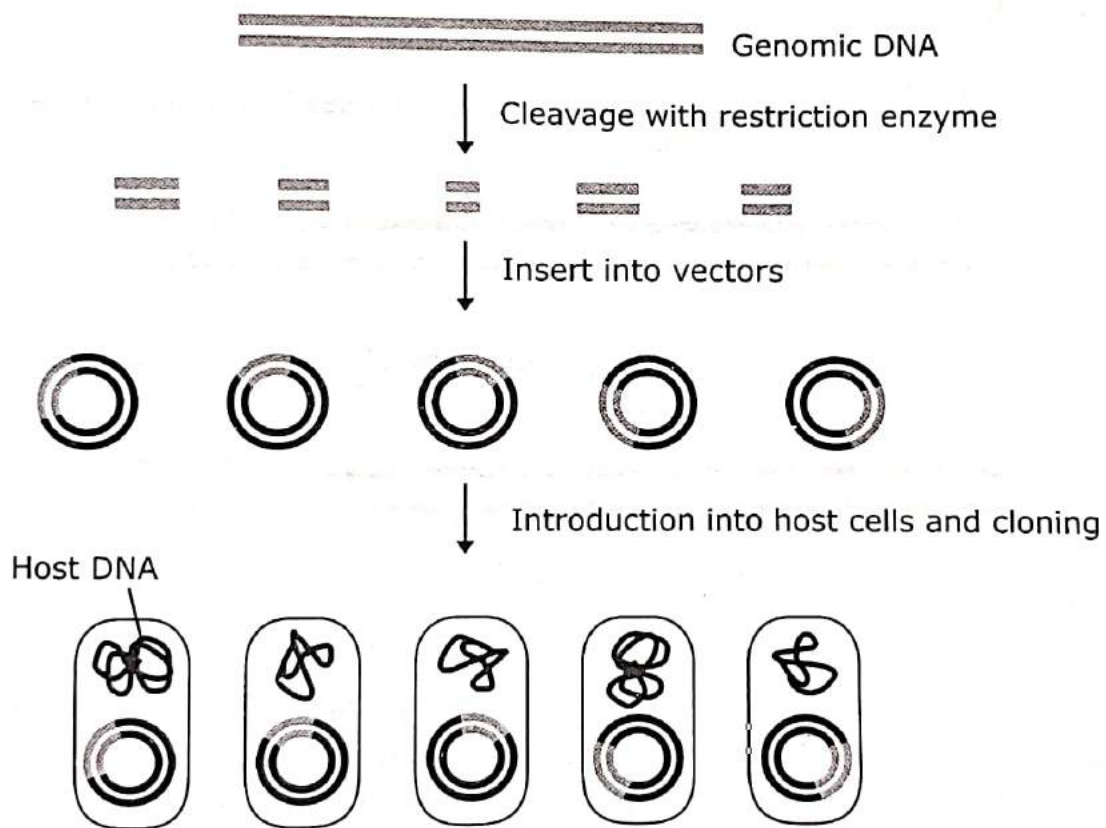


# DNA library (Genomic library)

A library encompassing an entire genome is called genomic library. The DNA is stored in a population of identical vectors, each containing a different insert of DNA. In order to construct a genomic library, the organism's DNA is extracted from cells and then digested with a restriction enzyme to cut the DNA into fragments of a specific size. The fragments are then inserted into the vector using DNA ligase. Next, the vector DNA can be taken up by a host organism - commonly a population of E. coli or yeast, with each cell containing only one vector molecule. Using a host cell to carry the vector allows for easy amplification and retrieval of specific clones from library for analysis.

There are several kinds of vectors available with various insert capacities. Some of the vectors commonly used for genomic libraries with its insert size are as follows -

<u>Vector</u>	<u>Insert size (in kilobase pairs)</u>
Plasmids	upto 10 <del>kb</del>
Phage lambda( $\lambda$ )	upto 25
Cosmids	upto 45
Bacteriophage	70 to 100
Bacterial artificial chromosome (BAC)	120 to 300
Yeast artificial chromosome (YAC)	250 to 2000



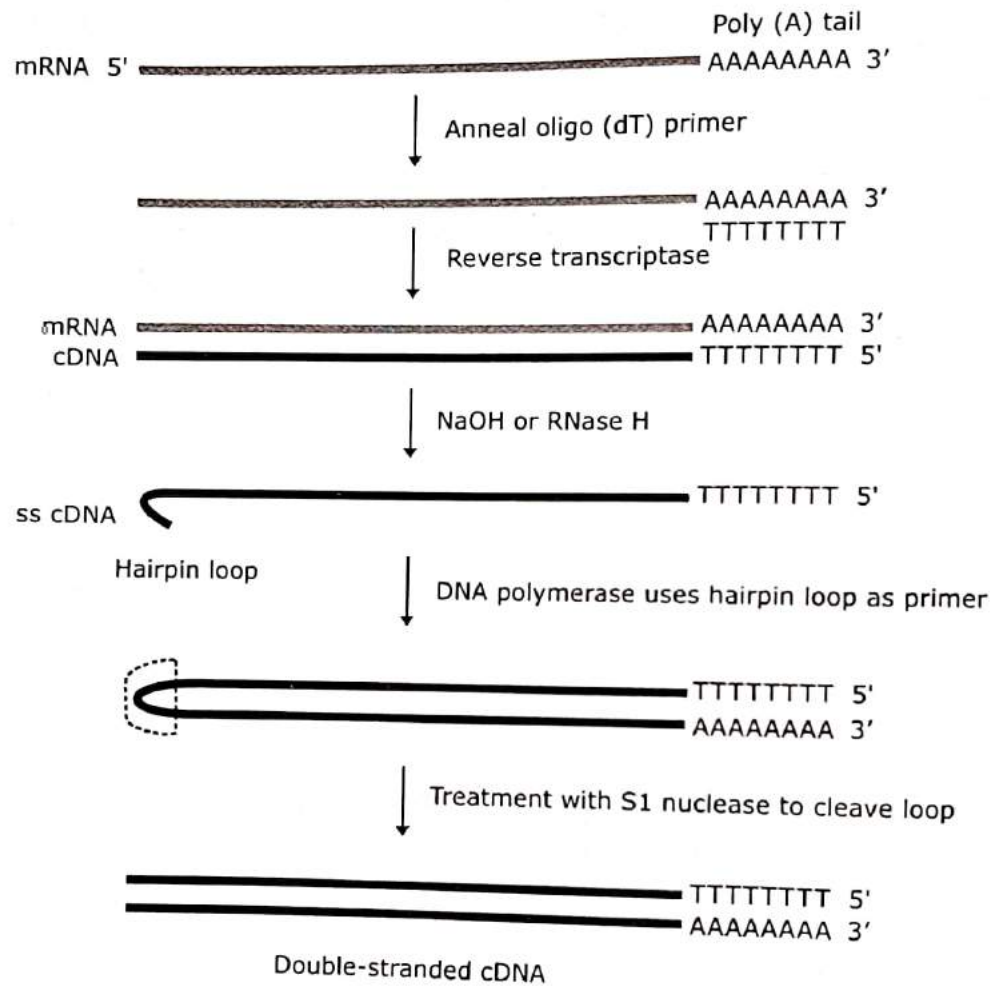
**Figure 2.19** A library of genomic clones.

(3)

Genomic libraries are commonly used for sequencing applications. They have played an important role in the whole genome sequencing of several organisms including the human genome and several model organisms.

### cDNA library

cDNA (complementary DNA) is synthetic DNA made from mRNA with the use of enzyme called reverse transcriptase. With the use of an mRNA as a template, reverse transcriptase synthesizes a single-stranded DNA molecule that can then be used as a template for double-stranded DNA synthesis. The double stranded cDNA molecules synthesized by the activity of reverse transcriptase and DNA polymerase can be inserted into a plasmid or virus vector and cloned. Each clone obtained in this way is called a cDNA clone, and the entire collection of clones derived from an mRNA constitutes a cDNA library. In eukaryotic cells, the mature mRNA is already spliced, hence the cDNA produced lacks introns and can be readily expressed in a bacterial cell. While information of cDNA libraries is a powerful and useful tool, the libraries lack information about enhancers, introns and other regulatory elements found in a genomic DNA library.

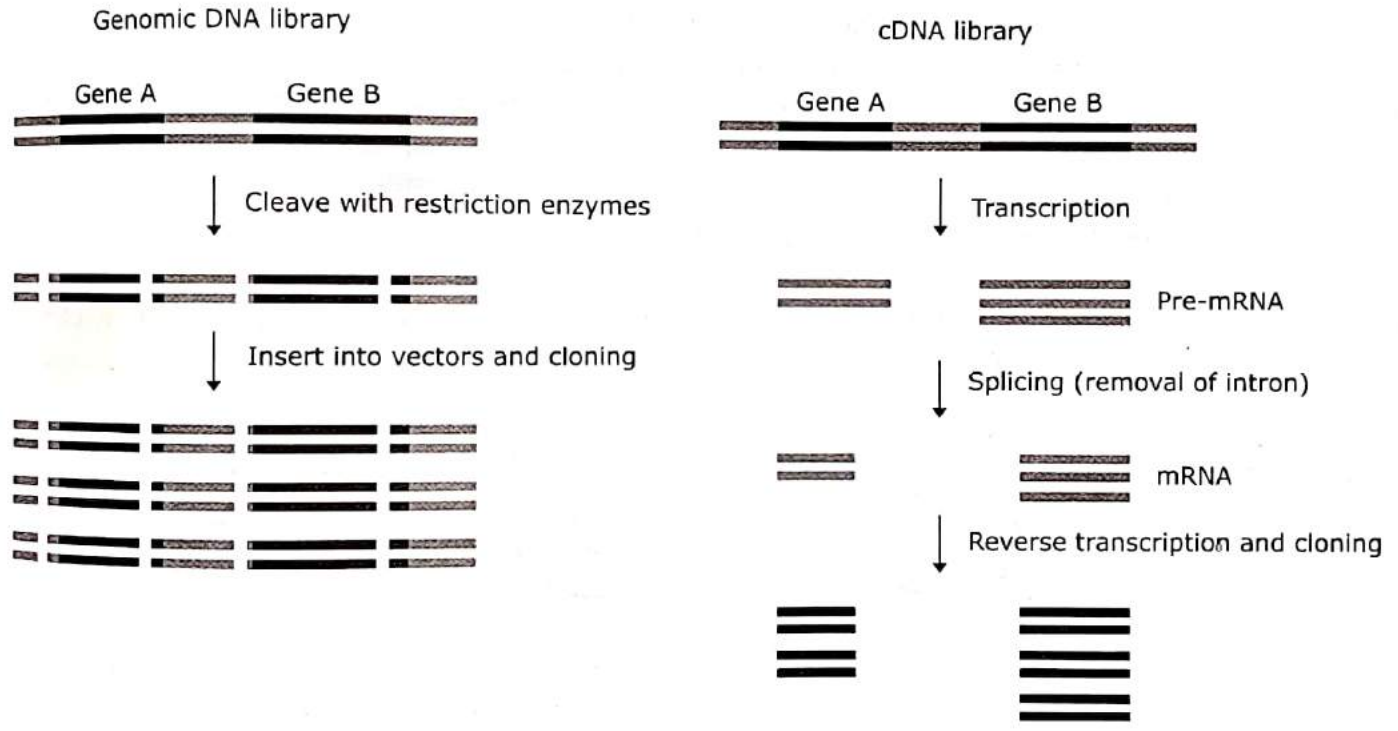


**Figure 2.20** The synthesis of double-stranded cDNA from mRNA. A short oligo(dT) chain is hybridized to the poly(A) tail of an mRNA strand. The oligo(dT) segment serves as a primer for the action of reverse transcriptase, which uses the mRNA as a template for the synthesis of a complementary DNA strand. The resulting cDNA ends in a hairpin loop. When the mRNA strand has been degraded by treatment with NaOH or RNase H the hairpin loop becomes a primer for DNA polymerase I, which completes the paired DNA strand. The loop is then cleaved by S1 nuclease (which acts only on the single-stranded loop) to produce a double-stranded cDNA molecule. Adapted from D. Watson, J. Tooze, and D. T. Kurtz, *Recombinant DNA: A Short Course*.

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### Differences between cDNA library and genomic DNA library

Unlike a genomic library, which is complete and contains coding and noncoding DNA, a cDNA library consists only of coding DNA sequences. This specificity offers considerable advantages over genomic DNA. In the figure 2.21, differences between cDNA library and genomic DNA library have been depicted. In this figure, gene A is infrequently transcribed while gene B is frequently transcribed, and both genes contain introns. In the genomic DNA clones both the introns and the non-transcribed DNAs are included, and most clones will contain only part of the coding sequence of a gene. In the cDNA clones, the intron sequences have been removed by RNA splicing during the formation of the mRNA, and a continuous coding sequence is, therefore, present.



**Figure 2.21** The differences between cDNA library and genomic DNA library.