GENE LIBRARY

A gene library is a collection of different DNA sequences present in an organism which is cloned in vector for ease of purification, storage and analysis. Just like a library of books, a good gene library represents all the genes present in the genome of the said organism.

Based on the type of DNA used for construction of library there are two types of libraries

1. Genomic Library
2. cDNA Library

A genomic library is a collection of clones sufficient in number to be likely to contain every single gene present in a particular organism. Genomic libraries are prepared by purifying total cell DNA, and then making a partial restriction digest, resulting in fragments that can be cloned into a suitable vector (Figure 8.5), usually a  replacement vector, a cosmid, or possibly a yeast artificial chromosome (YAC), bacterial artificial chromosome (BAC) or P1 vector.

Construction of Genomic Library

* Isolation of total genomic DNA from an organism and suitable vector DNA
* Restriction digestion of genomic DNA of organism and Vector with same restriction endonuclease to generate complementary cleavage site for ligation.
* Ligation of fragmented DNA in vector
* Transformation of Host cells (generally bacteria) with recombinant vector that may harbor rDNA.



In this figure construction of genomic library in a cosmid vector is shown. Total cell DNA is digested with Sau3A RE and digested fragments of approx. 35 Kb are inserted in cosmid vectors (Plz read about cosmids which was explained in theory class).

cDNA Library- This type of library is a collection of all the genes being expressed in a tissue at any particular stage of development. Thus it represents pool of total mRNA which formed in cell. Since mRNA are highly unstable, they are converted into cDNA and then inserted into a suitable vector.

Steps of cDNA library

* Isolation of total mRNA tissue
* Synthesis of cDNA from mRNA
* Ligation if cDNA into a vector
* Introduction in a host cell bacteria
* Screening and maintenance of clones



**Steps involved in cDNA synthesis**

* Isolation of mRNA from total RNA - mRNA has poly A tail at its 3’ end. Oligo dT beads are added to total RNA to fish out only mRNA. Due to mutual exclusive affinity, mRNA binds to the poly-T beads which is separated from rest of the RNA.
* An oligo dT primer is used with mRNA as template to prepare first strand of DNAwith the help of reverse transcriptase and dNTPs.
* After the synthesis of first strand, terminal transferase is used to add C nucleotides on 3‟of both mRNA and newly synthesized firsr strand of DNA. 
* DNA:RNA hydrid is treated with RNase H to produce nicks at multiple sites. Then DNA polymerase is used to perform DNA synthesis using multiple fragment of RNA as primer to synthesize new DNA strand. This method produces blunt end duplex DNA product.

**Screening of library to identify specific clones**

* Hybridization Probing-

Complementary nucleic acid strands hybridize to each other- Nucleic acid hybridization can be used to identify a particular recombinant clone if a DNA or RNA probe, complementary to the desired gene, is available.



A single stranded DNA probe can bind to single stranded clone. Similarly RNA can bind to denatured DNA in a clone.

* Colony and Plaque Hybridization probing- This technique is used to identify recombinant DNA molecule present in a bacterial colony or plaque formed by viruses.

Colonies or plaques are transferred on a nitrocellulose membrane and the they are treated with chemicals to remove all contaminants other than DNA. DNA also gets denatured and become single stranded. The nitrocellulose membrane is baked at 80oC to fix DNA

The probe (DNA ) is denatured, becomes single stranded, labeled with radioactivity or some enzyme and is applied to the membrane. The single stranded probe binds to the complimentary clone DNA present on membrane (hybridisation).

After a period to allow hybridization to take place, the filter is washed to remove unbound probe, dried, and the label detected in order to identify the colonies or plaques to which the probe has become bound.



**Application of GENE libraries**

**Genomic library**

* Required in DNA sequencing of genome of an organism
* Identification of genes which are silent in host
* Identification of Novel gene which are of interest
* Study the function of regulatory sequences
* To understand the complexity of a genome

cDNA library

* cDNA clones differ from genomic clones in lacking the introns present in split genes, and have the advantage of being capable to be expressed in bacteria, which do not have the machinery to process the eukaryotic mRNA.
* There are far less number of cDNA clones in a bank than in a genomic library, which makes easier to look for a desired gene.
* The cDNA contains sequence only for coding protein.
* Using the reverse transcriptase quantification PCR, the amount of the particular transcript or mRNA or the gene of our interest can be estimated.
* It is also used for gene cloning and transformation experiments.
* The cDNA library is also used in the expression of eukaryotic DNA into prokaryotes.