment to donor fragment, vector fragment to vector fragment, and, most important, vector fragment to donor fragment, which can be selected for. Recombinant DNA associations form spontaneously in the above manner, but these associations are not stable because, although the ends are paired, the sugar-[phosphate backbone](#) of the DNA has not been sealed. This is accomplished by the application of an enzyme called DNA [ligase](#), which seals the two segments, forming a continuous and stable double helix.

The mixture should now contain a population of vectors each containing a different donor insert. This solution is mixed with live bacterial cells that have been specially treated to make their cells more permeable to DNA. Recombinant molecules enter living cells in a process called [transformation](#). Usually, only a single recombinant molecule will enter any individual bacterial cell. Once inside, the recombinant DNA molecule replicates like any other [plasmid](#) DNA molecule, and many copies are subsequently produced. Furthermore, when the bacterial cell divides, all of the daughter cells receive the recombinant plasmid, which again replicates in each daughter cell.

The original mixture of transformed bacterial cells is spread out on the surface of a [growth medium](#) in a flat dish (Petri dish) so that the cells are separated from one another. These individual cells are invisible to the naked eye, but as each cell undergoes successive rounds of [cell division](#), visible colonies form. Each colony is a cell clone, but it is also a DNA clone because the recombinant vector has now been amplified by replication during every round of cell division. Thus, the Petri dish, which may contain many hundreds of distinct colonies, represents a large number of clones of different DNA fragments. This collection of clones is called a [DNA library](#). By considering the size of the donor genome and the average size of the inserts in the recombinant DNA molecule, a researcher can calculate the number of clones needed to [encompass](#) the entire donor genome, or, in other words, the number of clones needed to [constitute](#) a genomic library.

Another type of library is a [cDNA library](#). Creation of a cDNA library begins with [messenger ribonucleic acid](#) (mRNA) instead of DNA. Messenger RNA carries encoded information from DNA to [ribosomes](#) for [translation](#) into [protein](#). To create a cDNA library, these mRNA molecules are treated with the [enzyme reverse transcriptase](#), which is used to make a DNA copy of an mRNA. The resulting DNA molecules are called complementary DNA (cDNA). A cDNA library represents a sampling of the transcribed genes, whereas a genomic library includes untranscribed regions.

Both genomic and cDNA libraries are made without regard to obtaining functional cloned donor fragments. Genomic clones do not necessarily contain full-length copies of genes. Furthermore, genomic DNA from [eukaryotes](#) (cells or organisms that have a [nucleus](#)) contains introns, which are regions of DNA that are not translated into protein and cannot be processed by bacterial cells. This means that even full-sized genes are not translated in their entirety. In addition, eukaryotic regulatory signals are different from those used by [prokaryotes](#) (cells or organisms lacking internal membranes—i.e., [bacteria](#)). However, it is possible to produce expression libraries by slicing cDNA inserts immediately [adjacent](#) to a bacterial

promoter region on the vector; in these expression libraries, eukaryotic proteins are made in bacterial cells, which allows several important technological applications that are discussed below in [DNA sequencing](#).

Several bacterial viruses have also been used as vectors. The most commonly used is the [lambda phage](#). The central part of the lambda genome is not essential for the [virus](#) to replicate in *Escherichia coli*, so this can be excised using an appropriate restriction enzyme, and inserts from donor DNA can be spliced into the gap. In fact, when the phage repackages DNA into its protein capsule, it includes only DNA fragments the same length of the normal phage genome.

[Vectors](#) are chosen depending on the total amount of DNA that must be included in a library. Cosmids are engineered vectors that are [hybrids](#) of plasmid and phage lambda; however, they can carry larger inserts than either pUC plasmids (plasmids engineered to produce a very high number of DNA copies but that can accommodate only small inserts) or lambda phage alone. Bacterial artificial chromosomes (BACs) are vectors based on F-factor (fertility factor) plasmids of *E. coli* and can carry much larger amounts of DNA. [Yeast artificial chromosomes](#) (YACs) are vectors based on autonomously replicating plasmids of *Saccharomyces cerevisiae* (baker's yeast). In [yeast](#) (a eukaryotic organism) a YAC behaves like a yeast chromosome and segregates properly into daughter cells. These vectors can carry the largest inserts of all and are used extensively in [cloning](#) large genomes such as the [human genome](#).

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