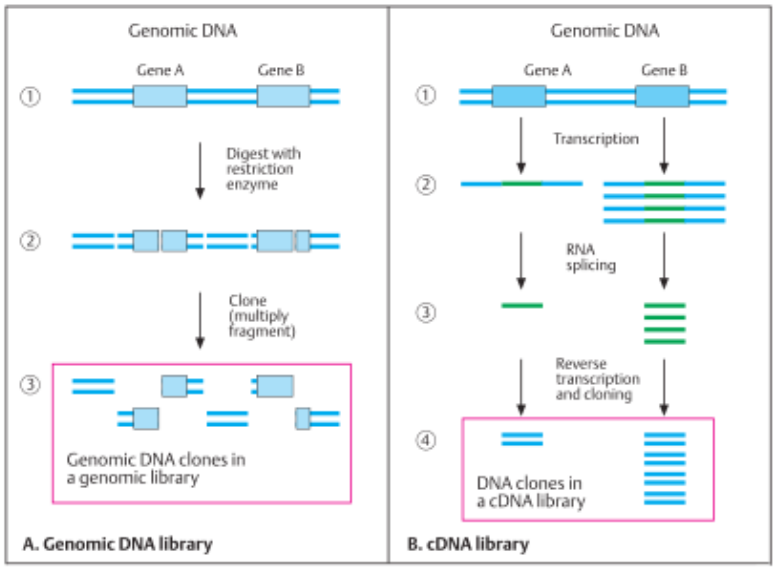
DNA Libraries

A DNA library is a collection of DNA fragments that in their entirety represent the genome, that is, a particular gene being sought and all remaining DNA. It is the starting point for cloning a gene of unknown chromosomal location. To produce a library, the total DNA is digested with a restriction enzyme, and the resulting fragments are incorporated into vectors and replicated in bacteria. A sufficient number of clones must be present so that every segment is represented at least once. This is aquestion of the size of the genome being investigated and the size of the fragments. Plasmids and phages are used as vectors. For larger DNA fragments, yeast cells may be employed. There are two different types of libraries: genomic DNA and cDNA.

Genomic DNA library

Clones of genomic DNA are copies of DNA fragments from all of the chromosomes (1). They contain coding and non coding sequences. Restriction enzymes are used to cleave the genomic DNA into many fragments. Here four fragments are schematically shown, containing two genes, A and B (2). These are incorporated into vectors, e.g., into phage DNA, and are replicated in bacteria. The complete collection of recombinant DNA molecules, containing all DNA sequences of a species or individual, is called a genomic library. To find a particular gene, a screening procedure is required (see B).



Genomic DNA and cDNA library

cDNA library

Unlike a genomic library, which is complete and contains coding and non coding DNA, a cDNA library consists only of coding DNA sequences. This specificity offers considerable advantages over genomic DNA. However, it requires that mRNA be available and does not yield information about the structure of the gene. mRNA can be obtained only from cells in which the respective gene is transcribed, i.e., in which mRNA is produced (1). In eukaryotes, the RNA formed during transcription (primary transcript) undergoes splicing to form mRNA. Complementary DNA (cDNA) is formed from mRNA by the enzyme reverse transcriptase (3). The cDNA can serve as a template for synthesis of a complementary DNA strand, so that complete double-stranded DNA can be formed (cDNA clone). Its sequence corresponds to the coding sequences of the gene exons. Thus it is well suited for use as a probe (cDNA probe). The subsequent steps, incorporation into a vector and replication in bacteria, correspond to those of the procedure to produce a genomic library. cDNA clones can only be won from coding regions of an active (mRNA-producing) gene; thus, the cDNA clones of different tissues differ according to genetic activity. Since cDNA clones correspond to the coding sequences

of a gene (exons) and contain no noncoding sections (introns), cloned cDNA is the preferred starting material when further information about a gene product is sought by analyzing the gene. The sequence of amino acids in a protein can be determined from cloned and sequenced cDNA. Also, large amounts of a protein can be produced by having the cloned gene expressed in bacteria or yeast cells.

Screening of a DNA library

Bacteria that have taken up the vectors can grow on an agar-coated Petri dish, where they form colonies (1). A replica imprint of the culture is taken on a membrane (2), and the DNA that sticks to the membrane is denatured with an alkaline solution (3). DNA of the gene segment being sought can then be identified by hybridization with a radioactively (or otherwise) labeled probe (4). After hybridization, a signal appears on the membrane at the site of the gene segment (5). DNA complementary to the labeled probe is located here; its exact position in the culture corresponds to that of the signal on the membrane (6). A probe is taken from the corresponding area of the culture (5). It will contain the desired DNA segment, which can now be further replicated (cloned) in bacteria. By this means, the desired segment can be enriched and is available for subsequent studies.

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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.

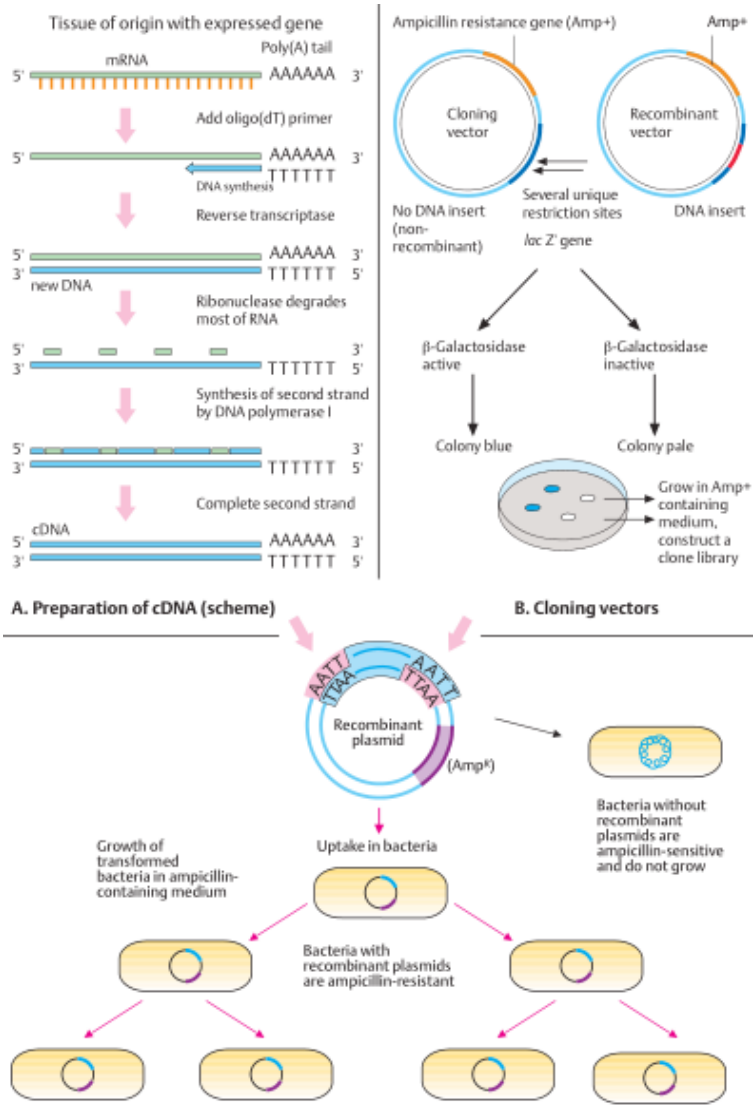
Cloning vectors

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).

cDNA cloning

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