cDNA cloning

cDNA is a single-stranded segment of DNA that is complementary to the mRNA of a coding DNA segment or of a whole gene. It can be used as a probe (cDNA probe as opposed to a genomic probe) for the corresponding gene because it is complementary to coding sections (exons) of the gene. If the gene has been altered by structural rearrangement at a corresponding site, e.g., by deletion, the normal and mutated DNA can be differentiated. Thus, the preparation and cloning of cDNA is of great importance. From the cDNA sequence, essential inferences can be made about a gene and its gene product. <fn>Watson, J.D., et al.: Molecular Biology of the Gene, 3rd ed. Benjamin/Cummings Publishing Co., Menlo Park, California, 1987.</fn>

Preparation of cDNA

cDNA is prepared from mRNA. Therefore, a tissue is required in which the respective gene is transcribed and mRNA is produced in sufficient quantities. First, mRNA is isolated. Then a primer is attached so that the enzyme reverse transcriptase can form complementary DNA (cDNA) from the mRNA. Since mRNA contains poly(A) at its 3′ end, a primer of poly(T) can be attached. From here, the enzyme reverse transcriptase can start forming cDNA in the 5′ to 3′ direction. The RNA is then removed by ribonuclease. The cDNA serves as a template for the formation of a new strand of DNA. This requires the enzyme DNA polymerase. The result is a double strand of DNA, one strand of which is complementary to the original mRNA. To this DNA, single sequences (linkers) are attached that are complementary to the single-stranded ends produced by the restriction enzyme to be used. The same enzyme is used to cut the vector, e.g., a plasmid, so that the cDNA can be incorporated for cloning.

Cloning vectors

The cell-based cloning of DNA fragments of different sizes is facilitated by a wide variety of vector systems. Plasmid vectors are used to clone small DNA fragments in bacteria. Their main disadvantage is that only 5–10 kb of foreign DNA can be cloned. A plasmid cloning vector that has taken up a DNA fragment (recombinant vector), e.g., pUC8 with 2.7 kb of DNA, must be distinguished from one that has not. In addition, an ampicillin resistance gene (Amp +) serves to distinguish bacteria that have taken up plasmids from those that have not. Several unique restriction sites in the plasmid DNA segment where a DNA fragment might be inserted serve as markers along with a marker gene, such as the lacZ gene. The uptake of a DNA fragment by a plasmid vector disrupts the plasmid's marker gene. Thus, in the recombinant plasmid the enzyme ! -galactosidase will not be produced by the disrupted lagZ gene, whereas in the plasmid without a DNA insert (non recombinant) the enzyme is produced by the still intact lacZ gene. The activity of the gene and the presence or absence of the enzyme are determined by observing a difference in color of the colonies in the presence of an artificial substrate sugar. Beta-Glactosidase splits an artificial sugar (5-bromo-4-chloro-3-indolyl! D -galactopyranoside) that is similar to lactose, the natural substrate for this enzyme, into two sugar components, one of which is blue. Thus, bacterial colonies containing non recombinant plasmids with an intact lacZ gene are blue. In contrast,coloniesthathavetakenuparecombinant vector remain pale white. The latter are grown in a medium containing ampicillin (the selectable marker for the uptake of plasmid vectors). Subsequently, a clone library can be constructed. (Figure adapted from Brown, 1999).

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Only those bacteria become ampicillin resistant that have

incorporated a recombinant plasmid. Recombinant plasmids, which contain the gene for ampicillin resistance, transform ampicillinsensitive bacteria into ampicillin-resistant bacteria. In an ampicillin-containing medium, only those bacteria grow that contain the recombinant plasmid with the desired DNA fragment. By further replication in these bacteria, the fragment can be cloned until there is enough material to be studied. (Figures after Watson et al., 1987).

cDNA cloning principle