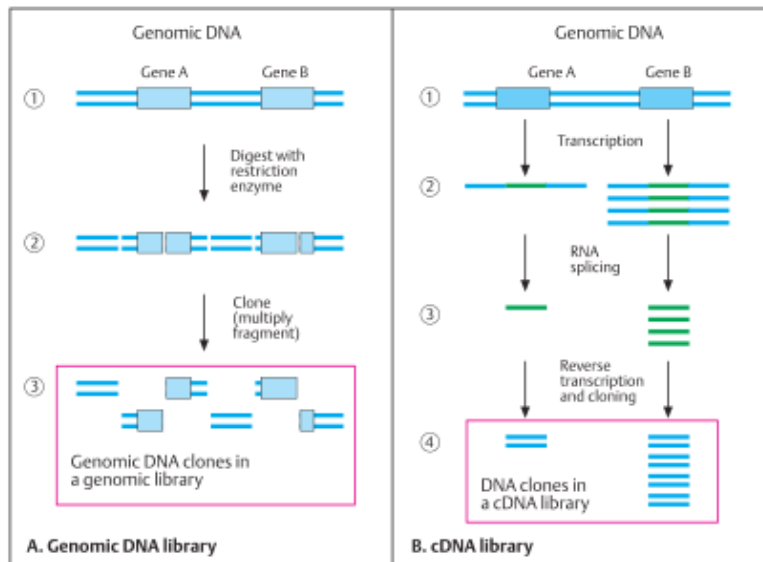


DNA Libraries

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 a question 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.